2.1. PULMONARY EDEMA

Pulmonary edema is swelling and/or fluid accumulation in the lungs (Mattu et al., 2000; Ware et al., 2005) accompanied by severe respiratory distress, cold sweat, cyanosis, elevated blood pressure, palpitations and crackles over the lungs with oxygen saturation frequently less than 90% on room air prior to treatment (Perinea et al., 2003; Swedberg et al., 2005). It is defined as the transfer of fluid from intravascular compartment into the interstitium of the lungs and alveolus (Timothy et al., 2007), which occurs when the alveoli fill up with excess fluid seeped out of the blood vessels in the lung instead of air. Sometimes, this can be referred to as "water in the lungs" when describing the condition to patients.

Pulmonary edema, a major manifestation of left ventricular heart failure, renal insufficiency, shock, diffuse alveolar damage and lung hypersensitivity states, is a significant medical problem world-wide and can be life-threatening with a 12% in-hospital and 40% out-patient mortality (Roguin and Behar, 2000; Yang et al., 2010). In India pulmonary edema has turned into a major public hazard with incidences of respiratory problems are likely to increase in coming years due to increase in exposure to environmental pollutants and chemical accidents. Pulmonary edema can cause problems with the exchange of gas (oxygen and carbon dioxide), resulting in breathing difficulty and poor oxygenation of blood (Mattu et al., 2005; Swedberg et al., 2005).

2.2. TYPES OF PULMONARY EDEMA

Pulmonary edema is either cardiogenic, due to failure of the heart to remove fluid from lung circulation, or non-cardiogenic, which is due to direct injury to lung parenchyma.

2.2.1 Cardiogenic Pulmonary Edema

Cardiogenic pulmonary edema is also called as hydrostatic or high pressure pulmonary edema. It may be defined as the leakage of fluid from the pulmonary capillaries and venules into the alveolar spaces as a result of increased hydrostatic pressure due to an inability of the left ventricle to effectively handle its pulmonary venous return. Cardiogenic pulmonary edema may be caused by myocardial infarction, hypertension, valvular heart disease, and exacerbation of
chronic systolic or diastolic heart failure, cardiomyopathies. Occult diastolic dysfunction can also manifest as cardiogenic pulmonary edema.

Cardiogenic causes of pulmonary edema results from high pressure in the blood vessels of the lung due to poor heart function. As the heart fails, pressure in the vein going through the lungs starts to rise as a result of which fluid is pushed into the air spaces (alveoli) interrupting normal oxygen movement through the lungs, resulting in shortness of breath. This type of pulmonary edema is a complication of heart attack, leaking or narrowed heart valves (mitral or aortic valves), or any disease of the heart that either results in weakening or stiffening of the heart muscle i.e. cardiomyopathy (O'Brien and Falk, 2009). In cardiogenic pulmonary edema, a high pulmonary capillary pressure (as estimated clinically from the pulmonary artery wedge pressure) is responsible for the abnormal fluid movement (Adair, 2001).

Congestive heart failure due to poor heart pumping function (arising from various causes such as arrhythmias and diseases or weakness of the heart muscle), heart attacks, or abnormal heart valves can lead to accumulation of more than the usual amount of blood in the blood vessels of the lungs (Massie, 2011; National heart and lung disease, DCI, 2008). This can, in turn, causes the fluid from the blood vessels to be pushed out to the alveoli as the pressure builds up. Most patients with acute pulmonary edema are not acutely fluid overloaded and their sudden decompensation results from a combination of ventricular dysfunction and elevated peripheral vascular resistance.

2.2.2 Non-cardiogenic Pulmonary Edema

Non-cardiogenic pulmonary edema (NPE) is also called as acute respiratory distress syndrome (ARDS) or acute lung injuries (ALI) or permeability-induced pulmonary edema (PPE). NPE is defined as the radiographic evidence of alveolar fluid accumulation without hemodynamic evidence to suggest a cardiogenic etiology i.e., pulmonary wedge pressure of ≤18 mmHg (Givertz et al., 2005). As is the case with cardiogenic pulmonary edema, the accumulation of fluid and protein in the alveolar space leads to decreased diffusing capacity, hypoxemia, and shortness of breath. Fluid leak from the capillaries in lungs air sacs leads become more
permeable or leaky, even without the buildup of back pressure from heart (Bates, 2007; Luks, 2008). In that case, the condition is known as NPE because heart is not the cause of the problem (Fig. 2.1). It may also be caused by damage directly to the lung, such as that caused by poisonous gas or severe infection. This entity was first recognized and described by the military in relation to battlefield casualties in World War I and World War II (Perina, 2003).

NPE is caused by changes in permeability of the pulmonary capillary membrane as a result of a direct or an indirect pathologic insult (Parker and Yoshikawa, 2002). The alveolar-capillary membrane becomes damaged and leaky this allows water and proteins to move freely from the intravascular to the interstitial space. Thus, the concentration of protein is almost identical in these two compartments. This situation is in contrast to that of the patient with cardiogenic pulmonary edema, in whom the protein concentration in the interstitial fluid is substantially less than within the intravascular space (Fein et al., 1979). NPE is the most prominent feature of ARDS in which lungs suddenly fills with fluid and inflammatory blood cells.

![Fig. 2.1: Showing; A) normal lung, B) lungs with cardiogenic and C) non-cardiogenic pulmonary edema](image_url)

Fig. 2.1: Showing; A) normal lung, B) lungs with cardiogenic and C) non-cardiogenic pulmonary edema
2.2.2.1 Interstitial Pulmonary Edema
Interstitial edema is a condition of abnormally large fluid volume in the circulatory system or in tissues between the body's cells (interstitial spaces). Interstitial edema, may occur as a result of aberrant changes in the pressures (hydrostatic and oncotic) acting across the micro vascular walls, alterations in the molecular structures that comprise the barrier to fluid and solute flux in the endothelial wall that are manifest as changes in hydraulic conductivity and the osmotic reflection coefficient for plasma proteins, or alterations in the lymphatic outflow system (Dongaonkar et al., 2008). Excessive accumulation of interstitial fluid is generally viewed as detrimental to tissue function because edema formation increases the diffusion distance for oxygen and other nutrients, which may compromise cellular metabolism in the swollen tissue (Reed et al., 2010).

2.2.2.2 Clinical Pulmonary Edema
It is caused due to direct pathologic processes which lead injuries in the lung and alveolar epithelium. The major pathophysiologic abnormality is increased vascular permeability to proteins, resulting in protein-rich fluid accumulation in the alveolar air sacs (Perina, 2003). Oxygenation is further hampered by decreased surfactant production secondary to cellular damage causing respiratory distress that may progress rapidly to respiratory failure.

2.2.2.3 Sub-clinical Pulmonary Edema
Strenuous exercise may cause progressive and proportional hemodynamic overload damage to the alveolar membrane. Some studies demonstrated flood of the interstitial space and consequent increase in pulmonary water content, but most of them were able to show this through indirect signs of interstitial edema.

2.2.2.4 Hemorrhagic Pulmonary Edema
Hemorrhagic pulmonary edema is an acute, catastrophic event characterized by discharge of bloody fluid from the upper respiratory tract or the endotracheal tube massively in the lungs, and has a high mortality rate. Although the pathogenesis is uncertain, it is probable that in
hemorrhagic pulmonary edema the hematocrit is decreases in blood and the concentration of small proteins is higher than in plasma. Vasoconstriction due to hypoxia and hyper adrenergic state is among possible factors for the development of such edema (Marcherutiene et al., 2008). Subsequently, there is frank bleeding into the pulmonary interstitial and alveolar spaces. Contributing factors include factors that favor increased filtration of fluid from pulmonary capillaries (e.g., low concentration of plasma proteins, high alveolar surface tension, lung damage, hypervolemia). Immediate treatment of hemorrhagic pulmonary edema should include tracheal suction, oxygen and positive pressure ventilation.

2.3 CAUSES OF NON-CARDIOGENIC PULMONARY EDEMA

Non-cardiogenic pulmonary edema/ acute inhalation injury is not an uncommon condition. There are certain high risk groups but it may occur at various places including home or workplace. In addition to individual susceptibility, the characteristics of inhaled substances such as water solubility, size of substances and chemical properties may affect disease severity as well as its location. Inhaled substances may directly injure the pulmonary epithelium at various levels of the respiratory tract, leading to a wide range of disorders from tracheitis and bronchiolitis to pulmonary edema. They may also be absorbed, resulting in systemic toxicity (Gorguner and Akgun, 2010).

The causes of NPE are diverse and myriad. NPE can be caused by the different agents including drowning, acute glomerulonephritis, fluid overload, aspiration, inhalation injury, allergic reactions, inhalation of toxic gases, blood transfusion adverse reactions, non-thoracic trauma, smoke inhalation, severe infection, acute radiation pneumonia, exposure to petrochemicals, acute pulmonary infections, sepsis, and high altitude pulmonary edema (Bates, 2007; Goodman, 1996; Luks, 2008; Shaw and Ihle, 1997). NPE may also be caused by a group of medical and surgical disorders in which factors other than elevated capillary pressure are responsible for protein and fluid accumulation in the alveoli (De Nofrio and Evan, 1999).
2.3.1 Toxic Chemicals Inhalation
The inhalation of toxic gases or vapors is capable of resulting in pulmonary edema. Chemical agents which attack lung tissues, primarily causing pulmonary edema, are classified as lung-damaging agents (choking agents) because irritation of the bronchi, trachea, larynx, pharynx, and nose may occur. Chemicals such as ammonia, chlorine, hydrogen cyanide, methyl isocyanate (MIC), and phosgene are widely used in industry and frequently transported (Ciottone, 2008). Warfare agents (sulfur mustard, nitrogen mustard), blister agents and certain systemic agents may also injure the respiratory tract. Gases such as ammonia (NH₃) and hydrogen chloride (HCl) that are highly soluble in water and may be very irritating to tissues in the upper respiratory tract producing injury to lungs and the respiratory tract (Lillie, 2005). The inhalation of toxic gases or vapours with a caustic or irritant action, or containing particles, however, usually adds on an obstructive syndrome, similar to a severe asthmatic attack. The principal mechanism of pulmonary edema by the inhalation of toxic gases or vapors is related to an increase in alveolo-capillary permeability.

2.3.2 Smoke Inhalation
Smoke inhalation injury, either by itself or in the presence of a burn, is now well-recognized to result in severe lung-induced morbidity and mortality. The most common cause of death in burn centers is respiratory failure (Kinsella et al., 1991; Park et al., 2003; Traber and Pollard, 2002). Smoking continues to be a major cause of chronic obstructive pulmonary disease (COPD), as well as of many other pulmonary diseases. Smoke contains chemicals that damage the membrane between the air sacs and the capillaries, allowing fluid to enter the lungs.

2.3.3 Lung Infections
Lung injury is a broad descriptor that can be applied to conditions ranging from mild interstitial edema without cellular injuries to massive fatal destruction of the lung. Lung is the most susceptible organ to injuries in terms of high vasculature, large surface area and its direct interaction with the atmosphere (Chiang et al., 2011; Rahman et al., 2006). When pulmonary edema results from lung infections, such as pneumonia, the edema occurs only in the part of lung that is inflamed.
2.3.4 Kidney Diseases
When kidneys can not remove waste effectively, excess fluid can build up. Kidney failure and inability to excrete fluid from the body can cause fluid build-up in the blood vessels, resulting in pulmonary edema.

2.3.5 Adverse Drug Reactions
Many drugs ranging from narcotics such as heroin to diabetes medications and chemotherapy drugs are known to cause non-cardiac pulmonary edema. Aspirin overdose or chronic high dose use of aspirin can lead to aspirin intoxication, especially in the elderly, which may cause pulmonary edema. Narcotic overdose on heroin or methadone can lead to pulmonary edema within two hours of drug injection (Sporer, 1999).

2.4 UNUSUAL FORMS OF NON-CARDIOGENIC PULMONARY EDEMA
There are also some other unusual types of non-cardiogenic pulmonary edema, often with unclear pathophysiology, which have been described below:

2.4.1 Reperfusion Pulmonary Edema
Reperfusion pulmonary edema appears to represent a form of high-permeability lung injury that is limited to those areas of lung from which proximal thromboembolic obstructions have been removed. It may appear up to 72 hours after surgery and is highly variable in severity, ranging from a mild form of edema resulting in postoperative hypoxemia to an acute, hemorrhagic and fatal complication (Fremont et al., 2007; Visvanathan et al., 2005; Westreich et al., 2006).

2.4.2 Re-expansion Pulmonary Edema
The incidence of edema appears to be related to the rapidity of lung re-expansion and to the severity and duration of lung collapse. This may happen in cases when the lung collapses (pneumothorax) or a large amount of fluid around the lung (pleural effusion) is removed, resulting in rapid expansion of the lung which results in pulmonary edema on the affected side (unilateral pulmonary edema). Re-expansion pulmonary edema usually occurs unilaterally after rapid re-expansion of a collapsed lung in patients with a pneumothorax; it may also occur
following evacuation of large volumes of pleural fluid (>1.0 to 1.5 liters) and after removal of an obstructing tumor (Stawicki et al., 2008).

2.4.3 Acute Respiratory Distress Syndrome
Acute respiratory distress syndrome (ARDS) is an inflammatory disease initiated by a wide variety of systemic and/or pulmonary insults that leads to disruption of the alveolar-capillary unit and to a breakdown in the barrier and gas exchange functions of the lung. Accumulation of pulmonary edema can result when either the net transcapillary filtration pressure or the permeability of the pulmonary microvascular endothelial barrier increases, such as in acute or chronic pulmonary hypertension or in acute respiratory distress syndrome.

2.4.4 High Altitude Pulmonary Edema
High altitude pulmonary edema (HAPE) can happen due to rapid ascent to high altitudes of more than 10,000 feet and develops within one to three days of a rapid transition from low to high altitude (Hultgren, 1996). The incidence and severity of this disorder depend upon the altitude the patient has achieved, the speed of ascent, age of the patient, and the amount of exercise performed. HAPE is commonly seen in climbers and skiers who ascend to high altitude without previous acclimatization. A working hypothesis of the etiology of HAPE suggests that hypoxia causes pulmonary vasoconstriction that is initially extensive but not uniform. The result is over perfusion of the remaining patent vessels, with transmission of the high pulmonary artery pressure to the pulmonary capillaries. Dilatation of the capillaries and high flow result in capillary injury, with leakage of protein and red cells into the alveoli and airways. Affected patients often have fever, tachypnea, fatigue, chest discomfort, cough, rales, dyspnea, and cyanosis.

2.4.5 Neurogenic Pulmonary Edema
Neurogenic pulmonary edema can occur due to variety of neurologic disorders and procedures, including head injury, intracranial surgery, grand mal seizures, subarachnoid or intracerebral hemorrhage, and electroconvulsive therapy (Simon, 1993). The clinical presentation is characterized by hypoxia, tachypnea, tachycardia, diffuse rales, and large amounts of frothy
sputum or hemoptysis (Kandatsu et al., 2005; Leal Filho et al., 2005). It is associated with intense and widespread activation of the sympathetic nervous system that induces remarkable hemodynamic alterations (e.g., systemic and pulmonary hypertension, with peripheral, pulmonary, and microvascular vasoconstriction). However, it is uncertain whether the hemodynamic changes produce a pulmonary capillary leak through pressure-induced mechanical injury to the pulmonary capillaries, or whether there is some direct nervous system control over pulmonary capillary permeability (Sedy et al., 2008). The neuro-effector site for neurogenic pulmonary edema appears relatively well established in regions about the caudal medulla, where nuclei regulating systemic arterial pressure and afferent and efferent pathways to and from the lungs are located.

2.4.6 Pulmonary Embolism
Pulmonary emboli can cause pulmonary edema by causing injuries to pulmonary and adjacent pleural systemic circulations, elevating hydrostatic pressures in pulmonary veins and/or systemic veins, and perhaps by lowering pleural pressure due to atelectasis. Pulmonary emboli may also decrease the exit rates of pleural fluid by increasing the systemic venous pressure (thereby hindering lymphatic drainage) or possibly by decreasing pleural pressure (thereby hindering lymphatic filling). The observation that majority of effusions following pulmonary emboli are exudates suggests an important role for vascular injury; however, approximately 20 percent of these effusions are transudates, suggesting that hydrostatic changes can also be important.

2.4.7 Pulmonary Hypertension
Pulmonary hypertension is a heterogeneous disease, in which cardiac (left heart failure, valvular abnormalities) and/or pulmonary diseases (COPD, hypoxia, pulmonary embolism) as well as primary pathological changes of the pulmonary vasculature lead to an elevation of pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP). It involves the vasoconstriction or tightening of blood vessels connected to and within the lungs which is characterized by an elevation of the arterial pressure and vascular resistance within the pulmonary circulation (Budhiraja et al., 2004). Vasoconstriction, vascular remodeling and thrombosis all contribute to increased pulmonary vascular resistance. Pulmonary hypertension is associated with lung disease
and/or hypoxemia COPD, interstitial lung disease, sleep-disordered breathing, alveolar hypventilation disorders, chronic exposure to high altitude, and developmental abnormalities (Chaouat et al., 2005; Humbert et al., 2004). Pulmonary hypertension is caused by a variety of acute and chronic pulmonary diseases with an increase in pulmonary arterial pressure. In contrast to secondary pulmonary hypertension, primary pulmonary hypertension is not related to a known underlying disease. The most common clinical signs of pulmonary hypertension are dyspnea and fatigue (Rich et al., 1987).

2.5 SIGN AND SYMPTOMS OF NON-CARDIOGENIC PULMONARY EDEMA

Non-cardiogenic pulmonary edema is characterized by symptoms like extreme shortness of breath or difficulty in breathing, feeling of suffocation, "air hunger" or drowning, wheezing or gasping for breath, anxiety, restlessness, excessive sweating, a sense of apprehension, cough that produces frothy sputum that may be tinged with blood, excessive sweating and pale skin (Sartori, 2010). A classical sign of NPE is the production of pink frothy sputum which leads to coma and even death (Ahrens et al., 2008; Kim et al., 2012). In gradually developing pulmonary edema, symptoms like nocturia (frequent urination at night), ankle edema, orthopnea and paroxysmal nocturnal dyspnea occur. Additional symptoms that may be associated with this disease include, nasal flaring, coughing up blood, inability to speak and decrease in level of awareness.

A number of insults involving damage to the integrity of the alveolo-capillary membrane with subsequent fluid accumulation in the airspaces of the lung results in increased permeability to plasma. Fluid accumulated in alveolar spaces in lungs disrupts the function of pulmonary surfactant, which causes micro-atelectasis and impaired gas exchange (Grommes and Soehnlein, 2011). In early stages, the fluid retention is confined to the lower lobes. However, with advanced edema all lung lobes may be involved acquiring a rubbery gelatinous consistency (Cotran et al., 1998). Ultimately, regional variations in pulmonary perfusion, ventilation/perfusion (V/Q) mismatch with shunting of blood through unventilated alveoli, and an increased alveolar-arterial oxygen gradient occurs (Raja, 2007). Histologically, these changes have been termed diffuse alveolar damage of lung tissue which shows the edema fluid first accumulating in septal capillaries with widening of the septa. Pathologic sectioning of the lung shows free escape of a
mixture of air and fluid in the form of frothy, sanguineous fluid. Further progression in pulmonary edema involves proteinaceous fluid escaping into the alveolar sacs, which is no longer retained in the histologic section. The alveolar fluid appears as a granular pink coagulate (Fig. 2.2).

![Normal Lung and Edematous Lung](image)

**Fig. 2.2:** Lungs showing; A) normal lung and B) edematous lung

### 2.6 DIAGNOSIS

Prompt diagnosis of NPE is important to avoid life-threatening complications (Kim et al., 2012), because the treatment may vary considerably depending upon the pathophysiologic mechanism at work. The most common way of detecting lung abnormalities is with a standard chest x-ray. A better insight of pulmonary diseases can be achieved by computed tomography (CT) imaging. It is valuable for the evaluation of morphological changes and regional pulmonary functional tests. However, CT-imaging does not supply any functional information of the lungs and it exposes the body to ionizing radiation. Chest radiography and other tests are key to establishing the diagnosis and distinguishing between the two types of pulmonary edema i.e. cardiogenic and non-cardiogenic pulmonary edema (Fig. 2.3). The radiographic abnormality most characteristic of NPE is an increased interstitial or alveolar opacity, most commonly in the caudo-dorsal lung fields. This finding is in contrast to the opacity seen in radiographs of patients with cardiogenic pulmonary edema (Presley, 2006). The correct diagnosis relies on both clinical and radiological
findings despite some overlap in the clinical and imaging findings between the different causes. A complete physical examination should be performed, but extreme caution should be taken to avoid unnecessarily stress (Presley, 2006). Even when the history and physical examination are supportive of NPE, the diagnosis is often made based on radiographic findings. Blood cell count, serum chemistry profile, urinalysis, pulse oximetry, and blood gas analysis are also performed when the patient has been stabilized (Kakouros and Kakouros, 2003; Presley, 2006).

![Chest radiographs showing a) cardiogenic pulmonary edema and b) Non-cardiogenic pulmonary edema (Perina, 2003)](image)

2.7 PATHOPHYSIOLOGICAL MECHANISM

Non-cardiogenic pulmonary edema/ acute lung injuries is a broad descriptor that can be applied to conditions ranging from mild interstitial edema without cellular injury to massive and fatal destruction of the lung. Numerous underlying disease processes have been associated with NPE, including systemic inflammation and severe neurologic stimulation (Lee et al., 2006; McComb et al., 2008; Mangalmurti et al., 2008). NPE commonly develops within 24 h of onset of the initial insult or disease process, but presentation may be delayed up to 5 days (Perinea, 2003). The pathophysiology of this form of pulmonary edema is unknown; a combination of direct toxicity of the drug, hypoxia, and acidosis secondary to hyperventilation and/or cerebral edema has been proposed. Resolution of this form of pulmonary edema is rapid once hyperventilation and hypoxia are reversed through assisted ventilation.
A number of pathological conditions alter the integrity of alveolar epithelial barrier and lead to patient morbidity and mortality (Okudan et al., 2004; 2005). An initial and rapid increase in pulmonary vascular pressure due to pulmonary vasoconstriction or pulmonary blood flow can lead to pulmonary micro-vascular injuries leading to increase in vascular permeability which consequently results in edema formation. There are two major components that contribute to the pathogenesis of NPE; i) elevated intravascular pressure (> 25 mmHg) and, ii) pulmonary capillary leak (Adair, 2001). Therefore, both hemodynamic cardiogenic and non-cardiogenic components exist. These components often work in tandem, as in pulmonary edema after epileptic convulsions or intracranial pressure elevation. The hemodynamic component is relatively brief and may unmask pure NPE such as that seen in seizures. Whether the hemodynamic changes produce a pulmonary capillary leak through pressure-induced mechanical injury to the pulmonary capillaries or whether some direct nervous system control over pulmonary capillary permeability exist remains uncertain.

However, anything that increases oncotic pressure outside blood vessels (for example, inflammation), or reduces oncotic pressure in the blood (states of low plasma osmolality, for example, cirrhosis) will cause edema. Increased hydrostatic pressure inside the blood vessel (e.g., in heart failure) will have the same effect (McMurray et al., 2005). When permeability of the capillary walls increases, more fluid tends to escape out of the capillary, which can happen due to inflammation. The respiratory epithelium is a selectively permeable barrier separating the airways and air spaces from the sub-mucosa and interstitium of the lungs and the pulmonary vasculature. In acute lung injury the integrity of endothelium is damaged, and the endothelium-dependent relaxation is selectively injured (Imai et al., 2005). Alveolar fluid clearance mechanisms are inhibited in several models of lung injury even when the injury to the distal lung epithelium is not associated with gross disruption of the alveolo-capillary barrier (Olivera et al., 1994; Sznajder et al., 1995). The clinical scenario is very similar in most patients once membrane damage has occurred, regardless of the etiology. In the context of our study effect of inhaled irritant gases shall depend on the extent and duration of exposure, and on the specific agent. A complete understanding of the etiologies and mechanisms of development of NPE is necessary to help outline a proper prophylactic/ therapeutic plan for patients that may suffer this serious complication.
2.8 OXIDATIVE STRESS AND LUNG INJURIES

Inhaled lung toxicants cause tissue damage via diverse mechanisms, many of which involve activation of cell survival and apoptosis pathways. Tissue injury almost invariably leads to oxidative stress. One of the key areas of recent interest is the role that oxidative and nitrite stress plays in mediating the response to toxicants via the cytotoxic pathway. Oxidative and nitrite species can impact such pathways at several key points. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can modulate survival signaling molecules such as C-Jun N-terminal kinase and p38 mitogen activated protein (MAP) kinase (Aslan et al., 2008; Robert et al., 2009).

Reactive oxygen species have also been suggested to play a role in smoking induced COPD (Rahman and MacNee, 1996), and human lung fibroblasts recruit inflammatory cells by chemotactic activities in response to smoke extract (Sato et al., 1999). Increased oxygen burden in the lungs may arise due to accumulation of inflammatory cells in the lower respiratory tract, including macrophages and neutrophils. These cells show an exaggerated generation of O2^- and 'OH in patients with acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and pneumoconiosis (Crystal, 1991; Rahman and MacNee, 1996). Free radical reactions have been suggested to play a contributory role in the fibrogenesis either directly or through inflammatory responses (Poli and Parola, 1997).

Similarly RNS such as nitric oxide (NO) are able to cause DNA damage, leading to p53 stabilization and engagement of apoptosis pathways. There has been a considerable body of evidence over the last few years to suggest that oxidative stress associated with excessive production of ROS/ RNS is a fundamental mechanism of lung damage. Oxidative stress culminates due to an imbalance between prooxidants and antioxidants and consequent excessive production of ROS. Reactive oxygen species are biphasic, playing a role in normal physiological processes and are also implicated in a number of disease processes, whereby they mediate damage to cell structures, including lipids, membranes, proteins, and DNA (Folkerts et al., 2001). Furthermore, increased production of ROS by activated neutrophils and decreased antioxidant capacity has been suggested to play a central role in the pathogenesis of acute
respiratory distress syndrome (Chabot et al., 1998). However, in certain physiological processes, ROS have been shown to be beneficial and play a role in cell signalling, induction of mitogenic responses, immune defence, cellular senescence, apoptosis, and breakdown of toxic compounds (Bergendi et al., 1999).

2.8.1 Free Radical Damage
Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins, and enzymes present in the body. The lung is the only organ in the entire human architecture, which has the highest exposure to atmospheric oxygen. Therefore lung vasculature is a major target of oxidative stress, playing a critical role in the pathogenesis of acute lung injuries following pulmonary edema. The lung and respiratory system are particularly vulnerable to free radical damage for a number of reasons such as high concentrations of peroxidisable lipids, low levels of protective antioxidants, high oxygen consumption, and high levels of iron that act as pro-oxidant under pathological conditions. The membrane lipids in the lung contain high levels of polyunsaturated fatty acid side chains, which are prone to free radical attack, and are readily peroxidisable, contributing to structural and functional perturbations of the membrane and cell function (Rahman, 2006). The lung also consumes large quantities of total oxygen during the process of blood purification for its relatively small weight, contributing further to the formation of reactive oxygen species. It has been estimated that up to 2% of the oxygen consumed by healthy mitochondria is converted to superoxide, and this amount is higher in damaged mitochondria. The lung is therefore vulnerable to oxidant damage because of its location, anatomy and function (Crystal, 1991). Lung epithelium is constantly exposed to oxidants generated internally as a part of normal metabolism, as well as to oxidants in the ambient air, including; ozone, nitrogen dioxide, diesel exhaust and cigarette smoke (Li and Trush, 1994; Liu et al., 2009) (Fig. 2.4).
Intracellular reactive oxygen species:
$O_2^{•-}$ = superoxide anion, NO= nitric oxide, $H_2O_2$= hydrogen peroxide, $'OH$= hydroxyl radical, $NO_2$= nitrogen dioxide, $ONOO^-$ peroxynitrite, $Fe^{2+}$ = ferrous ion, GPx = glutathione peroxidase.

### 2.8.2 Reactive Oxygen Species

The main cellular sources of ROS in the lung include not only neutrophils, eosinophils and alveolar macrophages (Kinnula et al., 1995), but also alveolar epithelial cells, bronchial epithelial cells and endothelial cells (Holland et al., 1990; Kinnula et al., 1992a, b). The generation of ROS in lungs is enhanced after exposure to numerous exogenous chemical and physical agents, which include mineral dusts, ozone, nitrogen oxides, ultraviolet and ionizing radiation (Janssen et al., 1993), and tobacco smoke (Church and Prior, 1985). Oxidant stress can lead to peroxidation of membrane lipids, depletion of nicotinamide nucleotides, rise in intracellular calcium ions, cytoskeleton disruption, and DNA damage (Halliwell and Aruoma, 1991).
2.8.3 Reactive Nitrogen Species

Reactive nitrogen species (RNS) are also generated under normal physiological and pathological conditions. The nitric oxide radical (NO’) is generated in biological tissues by specific nitric oxide synthases (sNOS) (Ridnour et al., 2004). Generation of NO’ and O$_2^-$ favors the production of a toxic reaction product, peroxynitrite anion (ONOO’), a very powerful oxidant (Beckman and Koppenol, 1996). NO’ regulates neural signaling, blood pressure, smooth muscle relaxation and immune surveillance. NO’ is both aqueous and lipid soluble, readily diffusing through the cytoplasm and plasma membranes. During the inflammatory process, immune cells produce O$_2^-$ and NO’, which react to produce the peroxynitrite anion (ONOO’), a potent oxidising agent that can cause DNA fragmentation (Beckman, 1990; Sestili et al., 2000) and lipid peroxidation (Radi et al., 1991; Rubbo et al., 1994).

2.8.4 Role of Inflammation

Inflammation is a characteristic response to tissue injury and involves the release of a large number of mediators that not only increase microvessel permeability and cause vasodilatation, but also act to attract leukocytes to the damaged tissue. The abnormal inflammatory response of the lungs to noxious particles and gases produce many effects in the airways, including bronchoconstriction, plasma exudation, mucus secretion and attraction and activation of inflammatory cells that are in turn responsible for the function impairments experienced by the patients. Inflammation is one of the causes of pulmonary arterial endothelial cell injury and increase of pulmonary artery pressure. Secretion of inflammatory factors may lead to damage in permeability of lung vascular endothelial cells (Kluge et al., 2011). Respiratory diseases such as asthma, chronic pulmonary obstructive disease (COPD), fibrosis, acute lung injuries including pulmonary edema involve a complex interaction of many different inflammatory and structural cell types, all of which release a variety of mediators that are involved in the genesis of the clinical manifestations of the disorders.

Inflammation has been implicated in the pathogenesis of experimental pulmonary edema models and the recruitment of inflammatory cells appears to worsen lung injury (Iadecola and Anrather, 2011). Many studies demonstrate that pulmonary edema is associated with the infiltration of
inflammatory cells to the alveolar region (Terao et al., 2008). It is histologically characterized by the infiltration of leukocytes, mainly polymorphonuclear leukocytes, monocytes/macrophages and astrocytes (Pantoni et al., 1998). Activation of these resident cells population along with immune cells stimulates the production and release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 and the induction of iNOS and COX-2, and the activation of enzymes such as NADPH oxidase from the lung tissue (Carvalho-Tavares et al., 2011). In this inflammatory environment, type II endothelial cells increase their expression on cell surface adhesion molecules that mediate recruitment of leukocytes and platelets to the alveolar region (Stanimirovic et al., 1997). The hallmark of pulmonary inflammation is neutrophil infiltration (Weston et al., 2007), which are known to release injurious mediators (Tomita and Fukuuchi, 1996). They also contribute to ROS generation (’O2 & H2O2) via NADPH oxidase (Ellis et al., 1998). Ischemia- or sepsis-induced release of cytokines, such as interleukin-1 or tumour necrosis factor, may also play an important role in increasing in the pulmonary capillary permeability, at least in part via the recruitment of neutrophils.

Monocytes (white blood cells that become particle-ingesting macrophages once they enter another tissue) are the primary inflammatory cell type that infiltrates early atherosclerotic plaques. Oxidized LDL promotes endothelial cell damage and increases the expression of adhesion molecules on endothelial cell surfaces allowing circulating monocytes to attach. Adhesion of circulating monocytes to the surface of intact endothelial cells appears to be an early event in the development of edema.

Numerous cytokines involved in the activation of inflammatory cells and pathogenesis of lung fibrosis are transforming growth factor-β, tumour necrosis factor-α, platelet derived growth factor, insulin-like growth factor-1, endothelin-1, interleukin-1, interleukin-8, and interleukin-4 (Gharaeet-Kermani et al., 2001), while interferon-γ (Coker and Laurent, 1998) and one of its major inducers, interleukin-12, may have antifibrotic effects. These cytokines/ growth factors are directly or indirectly regulated by ROS. Hyperoxia is known to cause oxidant injury and fibrosis in animals, and in humans it has been implicated as one of the major reasons for bronchopulmonary dysplasia (Jobe and Bancalari, 2001), a chronic lung disease in preterm
infants, and adult respiratory distress syndrome (Matthay and Martin, 2010). These data provide direct support for the idea that macrophages and inflammatory mediators such as ROS and reactive nitrogen species can contribute to tissue damage in lung injury induced by pulmonary irritants.

Fig. 2.5: Showing architectures of lungs; a) Normal, and b) Acutely injured

Inflammation occurring at a single site causes leukocyte activation and release of numerous cytokines, oxygen metabolites, and other mediators of inflammation. These inflammatory mediators may trigger the activation of complement and coagulation cascades as well. As the inflammatory and coagulation cascades intensify, the imbalance of anti-inflammatory and anticoagulation factors may result in a global or systemic inflammatory process. As a result of this endothelial damage, an increased permeability of the capillaries allows for the flux of plasma proteins as well as inflammatory mediators into the alveolar and interstitial compartments of the lungs (Fig. 2.5). This flux of fluid results in pulmonary edema and, if severe, may cause an acute life-threatening pulmonary compromise known as acute respiratory distress syndrome or acute lung injury.
The development of lung inflammation is co-ordinated by the activation, expression and secretion of numerous pro-inflammatory mediators from the lung parenchyma and vascular cells including cytokines, leukotriens and adhesion molecules (Giulian et al., 1993; del Zoppo et al., 2000). Pro-inflammatory cytokines, such as TNF-α and IL-1β (Salminen et al., 1995), and anti-inflammatory cytokines, such as IL-6 and IL-10 (Vila et al., 2003; Sotgiu et al., 2006) are released by the injured lung cells (Fig. 2.6). These and other inflammatory mediators are released by phagocytic leukocytes and infiltrating macrophages in the lung (Benoit et al., 2008; Laskin and Laskin, 2001; Libby, 2007).

Fig. 2.6: Mechanism of inflammation of airway (Source; National Heart, Lung, and Blood Institute, 2007)

2.9 ANTIOXIDANT SYSTEM IN THE LUNGS
Antioxidants are exogenous (natural or synthetic) or endogenous compounds that reduce generation of ROS by acting in several ways including removal of \(^{1}O_2\), scavenging ROS or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation. Cellular antioxidant defence is classified into two categories: non-enzymatic and enzymatic (Table 2.1). Primary non-enzymatic antioxidants are vitamin C, vitamin E and ubiquinol etc., in addition to thiol containing antioxidants such as reduced glutathione (GSH), which directly scavenge ROS (Fig. 2.7). Enzymatic antioxidant enzymes include superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), and catalase.
Table 2.1: Cellular Antioxidant Defense Systems

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Cu-Zn SOD (cytosol), Mn SOD (mitochondria), GSH peroxidase, GSH-S- transferase, GSSG reductase, Catalase, Quinone reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repair System</td>
<td>Methionine sulphoxide reductase, DNA repair, Proteolysis of oxidised proteins, Phospholipase A2 acyl transferase</td>
</tr>
<tr>
<td>Ion sequestration</td>
<td>Transferrin, Ferritin, Lactoferrin, Ceruloplasmin, Metallothioneins</td>
</tr>
<tr>
<td>Small molecules</td>
<td>Ascorbate, GSH, Bilirubin, α- tocopherol, Ubiquinol, Urate, Carotenoids</td>
</tr>
</tbody>
</table>

Ideally, there is a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/quench these free radicals, preventing them from causing deleterious effects in the body (Nose, 2000). The antioxidant defence systems in the body can only protect the body when the amount of free radicals is within the normal physiological level. But when this balance is shifted more towards free radicals, it leads to oxidative stress, which may result in tissue injury and subsequent diseases (Finkel and Holbrook, 2000). There are various antioxidant defence mechanisms present in the lung such as superoxide dismutase, catalase, glutathione peroxidase, and other reductants (glutathione, ascorbate, and alpha-tocopherol). Reduced levels of antioxidants, which include decreased levels of reduced glutathione in the broncho-alveolar lavage fluid (Pacht et al., 1991), decreased levels of ascorbate and ubiquinol-10, presence of oxidized glutathione in alveolar fluid (Bunnell and Pacht, 1993; Cross et al., 1990), and elevated levels of hydrogen peroxide in expired gas and urine of acute lung injury patients (Mathru et al., 1994), indicate an increased oxidant stress and a compromised antioxidant system in patients with acute lung/ acute respiratory distress syndrome.
2.9.1 Superoxide Dismutase

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (Zelko et al., 2002). Superoxide (O$_2^-$) is the primary ROS and generates H$_2$O$_2$ by dismutation. Superoxide is produced in tissues via a number of enzymatic reactions and common cellular sources of O$_2^-$ include auto-oxidation of small molecules, including hemoglobin and myoglobin, mitochondrial components and oxidative enzymes, e.g. nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, cyclooxygenases, and oxidation of unsaturated fatty acids (Lenaz et al., 2002). SOD is present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). Reactive oxygen metabolites have long been implicated in the development of lung injuries. ROS are usually scavenged by antioxidant enzymes, primarily by superoxide dismutase (Fig. 2.8). Thus, SOD is an important antioxidant defense in nearly all cells exposed to oxygen (Warner et al., 2004). Based on the metal ion requirements and the anatomical distribution three major endogenous superoxide dismutases exist in mammalian lung cells: Copper–zinc superoxide dismutase (CuZn-SOD) (SOD1), magensse superoxide dismutase (Mn-SOD) (SOD2) and extracellular SOD (EC-SOD) (SOD3) (Chan, 1996). Cu, Zn-SOD over expression reduces endothelial damage resulting from lung injuries (Yang et al., 1994). Superoxide radicals (O$_2^-$•) generated during oxidative metabolism, are neutralized to water via a two-step process involving superoxide dismutase (SOD) in the first step, and both or either glutathione peroxidase (GPx) and catalase in the second step.

![Fig. 2.7: Oxidants and anti-oxidants system in lungs](image-url)
2.9.2 Lipid Peroxidation

Lipid peroxidation (LPO) is one of the major outcomes of free radical-mediated injury that directly damages biological membranes and generates a number of secondary products that may cause lung injuries (Halliwell and Chirico, 1993). Lipid peroxidation has been defined as the oxidative deterioration of polyunsaturated lipids, i.e. those lipids containing more than two carbon-carbon double covalent bonds (Aruoma et al., 1991) (Fig. 2.8).

Several experimental evidences indicate that extensive lipid peroxidation results in loss of membrane integrity, impairment of the function of membrane-transport proteins and ion channels, disruption of cellular ion homeostasis and eventual rupture leading to release of cell and organelle contents such as lysosomal hydrolytic enzymes (White et al., 2000). This process proceeds by free radical chain reaction mechanism. Increased nitric oxide concentrations associated with pulmonary edema may have dual effects on lipid peroxidation. Reaction of nitric oxide with superoxide causes the formation of peroxynitrite that initiates lipid peroxidation via reaction of lipids with its decomposition products, hydroxyl radical and nitrogen dioxide (Brookes et al., 1998). In contrast, nitric oxide itself may directly inhibit lipid peroxidation by intercepting alkoxy and peroxy radical intermediates thereby terminating chain propagation reactions (Nicolescu et al., 2002; Niziolek et al., 2003).

![Fig. 2.8: Basic mechanism of reactive oxygen species mediated lipid peroxidation and free radical scavenging](image-url)
2.9.3 Glutathione System

The glutathione system includes glutathione peroxidases, glutathione reductase, and glutathione S-transferase which is found in animals, plants and microorganisms (Creissen et al., 1996). The tripeptide of glutathione (GSH; γ-L-glutamyl-L-cysteinyl-glycine) is one of the most abundant intracellular thiol in cytosol, nuclei and mitochondria representing the major soluble antioxidant in these cell compartments (Meister, 1981). GSH is synthesized in cytosol by consecutive reactions of two enzymes: γ-glutamylcysteine (γGlu-Cys) synthetase and GSH synthetase. In nucleus, GSH maintains the redox state of critical protein sulfhydryls that are necessary for DNA repair and expression. Oxidized glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism (Chi et al., 2005). GSH plays an important role in the protection of cells from oxidative damage by reducing disulphide groups of proteins and other cellular molecules, or by scavenging free radicals and active oxygen species (McLennan et al., 1991). The product of the oxidation of GSH is glutathione disulfide (GSSG).

GSH has a major intracellular antioxidant activity of being involved in detoxification of peroxides and electrophilic toxins. Glutathione peroxidase is one of the most abundant antioxidant enzymes and is a very efficient scavenger of hydrogen peroxide. GSH is regenerated from GSSG within the cells in a reaction catalyzed by glutathione reductase (Fig. 2.9). This enzyme regenerates GSH by transferring reducing equivalent from NADPH to GSSG. NADPH regeneration during GSH redox cycling in the lung depends on NADPH–regenerating enzymes such as glucose-6-phosphate dehydrogenase (G6PDH).

2.9.4 Catalase

Catalase, an antioxidant enzyme like superoxide dismutase (SOD) and glutathione peroxidase is produced naturally within the body (Liddell et al., 2004). Catalase is one of the most efficient enzymes found in nearly all living organisms exposed to oxygen (del Rio et al., 1992); each catalase molecule can convert millions of hydrogen peroxide molecules every second. It catalyzes the decomposition of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani et al., 2004) (Fig. 2.9). Catalase also uses hydrogen peroxide to
break down potentially harmful toxins in the body, including alcohol, phenol, and formaldehyde. It also helps to prevent the conversion of hydrogen peroxide to hydroxyl radicals, potentially dangerous molecules that can attack on membrane, protein and damage DNA.

![Fig. 2.9: Anti-oxidant enzyme mediated ROS scavenging mechanism](image)

### 2.9.5 Nitric Oxide Synthase

Nitric oxide may also serve as an antioxidant against products of the Fenton reaction. Nitric oxide synthase (NOS), an enzyme that produces nitric oxide, is expressed in a number of lung pathologies, including acute lung injuries/edema (Agorreta et al., 2003). Nitric oxide (NO) is enzymatically synthesized from L-arginine, requiring a number of cofactors, namely, NADPH, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), tetrahydrobiopterin, as well as calmodulin (Moncada and Higgs, 1995). Molecular oxygen is also used in this reaction, which proceeds via the synthesis of N-hydroxyarginine and results in the formation of citrulline in addition to NO. The three isoforms of mammalian NOS: endothelial NOS, neuronal NOS and inducible NOS, are products of distinct genes and share 50-60% homology (Alderton et al., 2001). All three isoenzymes have been found in the lung (Guo, 1995; Kobzik, 1993; Xue, 1994) and mediate a wide variety of biological events in the respiratory system, such as bronchodilation (Nijkamp and Folkerts, 1995), pulmonary vasodilation (Adnot et al., 1995; Bigatello, 2000), and cytotoxic effects (Hibbs et al., 1988). The relevance of nitric oxide was increased with the report that diffusion-limited reaction between superoxide and nitric oxide
gives rise to peroxynitrite (Beckman et al., 1990). The highly reactive peroxynitrite provided a
deanistic basis for oxidative stress derived from increased nitric oxide production caused by
acute lung injuries (Kristof et al., 1998; Sittipunt et al., 2001). Nitric oxide has also been shown
to inhibit mitochondrial respiration via competition with oxygen for cytochrome oxidase (Brown
and Borutaite, 1999) and play a role in the initiation of apoptosis. Although little has been
reported on efforts to bring nitric oxide inhibitors to clinical investigation, there is no doubt that
nitric oxide plays a pivotal role in mediating oxidative stress (Mikkelsen and Wardman, 2003).

2.10 CURRENT TREATMENT OF NON-CARDIOGENIC PULMONARY EDEMA

Non-cardiogenic pulmonary edema is a medical emergency requiring immediate care. Although
pulmonary edema can sometimes prove fatal, the morbid effect is reduced when prompt
treatment for pulmonary edema is received along with therapy for the underlying problem. Most
patients with non-cardiogenic pulmonary edema have impaired ability to clear the edematous
fluid from lungs. There are currently no known measures to correct the permeability abnormality
in non-cardiogenic pulmonary edema; clinical management involves primarily supportive
measures to maintain cellular and metabolic function while waiting for the acute lung injury to
resolve. Mechanical ventilation is one of the important means to treat acute respiratory distress
syndrome/ acute lung injuries, but such patients have many complications, such as pulmonary
edema, loss of type I alveolar epithelium and uneven distribution of gas caused by alveoli
collapse. Additional measures include antibiotics for infection, maintenance of adequate
nutrition, and hemodynamic monitoring when necessary to guide fluid management and
cardiovascular support (Fulkerson, 1996). Several clinical studies have shown improved
pulmonary function and outcome in patients in whom the pulmonary artery wedge pressure falls
as a result of diuresis or fluid restriction (Simmons et al., 1987; Humphrey et al., 1990).

Pharmacologic therapy of non-cardiogenic pulmonary edema and ARDS has centered on
inhibition of the inflammatory process. Corticosteroids, antioxidants, ketoconazole, non-steroidal
anti-inflammatory (NSAID) drugs, prostaglandin E, anti-endotoxin and anti-cytokine therapy,
exogenous surfactant, and pentoxifylline have been examined in clinical trials (Anzueto et al.,
Administering oxygen is the first step in the treatment for pulmonary edema. Depending on condition and the reason for pulmonary edema, a patient may also receive one or more of the following medications:

- **Preload reducers**: Nitroglycerin and diuretics, such as furosemide (Lasix), to treat pulmonary edema. These medications dilate the veins in lungs and elsewhere in body, which decreases fluid pressure going into heart and lungs.

- **Morphine (Astramorph, Roxanol)**: This narcotic, for years a mainstay in treating cardiac pulmonary edema, may be used to relieve shortness of breath and associated anxiety. But some doctors believe that the risks of morphine may outweigh the benefits and that it is more appropriate to use other, more effective drugs.

- **Afterload reducers**: These drugs dilate the peripheral vessels and take the pressure load off the left ventricle. Some examples of afterload reducer medications include nitroprusside (Nitropress), enalapril (Vasotec) and captopril (Capoten).

- **Aspirin**: Aspirin helps in thinning the blood so that it moves through the small blood vessels more easily.

- **Blood pressure medications**: When high blood pressure exists consistently, the chances of development of pulmonary edema increases. Medication is required to manage high blood pressure.

### 2.10.1 Oxygen Supplementation

Oxygen supplementation may be provided in several ways. The easiest method is to administer oxygen by mask or to give flow-by oxygen, which is very useful for short periods, especially while performing a cursory physical examination at presentation. With this technique, high fractions (up to 100%) of inspired oxygen ($\text{FiO}_2$) may be reached with oxygen rates of 100 to 200 ml/kg/min (room air provides approximately 20% $\text{FiO}_2$).

### 2.10.2 Diuretics

Much debate has arisen concerning the use of fluids and diuretics as therapy for acute lung injuries. In patients with hemodynamic or cardiogenic pulmonary edema, the mainstay of therapy
following oxygen supplementation is a diuretic such as furosemide to help decrease both the overall fluid volume and the increased hydrostatic pressure in the pulmonary vasculature. Furosemide is also believed to directly affect the alveolar epithelium’s ability to pump fluid out of the airspace. Fluid administration is generally contraindicated in patients with hemodynamic or cardiogenic pulmonary edema, unless the patient is severely dehydrated. In non-cardiogenic edema, increased fluid volume resulting in increased hydrostatic pressure is not the mechanism that causes edema formation. Therefore, the use of diuretics such as furosemide in these patients may contribute to systemic hypovolemia, actually worsening the patient’s condition. In patients with severe endothelial damage, however, the oncotic pressure of the pulmonary capillaries is decreased as the result of protein leakage into the interstitial and alveolar compartments; therefore, the main determinant of net fluid flux is hydrostatic pressure (Bateman, 2003.). In other words, the amount of fluid exuding from the damaged capillary is determined by the overall volume of fluid passing through the vessel. For this reason, some clinicians advocate the use of furosemide in a low-dose (0.1 mg/kg/hr) constant-rate infusion to help resolve the edema (Shell, 2004).

2.10.3 Fluid Therapy

Fluid therapy in patients with NPE is also complex. Although crystalloids may be required to maintain the patient’s hydration status, most critical care experts recommend colloids e.g., hetastarch, for the treatment of NPE. Because most synthetic colloid particles are larger than albumin particles, colloids may act to increase the oncotic pressure in the pulmonary capillaries, resulting in decreased efflux of fluid into the interstitial spaces. However, if the pores in the damaged endothelium are large enough for the colloids to escape into the interstitium, administration of these products may confound the pulmonary edema significantly.

2.11 LIMITATIONS IN CURRENT THERAPY

Specific prophylactics to prevent chemically induced lung injuries due to respiratory airway toxins are not available at present. Analgesic medications, oxygen, humidification, and ventilator support currently constitute standard therapy. In fact, mechanical ventilation has remained the therapeutic mainstay for acute inhalation injury (do Pico, 1995; Matthay and Zemans, 2011). Hemorrhaging, signifying substantial damage to the lining of the airways in lungs can occur with exposure to highly corrosive chemicals and may require additional medical interventions.
Corticosteroids are sometimes administered, along with bronchodilators to treat bronchospasms. Several drugs that have been approved by the FDA for other indications hold promise for treating chemically induced pulmonary edema.

Drugs that reduce the inflammatory response, promote healing of tissues, and prevent the onset of pulmonary edema or secondary inflammation may be used following severe injury to prevent chronic scarring and airway narrowing (Johnson and Matthay, 2010). These include β2-agonists, dopamine, insulin, allopurinol, and non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen. Inhaled and systemic forms of β2-agonists used in the treatment of asthma and other commonly used medications, such as insulin, dopamine, and allopurinol have although been found to be effective in reducing pulmonary edema in animal models, but require further clinical studies. However, slow action, severe systemic effect on high doses, small fraction of aerosolized particles delivered to proximal airways, with even a smaller fraction reaching the distal airways are some of the main limitations of the currently available treatment for acute lung injuries.

2.12 PULMONARY DRUG DELIVERY

2.12.1 Basic Anatomy of Lungs

Lungs are the vital organs of body system responsible for gaseous exchange within the body, which specifically include absorption of oxygen by the blood with the resultant loss of carbon dioxide. Gas exchange occurs in a vast number of alveoli in the lungs, where a tissue the thickness of only one cell separates blood from air. The thin, fragile nature of this tissue makes the lungs especially susceptible to absorption of toxicants and to direct damage from toxic substances. The anatomical organization of the respiratory tract (characterized by extensive bifurcation) and aerosol characteristics of inhaled drug molecules (especially particle size) generally determine the efficacy of any pulmonary drug administration. The respiratory tract comprises the conducting and respiratory regions. The conducting region essentially consists of nasal cavity, nasopharynx, bronchi, bronchioles, and terminal bronchioles (Faller et al., 2004) (Fig. 2.10). Airways distal to the bronchioles and the alveoli constitute the respiratory region, where rapid solute exchange takes place. Two major airways (bronchi) carry air into lungs.
These airways subdivide into smaller airways (bronchioles) that finally end in clusters of tiny air sacs called alveoli. These air sacs inflate like miniature balloons every time when a person inhales. Wrapped around each air sac are capillaries that connect the arteries and veins in the lungs.

Fig. 2.10: Showing the anatomy of alveoli and lungs

2.12.2 Significance of Pulmonary Drug Delivery

In medicine, pulmonary drug delivery has primarily been used as a modality for treating local respiratory diseases. Over the last 20 years the research focus within the field of respiratory drug delivery has broadened to include a wider range of potential applications for inhalation by delivering drugs not just onto the airway but across it (Gonda, 2000; Sanjar and Mathews, 2001). This change in thinking has been catalyzed by a number of factors including the emergence of bio-therapeutics with their associated delivery issues and a greater understanding of the absorption properties of the lungs. The physiology and anatomy of the lung offer a unique portal for drug delivery. Its large surface area, decreased metabolic capacity (relative to the gastrointestinal tract), a relatively thin alveolar epithelium in the lower airways, and a rich blood supply have been found to facilitate rapid drug absorption and adequate pulmonary bioavailabilities (Patton and Bryan, 2007).
The particle size and solubility are the two main factors influencing pulmonary drug deposition. The nature of drug aerosol droplets is dependent on its mass median aerodynamic diameters (MMAD), which is a function of particle size, shape and density. Particle charge and air velocities within the airways are also important attributes. Strict control of MMAD of the particles ensures reproducibility of aerosol deposition and retention within desired regions of the respiratory tract. The size of the aerosol droplets or particle containing solution are generally between 1 and 5 µm in diameter to permit the medication to reach the broncho-pulmonary mucosal surface, and thus most inhaled products are formulated with a high proportion of drug in this size range (Chrystyn, 1997). Particles having MMAD greater than 5µm tend to deposit by impaction in the oropharynx and the large airways. Whereas on one hand, particles above 2µm rarely reach the alveoli, where the conditions for absorption are greatest; particles below 0.5µm are exhaled without deposition in the lungs (Fig. 2.11). The fate of inhaled particles is particularly important for formulations intended to provide drug release over prolonged periods. Insoluble particles depositing in the bronchi and bronchioles are cleared from the lungs by mucociliary clearance depending on the depth of penetration into lungs.

Fig. 2.11: Diagrammatic representation of particles deposition in lungs according to size
2.12.3 Advantages of Nano/sub-micronized Formulations in Inhalation

Nano-medicine is an emerging field for pulmonary administration for systemic as well as local action. The pulmonary route of administration by nanoparticles has been used for many years for the local treatment of lung diseases. The human respiratory tract can have a profound impact on the behavior of inhaled hygroscopic pharmaceuticals used for the prophylaxis and treatment of airway diseases depending upon their particle size. This fact should be actively integrated into current drug-delivery protocols. Institute (Institute of Nuclear Medicine & Allied Sciences (INMAS), DRDO, Delhi, has introduced a concept named, Inhalation Therapy for Alveolar Deposition (ITAD), where-in submicron sized aerosol particles are produced using large spacers employing the re-entry principle, & using ethanol as an excipient.

To provide sustained delivery of drug within the lungs in excess of a few hours requires the particles to be deposited in the alveoli, so as to avoid clearance by mucociliary action. Previous studies reported from our laboratory and elsewhere have shown that submicron size drug particles are deposited deeper into the lung parenchyma compared with micronized drugs thereby increasing their efficacy (Ali et al., 2009; Bhavna et al., 2009; Kumar et al., 2011; Sultana et al., 2011). When compared with conventional micro particulate drug formulations, nano particulate drugs have several advantages such as increased bioavailability, faster onset of action, dose uniformity, and reduction in fasted and fed variability (Ahmed, 2006).

2.13 AEROSOL GENERATION AND DELIVERY

Drugs can be inhaled as aerosols, which are airborne suspensions of fine particles. The particles can be comprised of either liquid droplets, or solids that remain suspended long enough to permit absorption deep into the lungs. There is increasing interest in the potential of the respiratory tract as a route of drug administration as a result of advances in biotechnology and nano-technology. Alongside the pharmaceutical developments, inhalation devices are being improved to deliver doses more accurately and more efficiently to their sites of action. The inhalation dosage technology has primarily focused on two parallel development pathways: fabrication of novel inhaler devices with enhanced deposition efficiency, and improvement in the existing inhalation formulations themselves. Pulmonary drug administration imposes stringent requirement on the
delivery device; since the particle size of inhaled drug greatly influences its localization and thus the degree of its therapeutic effect. Therapeutic aerosols are generated and delivered to lungs by three types of device; nebulizers, pressurized metered dose inhalers and dry powder inhalers (Fig. 2.12).

2.13.1. Drug Delivery Using Nebulizers
Nebulizers are used for converting aqueous solutions and suspensions into respirable droplets. There are two basic types of nebulizers: jet nebulizers, which rely on a stream of air to generate the aerosol, and ultrasonic nebulizers in which droplets are produced by the high frequency vibration of a piezoelectric crystal. Nebulizers are useful for the delivery of relatively high dose treatments to patients with severe chronic obstructive pulmonary disease or asthma. Nebulizers are the preferred choice of many physicians for the therapy of acute asthma in an emergency care unit or for treating patients with severe asthma at home. They are also commonly used for the administration of antibiotics (Mukhopadhyay et al., 1996; Webb and Dodd, 1997).

Fig. 2.12: Schematic representation of various devices used for pulmonary drug delivery
Additionally, nebulization is being used to deliver more complex dispersed systems such as liposomes or microspheres. These advantages of nebulization are however tempered by the large size, more obtrusive dosing, constant cleaning requirements, and unit-dose nature of nebulizers. Perhaps, the most important limitation to their expanded use is the fact that nebulization therapy usually requires a nebulizing device, respiratory solution or suspension, auxiliary tubing, and mouthpieces or face masks. These components are often assembled and used arbitrarily, making it almost impossible to specify the actual dose of drug inhaled by the patient. This leads not only to inter device variability but also to a degree of variability within the same device design (Alvine et al., 1992).

However, the CFC content in pressurized metered dose inhalers (pMDIs) and perceived technical difficulties associated with the development of DPIs have led to a resurgence of interest in nebulizers. The popularity of nebulizer use is currently increasing due to their ability to generate small droplets capable of penetrating deeply into the lung, their high dose-delivery capacity, miniaturization of hardware, and the development of high-output nebulizers, permitting shorter treatment times and increased efficiency of drug delivery to the patient. Coordination between aerosol generation and breathing, which is required for successful pMDI use, is not essential for nebulizers, making them useful for treating hospitalized, very young, and elderly patients (Clay and Clarke, 1987; Johnson and Matthay, 1989).

2.13.2 Inhalation Assemblies for Pre-Clinical Evaluation of Drugs

Inhalation assemblies where only the mouth/ nose of rat/ rabbit is exposed to the drug aerosol (partial exposure chamber) and where the aerosol delivery is directed to the respiratory system alone without release of drug into the atmosphere around the animal are available at selected places only. In view of this limitation, closed chamber method for exposure of drug aerosols (whole body exposure chamber) for toxicity studies was chosen. This results in incremental enhancement in drug concentration in the respirable air leading to higher toxicity profile and therefore indirectly generating more safety data, recycling through re-breathing ensures smaller particles leading to deeper penetration and therefore higher level of drug concentration in lung.
tissues in comparison to partial exposure chamber. After stopping the nebulization process, continued presence of drug aerosols in significant concentration ensures higher level of exposure than recorded. Drug aerosols settled on fur, skin or on surrounding surfaces are likely to be breathed in for a long time (hours) due to unsetting and being air borne again and again, ensuring higher level of exposure than recorded. All these factors ensure higher drug exposure than intended and if still no local toxicity effects are seen, it signifies even higher safety margins, as documented.

2.14 NON-INVASIVE METHODS IN DRUG DEVELOPMENT

Current techniques to evaluate the efficacy of potential treatments for airways diseases in small animal models are generally invasive and terminal. To study specific aspects of human respiratory diseases and its treatment, it is necessary to measure the induced symptoms and the impairment of lung function in living animals (Beckmann et al., 2007). The inflammatory status of the lung is routinely inferred from post mortem analyses of broncho-alveolar lavage (BAL) fluid. Occasionally, time consuming histological analysis is also performed. Simple non-invasive imaging techniques can be used to study the changes in lung micro vascular and alveolar permeability to proteins in vivo and the movement of fluids in the lungs.

There are large numbers of non-invasive imaging techniques available which can be used to demonstrate the behavior of drugs in vivo. The older imaging modalities such as X-ray and CT image the anatomy (structural imaging) whereas the new modalities like SPECT, PET, and Animal MRI image a function or a process. Sequential images of the structural imaging modalities show movement of internal body organs, while sequential imaging by functional imaging modalities represents dynamics or a physiological or a biochemical process. For example, an X-ray or a CT cannot differentiate between a live or a recently dead bone or brain, but a PET or a SPECT scan will be vastly different in the two situations. In the present study, two state of the art non-invasive imaging modalities; viz., Gamma Scintigraphy and Animal-MRI were extensively used for pre-clinical drug evaluation.
2.14.1 Gamma Scintigraphy
The non-invasive imaging technique of gamma scintigraphy was developed originally for use in diagnostic tests in nuclear medicine (David, 2005). Specific radiopharmaceuticals which localize in different organs and which are visualized by gamma camera (Fig. 2.13) are used to provide vital information about the structure and function of various body systems. In contrast to radiological imaging that is based on transmission of radiation, gamma scintigraphy it uses emitted radiation. It is increasing being used as the method of choice for investigating the fate of pharmacological dosage form in pre-clinical and clinical studies and demonstrating how a delivery system is behaving in vivo and whether it is behaving as intended or not (Brooks, 2005; McEwan et al., 2008; Youngho, 2008). It is the only non-invasive method currently capable of providing human data on total and regional lung deposition and mucociliary clearance.

Fig. 2.13: Gamma Camera SPECT (Symbia T-2, Siemens, Germany)

Technetium-99m diethylene triamine penta acetic acid (Tc-99m DTPA) aerosol inhalation scintigraphy is a simple, easy, sensitive and non-invasive method to assess disorders of alveolar-
capillary barrier permeability, secondary to epithelial damage (Suskind, 1996). DTPA clearance rate is a reliable index of alveolar epithelial permeability, and is a highly sensitive marker of pulmonary epithelial damage, even of mild degree which can be used as a measurement of the integrity of the alveolar capillary barrier (Okudan et al., 2004; Pinheiro et al., 2003). The measurement of pulmonary radioaerosol clearance may indicate the presence of various interstitial diseases and is based on the ability of the alveoli to absorb liquid particles and rapidly remove them (Agnew, 1984). The radiotracer, i.e., $^{99m}$Tc-DTPA which is used for this purpose is a hydrophilic molecule with very low lipid solubility. It diffuses across the intraepithelial pores of the epithelium after deposition in the alveoli with no apparent active transport mechanism. Alterations in lung clearance of $^{99m}$Tc-DTPA aerosol have been shown in persons who smoke and in those with various lung disorders (Kaya et al., 2003).

### 2.14.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is used in radiology to visualize detailed internal structures. MRI uses no ionizing radiation unlike CT scans and traditional X-rays; instead it uses a powerful magnetic field. MRI is relatively a new technology, with animal MRI being the latest advancement in the field. MRI is used to image every part of the body, and is best imaging modality which is particularly useful for tissues with many hydrogen nuclei and little density contrast, such as imaging of lungs, brain, muscles, heart, and cancer (Holzgrabe et al., 2000; Pellecchia et al., 2002). Our body is largely composed of water molecule which has two hydrogen nuclei or protons. When a person goes inside the powerful magnetic field of the scanner the magnetic moments of some of the protons changes, and aligns with the direction of the field. This causes the nuclei to produce a rotating magnetic field detectable by the scanner and this information is recorded to construct an image of the scanned area of the body. As the intensity and duration of application of the field increases, more aligned spins are affected. After the field is turned off, the proton decay to the original spin-down state and the difference in energy between the two states is released as photon which produces the electromagnetic signal.
that the scanner detects. An image can be constructed because the protons in different tissues return to their equilibrium state at different rates, which is a difference that can be detected. MRI is primarily a clinical diagnostic tool; however, in the past ten years significant developments have been achieved in imaging small animals as well. Animal MRI (Fig. 2.14) can assess effects of inflammation, mucus secretion (Beckmann et al., 2001), airway and vascular remodeling (Tigani et al., 2007), and parenchymal destruction (Karmouty et al., 2006) in the rat lung, serving as an important tool for models covering a variety of respiratory diseases. Further the effect of therapy can be followed in the same animal.

Fig. 2.14: Animal MRI System (Bruker, Biospec 70/30USR)

2.14.3 Pulse Oximeter

In case of lung disease, pulmonary function tests (PFTs) can be vital for measuring lung function. Spirometry is the most common way of measuring lung function and investigating lung diseases, where the volume and/or flow of air that can be inhaled and exhaled are examined. However, this method only provides information on a global scale, when disorders of the lung
have reached more advanced stages. PFT is a relative insensitive method. Pulse oximeter (Fig. 2.15) indirectly measures the oxygen saturation in blood (SpO₂) and pulse rate as opposed to measuring oxygen saturation directly through a blood sample. The adverse effects of any inhalation toxicant on the lungs can easily be assessed by measuring basic pulmonary functions (SpO₂ and Pulse rate). Detection of any change in SpO₂ and pulse rate, before they become irreversible, can therefore be important biomarkers and necessary steps can then be taken to prevent further deterioration of the cardiopulmonary system.

![Fig. 2.15: Finger Tip Pulse Oximeter (TR-800)](image)

2.15 THERAPEUTIC AGENTS EVALUATED UNDER PRESENT STUDY

Investigation and characterization of respiratory diseases are motivated by the fact that these illness are among the most prevalent and most rapidly expanding problems in medicine worldwide. Obviously there is a demand for new therapies in this area, leading to a growing interest for research in this field. The present work is part of our institute’s endeavor to primarily develop suitable antidote drug formulations that can reduce the various lung toxicity markers such as toxic pulmonary edema, tracheitis, and bronchitis subsequent to inhalation of various toxicants. Under this drug development program, three novel inhalation based drug formulations; viz. (a) Alphaketoglutaric Acid, (b) Sodium Nitrite, and (c) Fluticasone propionate have been evaluated for their toxicity and efficacy in suitable animal models as part of pre-clinical research.

We have developed several nano-based inhalation formulations, including dry powder inhalation and nebulizable respiratory fluid formulations, for various clinical applications, and shown their
distinct clinical advantage over the conventional available technology. An example is nano-
salbutamol, that has been shown extremely useful in treating acute mountain sickness through
user studies in Ladakh, and continuing studies suggests a beneficial role in bronchial asthma,
COPD, pulmonary hypertension and interstitial lung diseases.

2.15.1 Alpha Ketoglutaric Acid (AKG)

**Drug Profile**

<table>
<thead>
<tr>
<th>Name</th>
<th>Alpha Ketoglutaric Acid (2-Ketoglutaric acid 2-Oxoglutaric acid, Oxoglutaric acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Formula</td>
<td>$C_5H_5O_6$</td>
</tr>
<tr>
<td>Structural Formula</td>
<td><img src="image" alt="Structural Formula" /></td>
</tr>
</tbody>
</table>

**Description**

Alpha ketoglutaric acid (AKG), an important biological compound is one of two ketone
derivatives of glutaric acid. AKG act as a dietary supplement and is recommended for use in
chronic fatigues and in metabolic deficiencies often diagnosed by amino acids analysis. It is
available over-the-counter in the USA as an energy supplement (Sultana et al., 2011). AKG is a
keto acid produced by de-amination of glutamate and a key intermediate in the Kreb’s cycle,
coming after isocitrate and before succinyl CoA steps. It is an important derivative of glutamine
which is considered as a crucial molecule in transmembrane amino acid transport, protein
metabolism, and in gene and cellular redox regulation (Filip et al., 2008; Wernerman et al.,
1999).

AKG also serves as a natural scavenger of ammonium ions, facilitates its conversion to amino
acids and protein and reduces levels of ammonia in the body. It has a beneficial effect on
nitrogen metabolism and is one of the most important nitrogen transporters in metabolic
pathways (Schlegel et al., 2002; Wiren et al., 2002). Currently AKG is also being pursued widely as a cyanide antidote (Bhattacharya and Vijayaraghavan, 1991; Bhattacharya, 2000; Hume et al., 1996). Since it is a scavenger of amino groups, AKG is considered a natural detoxifier of active nitrogen. Thus, it seemed logical to us to believe that AKG inhalation might have therapeutic effect against toxic effects of inhaled ammonia aerosols.

2.15.2 Sodium Nitrite

**Drug Profile**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sodium Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Formula</td>
<td>NaNO₂</td>
</tr>
<tr>
<td>Structural Formula</td>
<td><img src="image" alt="Structural Formula" /></td>
</tr>
<tr>
<td>Molar Mass</td>
<td>69.0 g/mol</td>
</tr>
</tbody>
</table>

**Description**

Sodium nitrite has been used in human and veterinary medicine as a vasodilator, bronchodilator as well for the treatment of angina (Martindale, 1989). It has vasodilator property due to on-site production of nitric oxide (NO) when administered locally, which causes vasodilation by relaxing vascular smooth muscle in pulmonary microvasculature. It is used to treat pulmonary hypertension (Kruszyna et al., 1985). Other mechanisms, including vasodilatation with changes in local capillary blood flow, have been postulated to account for part or all of the antidotal action of sodium nitrite (Cohen and Guzzardi, 1984; Way et al., 1984). Sodium nitrite is the standard treatment for cyanide poisoning along with sodium thiosulphate. In the present work respiratory formulation of sodium nitrite was evaluated against passive cigarette smoke induced non-cardiogenic pulmonary edema/ acute lung injuries.
2.15.3 Fluticasone Propionate

**Drug profile**

**Name**  
Fluticasone propionate

**Structural Formula**

![Structural Formula Image]

**Molecular Formula**  
C_{25}H_{31}F_3O_5S

**Molar Mass**  
500.6 g/mol

**Description**

Fluticasone propionate is a corticosteroid which has a high affinity for glucocorticoid receptor with negligible binding to mineralocorticoid, estrogen, progesterone and androsterone receptors. It is not suitable for oral administration as it is subjected to almost 100% inactivation in the liver (Hochhaus, 2004). In common with other intranasal and inhaled steroids, Fluticasone propionate has anti-inflammatory and immunosuppressant activity, which is probably due to actions at several sites including reducing cytokine release from activated lymphocytes and macrophages leading to decreased eosinophil accumulation and activation, and decrease in number of airway mucosal mast cell (Ahmet et al., 2011). Inhaled steroids also decrease airway edema formation, upregulate β_2-adrenoceptor activity and decrease prostaglandin and leukotriene formation by an effect on phospholipase A_2. Fluticasone propionate is reported to have protective effects even at lower doses in interstitial lung diseases. It is very poorly water soluble (<1 g/L) corticosteroid and displays a high degree of binding to lung tissue (Davies and Feddah, 2003; Fuller et al., 1995). We have evaluated the safety and efficacy of developed respiratory formulation of Fluticasone propionate against smoke induced oxidative stress and lung injuries.