Idiopathic pulmonary fibrosis (IPF) is a chronic progressive and ultimately fatal lung disease of unknown aetiology and is reported to be refractory to the existing medical therapy (ATS, 2000). The poor prognosis and hence, unexpected fatality makes IPF among the dreaded pulmonary diseases in the present time. Moreover, IPF is cited as one of the most frequent interstitial lung diseases, which is characterized by the histological pattern of usual interstitial pneumonia (ATS, 2000).

In the extensive literature on idiopathic pulmonary fibrosis, controversy prevails as who first documented the disease. Hamman and Rich are generally recognized as the first to describe idiopathic pulmonary fibrosis as a new clinical and pathological entity. However, several earlier papers published in German have reported necropsy findings consistent with idiopathic pulmonary fibrosis from a contemporary point of view (Homolka, 1987).

Von Buhl (1872) argued that desquamative pneumonia is a distinct form of lung disease, with degeneration and desquamation of alveolar and bronchiolar epithelium. His histological findings included infiltration of the lung stroma by spindle and star cells (i.e., fibroblasts) and an excess of connective tissue. For more chronic changes he used the term ‘chronic interstitial pneumonia’. He considered tuberculosis or syphilis as the probable aetiological agent.

Rindfleisch (1898) published a conclusive necropsy finding of idiopathic pulmonary fibrosis in a 40-year-old clergyman who had progressively worsening cough and dyspnea. He noticed a hypertrophied right ventricle and small, stiff lungs without pleural adhesions. The lung interstitium contained an enormous amount of fibrous tissue with round cells, as well as multiple cystic spaces lined by a single layer of epithelium and connected to small bronchioli. The term he used to describe the fibrous and cystic changes was ‘cirrhosis cystic pulmonum’.

However, even though there are certain important findings reported by a myriad of scientists the most important contribution came from Hamman and Rich when they first
published their observations on idiopathic pulmonary fibrosis in the year 1933 (Hamman and Rich, 1933). A testimony to their growing experience with the disease was their additional publications for the next one decade or so (Hamman and Rich, 1935; Hamman and Rich, 1944). The main contribution of Hamman and Rich was the recognition and detailed description of the clinical and pathological features of a form of lung disease that they called ‘acute diffuse interstitial fibrosis of the lungs’. In their cases the disease was clinically characterized by extreme dyspnea, cyanosis and cough. Death occurred between 31 days and 24 weeks after the first admission to hospital. Histologic findings included alveolar edema, erythrocytes, hyaline membrane formation and cuboidal proliferation of alveolar cells. Interstitial infiltrates included occasional leukocytes and in three of four cases there were an excessive number of eosinophils. Excessive proliferation of fibrous tissue in the interstitium was described as a striking histologic feature in all cases. In some cases they also observed necrosis of alveolar and bronchiolar walls.

With further pathologic analysis, several distinct types of pulmonary fibrosis were described, and the terms diffuse fibrosing alveolitis, diffuse interstitial fibrosis and idiopathic pulmonary fibrosis were introduced to describe a more insidious, yet still debilitating form of chronic pulmonary fibrosis (Scadding and Hinson, 1967; Crystal et al., 1976). Currently, idiopathic pulmonary fibrosis is considered the most common and severe form of pulmonary fibrosis, with a startling median survival of nearly three years, with no proven effective therapy and with lung transplantation remaining the only viable intervention in end-stage disease (Raghu et al., 2011).

Moreover, it is now well recognized that the natural history of idiopathic pulmonary fibrosis is unknown and the onset of symptoms is gradual, starting usually with non-productive cough and exertional dyspnea. With involvement of larger areas of the lung, severe dyspnea at rest and signs of right heart failure develop (ATS, 2002). It has been reported that only in few cases the clinical state is preserved for a period of several years, but in majority of patients health deteriorate more rapidly and during acute exacerbation chances of mortality remain high (ATS, 2002). The prevalence of idiopathic pulmonary fibrosis is estimated at 20/100,000 for males and 13/100,000 for females, and survival time from diagnosis ranges from 2 to 4 years (Kim et al., 2006).
Because of stealthy rapid progression, most patients present at an advanced stage of disease. Histological characteristics include remodelling of lung architecture with fibroblastic foci and “honeycombing”. The lung involvement is patchy with a predominantly basal and subpleural pattern of matrix deposition and tissue distortion within the pulmonary interstitium, leading to impaired gas transfer and respiratory failure (ATS, 2002; Zhao et al., 2010). Treatment options for pulmonary fibrosis are limited. The clinical management focuses on treatment of complications (e.g. right heart failure, infections, etc.), supportive care and in few cases involves lung transplantation. Anti-inflammatory drugs such as prednisone may carry symptomatic relief, but they do not appear to halt progression of fibrosis and their beneficial effects in idiopathic pulmonary fibrosis remain in question. Cytotoxic drugs (cyclophosphamide, azathioprin, etc.) have not been shown to improve lung function or life expectancy and may be associated with harmful side effects (Moeller et al., 2008).

**Pathogenesis of pulmonary fibrosis**

The review of literature so far made it apparent that the underlying mechanisms for the onset and progression of fibrosis or even the natural history of idiopathic pulmonary fibrosis are poorly understood. Epithelial damage, tissue injury and inflammation are clearly involved in the initiation of repair, but how much these initiating events contribute to the chronicity and progression of disease is unknown (Moeller et al., 2006).

One hypothesis suggests a role for resident intrapulmonary fibroblasts, responding to a variety of stimuli such as transforming growth factor beta (TGF-β) and differentiating into myofibroblasts, with matrix deposition and scar formation as a result. Repeated microinjuries associated with inflammatory processes are thought to be triggers of progression of the fibrotic response (Ask et al., 2006). However, the importance of an ongoing inflammatory component raises questions since anti-inflammatory therapy evoked no significant reversal in the clinical setting associated with idiopathic pulmonary fibrosis (Cook et al., 2002). On the basis of the clinical and histopathologic findings in human disease, it is likely that inflammation is important but dispensable (Selman et al., 2002). However, the involvement of excessive extra-cellular matrix deposition and abnormal wound healing is widely accepted (Selman et al., 2001).

Several factors, including age, genetic susceptibility and environmental agents are known to contribute to lung fibrosis. There are other conditions that might also cause
lungen scarring. The lung insult as a result of other conditions is often referred to as “pulmonary fibrosis” and these conditions include the following:

- Diseases, like rheumatoid arthritis and sarcoidosis
- Medicines, such as those used for certain heart conditions
- Breathing in mineral dusts, such as asbestos or silica
- Allergies or overexposure to dusts, animals or moulds.

There are many names for this condition, such as “bird breeder’s lung,” “farmer’s lung,” or “humidifier lung.” These conditions are called hypersensitivity pneumonitis (Mannino et al., 1996; Baumgartner et al., 2000; Xaubet et al., 2003; Yang et al., 2007).

The suggested mechanisms involved in progressive fibrosis are summarized in figure 1.

![Figure 1. Schematic representation of pathways leading to the progression of fibrosis. Tissue damage initiates inflammation and repair, resulting usually in normal tissue. Repeat injury and inflammation can progress to excessive matrix deposition and scar. Other inflammation independent pathways may involve aberrant epithelial-fibroblast interactions, epithelial mesenchymal transition (EMT), mesenchymal precursor cells (MPC) or autocrine pathways of stimulation resulting in progressive scar formation [Adopted from Moeller et al., 2006].](image-url)

An increased interest exists in mesenchymal cells and in particular, in the fibroblastic foci that are associated with disease progression (Kasai et al., 2005). These fibroblastic foci are also associated with increased levels of active transforming growth factor beta (TGF-ß) in the fibrotic lung. This cytokine is an important mediator of fibroblast differentiation into the myofibroblast phenotype (Hashimoto et al., 2001). Over expression of active TGF-ß produces lung fibrosis in animals (Sime et al., 1997). TGF-ß
is a major regulator of wound repair and a stimulant of reactive oxygen species production in fibroblasts (Waghray et al., 2005). Oxidative stress is often defined as an imbalance between reactive oxygen species production and antioxidant defences. Oxidative stress can deregulate cell signalling (Rahman et al., 2006) and is a potential target for the development of therapeutics to treat lung fibrosis (Kinnula et al., 2005).

**Oxidative Stress and Lung Fibrosis**

Part of the altered alveolar environment in lung fibrosis involves oxidative stress that is driven by an imbalance between oxidant production and antioxidant defences. Reactive oxygen species are normal byproducts of cellular metabolism and are continually produced at low levels under basal conditions. Biologically, the reactive oxygen species superoxide (O$_2^-$) is commonly generated from the uncoupling of the cellular electron-transport systems (McCord and Fridovich, 1978). Superoxide can also rapidly react with nitric oxide (NO) to form the strong oxidizing and nitrating agent, peroxynitrite (ONOO$^{-}$). The reactive oxygen species hydrogen peroxide (H$_2$O$_2$) is generated directly from O$_2^-$ through a rapid dismutation reaction that can occur either enzymatically with superoxide dismutases or spontaneously. This means that wherever O$_2^-$ is generated, formation of H$_2$O$_2$ also occurs. In addition, H$_2$O$_2$ is formed enzymatically as a byproduct of lipid metabolism in peroxisomes (Langan et al., 2006). H$_2$O$_2$ is stable at biologic pH and easily crosses lipid membranes. Hydrogen peroxide can participate in hydroxyl radical (HO$^\cdot$) formation in the presence of metals (Gutteridge, 1994). H$_2$O$_2$ readily reacts with thiol functional groups and this type of reaction is proposed to be a key mechanism by which reactive oxygen species modulate cell-signalling events (Dickinson and Forman, 2002).

The impact of reactive oxygen species may be especially important in the lung because of its large surface area and its exposure to higher oxygen levels than other tissues. The lung counters this with a formable array of antioxidant defense systems, starting with high levels of antioxidants in the epithelial lining fluid (Cantin et al., 1987). Glutathione is a major water-soluble antioxidant thiol in the lung epithelial lining fluid (Cantin et al., 1987), and its levels are lower in subjects with idiopathic pulmonary fibrosis (Cantin et al., 1989). The lung also has a number of antioxidant enzyme systems including superoxide dismutase, catalase and glutathione peroxidase (Rahman et al., 2006). Overexpression of many of these antioxidant enzyme systems is protective against lung...
fibrosis (Gao et al., 2008). Many of these antioxidant systems are upregulated during lung fibrosis via the nuclear factor erythroid 2–related factor 2 (Nrf2) a redox-sensitive transcription factor (Itoh et al., 1999) that when deficient, enhances lung fibrotic responses (Cho et al., 2004). It is likely that inadequate antioxidant adaptive responses play a key role in lung fibrosis.

When reactive oxygen species production and antioxidant defences are mismatched, an increase in reactive oxygen species steady state levels leads to an increase in the oxidation of cellular macromolecules. Reactive oxygen species are difficult to measure directly and often are assessed by measuring oxidative footprints in fluids and tissues, such as markers of protein, lipid and DNA oxidation. Subjects with idiopathic pulmonary fibrosis have increased levels of oxidized proteins in their epithelial lining fluid that correlate with the percentage of neutrophils in the epithelial lining fluid (Behr et al., 1991; Lenz et al., 1996; Rottoli et al., 2005). Idiopathic pulmonary fibrosis subjects are also reported to have lower antioxidant capacity in their epithelial lining fluid than do healthy subjects (Rahman et al., 1999). Moreover, IPF subjects have higher levels of exhaled ethane (a marker of lipid peroxidation) than do normal subjects and these levels are inversely correlated with partial pressure of oxygen (PaO₂) in arterial blood (Kanoh et al., 2005).

**Animal models of pulmonary fibrosis**

Animal models play an important role in the investigation of diseases and many models are established to examine pulmonary pathobiology. Nevertheless, chronic diseases are more difficult to model. The situation with idiopathic pulmonary fibrosis is even more complicated, since the etiology and natural history of the disease is unclear and no single trigger is known that is able to induce “idiopathic pulmonary fibrosis” in animals. Different models of pulmonary fibrosis have been developed over the years. Most of them mimic some, but never all features of human idiopathic pulmonary fibrosis, especially the progressive and irreversible nature of the condition. A large majority of lung fibrosis animal models involve the overproduction of oxidants and the fibrotic effects are potentiated in antioxidant-deficient animals. However, a number of drugs are known to produce lung fibrosis in humans and animals (Zimmerman et al., 1984; Kehrer et al., 1986). Many of these drugs are chemotherapeutic agents that stimulate oxidative stress. Ionizing radiation is also a well-characterized method of producing lung fibrosis.
in animals, as well as a known adverse effect of cancer radiation treatment (Coggle et al., 1986). A number of environmental exposures produce lung oxidative stress and fibrosis, including exposure to asbestos and silica (Yamano et al., 1995; Gulumian, 1999). In addition, known cytokines, such as TGF-β, when overproduced, result in lung fibrosis. Most of these models have been shown to stimulate lung-injury responses and oxidative stress. An ideal animal model would mimic human disease as closely as possible, be highly reproducible and consistent, easy to perform, not too costly and widely accessible.

Advantages of animal models in general, as opposed to in vitro studies, are the ability to replicate the complex genetic, biochemical and environmental interactions in pulmonary fibrosis in lung tissue, whereas in vitro systems are limited to investigate specific cellular or molecular responses. However, no ideal experimental model of idiopathic pulmonary fibrosis, representing all aspects of human disease, exists. Major histologic and biochemical changes in fibrotic tissue can be replicated satisfactorily, whereas the slow progressive character of human disease, an aspect that is poorly understood, is more difficult to mimic. A major limitation with currently available animal models of lung fibrosis is that they do not closely mimic human interstitial pneumonias and many spontaneously resolve over time (Chua et al., 2005).

Common methods include radiation damage, instillation of bleomycin, silica or asbestos and transgenic mice or gene transfer employing fibrogenic cytokines.

**Irradiation model**

In humans, irradiation-induced lung fibrosis occurs as complication of treating thoracic malignancies such as esophageal and bronchial carcinomas, lymphomas or total body irradiation for bone marrow transplantation (Gross and Hunninghake, 2001). TGF-β has been shown to be involved in acute and chronic radiation-induced fibrosis (Epperly et al., 2006).

Ionizing radiation produces fibrotic responses and generates hydrogen atom radical H·, hydroxyl radical OH·, and hydrated electrons from the ionization of water in tissues. All three of these species are highly reactive and can generate and propagate a cascade of different reactive oxygen species mediated DNA damage and induction of TGF-β. Whole-body radiation decreases the levels of endogenous antioxidants and increases markers of lipid oxidation in animals and humans (Clemens et al., 1989; Arterbery et al.,
1994). Increased oxidative stress has been reported in radiation pneumonitis in humans (Jack et al., 1996) and in radiation-induced lung injury in rats (Vujaskovic et al., 2001; Fleckenstein et al., 2007). Several animal hemithoracic irradiation models of lung fibrosis have been developed and used to screen compounds for antifibrotic effects.

Both catalytic and scavenger antioxidants have been shown to attenuate radiation-induced lung injury and fibrosis in animals. Radiation-induced lung fibrosis is worsened in antioxidant-deficient animals (Thanislass et al., 1995; Epperly et al., 2000) and attenuated in superoxide dismutases-overexpression models (Malaker and Das, 1988; Epperly et al., 2000; Kang et al., 2003).

Irradiation models present a reliable tool for induction of fibrosis, as the radiation effect is dependent on dose and volume and therefore, predictable. The long period of time needed to see fibrotic changes comes close to human disease; however, this represents a practical limitation of the model (Moeller et al., 2006).

**Gene overexpression models - Fibrogenic cytokines**

A second approach to models of fibrosis involves transgenic modulation to produce animals with genetic defects, such as tissue specific overexpression of cytokines and growth factors or other extracellular matrix components, leading to downstream activation of specific cytokine pathways. Conventional constitutive transgenic models do not fit well with the adult nature of the human disease, so most useful data makes use of tissue specific inducible transgenic systems to provide temporal and spatial control over transgene expression, initiating or terminating it rapidly and reversibly. The most widely used inducible transgenic system for the lung is based on the tetracycline-controlled transcriptional regulator controlling pneumocyte specific gene promoter sequences (Zhu et al. 2002; Lee et al. 2003). Transient transgenic models, using adenoviral vector mediated cytokine gene transfer to bronchial, bronchiolar and alveolar epithelium, have been successfully developed (Sime, et al., 1997; Kolb, et al., 2001; Kolb et al., 2002) and can be applied to all ages of rodents (Gauldie et al., 2003). Both of these transgenic systems have provided data on several key molecular regulators of the fibrotic process, along with data showing other molecules which apparently do not directly contribute to the fibrotic process.
A number of cytokines have been shown to stimulate fibrotic events and include TGF-β, tumor necrosis factor alpha, platelet-derived growth factor, connective tissue growth factor, endothelin, granulocyte–macrophage colony-stimulating factor, interleukin (IL-1β), IL-6, IL-10, and IL-13 (Ask et al., 2006). The best studied of these various cytokines in lung fibrosis is TGF-β produced by a variety of different cell types namely thrombocytes, macrophages, lymphocytes, epithelial and endothelial cells and fibroblasts. TGF-β is secreted to the extracellular space in an inactive form, bound to latency associated peptide (LAP), which is also bound to latent TGF-β-binding protein (LTBP) and is directly connected to the extracellular matrix (Leitlein et al., 2001). Multiple enzymes, extracellular proteases or physical/chemical factors such as ionizing radiation, are able to activate TGF-β (Sheppard, 2001). TGF-β isoforms have a number of effects on cellular responses including modulating cell growth, migration, differentiation and apoptosis (Safayhi et al., 1985). TGF-β induces myofibroblast differentiation, extracellular matrix synthesis and inhibits extracellular matrix breakdown (Zhang and Phan, 1996). TGF-β1 is abundant in bronchoalveolar lavage fluid and present in fibroblastic foci biopsies from idiopathic pulmonary fibrosis subjects (Broekelmann et al., 1991). Overexpression of TGF-β1 in animals induces a progressive lung fibrosis that is largely independent of inflammation (Smith, 1971). TGF-β1 produces oxidative stress by the induction of reactive oxygen species production and a decrease in expression of cellular antioxidants (Koli et al., 2008). TGF-β1 induces reactive oxygen species production by activation of NADPH oxidases and through mitochondrial dysfunction (Thannickal and Fanburg, 1995; Sturrock et al., 2006). Very few studies have been reported on the effects of antioxidants in this relatively new animal model of lung fibrosis.

**Fibrogenic environmental agents**

A number of environmental dust and fibre exposures have been associated with the development of lung fibrosis (Mossman et al., 1991). Both silica and asbestos exposures produce lung fibrosis in animals (Driscoll et al., 1995; Kawanami et al., 1995) and pneumoconiosis in humans (Ross and Murray, 2004). Both silica and asbestos produce injury and oxidative stress in the lungs of animals leading to lung fibrosis (Abidi et al., 1999).
Several asbestos and silica induced animal models of lung fibrosis have been developed. Silica and asbestos-induced lung fibrosis are worsened in antioxidant-deficient animals (Lombard-Gillooly and Hubbard, 1993; Fattman et al., 2006) and attenuated in catalase overexpression models (Mossman et al., 1990). Catalytic and scavenger antioxidants have been shown to attenuate asbestos- and silica-induced lung injury and fibrosis in animals. Catalytic antioxidants have been shown to have protective effects against silica induced injury (Day, 2008).

**Fibrogenic drugs**

Paraquat is a redox-active herbicide that also is known to produce fatal pulmonary fibrosis in humans (Toner et al., 1970; Copland et al., 1974) and animals (Smith, 1971; Smith et al., 1974). Paraquat is thought to redox cycle with cellular enzymes to produce the paraquat cation radical that rapidly reacts with oxygen to form $\text{O}_2^-$ (Bus et al., 1976; Adam et al., 1990; Gray et al., 2007). Paraquat produces lung oxidative stress in animals (Kornbrust and Mavis, 1980; Brigelius et al., 1986; Dusinska et al., 1998; Wilhelm et al., 1999; Adachi et al., 2003) and humans (Minakata et al., 1993; Ishii et al., 2002). Both catalytic and scavenger antioxidants have been shown to attenuate paraquat induced lung injury and fibrosis in animals. Administration of superoxide dismutases has been shown to attenuate paraquat-induced lung injury in vitro (Ishii et al., 2002) and in vivo (Wasserman and Block, 1978; Ogata et al., 1994).

The antiarrhythmic drug amiodarone produces lung fibrosis in humans (Sobol and Rakita, 1982) and animals (Cantor et al., 1984). Some data suggest a role for oxidative stress in amiodarone-induced lung fibrosis. Amiodarone inhibits mitochondrial complex I and II respiration and produces mitochondrial dysfunction in lung epithelial cells and macrophages (Bolt et al., 2001). In the ventilated perfused rabbit lung system, amiodarone increases the levels of reactive oxygen species and oxidized glutathione (Kennedy et al., 1988). Further studies have revealed that amiodarone is metabolized to an aryl radical that may give rise to other reactive oxygen species (Vereckei et al., 1993; Nicolescu et al., 2007). Both catalytic and scavenger antioxidants have been shown to attenuate amiodarone-induced lung injury and fibrosis in animals.

**Bleomycin model**

Bleomycin, first discovered in 1962 is a glycosylated linear nonribosomal peptidechemotherapeutic antibiotic, produced by the bacterium “*Streptomyces*
verticillus” (Umezawa, 1967; Adamson, 1976). Its use in animal models of pulmonary fibrosis is based on the fact that fibrosis is one of the major adverse effects of bleomycin in human cancer therapy. Bleomycin plays an important role in the treatment of lymphoma, squamous cell carcinomas, germ cell tumors and malignant pleural effusion, where it is injected intrapleurally. It is believed that bleomycin acts by causing single and double-strand DNA breaks in tumor cells and thereby interrupting the cell cycle. This happens by chelation of metal ions and reaction of the formed pseudoenzyme with oxygen, which leads to production of DNA-cleaving superoxide and hydroxide free radicals (Claussen and Long, 1999). An overproduction of reactive oxygen species can lead to an inflammatory response causing pulmonary toxicity, activation of fibroblasts and subsequent fibrosis (Grande et al., 1998; Chaudhary et al., 2006). Bleomycin hydrolase, a bleomycin inactivating enzyme, critically influences the effects of this drug on different tissues. The lungs maintain low levels of the enzyme and therefore, are more susceptible to bleomycin-induced tissue injury (Sebti et al., 1989).

Pulmonary side effects in patients are dose-dependent, age-related and occur more often in the presence of pre-existing pulmonary diseases or smoking. Lung toxicity develops in approximately 10% of patients receiving bleomycin and is clinically associated with cough, dyspnea, fever, cyanosis and deterioration of lung function parameters. Within weeks to months this response might progress to pulmonary fibrosis in nearly 1% of patients (Compendium of Pharmaceuticals and Specialties, 2006).

Bleomycin as an agent to induce experimental lung fibrosis was first described in dogs (Fleischman et al., 1971), later in mice (Adamson and Bowden, 1974), hamsters (Snider et al., 1978) and rats (Thrall et al., 1979).

It causes inflammatory and fibrotic reactions within a short period of time, even more so after intratracheal instillation. The initial elevation of pro-inflammatory cytokines (interleukin-1, tumor necrosis factor-α, interleukin-6, interferon-γ) is followed by increased expression of pro-fibrotic markers (transforming growth factor-β1, fibronectin, procollagen-1) with a peak around day 14. The “switch” between inflammation and fibrosis appears to occur around day 9 after bleomycin challenge (Chaudhary et al., 2006).
It has been reported that histological hallmarks, such as intra-alveolar buds, mural incorporation of collagen and obliteration of the alveolar space are present in bleomycin-treated animals similar to idiopathic pulmonary fibrosis patients (Usuki, 1995). This observation has led to the assumption, that bleomycin reproduces typical features of the human disease and hence, the use of this model has become very popular. Further, the bleomycin model has the advantage that it is quite easy to perform, widely accessible and reproducible, and therefore, fulfils important criteria expected from a good animal model. Fairly consistent dosages have been established for each species to achieve a fibrotic response and dependent on the route of administration, different fibrotic patterns develop. Intratracheal instillation of bleomycin, the standard route of administration, results in bronchiocentric accentuated fibrosis, whereas intravenous or intraperitoneal administration induces subpleural scarring similar to human disease (Chua et al., 2005). The bleomycin model has contributed tremendously to elucidate the roles of cytokines, growth factors and signalling pathways involved in pulmonary fibrosis. For instance, it has helped to determine TGF-β as one of the key factors in the development of pulmonary fibrosis (Zhao et al., 2002).

However, despite undisputed qualities and some similarities in histological alterations, the bleomycin model has significant limitations with regard to understanding the progressive nature of human idiopathic pulmonary fibrosis. As mentioned, bleomycin causes an inflammatory response, triggered by overproduction of free radicals, with induction of proinflammatory cytokines and activation of macrophages and neutrophils, thus resembling acute lung injury in some way. The subsequent development of fibrosis, however, is at least partially reversible, independent from any intervention (Izbicki et al., 2002).

The above review of literature suggests that the standard pharmacological agent for induction of experimental pulmonary fibrosis in animals is bleomycin hence the same was used as an inducing agent to develop the animal model in the present study. A schematic representation of the major cellular events leading to lung fibrosis is depicted in figure 2.
Figure 2. Pictorial representation of sequence of events in bleomycin-induced pulmonary fibrosis. After administration of bleomycin, there is the onset of an acute inflammatory response lasting up to 8 days, followed by fibrogenic changes resulting in deposition of matrix and distortion of lung structure out to 28 or 35 days. Treatments during the first seven days would be considered “preventive” while treatments during the later stages after days 7–10 would be considered “therapeutic” [adopted from Moeller et al., 2008]

The entire current animal models of lung fibrosis have clear involvement of inflammation and reactive oxygen species in their pathogenesis. The evidence of inflammation and redox imbalance in lung fibrosis is substantial and thus the rationale for testing anti-inflammatory and antioxidants as potential new therapeutics for lung fibrosis is appealing. In the literature there are number of compounds which have antioxidant effect and have effectively attenuated the bleomycin induced fibrosis. Soumyakrishnan, and Sudhandiran, (2011) reported that the isoflavone daidzein possesses anti-fibrotic effect against bleomycin induced fibrosis in rats. The garlic
derived antioxidant diallyl sulfide effectively thwarted bleomycin induces pulmonary fibrosis (Kalayarasan et al., 2008). Also there are ample evidences of anti-inflammatory drugs attenuating the bleomycin induced fibrosis. Arafa and co-workers (2007) reported the anti-fibrotic action of meloxicam, a non steroidal anti inflammatory drug, in Swiss albino mice. Another anti inflammatory drug namely montelukast has been known to arrest the development of bleomycin-induced pulmonary fibrosis in mice (Shimbori et al., 2011). The dexamethasone, a corticosteroid and a drug used to reduce inflammation in many conditions was found to delay bleomycin-induced lung fibrosis in rats (Chen et al., 2006). An anti-inflammatory Chinese herbal formulation feining was reported to be effective against bleomycin-induced pulmonary fibrosis Sprague–Dawley rats (Liang et al., 2011).

The detrimental role of reactive oxygen species in many disease states has led to the development of new antioxidants. One such group of compounds with potential antioxidant property is the flavonoids present in fruits and vegetables, of which quercetin (3’,3’,4’,5,7-pentahydroxyflavone) (Figure 3) has attracted much attention for its beneficial health effects (Hollman and Katan, 1999; Skibola and Smith, 2000; Boots et al., 2008; Jagtap et al., 2009). Quercetin, a typical flavonoid ubiquitously present in fruits and vegetables, such as onion, tea, apples and berries. It exhibits antioxidative, anti-inflammatory and vasodilating effects, and has been proposed to be a potential anti-cancer agent (Erlund, 2004).

![Figure 3. Molecular structure of quercetin- 3,3’,4,5,7-Pentahydroxyflavone (from Kroon et al., 2004).](image-url)
Chemistry of quercetin

Flavonoids are characterized by 2 benzene rings (A and B) which are connected by an oxygen-containing pyrene ring (C). The three rings are planar and the molecule is relatively polarized. Three intermolecular hydrogen bonds are observed: two with the carbonyl group and the other between the hydroxyl groups in ring B (Mendoza-Wilson and Glossman-Mitnik, 2004). Analysis of the structure–activity relationships showed that the physical properties of quercetin are determined by its chemical structure. Quercetin can participate in the complex reactions with metals, affecting the transportation, reactivity, bioavailability and toxicity of metal ions. It possesses three possible chelating sites in competition which could be classified in the following way: catechol >α-hydroxycarbonyl>β-hydroxycarbonyl (Cornard et al., 2005).

Quercetin possesses all the structural elements characteristic of an anti-oxidant: (1) an ortho-dihydroxy or catechol group in ring B, (2) a 2, 3-double bond, and (3) the 3- and 5-OH groups with the 4-oxo group (Bors et al., 1990; Silva et al., 2002).

Pharmacokinetics of quercetin

At present, the pharmacokinetics of quercetin has not been fully characterized, although a number of studies have been carried out both in animals and humans. Flavonoid glycosides from diet are believed to pass through the small intestine be hydrolyzed to aglycone by enterobacteria in the caecum and colon and absorbed into epithelial cells via lipophilicity-dependent simple diffusion (Bokkenheuser et al., 1987). Quercetin glucosides can also be directly absorbed via the sodium-dependent glucose transporter-1 or excreted into the lumen via multidrug resistance protein 2 (Murota and Terao, 2003). After their facilitated uptake by means of carrier-mediated transport, quercetin glycosides are often hydrolyzed by intracellular β-glucosidases (Nemeth et al., 2003). The intestinal lactase phlorizin hydrolase displays a specific activity towards flavonoid glycosides (Day et al., 2000). Hydrolysis to aglycone by enterocytes or enterobacteria is crucial for the efficient absorption of quercetin glucosides in the intestinal tract (Nemeth et al., 2003).

Quercetin absorbed from the intestinal lumen is mostly converted to conjugated metabolites before entering circulation, and the major metabolites present in human plasma are quercetin 3′-O-β-D-glucuronide (Q3′GA) and quercetin 4′-O-β-D-glucuronide.
Some metabolites still possess considerable activity, including Q3GA, Q3’GA and Q4’GA (Williamson et al. 2005).

Regarding the tissue distribution, a recent study observed that quercetin is found concentrated in lungs, testes, kidneys, thymus, heart and liver, with the highest concentrations of quercetin and its methylated derivatives detected in the pulmonary tissue (de Boer et al. 2005).

Urinary elimination of quercetin is not the main excretion routes in human subjects or in rats. A substantial portion of the metabolites may be excreted in the bile (Murota and Terao, 2003). Quercetin can undergo microbial degradation in the colon to phenolic acids and CO$_2$, which is exhaled in the breath (Abrahamse et al. 2005).

Beneficial health effects of quercetin against various oxidative stress related diseases have been documented (Flora, 2009). However, studies examining its potential pneumoprotective effects are limited. Therefore, it was of interest to determine the ameliorative role, if any, of quercetin against bleomycin-induced lung injury because of the former’s potent antioxidant activity.

Further, it is suggested that along with reactive oxygen species, inflammation also plays an important role in the development of lung fibrosis. From the earliest descriptions of patients with pulmonary fibrosis, cellular inflammation in the lung parenchyma has been a consistent pathologic finding (Hamman and Rich, 1935 and 1944; Scadding and Hinson, 1967; Crystal et al., 1976). Histological analysis has shown varied accumulations of lymphocytes, macrophages, plasma cells, eosinophils and neutrophils, and the presence of lymphoid follicles with germinal centres has been observed in many patients in the lung interstitium (Scadding and Hinson, 1967; Crystal et al., 1976). Also, the forerunner for bleomycin-induced pulmonary fibrosis has been considered the inflammatory response induced by the anti-neoplastic agent (Arafa et al., 2007).

The non-steroidal anti-inflammatory drug (NSAID), sulindac ([Z]-5-fluoro-2-methyl-1-[p-(methylsulfinyl)-benzylidene]indene-3-acetic acid), is well known for its anti-inflammatory activity, which is due to its ability to inhibit the cyclooxygenases enzymes thereby inhibiting prostaglandin synthesis (Vane et al., 1998).
The mechanism of action of NSAIDs involves reduction of prostaglandin synthesis by inhibition of cyclooxygenase enzyme (COX) enzyme through competitive antagonism for arachidonic acid binding to the COX. For a drug to be an effective competitive inhibitor for arachidonic acid binding to COX, the drug must possess both high lipophilic and acidic properties to mimic the natural substrate chemistry. Sulindac possesses a polar group in the lipophilic tail (Vane et al., 1998). Moreover, sulindac is a sulfoxide prodrug, upon consumption it is converted into the metabolites (figure 4) sulindac sulfide and sulindac sulfone (Duggan et al., 1977).

![Figure 4. Metabolism of Sulindac to the sulphide and sulphone derivatives (Gurpinar et al., 2013)](image)

**Pharmacokinetics of Sulindac**

Since all NSAIDs are highly lipophilic substances, members of the class share similar, if not identical, absorption properties. Drug absorption after oral administration is generally rapid and complete. Sulindac, and its sulfone and sulfide metabolites, are bound to plasma proteins, predominantly to albumin. In humans, after oral intake of sulindac, peak plasma concentration of this drug is reached after 1 h and after 2 to 4 h for its sulfide and sulfone metabolites. All three forms of sulindac undergo varying degrees of entero-hepatic recirculation. There are little data regarding the distribution of sulindac into human tissues and fluids (Davies and Watson, 1997).
The original findings of cellular inflammation in the lung have been supplemented with an extensive accumulation of scientific studies which have implicated numerous inflammation-related cytokines and cell surface molecules in profibrotic mechanisms (Barnes and Adcock, 2009). Stemming from the observations on inflammatory cells, cytokines, chemokines, and cell surface molecules, and the inflammation hypothesis has dominated the field of pulmonary fibrosis for nearly four decades (Homer et al., 2011; Wynn, 2011; Crystal et al., 2002). Therefore, it was thought pertinent to study the modulatory role, if any, of sulindac, a known cyclooxygenases inhibitor which has also reported to have antiradical effects too (Fernandes et al., 2003) on belomycin-induced pulmonary fibrosis in rats.

Moreover, as it has been observed in many complex disorders, it is likely that combination of molecules, rather than a single drug, will be more effective as therapeutic agent. Therefore, the present study was extended to evaluate the inhibitory effects of quercetin in combination with sulindac on bleomycin induced rat pulmonary fibrosis as quercetin possess an antioxidant while sulindac is well known for its anti-inflammatory activity. To the best of our knowledge, so far no scientific data is available regarding the combined effects of quercetin and sulindac on experimentally induced pulmonary fibrosis.