Chapter I

Introduction

and

Review of Literature
Introduction

Medicinal preparations derived from natural resources, particularly from plants, have been in extensive use since long time. Ancient literature gives detailed depictions about the practices and use of plants and their parts as medicines to cure a variety of diseases. Plants are used for medicinal purposes in many traditional therapies including Chinese, Kampo (Japan), European, Ayurveda, Unani and Siddha etc. last three being practised mainly in India. The people are getting cured by these medicines, though there is a lacuna in the understanding of the mechanism of action. Plants constitute a major fraction of such practices, along with metals and ‘bhasma’ etc.

Since centuries the plants/herbs have been used as medicines against human diseases and they are still popular among a large proportion of the world’s population. Since ages these drugs have demonstrated effectiveness against a variety of ailments with no or less side effects. Although synthetic drugs have gain popularity due to their fast and specific action, but they have been plagued by unwanted effects and toxicities. Now a days more emphasis has been given on the use of plant derived compounds. Low toxicity made them more favourable to the body.

Chemoprevention by such compounds/derivatives is one of the strategies to reverse, suppress or prevent the diseases. The term ‘chemoprevention’ was coined by Sporn in 1976. Prevention is the most effective way to reduce mortality by the cancer, and we know that lung cancer is responsible for most of the cancer deaths; that is why priority should be given to lung chemoprevention strategies. Plants contain phytochemicals and have shown antioxidant properties (Rahman et al., 2006). They can minimize the oxidative damage by scavenging free radicals or by restoring the anti-oxidant enzymes.
The concept of using natural or synthetic agents to reduce cancer risk is based on epidemiological and experimental studies showing that certain compounds may influence carcinogenesis. The relationship between diet and lung cancer has been extensively explored in epidemiological studies and there are many leads to support an association between a high intake of fruits and vegetables and a reduced risk of lung cancer (Block, 1992). Animal studies also do indicate that many plant constituents show protective role against cancer (Wattenberg et al. 1992, Banerjee et al. 2006).

A wide variety of plants including fruits, vegetables, tea, legumes, grains etc. are known to contain antioxidant components, mainly polyphenols. In various studies plants have shown their protective role against oxidative stress and consequent injuries and ailments. As oxidative stress is involved in development of various disorders, countering these effects by using exogenous antioxidants (plant polyphenols) is a well reported strategy (Bravo, 1998; Rahman, 2006). Most of the medicinal activities of plants are attributed to antioxidants/polyphenols present in these natural sources (Bravo, 1998; Eastwood, 1999). Reports suggest that the health benefits associated with consumptions of fruits vegetables and other plants including having medicinal properties, are linked to their polyphenol antioxidants. In various studies polyphenols are reported to scavenge reactive oxygen species (ROS), which include superoxide anion, hydroxyl radicals, hydrogen peroxide etc., and plays leading role in diminishing oxidative stress. They also exhibit protective activities against oxidative stress induced damages at cellular/tissue level and are suggested to have medicinal effects in various diseased conditions. Polyphenols execute their antioxidant activities not only by scavenging free radicals but other mechanisms as well. These plant products are reported to induce cell’s own, endogenous, antioxidants showing their biphasic role against oxidative stress and consequent injuries. They induce reduced glutathione (GSH) level and its redox cycle components glutathione reductase (GR) and glutathione peroxidise (GPx) etc.
GSH is an important reducing equivalent that participates not only in antioxidant activities in reduction of hydrogen peroxide (H$_2$O$_2$), but also in various detoxification reactions. It forms conjugates with toxic xenobiotic metabolites via a reaction that is catalyzed by enzyme glutathione S transferase. It also plays a role in intracellular signalling in cooperation with glutaredoxin. Polyphenols also maintain the level of antioxidant enzymes catalase, super oxide dismutase (SOD), GPx and GR, and reduce the lipid peroxidative damages to cell membrane.

Although preventive activities of most of the plant constituents, mainly polyphenols, are attributed to their antioxidant nature but they may have pro-oxidant effects simultaneously. Curcumin, resveratrol, catechins etc. are the most rigorously studied plant antioxidants and are reported to show disease preventive activities in-vitro as well as in-vivo. They are also reported to have pro-oxidant activities, still they continued to prove themselves as effective plant components in various studies. This kind of nature indicated towards some alternative mechanism of action of these phytochemicals, other than antioxidant activities. Now it is well reported that these and other plant constituents have their medicinal effects by altering various molecular events within the cell. By interfering with signalling pathways and alteration of gene expression, plant constituents have taken a big leap beyond the limits of mere antioxidant activities.

Inflammation plays a central role in the development of a vast array of diseases and it may be fatal for the patient in various cases. Checking inflammation by means of anti-inflammatory drugs can serve as a remedy for all the diseases associated with inflammation. Any inflammatory condition that is chronic and has emerged as a lethal ailment always arises as an initial acute inflammatory burst after exposure to a particular toxic agent. Both acute and chronic inflammatory conditions vary greatly on the basis of inflammatory cells and
cytokines involved. Treating acute inflammation is an easy process as compared to treatment of chronic inflammation. Therefore, like any other disease, treating inflammation is a better strategy. NF-κB plays a central role in regulation of both acute and chronic inflammatory processes. Various plants are reported to modify inflammatory processes by altering NF-κB activities. This activity is also associated with modulation of inflammatory components like inducible nitric oxide synthetase (iNOS) (Rahman, 2006). These properties can be exploited to treat inflammatory diseases in herbal therapies.

Various epidemiological studies have shown a negative correlation between consumption of fruits and vegetables, and occurrence of cancer. This kind of reports makes a basis of using naturally occurring phytochemicals against carcinogenic events (Gullcott et al., 2010). Phytochemicals are reported to mitigate carcinogenesis in various cancer models (Kim et al., 2010; Konijeti et al., 2010; Zou et al., 2010) and gives a way to study and use them for clinical conditions involving carcinogenesis. Alteration of various molecular pathways by plant constituents is suggested to involve in mitigation of various cancers including colorectal (Wondrak et al., 2010) and lung cancer (Karoor et al., 2010). This shows that plants and their constituents can have more than one mechanism, in addition to antioxidant activities, to reduce the chances of cancer occurrence.

Inhalation route is the most important one for both survival and exposure to toxicant. In the present environment the presence of toxic inhalants, which are released from chimneys, automobile exhausts, cigarettes, forest fires etc., is unavoidable. Survival without food and water is possible for several days or weeks, but the need of oxygen can not be avoided even for minutes. This makes the inhalation process so much important for the continuity of life. Exposure through inhalation route is more important in terms of interactions with the environment. The Lung works as an interface between body and the environment, however, skin also fulfil the same purpose, but the anatomical and physiological natures of these
organs are different. Lungs provide much larger surface area (~70 m²), for the exposure to external environment, than skin (1.73 m², in average adult human).

**Lung as a target for toxicities**

Human body is a complex organization of certain organs like heart, liver, lung and kidney etc. and any damage (on cellular, biochemical, or molecular level) can affect the whole system and lead to various pathological conditions. Lung is the organ that provides an interface for gaseous exchange between blood vessels and the outside environment. It provides an essentially continuous supply of oxygen to the body by a process known as respiration. Other than this function, lung is also involved in excretion of volatile harmful substances by exhalation, carbon dioxide being major product, and it also works as a reservoir for blood.

The respiratory system, located in the thoracic cavity, makes an interface between the circulatory system and the external environment, and it is responsible for gaseous exchange. Upper airways are involved in inhalation of air via the nasal cavity, pharynx and larynx through the lower airways (trachea, primary bronchi and bronchial tree) and small bronchioles and alveoli within the lung tissue.

Respiratory system can be divided into following three major components:

- Nasopharyngeal region
- Tracheobronchial region
- Pulmonary region

The nasopharyngeal region includes nares, nasal turbinates, glottis, epiglottis, pharynx and larynx. This region functions in removing the larger inhaled particles through filtration by
nasal hair and impaction in the turbinates. It also conditions the inhaled air by increasing the humidity and moderating the temperature. Physiological responses of the nasopharyngeal region can be altered in response to inhaled toxicants.

Conducting airways or the tracheobronchial region is responsible for maintaining the supply of inspired air to the alveolar region of the lung. Trachea and bronchus are the major components of this region; it ends at the terminal bronchioles. These airways are covered with a layer of goblet cells that secrete mucus, and ciliated columnar cells that, in combination with mucus, form a protective mucociliary coating. This region function in further conditioning of the inspired air and clearance of the inhaled particulate matter, by ciliary movement, that is trapped on the protective mucociliary layer.

Figure: Overview of respiratory tract anatomy (Source: Faller, The Human Body, 2004, Thieme).
Gaseous exchange occurs in the pulmonary region, which comprise approximately 80–90% of the total lung parenchyma. The region is an arrangement of bronchioles, alveolar ducts and alveoli. Capillaries, blood plasma, and other blood components are separated from the air space by means of a thin layer of tissue formed by epithelium, interstitial part, and endothelium.

As a whole lungs are divided into lobes. In humans lungs contain five lobes, two left and three right. The left lung is composed of the upper lobe, the lower lobe, the right lung is composed of upper, middle and lower lobes. Both right and left parts of the lungs are separated from each other by contents of the mediastinum and the heart. In rats and mice (Rodents) lungs contain five lobes, one left and four right.

Figure: The segments of the lung viewed from in front. The numbers in the circles indicate the segments of the lung 1–10 (segment 7 of the left lower lobe is missing). The broken lines demarcate the segments. (Source: Faller, The Human Body, 2004 Thieme).
The number and concentration of air pollutants is rapidly increasing in the present environment. Most of them are causing damages to the lung. A minor imbalance in atmospheric composition can disturb the physiology of the lung. Airborne toxicants are a matter of major concern regarding human health. They get direct entry into the lungs with the inhaled air during the normal process of breathing. Major inhalant toxicants include automobile exhausts, chimney exhausts, suspended particulate matter (SPM), certain gases, fumcs, cigarette smoke, smoke generated from forest fires etc. Automobile exhaust, cigarette smoke and SPM are causing more debilitating effects as people are exposed to these agents on a regular basis. These exposures are more common throughout the world.

Due to rapid industrialization and consequent environmental contamination our vital organs, such as liver, kidney and lungs are subjected to damage by various toxicants. Lung toxicities are very common due to direct interaction of lung alveolar epithelium with inhaled toxicants. The sensitivity of lung towards a variety of airborne toxic agents including carcinogens leads to a brisk inflammatory response and cancer. Lung carcinoma is one of the most commonly occurring malignancies with approximately 1.2 million new cases each year (Parkin et al., 2000). Various studies have been conducted in search of potent chemopreventive agents against lung carcinogenesis. The relationship between natural compounds and lung cancer incidences has been extensively explored in epidemiological studies and there are many positive indications to support an association between a high intake of fruits and vegetables and a decreased risk of lung cancer (Block et al., 1992). Smoking is associated with the most of the lung cancer cases and cessation of smoking is the most effective strategy to avoid lung carcinogenesis (WHO Tobacco Free Initiative, 2006). But only smoking cessation is not enough to cop up the problem, it certainly reduces the chances of getting lung ailments and cancer, further measurements are needed to avoid the actual problem. Plants and their constituents has shown promising role against lung cancer (Banerjee et al., 2006; Hecht,
induced by cigarette smoke carcinogens. Although various studies are available to prove natural phytochemicals as effective chemopreventive agents against lung toxicities induced by cigarette smoke, more studies are needed to search for a better agent.
Lungs are more susceptible to damage when exposed to toxicant via inhalation. Rich vasculature, large surface area and thin epithelium contribute to lung’s high sensitivity towards various kind of air borne toxicants. The most important effect of many toxic inhalants is to place an undue oxidative burden on the lungs. Studies on humans and animals provide strong evidences that the consequences of oxidative stress may be instrumental in initiating and propagating ailments such as chronic bronchitis, emphysema, fibrosis, and cancers (Crapo et al. 1992; Pastorino 1997; Witschi 1997). Alterations in lung epithelial cells may cause architectural and functional disruptions in the lung (Aoshiba and Nagai, 2003).

Presence of an ample amount of oxygen, in essentially continuous manner, increases the chances of occurrence of oxidative stress even after a minor insult by a toxicant. Skin also remains in direct contact with the environment but provide less interaction with the toxic components present in the ambience. It works more as a physical barrier; it has very much less surface area (1.73 m\(^2\), average body surface area of an adult human) than that of the lung (~70 m\(^3\)), it has less blood supply and an upper layer of dead cells as a physical obstruction for most of the hydrophilic xenobiotics. Only lipophilic substances can cross the skin and enter into the systemic circulation whereas in lungs there is a coating of a secreted fluid, containing water, which facilitate gaseous exchange and can trap hydrophilic toxicants.

Like any other organ lung also contain its own antioxidant machinery that includes enzymatic and non-enzymatic components. Catalase is an important enzyme that reduces hydrogen peroxide (H\(_2\)O\(_2\)) into water thus minimizing its involvement in Fenton’s reaction. Thus catalase indirectly reduces the production of hydroxyl free radicals; the most reactive free radical capable of damaging DNA by strand breaks (Maruyama et al. 2005). Glutathione (GSH) is a ubiquitous, essential tripeptide (L-\(\gamma\)-glutamyl-L-cysteinyl-glycine) containing a
sulfhydryl group that enables it to protect cells against oxidants, electrophilic compounds, and xenobiotics. GSH, which accounts for 90% of intracellular nonprotein thiols, is a key intracellular reducing agent and is implicated in immune modulation and inflammatory conditions (Meister et al. 1991). GSH also serves as a storage and transport form of cysteine and as a cofactor in several enzymatic reactions. Hence GSH is emerging as one of the fundamental antioxidant defense mechanisms in oxidant-induced lung injury and inflammation. Alterations in lung lining fluid GSH levels have been shown in various inflammatory conditions (Rahman and MacNee 1999).

Glutathione-S-transferase (GST) is the enzyme involved in detoxification of many carcinogens and reactive oxidants. It is a phase II enzyme involved in glutathione conjugation reactions. Glutathione peroxidise (GPx) functions in reduction of $H_2O_2$ so does the catalase, but GPx uses GSH as reducing equivalents. In this reaction GSH is oxidised to GSSG, which are no longer available for further reactions involving reduction of $H_2O_2$ and other detoxification reaction. Glutathione reductase (GR), an enzyme involved in reduction of GSSG into GSH, contributes to the availability of GSH in an appropriate manner so that cell could be protected against oxidants/toxicants. Cigarette smoking may affect critical detoxifying and regulatory enzymes such as GPx, glutathione reductase, and GST involved in the GSH redox system in lungs. (Rahman et al., 1996). These all enzymes involved in antioxidant defences play a major role in early events of cancer development. Measurement of these enzymes might be a good step in studying early events in cancer studies (Sultana et al. 2005).

Xanthine oxidase is a modified form of the ubiquitous enzyme xanthine dehydrogenase. It is a potential inducible intracellular source of reactive oxygen species (ROS), and plays a major role in formation of ROS and hydrogen peroxide ($H_2O_2$), which are highly damaging to the cells (Kuwabara et al. 2003, Lynch et al. 1988). ROS cause membrane damage (lipid...
peroxidation) and can damage to DNA, a role probably leading to cancer. ROS also induces inflammatory reactions in lungs; responsible for cancer promoting activities (Brody and Spira 2006).

Cigarette smoke is known to impose and cause initial damages by oxidative stress in lungs. Cigarette smoke is known to contain an estimated $10^{14}$ free radicals/puff and these damaging free radicals include tar semiquinone, which can produce H$_2$O$_2$ by the Fenton reaction (Nakayama et al., 1989; Pryor and Stone 1993; Zang et al., 1995). These are found to be relatively long lived and their persistence increases the chances of oxidative injuries in lung tissue and progression towards the disease development.

Oxidative stress imposed by cigarette smoking can result in destruction of the alveolar epithelium, leading to airway enlargement. Moreover, increased oxidative stress can induce inflammatory responses and trigger pro-inflammatory cytokines, which are increased in the lungs of smokers and patients having COPD (Kirkham and Rahman, 2006; Rahman, 2006(b)). Cigarette smoke induced oxidative stress is capable enough of inducing cellular injuries, inflammation and DNA damages that can lead to cancer development (Faux et al., 2009). The conditions like inflammation and oxidative stress are responsible for various kinds of damages to the lung epithelium. Inflammation, itself, exaggerates oxidative stress by recruiting inflammatory cells, which in turn produces reactive oxygen species (ROS). Inflammation is also associated with generation of nitric oxide (NO), a free radical gas, which participate in worsening the oxidant burden within the tissue when over produced. These oxidants induce cellular injuries in lung epithelium and may lead to permanent alterations in lung architecture and function. Repeated exposure of cigarette smoke can tilt the acute inflammatory responses towards chronic one. These chronic inflammatory conditions may have any of the diseases form that is known to have lethal effects on the patients. These include chronic obstructive pulmonary disease (COPD), emphysema, bronchitis,
bronchiolitis, pulmonary fibrosis etc. Cancer is also a chronic disorder that is associated with genetic alterations in oncogenes and tumor suppressor genes. Although inflammation is a protective response of the body but a close relation has been found between chronic inflammation and cancer development (Mantovani, 2008). Lungs are very much sensitive towards damages and show quick inflammatory responses and most of the lethal lung ailments are inflammatory. This relation between cancer and inflammation may be a potent reason why lung cancer deaths outnumber the deaths caused by any other cancer. Cigarette smoke is known to induce inflammatory cytokines (Kode et al., 2006; Yang et al., 2006) and also contain several known carcinogens. Induction of inflammatory cytokines by cigarette smoke is reported via activation of NF-κB (Yang et al., 2006). These properties make cigarette smoke a potent toxicant particularly for the induction of lung inflammatory diseases and cancer.

Inflammation is body’s protective response to cellular or tissue injuries. The function of this process is to eradicate and remove the pathogens, noxious agents and injured tissues, thereby promoting tissue repair. When this important and normally protective response occurs in an uncontrolled manner, it results in excessive cellular and tissue injuries that may lead to chronic inflammation and destruction of normal tissue. In case of lung ailments, inflammation plays a central role as most of the lethal lung diseases are inflammatory in nature. Inflammatory lung diseases are characterized by chronic inflammation (McNee, 2000) associated with oxidative stress. Oxidative stress conditions are proposed to be involved in tissue damage associated with chronic inflammation and consequent diseases (Rahman and McNee, 1996; Rahman et al., 1996, Morcillo, 1999). Many inflammatory cytokines are involved in this process.

Chronic inflammation has been shown to be involved in development of various cancers. Gastric inflammation by H. pylori, reflux esophagitis, hepatitis by viral infection, ulcerative
colitis, and cigarette smoking are all allied with chronic inflammation and an increased incidence of cancers. Various studies provide substantial evidences that inflammatory lung ailments such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by chronic inflammation and oxidative stress. Oxidants may play a role in induction of inflammation through activation of different kinases and transcription factors such as NF-κB and AP-1 (Caramori and Papi, 2004; Guo and Ward 2007; Hoshino and Mishima, 2008; MacNce, 2001).

Various transcription factors, such as NF-κB which is redox-sensitive, have been reported to be activated in epithelial and inflammatory cells during oxidative stress or inflammation, leading to the upregulation of a number of pro-inflammatory genes (Jansson-Hcininger, 1999). TNF-α is a ubiquitous pro-inflammatory cytokine and mediate most of the inflammatory events in the lungs and other tissues. It also plays a role in the development of chronic inflammation (Balkwill, 2009). TNF-α is an important inflammatory mediator in chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS) and is present in increased amounts in the bronchoalveolar lavage fluid (BALF) and sputum of COPD patients (Keating et al., 1996).

Alveolar macrophages are the native immune cells of the alveolar spaces, which are differentiated mast cells. They roam across the alveolar space and participate in cleaning inhaled pathogens and other particulate matter by a process known as phagocytosis. Whenever any insult occur to the lung they secrete pro-inflammatory cytokines and chemokines, and trigger the recruitment of neutrophils and other immunological cells (Tsushima et al., 2009) including T and B lymphocytes, and induce further activation of macrophages. Increased number of neutrophils in lavage fluids can be used as a marker of inflammation, mainly acute, in the lung. The latter event, that is activation of macrophages, is a crucial event in the progression towards chronic inflammation. IFN-γ plays a central role in
the activation of alveolar macrophages and chronic inflammation in lung (Rahman et al., 2006).

This inflammation can lead to degradation of elastin protein of lung parenchyma and consequent emphysema, with enlargement of air spaces, closure of small airways and loss of lung elasticity (Barnes, 2000). Studies show a strong link between cigarette smoking and lung inflammatory diseases (COPD) and cancer. Smokers who are suffering from COPD appear to get lung cancer more frequently (Brody and Spira, 2006).

![Diagram: Relation between cigarette smoke induced oxidative stress, inflammation and consequent COPD. (Source: Barnes, PJ. Chronic Obstructive Pulmonary Disease. NEJM. 2000; 343(4); 269-280.)](image)

The number and concentration of air pollutants is rapidly increasing in the present environment. Most of them are causing damage to the lung. Among them heavy metals,
organic solvents, automobile exhausts and tobacco smoke are notorious. Some of them contain genotoxic agents responsible for damaging DNA of the lung cells, which ultimately can lead to the formation of lung tumor and malignancies. With around 1.2 million new cases each year, lung cancer is one of the most commonly occurring malignancies (Parkin et al., 2001). Cigarette smoke is one of the major toxicants present in the environment.

Cigarette smoke is a well-known pneumotoxicant responsible for many inflammatory lung diseases, and cancer. This is because of presence of approximately 100 carcinogens, cancer promoters, mutagens etc. Tar content of the cigarette smoke contains thousands of chemicals including polyaromatic hydrocarbons (PAHs) and nitrosamines, which are known to cause cancer (Hoffmann et al., 2001; Hecht, 2002). These carcinogenic agents are included in a long list of more than 4000 chemicals that are released when cigarette is smoked (Church and Pryor, 1985). All types of tobacco products are consumed for nicotine only, which has pharmacological activities. It is the only component that has stimulatory effects on nervous system. However, people might not be aware about that large number of chemicals that are released when a cigarette is smoked, or those cancer causing agents present in all types of tobacco either smoked or smoke less.

Cigarette smoke also contains stable compounds that undergo redox reactions to form superoxide radicals, hydrogen peroxide, hydroxyl radicals etc. (Jaimes et al. 2004; Pryor and Stone, 1993). In this context cigarette smoke can cause severe oxidative damages in lungs. Studies suggest that oxidative burden placed by cigarette smoke play a role in the development of various lung ailments (Crapo et al. 1992; Brody and Spira, 2006). Cigarette smoke can alter the normal physiological functions of the lung epithelium by its cytotoxic activities (Aoshiba and Nagai, 2003). Exposure to cigarette smoke may cause an increase in epithelial permeability and inflammatory reactions, debility in antioxidant defenses and an increased risk of developing lung diseases (Carp and Janoff, 1978).
Bidi is the most popular form of smoking in India. Bidis are made by rolling crude tobacco in tendu or tamburni leaf (*Diospiros melanoxylon* L.) and tied with a coloured cotton thread. It is mainly manufactured in India. Other producers include Bangladesh, Pakistan, Nepal and Sri Lanka.

In India bidis constitute over 50% of the total tobacco consumption (Jha et al., 2008; Panchmukhi et al., 2008). During the past three decades bidi has gained popularity among the young population of many developed countries (CDC, 1999; Richter and Watson, 2008). The reason of its popularity is its more natural appearance, low price, availability in many flavours like chocolate, vanilla, mango, cherry etc. (Fisher, 2000; Yen et al., 2000). There is a misconception among youth that bidis are safer than conventional cigarettes. A survey conducted in India found that 12.5% of school students were bidi smokers (Sinha and Dikshit, 2008). Another survey in United States reported that 4.8% students were current bidi users (CDC, 2005).

Bidi smoking is a major health concern. They release greater amount of tar, nicotine and carbon monoxide than western branded cigarettes (Malson et al., 2001; Watson et al., 2003). Bidis are reported as a major cause of death in Indian men mainly with respiratory and vascular diseases (Gajalakshmi et al., 2003; Gupta and Mehta, 2000). Studies from India show an association between bidi and cancers of lung, oral, stomach and oesophagus. It is also involved in the development of chronic bronchitis, coronary heart disease and myocardial infarction (Pais et al., 1996; Rahman and Fukui, 2000; Sankaranarayanan, 1991).

“Herbal bidi” is an alternative form of bidi in which some herbs and non-tobacco contents such as ginseng, catnip and jasmine are wrapped in the same tendu leaf (*D. melanoxylon*). Herbal bidis are considered safer than tobacco filled bidis. Although both the types of bidis are considered harmful for the health but a few studies have been conducted on their toxicological aspects.
Benzo(a)Pyrene

A large fraction of the particulate part of tobacco smoke is constituted with tar and contains a complex mixture of constituents, (Dube et al., 1982) some of which are carcinogenic in nature. One of the constituents is benzo(a)pyrene [B(a)P], a polynuclear aromatic hydrocarbon (PAH) formed during the process of incomplete combustion of organic matter such as fossil fuel, garbage and plant parts. B(a)P has been classified by the International Agency for Research on Cancer as an animal carcinogen and a probable human carcinogen (class 2A) (IARC, 1987) with various routes including inhalation, oral and dermal absorption as the important one for entry into the body.

B(a)P is reported to alter cellular antioxidant levels (Lina et al., 2007) and play an initial role in oxidative status of the cell besides its known carcinogenic activities. It is also reported to show genotoxic effects in animal experiments after pulmonary exposure (Garry et al., 2003). However, no such reports are available related with humans but animal studies give a clear indication of probable role of B(a)P in lung cancer induction. In smokers this type of effect may be a consequence of cumulative action of other genotoxic agents present in tar (Hoffman et al., 2001; Hecht, 2002). Cigarette smoke constituents, including N-nitrosamines, polycyclic aromatic hydrocarbons (PAHs), and aromatic amines, are considered to be potent carcinogens accountable for many cancers, most notably lung cancer (Hecht, 2002). Metabolic activation of tobacco smoke associated PAHs like B(a)P and the DNA adducts formed have been proposed to be central to the carcinogenic process of PAH-induced cancers, including lung and oral cancer (Hecht, 2002; Rodu et al., 2004). Researchers have found a perceptible amount of B(a)P in tobacco smoke. It has been detected in concentrations ranging from 20-40 ng/cigarette in mainstream cigarette smoke to 40-79 ng/cigarette in sidestream smoke (US Department of Health and Human Services, 1989). In various studies B(a)P has been proved...
as a model toxicant to study the adverse effects on pulmonary system in rodents (Garry et al., 2003; Gunning et al., 2003; Wolterbeek et al., 1995).

**Bronchoalveolar Lavage Fluid (BALF) as a tool to study the effects on the lung**

Lung is a very susceptible organ as it remains in direct contact with the atmosphere. Any toxic agent present in the air gets direct entry into the lung and causes injuries to the cells, which directly affect the individual. To assess the level of injury by some specific agent, animal models are established in which rodent model is commonly exploited.

Lung alveolar Type-II cells release some surfactant to make the alveoli safer from the collapse and Clara cells also release a serous fluid to remove the excess of the surfactants. These two secretions make an internal environment of the lung, and play a major role in the lung defences (Savov et al. 2000).

When some toxicant insult occurs, the composition of these secretions is altered. Further some cytotoxicity and inflammation markers are also released. So with the help of lavage of the lung we can obtain these markers in the lavage fluid. For this bronchoalveolar lavage (BAL) method was established for the collection of bronchoalveolar lavage fluid (BALF), so that lung injury markers could be assayed by some biochemical and molecular methods (Henderson et al. 1985, Ameen et al. 2003).

Lactate dehydrogenase (LDH) and alkaline phosphatase (AP) are assayed in the BALF to evaluate the lung injury. Lactate dehydrogenase (LDH) is an intracellular enzyme, it catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD⁺. Its presence in extracellular space is considered as a biomarker of cytotoxicity. High level of LDH in BALF is an indication of lung injury (Warheit et al. 2006, Cermá et al. 2005).
Alkaline phosphatase is involved in dephosphorylation of cellular biomolecules. Increased level of alkaline phosphatase in BALF is considered to be an indicator of Type II lung epithelial cell toxicity (Warheit et al. 2006).

High content of protein in BALF is also a lung injury marker and indicates increased capillary permeability. This increased permeability allows many inflammatory cells (macrophages, neutrophils, polymorphonuclear cells) to enter in alveolar space thus invoking more intense inflammatory response. So an increase in total cell count in BALF is a primary indicator of inflammation. (Yamada et al. 2004, Ameen et al. 2003).