Introduction
INTRODUCTION

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death (American Cancer Society, 2016). Lung cancer is the leading cause of cancer deaths in the United States and will claim more lives this year than cancer of the breast, prostate, and colon combined (Lung Cancer Alliance 2015).

Lung cancer is a serious health problem in most developed countries and its incidence rate is profusely increasing. Lung cancer is a major cause of morbidity and mortality worldwide in both men and women, accounting for 29% of all cancers.

Lung cancer is the number one neoplasm in the world, both in terms of incidence and mortality. Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer death in both men and women. Lung cancer accounts for 13% (1.6 million) of the total cases and 18% (1.4 million) of the deaths in 2008.

Siegel et al. (2016) reviewed recent cancer data and estimated a total of 239,320 new cases of lung cancer and 161,250 deaths from lung cancer in the United States in 2010. The statistics reflect data from 2007 and therefore, likely underestimate the current lung cancer burden.

Lung Cancer

Lung cancer is the single most devastating cause of cancer-related death in developed countries and also rising at alarming rate in developing countries (Khuri et al.,
Lung cancer will continue to be a major cause of death throughout the world with in the foreseeable future, it is estimated that by 2030 lung cancer will be the sixth most common cause of death compared with its current ranking of ninth (Mathers and Loncar, 2006).

Lung cancer has been the most common cancer worldwide since 1985, both in terms of incidence and mortality. Globally, lung cancer is the largest contributor to new cancer diagnoses (1,350,000 new cases and 12.4% of total new cancer cases) and to death from cancer (1,180,000 deaths and 17.6% of total cancer deaths).

Approximately half (49.9%) of the cases now occur in developing countries whereas in 1980, 69% of cases were in developed countries. The estimated numbers of lung cancer cases worldwide has increased by 51% since 1985 (a 44% increase in men and a 76% increase in women). In the United States, cancer of the lung and bronchus ranks second in both genders, with an estimated 115,060 new cases in men (14% of all new cancers) and 106,070 in women (14% of all new cancers).
About 1,685,210 new cancer cases are expected to be diagnosed in 2016. This estimate does not include carcinoma in situ (noninvasive cancer) of any site except urinary bladder, nor does it include basal cell or squamous cell skin cancers because these are not required to be reported to cancer registries.

Estimated number of death for selected cancers by state, US, 2016

<table>
<thead>
<tr>
<th>State</th>
<th>All sites</th>
<th>Brain/nervous system</th>
<th>Female breast</th>
<th>Colon &amp; rectum</th>
<th>Leukemia</th>
<th>Liver¹</th>
<th>Lung &amp; bronchus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>10,650</td>
<td>300</td>
<td>670</td>
<td>920</td>
<td>430</td>
<td>420</td>
<td>3,260</td>
</tr>
<tr>
<td>Alaska</td>
<td>1,070</td>
<td>†</td>
<td>70</td>
<td>90</td>
<td>†</td>
<td>50</td>
<td>290</td>
</tr>
<tr>
<td>Arizona</td>
<td>11,800</td>
<td>360</td>
<td>780</td>
<td>980</td>
<td>510</td>
<td>550</td>
<td>2,830</td>
</tr>
<tr>
<td>Arkansas</td>
<td>6,830</td>
<td>170</td>
<td>430</td>
<td>600</td>
<td>260</td>
<td>260</td>
<td>2,190</td>
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<tr>
<td>California</td>
<td>59,060</td>
<td>1,760</td>
<td>4,400</td>
<td>5,180</td>
<td>2,560</td>
<td>3,600</td>
<td>12,230</td>
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<tr>
<td>Colorado</td>
<td>7,760</td>
<td>270</td>
<td>560</td>
<td>650</td>
<td>330</td>
<td>370</td>
<td>1,690</td>
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<tr>
<td>Connecticut</td>
<td>6,780</td>
<td>190</td>
<td>450</td>
<td>450</td>
<td>310</td>
<td>300</td>
<td>1,690</td>
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<tr>
<td>Delaware</td>
<td>2,050</td>
<td>50</td>
<td>130</td>
<td>150</td>
<td>80</td>
<td>100</td>
<td>600</td>
</tr>
<tr>
<td>Dist. of Columbia</td>
<td>980</td>
<td>†</td>
<td>90</td>
<td>90</td>
<td>†</td>
<td>80</td>
<td>210</td>
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<tr>
<td>Florida</td>
<td>42,600</td>
<td>1,080</td>
<td>2,880</td>
<td>3,500</td>
<td>1,770</td>
<td>1,870</td>
<td>11,960</td>
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<td>Georgia</td>
<td>15,840</td>
<td>460</td>
<td>1,260</td>
<td>1,500</td>
<td>620</td>
<td>730</td>
<td>4,700</td>
</tr>
<tr>
<td>Hawaii</td>
<td>2,480</td>
<td>†</td>
<td>130</td>
<td>230</td>
<td>90</td>
<td>150</td>
<td>570</td>
</tr>
<tr>
<td>Idaho</td>
<td>2,810</td>
<td>90</td>
<td>180</td>
<td>220</td>
<td>120</td>
<td>110</td>
<td>670</td>
</tr>
<tr>
<td>Illinois</td>
<td>24,080</td>
<td>600</td>
<td>1,660</td>
<td>2,030</td>
<td>1,010</td>
<td>930</td>
<td>6,540</td>
</tr>
<tr>
<td>Indiana</td>
<td>13,510</td>
<td>350</td>
<td>860</td>
<td>1,070</td>
<td>570</td>
<td>480</td>
<td>4,020</td>
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Leading sites of new cancer cases and deaths- 2016 Estimates

- **Estimated New Cases**
- **Estimated Deaths**
Etiology and Epidemiology of Lung Cancer

World Scenario

World-wide lung cancer is the most common cancer in term of incidence and mortality with 1.35 million new cases and 1.18 million death in 2002 (Youlden et al., 2008). Lung cancer deaths caused almost 18% of total cancer mortality (Parkin et al., 2005; Mathers and Loncar, 2006). Not all the cases of lung cancer are due to smoking but the role of passive smoking is increasingly being recognized as a risk factor for lung cancer leading to policy interventions to decrease undesired exposure of non smokers to other’s tobacco smoke, emission from automobiles, factories and power plants also pose potential risk in developing countries (Kabir et al., 2007; Parent et al., 2007; Chiu et al., 2006).

Lung cancer was considered to be rare in the beginning of the century (Parkin and Muir, 1992) but now it has reached almost epidemic proportion it is the leading cause of death in developed countries (Khuri et al., 2001). Lung cancer had a higher incidence among male worldwide than any other cancer in developing countries, among female lung cancer was the fourth most diagnosed cancer (Parkin et al., 2005). In women the epidemic is less advanced, most western countries are still showing a rising trend in incidence and mortality (Bray et al., 2004; Jemal et al., 2004). Lung cancer incidence with increased smoking incidence is expected to increase in the world few years notably in China and India (Behera and Balamugesh, 2004).

Lung cancer incidence rate were around twice as high in more developed countries (61/100,000 among males and 19/100,000 among females) compared with less developed countries (29/100,000 among male and 10/100,000 among females (GLOBOCAN, 2002). The shift towards a higher proportion of lung cancer cases in developing countries appear set to continue (Kanmangar et al., 2006; Alberg et al., 2005).
By the year 2025, 85% of the world smoker will live in less developed countries (Mackay, 1998). It is expected that around 70% of all tobacco related death will occur in the world’s poor and middle income nations compared with the current estimate of 50% (Mackay, 2006; Warner, 2005).

**Indian Scenario**

The National Cancer Registry Programme of the Indian council of medical research which collected data from six different parts of the country, both rural and urban areas showed varying figures in different areas (National Cancer Registry Programme, 1988-1989), where lung cancer was initially thought to be infrequent in India (Parkin et al., 2001).

International comparison of incidence of lung cancer with that seen in India showed a low figure (age adjusted rates of 66.5-100.4 in Europe and USA versus 2.0 to 14.6 per $10^5$ in Indian males the same is 16.1 to 33.3 versus 0 to 3.7 in females) because of the overall population size (Behera and Balamugesh, 2004). The population census data for India in 1991, 609,000 new cancer cases were estimated to have diagnosed in the country, this figure had increased to 806,000 by the turn of century, the estimated age standardized rates per 100,000 were 96.4 for males and 88.2 for females (Rathika Bobba and Yamin Khan, 2003).

There are nearly 101 billion smokers across the world and 80% of them are in developing countries. India has total of 240 million smokers, 194 million of these are male and 45 million as females (The Hindu Jun 01, 2003).

Lung cancer is the most common cancer among men in India with approximately 33,000 new cases every year (Cancer patients Aid association). Pattern of lung cancer in India vary from that of the Western European/USA population.

In India, Squamous cell carcinoma is the commonest variety as compared to the adenocarcinoma in the west and the diseased tend to occur early in India (51-60 years).
almost 90% of patients coming with lung cancer are smokers with the male to female ratio of 10:1 (Pathak et al., 2003). As of 1st July 2002 a total of 41,000 cases of lung cancer would have been diagnosed for that year in India as per the ICMR data from its cancer registry (National Cancer Registry Programme, 1988-1989) and it may steadily rise in the coming year.

The age-adjusted incidence rate of lung cancer is 62 per 100,000 men and women per year in the United States, with the incidence rate higher in men than in women (75.2 vs 52.3 per 100,000). Lung cancer in both genders tops the list on the number of estimated deaths yearly (85,600 ) or 28% of all cancer deaths for men, and 71,340, or 26% of all cancer deaths for women.

Over 4000 individual compound have been identified in tobacco including toxic, mutagenic and carcinogenic compound (Berlin 2002: Albert et al., 1991). While carcinogen directly cause cancer, toxic and mutagenic agent also contribute to the carcinogenic process leading directly to cancer for example Formaldehyde and Acrolein are not carcinogen themselves, but they may contribute to carcinogenic process by inhibiting lung clearance and there by prolonging contact of smoke compound with respiratory epithelium (Carolyn et al., 2001).

While toxic agent such as Hydrogen cyanide and Ammonia function similarly as prolonged exposure of tobacco smoke as in active smoker, provide adequate contact with compound for the initiation and eventual completion of the multistage carcinogenic process (Prakash and Gupta, 2001).

Etiology of Lung cancer

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Occupational carcinogens</th>
<th>Risk Definitely known</th>
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</table>
1. **Asbestos**
   - Insulation workers and shipyard workers are exposed to asbestos. There is some increase in risk of lung cancer after 10 years of exposure, substantial risk after 20 years of exposure. Concurrent smoking increases the risk to 90-fold.

2. **Arsenic**
   - Smelter workers and vineyard workers are exposed to arsenic. The risk is dose related. Lung cancers have upper lobe predominance and there may be multiple primaries.

3. **Nickel Refinery**
   - Squamous cell carcinoma in workers more common

4. **Radiation**
   - Uranium mining Oat cell carcinoma is more common

5. **Hematite mining**
   - Due to radon exposure

6. **Hard rock mining**
   - Chromium exposure in ore mining and pigment manufacturing: squamous cell is most common.

7. **Chloromethyl**
   - Oat cell carcinoma is most common

8. **Ethers**
   - Squamous and undifferentiated carcinomas most common.

### Classification of Lung Cancer

Human lung cancer can be divided into non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) based on histopathological features. About 80% of human lung cancers are NSCLC, and they are subdivided broadly into adenocarcinoma, squamous cell carcinoma (SCC), and large-cell carcinoma, of which adenocarcinoma is the most
prevalent and appears to be increasing in frequency, especially in women and nonsmokers (Husain and Kumar, 2005).

Non-Small Cell Lung Carcinoma (NSCLC)

NSCLC are heterogeneous group, there are three main sub-types: squamous cell lung carcinoma, adenocarcinoma and large cell lung carcinoma (Table 1.2). NSCLC accounts for 31.1% of lung cancer (Travis et al., 1995). Squamous cell carcinoma are predominantly linked to smoking (Sakurai et al., 2004). These tumors tend to grow in the centre of the lung and have the capacity to grow to large size (Beadsmoore and Screaton, 2003). Well differentiated squamous cell lung cancer often grow more slowly than other cancer types (Vaporciyan et al., 2002).

Adenocarcinomas usually originate in the periphery of the lung (Beadsmoore and Screaton, 2003) accounts for 29% of lung cancer. It is the most common form of lung cancer in non-smokers (Subramanian and Govindan, 2007). Smoking has been increasingly associated as a cause of adenocarcinoma in recent years (Yang et al., 2002).

A subtype of adenocarcinoma, the bronchiol alveloar carcinoma is more common in female passive smokers and may have different response to treatment (Raz et al., 2006).
cell lung carcinoma account for 10.7% of lung cancer. It is a fast growing form that develops near the surface of the lung (Veronesi et al., 2006). Often poorly differentiated and tend to metastasize early (Morandi et al., 2006).

Small Cell Lung Carcinoma (SCLC)

SCLCs are the most aggressive form of the disease, having greater potential to metastasis than other type of lung cancer, nearly all patients (over 95%) diagnosed with SCLC are current or ex smokers (Jackmann and Johnson, 2005).

Small cell lung carcinoma (SCLC) also called as oat cell carcinoma is less common it tend to arise in the larger airways (Primary and secondary bronchi) grow rapidly becomes quite large (Collins et al., 2007). The cell contain dense neurosecretory granules (which contain neuro endocrine hormones) gives an endocrine para neoplastic syndrome association (Rosti et al., 2006). While initially more sensitive to chemotherapy, if ultimately carries a worse prognosis and is often metastatic at presentation, they are diagnosed at late stage after metastatic (Chyczeweski et al., 2001). It is strongly associated with smoking (Barbone et al., 1997).

**Signs and Symptoms of Lung Cancer**

The symptoms of lung cancer include (Hamilton et al., 2005).

- Dyspnea (shortness of breath)
- Hemoptysis (Coughing up blood)
- Chronic coughing or change in regular coughing pattern
- Wheezing
- Chest pain or pain in abdomen
- Cachexia (weight loss), fatigue and loss of appetite
- Dysphonia (hoarse voice)
✓ Clubbing of the fingernails (uncommon)
✓ Dysphagia (difficulty swallowing)

**Diagnosis of Lung Cancer**

The lack of diagnostic tools for earlier detection and efficient treatment during the advanced stage of disease, prognosis of lung cancer is still poor, with statistics showing less than 15% surviving after 5 years of diagnosis (Greenlee et al., 2000).

➢ The history and Physical examination
➢ The chest x-ray
➢ CT (computerized axial tomography scan, or CAT scan) scans
➢ Magnetic resonance imaging (MRI)
➢ Positron emission tomography (PET)
➢ Bone scans
➢ Sputum cytology
➢ Bronchoscopy
➢ Mediastinoscopy, mediastinotomy
➢ Endoscopic Ultrasonography
➢ Throacentesis
➢ Thoracoscopy
➢ Needle biopsy (Fine Needle aspiration)
➢ Major surgical procedures

**Treatment of Lung Cancer**
The three primary forms of treatment for lung cancer are surgery, radiation and chemotherapy (Minna and Schiller, 2008; Schiller et al., 2007). One or more of these therapies may be used to treat lung cancer, depending upon the type of lung cancer and stage of the disease as well as age and health. Chemotherapy is widely accepted as the primary treatment for small and non small cell lung cancer (Edward et al., 2000; Goodman, 2002).

**Surgery**

Surgery is the most effective treatment for lung cancer when it is caught in the earlier stage, three major procedures are done to remove lung cancer. These vary from removing only the cancerous tissue and nearby tissue, to complete removal of lung depend upon the size and location of the tumor.

- Wedge resection (Segmental resection)
- Lobectomy
- Pneumonectomy

**Radiotherapy**

Radiotherapy is also called as radical radiotherapy (Arriagada et al., 2002), it is the most versatile treatment for non small cell lung cancer of any Stage because it does not require fitness for surgery or the ability to tolerate chemotherapy. Different type of radiotherapy include,

- External-beam radiation therapy
- Conventional (two dimensional) radiation therapy
- Three-dimensional conformal radiation therapy
- Intensity-modulated radiation therapy
- Internal radiation (Brachytherapy)

**WHO Response on Prevention**
In 2013, WHO launched the Global Action Plan for the Prevention and Control of Non communicable Diseases 2013-2020 that aims to reduce by 25% premature mortality from cancer, cardiovascular diseases, diabetes and chronic respiratory diseases by 2025. Some of the voluntary targets are most relevant for cancer prevention, including target 5 aimed at reducing the prevalence of tobacco use by 30%.

- Global action plan for the prevention and control of NCDs 2013-2020
- WHO and the International Agency for Research on Cancer (IARC), collaborate with other United Nations organizations within the UN Non communicable Diseases Interagency taskforce (2014) and partners too
- Increase political commitment for cancer prevention and control;
- Coordinate and conduct research on the causes of human cancer and the mechanisms of carcinogenesis
- Monitor the cancer burden (as part of the work of the Global Initiative on Cancer Registries GICR)
- Develop scientific strategies for cancer prevention and control;
- Generate new knowledge, and disseminate existing knowledge to facilitate the delivery of evidence-based approaches to cancer control;
- Develop standards and tools to guide the planning and implementation of interventions for prevention, early detection, treatment and care;
- Facilitate broad networks of cancer control partners and experts at global, regional and national levels;
- Strengthen health systems at national and local levels to deliver cure and care for cancer patients
✓ Provide technical assistance for rapid, effective transfer of best practice interventions to developing countries.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Types of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Surgery followed by radiation therapy.</td>
</tr>
<tr>
<td>II</td>
<td>Surgery, with chemotherapy before or after surgery, radiation therapy</td>
</tr>
<tr>
<td>III</td>
<td>A combination of surgery, chemotherapy, and/or radiation.</td>
</tr>
<tr>
<td>IV</td>
<td>Chemotherapy, radiation therapy (as a form of palliative treatment) to relieve pain or other symptoms affecting quality of life, targeted therapies.</td>
</tr>
</tbody>
</table>
**Chemical Carcinogens**

Carcinogens are a group of naturally occurring synthetic compound which are stable in the environment and require metabolic activation usually within the target cell to cause mutagenic and carcinogenic effect (Miller and Miller, 1981). About 81% of cancer result from exposure to naturally occurring man made environmental carcinogens. The hydrocarbons are widely distributed organic matter in the environment and also represent a very large group of carcinogens.

**Polycyclic Aromatic Hydrocarbons (PAHs)**

The most important class of compound of lung cancer risk is polycyclic aromatic hydrocarbon (PAHs). The PAHs and nitrosamines are the most important tobacco smoke carcinogens (Witschi et al., 2001). PAHs are ubiquitous environmental agents commonly believed to contribute significantly to human cancer. PAHs are formed in the process of incomplete combustion of organic material and are found widely in the environment (in the engine exhaust, cigarette smoke etc.). The most extensively studies and common PAH that exhibit strong carcinogenic property in experimental animals is benzo(a)pyrene (Travis et al., 1995).

**Benzo(a)pyrene (Chemical Carcinogen)**

Benzo(a)pyrene (B(a)P) is a prototypical polycyclic aromatic hydrocarbon, formed by the incomplete combustion of many organic materials and is ubiquitously present in our environment. Various anatomical sites of cancer are related to ingestion and inhalation of polycyclic aromatic hydrocarbons. It is highly carcinogenic and mutagenic. Its molecular formula C\textsubscript{20}H\textsubscript{12} and a molecular weight of 252.3. (CAS Reg. No. 50-32-8)
Properties

Benzo(a)pyrene is practically insoluble in water but is soluble in benzene, toluene, xylene and sparingly soluble in alcohol and methanol (Budavari et al., 1989). It exists as pale yellow crystal, has a boiling point of 496°C, a melting point of 178.1°C and a density of 1.35g/cm³ (EPA, 1991). It has a vapor pressure of 5.0 x 1⁻¹ torr and a log octanol/water coefficient of 6.04. Its readily absorbed by the oral, inhalation and dermal route of exposure.

B(a)P-Metabolism

B(a)P is metabolized initially by the microsomal cytochrome P₄₅₀ monooxygenase system to several arene oxides, which may rearrange spontaneously to phenol, undergo hydration to the corresponding trans-dihydrodiols or react covalently with glutathione, either spontaneously or in a reaction catalyzed by glutathione-S-transferases. One of the phenolic metabolites 6-hydrobenzo(a)pyrene is further oxidized to the 1,6-,3,6,or 6,12-quinones. The phenols, quinones and dihydrodiols can be detoxified by conjugation to glucuronides and sulfate esters and the quinones can also form glutathione conjugates. In addition to conjugation, the dihydrodiols undergo further oxidative metabolism. Benzo(a)pyrene 7,8-dihydrodiols is in part oxidized to 7,8-diol-9,10-epoxoide, a compound considered to be the ultimate carcinogenic metabolism of B(a)P (Alexandrov et al., 1993).
B(a)P-Carcinogenesis

The mechanism of B(a)P carcinogenesis has been reported to be related to DNA adduct formation during the initiation stages (Brauze et al., 1991; Albert et al., 1991). Benzo(a)pyrene is bio activated by cytochrome P4501A1 (CYP1A1) enzymes by binding to the aryl hydrocarbon receptor (AHR) in the cytosol. Upon the transformed receptor translocates to the nucleus where it dimerises with aryl hydrocarbon receptor nuclear translocator (ARNT) and then binds xenobiotic response elements (XREs) in DNA located upstream of certain genes. This process increase transcription of certain genes, notably CYP1A1, followed by increased CYP1A1 protein production (Whitlock, 1999). Which further enhance the formation of benzo(a)pyrene diol epoxide molecule, to acquire its mutagenic and carcinogenic properties. It is formed by three step enzymatic reaction the first step oxidized by cytochrome P4501A1 activation is the formation of B(a)P-7,8-epoxide (Shou et al., 1996), followed by hydrolysis by epoxide hydrolase (EH) to the B(a)P-trans-7,8 dihydrodiol (7,8-diol), which is further metabolized by CYP enzymes to the ultimate genotoxic (+)-B[a]P-r-7,r-8-dihydrodiol-t-9,10-epoxide (BPDE). The BPDE, are highly reactive electrophiles (Fig.1.5). Cytochrome P4501A1 (CYP1A1) and CYP1B1 have been shown to be the principal catalysts of metabolism of B(a)P and other polycyclic aromatic hydrocarbons (Slaga et al., 1979; Gelboin, 1980; Szeliga and Dipple, 1998).

Benzo(a)pyrene activates oxidative stress-induced cell proliferation and carcinogenesis by transcriptional elevation of several genes including c-jun, c-fos, c-myc and inducible nitric oxide synthase (iNOS). These genes are found to be activated by stress signals through the stimulation of tyrosine kinase, which in turn modulates downstream of events including the expression of nuclear proto oncogenes (Chang, 2000).

B(a)P- DNA adduct
Benzo(a)pyrene is metabolized to (±)-B[a]P-r-7,t-8-dihydrodiol-r-9,10-epoxide (BPDE), the ultimate carcinogen. BPDE isomers bind to the exocyclic-nitrogen of deoxyguanosine in DNA via trans-addition of the C-10 position in the epoxide molecule. This results in the formation of B(a)P-DNAs adducts is a critical event in lung tumourgenesis by B(a)P, and is responsible for the mutational activation of ras-oncogenes in B(a)P induced mouse lung tumors. This adduct may also cause activation of proto oncogenes in human large cell carcinoma and adenocarcinoma of the lung (Sticha et al., 2002; Kristna et al., 2002; Schwarz et al., 2001). X-ray crystallographic and nuclear magnetic resonance structure studies show that binding distorts the DNA (Volk et al., 2003). Inducing mutation by perturbing the double helical structure. This process disrupt the normal process of copying DNA and induces mutation which explains the occurrence of cancer after exposure.

**Interacting with DNA**

Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide is the carcinogenic product of three enzymatic reactions:

Benzo[a]pyrene is first oxidized by cytochrome P4501A1 to form a variety of products, including (+)benzo[a]pyrene-7,8-epoxide.

This product is metabolized by epoxide hydrolase, opening up the epoxide ring to yield (-)benzo (a) pyrene -7,8-dihydrodiol.

The ultimate carcinogen is formed after another reaction with cytochrome P4501A1 to yield the (+)benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide. It is this diol epoxide that covalently binds to DNA.
**B(a)P – Detoxification**

In addition to the B(a)P activating enzymes, a series of enzymes (Phase-11 enzymes) detoxify activated carcinogens, thus preventing their binding to DNA (Kiani and Dar, 1992). The induction of GST is an important response for the detoxification of xenobiotics. Other detoxification enzymes include UDP-glucuronyl transferase and Quinone reductase. The efficiency of these and other enzymes detoxify carcinogens is a critical factor in determining the carcinogenicity of a particular xenobiotic (Singh et al., 1998).

**Chemotherapy**

The chemotherapy drugs are used to kill cancer cells, most drugs are cytotoxic they work by preventing the formation of new DNA or by blocking some other essential function in the cells or by apoptosis (Belany and Langer, 2002). It kills only a certain fraction of cells growing in large tumor at any one time, the cells that are at a certain stage in cell cycle (Schiller et al., 2002). Chemotherapy for the treatment of cancer is associated with various side effect such as cardiotoxicity, nephrotoxicity, neurotoxicity etc. Ideally chemotherapeutic drug should specifically target only cancer cell. In practice the use of chemotherapy is marked by various factors, including systemic toxicity due to a lack of
specificity resulting in damage to normal functioning cells which are involved in vital functions. (Nishimura et al., 2007) Recently it has been found that plant monoterpenes are potential compounds, which can be used for various ailments including cancer, the major advantage of compounds derived from plant origin is their cytotoxicity, specifically to aggressively proliferating cancer cells without harming the surrounding normal cells.

**Mechanism of Cancer Chemopreventive Agents**

Chemopreventive agents, especially substance derived in the diet when Dr. Michael Sporn coined for the first time the term “chemoprevention” referring to the activity of natural forms of vitamin A in preventing the development and progression of epithelial cancer (Sporn et al., 1976). He originated a novel field in cancer research Chemoprevention, a relatively new area of cancer research relies on the potential for modulating the process of carcinogenesis, through the use of defined chemical agents to reduce the incidence of cancer in a given population (Miller and Miller, 1981). Cancer chemopreventive can be defined as prevention of cancer by the administration of one or more chemical entities, either as individuals drugs or as naturally occur in constituents of the diet (Morse and Stoner, 1993). The underlying hypothesis of prevention is that, carcinogenesis is the stepwise accumulation of genetic and epigenetic changes that result in a cell with a malignant phenotype. Chemoprevention strategies can be classified into three category.

- Primary prevention of cancer in healthy individuals who are at high risk it is possible to inhibit mutation and cancer inside cell (e.g., by modifying transmembrane transport, modulating metabolism, blocking reactive species, inhibiting cell proliferation, maintaining DNA structure, modulating DNA metabolism and repair and controlling gene expression).
Secondary prevention of cancer in individuals with premalignant lesions it is possible to inhibit tumor progression via the same mechanism and in addition by affecting the hormonal status and the immune system in various ways, and by inhibiting tumor angiogenesis.

Tertiary prevention aimed at cancer patients after therapy it exploits similar mechanism, it is also possible to affect cell-adhesion molecules, to activate antimetastasis genes, and to inhibit proteases involved in basement membrane degradation (Flora and Ferguson, 2005).

Consequently chemopreventive therapy, or use of chemical agents to block or reverse carcinogenesis, has great appeal for lung cancer prevention (Destefani et al., 2002). The three major type of chemopreventive agent are (a) Inhibitors of carcinogen formation (b) Blocking agent (c) Suppressing agent (Wattenberg, 1985). while suppressing agent can be described more specifically as inhibitors of tumor promotion/progression, where nobiletin belong to this type.

**Dietary Chemoprevention**

According to recent large epidemiologic studies, protection of lung cancer is associated with higher dietary intake of fruits and vegetables (Tan and Spivack, 2009). Dietary phytochemicals has proven efficacy in cancer chemoprevention both *in vitro* and *in vivo* models (Russo et al., 2005; Nishino et al.,2000). Epidemiological evidences has suggested that vegetables and fruits have a role in cancer prevention (Cherng et al., 2007). Many phytochemicals present in a diet, rich in fruit and vegetables and have been proposed as potential chemopreventive agents. These compounds can be divided into two main groups: cancer-blocking and cancer-suppressing agents. The former prevent carcinogens to hit their cellular targets (initiation) by several mechanisms: enhancing carcinogen
detoxification, modifying carcinogen uptake and metabolism, scavenging reactive oxygen species (ROS) and other oxidative species, enhancing DNA repair. While cancer suppressing agents inhibit cancer promotion and progression after the formation of preneoplastic cells by interfering with cell cycle regulation, signal transduction, transcriptional regulation, and apoptosis (You and Bergman, 1998; Surh, 2003; Greenwald, 2002). Several epidemiologic studies suggest a positive association between consumption of a diet rich in fruits and vegetables and a lower incidence of stomach, esophagus, lung, oral cavity and pharynx, endometrium, pancreas, and colon cancers (Mathew et al., 2004; Reddy et al., 2003; Block et al., 1992).

**Phytochemicals in Chemotherapy**

Accordingly to a more modern and complete definition, chemoprevention includes the use of natural or pharmacological agents to suppress, arrest or reverse carcinogenesis, in early stages (Sporn and Suh, 2002). The need of new agent with novel mechanism of action to prevent cancer, is perhaps the most urgent need in the field of chemoprevention. In recent years, considerable attention was given on dietary intake of phytochemicals.

Phytochemicals are natural bioactive compound found in plant foods that have potent properties to protect against disease. Epidemiological studies, have consistently shown that regular consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic disease such as cancer and cardiovascular disease (Moiseeva and Manson, 2009; Rui Hai Liu, 2004). Fruits and vegetables are most effective against those cancer that involve epithelial cells such as cancer of the lung, cervix, esophagus, stomach and pancreas (Tavani and Vecchia, 1995). Higher intake of fruits provided reduced risk of many epithelial cancer and less mortality from cancer (Steinmetz and potter, 1991; Ziegler,
Phytochemicals “the super nutrients” of the 21st century are effective in the prevention and treatment of cancer.

**Phytochemicals have an anti carcinogenic action by,**

- Slowing cell proliferation by interfering with the cell cycle
- Inducing apoptosis
- Inhibiting phase I (enzyme that convert harmless substance into carcinogen)
- Inducing phase II (enzymes that attack carcinogen to molecules that facilitate speedy excretion)

Phytochemicals such as carotenoids, phenolic compounds and terpenoids suppress experimental carcinogenesis in various organs (Nishino et al., 2000). Phytochemicals are reported to exhibit a wide variety of biological activities including antioxidants and free radical scavenging activities (Beutner et al., 2001). Citrus fruits are particularly high in a class of phytochemical known as limonoids (Hasegawa and Miyako, 1996).

Since numerous epidemiological as well as experimental studies gave positive correlation between reduced risk of cancer and intake of phytochemicals (Ramakrishnan et al., 2007). The use of plant-based natural product as chemopreventive agents is drawing a lot of attention and considered to be practically beneficially in cell/tissue based system and animal model systems (Selvendiran et al., 2006). Henceforth the search for the new chemopreventive and anti-tumor agents that are more effective and less toxic has kindled great interest in dietary phytochemicals (Kamaraj et al., 2007).

**Flavonoids**

Citrus fruits are a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants (Y. Nogata et al., 2006). Citrus flavonoids are generally categorized into two groups, flavanone glycosides (hesperidin, naringin and neohesperidin)
and polymethoxylated flavones (nobiletin, tangeretin and sinensetin) (E. J. Middleton et al., 2000).


Recent studies of the polymethoxylated flavones, nobiletin and tangeretin, have focused especially on anti-inflammatory, anti-tumor and anti-carcinogenic activities (A. Murakami et al., 2000, H. Nishino et al., 2004, Y. Iwase et al., 2001, A. Murakami and H. Ohigashi et al., 2006, A. Eguchi et al., 2006). For example, nobiletin has been reported to be a novel, and promising anti-inflammatory agent, inhibiting the activity of nuclear factor-kappa B (NF-kappa B) and suppressing bone resorption and the generation of reactive oxygen species (ROS) (S. Y. Choi et al., 2007, S. Harada et al., 2011).

**Nobiletin**

**Chemistry**

Molecular formula: C_{21}H_{22}O_{8}

**Nobiletin is an Promising Anticancer Flavonoid**

Nobiletin, a citrus polymethoxylated (six methoxy groups) flavonoid that is highly contained in the peels of citrus fruits, exerts a variety of beneficial effects including anti-proliferative effects in cancer cells, repressive effects in hyperlipidemia and hyperglycemia and ameliorative effects in dementia at in vitro and in vivo levels.
Characteristics of nobiletin-mediated alteration of gene expression was seen in cultured cell lines (Kiyomitsu Nemoto et al., 2013).

Nobiletin exerts a wide variety of beneficial activities, including anti-dementia (Nagase et al., 2005), anti-tumor (Murakami et al., 2000), anti-metabolic syndrome (Yamamoto et al., 2009), including anti-obesity (Sato et al., 2003), anti-hyperlipidemia (Andrews et al., 2013), and anti-diabetes (Gao et al., 2012) and anti-inflammatory (Park et al., 2012) activities at in vivo and in vitro levels. It has therefore become increasingly important to clarify the mechanisms of Nobiletin-mediated biological effects, including adverse (toxic) effects.

The in vitro anti-cancer mechanisms of nobiletin found prior to the in vivo study included apoptosis nobiletin induced cell cycle arrest atG2/M phase in A549 cells, inhibition of Bcl-2 protein expression, increase of Bax and p53 protein expression and elevated protein ratio of Bax/Bcl-2 (G. Luo et al., 2008).

The metabolite identification of nobiletin in mouse urine has concluded that it undergoes mono-demethylation (30- and 40-demethyl nobiletin) and di-demethylation (30,40-didemethylnobiletin) metabolic pathway.

Nobiletin exerts a wide variety of biological effects, at least partly, through induction of endoplasmic reticulum stress and suppressions of oxidative stress and cell proliferation

Structure of Nobiletin
Nobiletin has certain inhibitory effects on the proliferation of lung cancer cells both in vivo and in vitro. The mechanism may be related to up-regulation of Bax and Caspase-9 and down-regulation of Bcl-2 (Luo et al., 2009).

Nobiletin, a major component of polymethoxyflavones in citrus fruits, has a broad spectrum of health beneficial properties including anti-inflammatory and anti-carcinogenic activities. The metabolite identification of nobiletin in mouse urine has concluded that it undergoes mono-demethylation (30- and 40-demethyl nobiletin) and di-demethylation (30,40-didemethylnobiletin) metabolic pathway.

Biological screening of Nobiletin and its metabolites has revealed that the metabolites possess more potent anti-inflammatory activity than their parent compound. The study of health promoting property of Nobiletin (5, 6, 7, 8, 30, 40-hexamethoxyflavone), one of the major components of polymethoxyflavone family in citrus fruits, has made tremendous progress.

Both in vitro and in vivo data have shown that Nobiletin has anti-inflammatory (Lin et al., 2003) and anti-carcinogenic activities (Kandaswami et al., 2011). It has been found
that the properties of Nobiletin are very similar to those of dexamethasone, an anti-inflammatory drug.

Choi et al., 2007 have shown that the anti-inflammatory activities among 20 citrus fruit peel extracts are significantly and positively correlated with the content of nobiletin. Nobiletin contributes to pharmacological activities such as anti-cancer and anti-inflammatory effects (Ishiwa et al., 2000; Murakami et al., 2000; Lin et al., 2003; Murakami et al., 2003; Tanaka et al., 2004; Murakami et al., 2005; Wu et al., 2006).

**Nobiletin Inhibits the DNA-Binding Activity by NFkB Pathway**

Nobiletin, a polymethoxylated flavone is recognized as a promising anti-inflammatory and anti-tumor agent (Kandaswami et al.,1991; Kawai et al.,1999; Wu et al.,2006). It has been shown that nobiletin inhibits LPS-induced NF-kB transcriptional activation in mouse macrophages (Murakami et al., 2005. It has been shown nobiletin inhibits NF-kB activation by targeting the DNA-binding activity. Nobiletin inhibited the transactivation of NF-kB and DNA-binding activity of p50/p65, the heterodimer of NF-kB, following LPS stimulation.

Decreased p50/p65 nuclear binding in nobiletin treated cells is of particular interest because NF-kB with this subunit composition potently trans-activated the target genes (Baeuerle and Henkel, 1994). The release of cytosolic NF-kB from the NF-kB–IκB complex requires phosphorylation of IκB by the IKK multiprotein complex, followed by ubiquitination and degradation by the 26S proteasome to generate transcriptionally active NF-kB that undergoes rapid translocation to the nucleus for the DNA binding (Baldwin, 1996).

Although there are a number of IκB proteins, IκBk is the primary regulator of rapid signal-induced activation of NF-kB. After regradation, the cytoplasmic IκB is rapidly
replenished by an accelerated production of the protein which is, at least in part, transcriptionally regulated (Sun et al., 1993). Soo-Youn Choi et al., 2007 showed that nobiletin did not affected LPS-induced phosphorylation and degradation of IκBκ protein, and LPS-induced nuclear translocation of NF-κB.

It indicates that the inhibitory effect of nobiletin on the production of pro-inflammatory mediators must involve transcriptional regulation through suppression of NF-κB DNA-binding activity.

Nobiletin itself is unlikely to react directly with NF-κB given that direct addition of nobiletin to nuclear extracts did not inhibit DNA binding. This suggests that either a metabolite of nobiletin is responsible for the effect or that a nobiletin-induced product drives the inhibition of the interaction between NF-κB and DNA.

PDTC (an NF-κB inhibitor) and herbimycinA (a tyrosine kinase inhibitor) have been shown to block DNA binding by covalent modification of the NF-κB p50 subunit in EL4. NOB-1 cells (Mahon and O’Neill, 1995). Brennan and O’Neill (1996) have shown that PDTC increases the oxidation of GSH to GSSG, leading to the formation of a mixed sulphide with NF-κB, thereby inhibiting DNA binding. Further research is required to elucidate the precise mechanism of nobiletin-dependent modulation of the DNA binding of the NF-κB protein.

LPS-triggered inflammatory responses induce ROS production via the activation of NADPH-oxidase in macrophages (DeLeo et al., 1998). Furthermore, ROS stimulates the redoxbased activation of NF-κB and pro-inflammatory cytokine gene transcription (Leeper-Woodford and Detmer, 1999; Kabe et al., 2005). LPS does not increase acutely ROS production (within 120 min) but does induce ROS generation after 8–48 h. LPS induces NF-κB activation in a ROS-independent manner. ROS are required for the hypoxic activation of NF-κB and TNF-κ gene transcription but not for the LPS-induced activation of NF-κB.
(Chandel et al., 2000; Thakur et al., 2006). Early results have been shown that nobiletin has the ability to alleviate oxidative stress by inhibiting LPS-mediated ROS production by RAW 264.7 cells.

Nobiletin purified from the fruit peel of *Citrus sunki* Hort. ex Tanaka, inhibits the expression of inflammation associated genes by targeting the DNA-binding activity without interfering with the nuclear translocation of NF-kB. S.Y. Choi et al. 2007 proves that nobiletin may inhibit the redox-based NF-kB activation by suppressing LPS-induced ROS production in RAW 264.7 cells. These properties may provide a potential mechanism that explains the anti-inflammatory activity of nobiletin.

**Nobiletin Inhibits the Cell Cycle Arrest at G2/M phase**

Nobiletin could induce apoptosis in A549 cells. In addition, Nobiletin induced cell cycle arrest at G2/M phase in A549 cells. The tumor suppressor gene, p53, functioned as a cellular emergency response system to induce cell growth arrest or apoptosis. (Bunz F et al 1998, Karpinich NO et al.,2002) When DNA damage occurred, p53 plays a key role in preventing DNA replication and inducing cell cycle arrest at G1/S phase or G2/M phase (Ciciarello M et al.,2001, Zhang HY et al.,2004).

If DNA repair is ineffective, apoptosis will be induced (Fukazawa Tet al.,2003). However, the p53 gene is often mutated in many tumor cells and the mutations contribute to genomic instability impaired cell cycle regulation and inhibition of apoptosis (Benchimol S et al.,2001, Haupt Y et al.,1997) But unlike the majority of human lung carcinoma cell lines, A549 cells carry a wild-type p53 gene and did not show mutation in p53 gene(Lehman TA et al.,1991).

Over expression of the wild-type p53 gene has shown to suppress the growth of human tumor cell lines. (Mercer WE et al.,1990, Baker SJ et al.,1990) Nobiletin augmented
the expression of p53 protein. The accumulation of p53 is a critical step for p53 activation. p53 protein might act as a major activator for G2/M phase arrest and apoptosis promotion in A549 cells. The mechanisms by which p53 regulates apoptosis are not completely understood, but one mechanism involves activating the mitochondria-regulated death pathway by increasing gene expression of pro-apoptosis gene of Bcl-2 family and inhibiting the expression of anti-apoptotic gene (Oren M et al.,2003, Green DR et al.,2004).

**Nobiletin Regulated the Expression of Apoptotic and Proapoptotic Proteins**

The DNA binding domain of wild-type p53 protein interacts with Bcl-2 to promote Bax induced outer mitochondrial membrane permeabilization, while p53 also is a direct transcriptional activator of the Bax gene. (Butt AJ et al.,2000, Miyashita et al.,1995) Bcl-2 is overexpression in high percentage of human non small cell lung cancers. (Pezzella F et al.,1993, Reed JC 1997). Over expression of Bcl-2 may result in accumulation of cells in the G0 phase of cell cycle division and contribute to chemoresistance (Reed JC 1997).

Nobiletin inhibited the growth of A549 cells and arrested cell cycle at G2/M phase through activation of p53 in response to DNA damage. Gang Luo et al., 2008 have been shown that Nobiletin may induced cell apoptosis mediated by p53, through downregulation of Bcl-2 and upregulation of Bax.

**Apoptosis by Intrinsic and Extrinsic Pathways**

Extrinsic or cytoplasmic pathway and intrinsic or mitochondrial pathway. One of the most important regulators of the intrinsic pathway is the Bcl-2 family proteins. Bax and Bcl-2 are known to function upstream of Caspases to regulate apoptosis induced by various stimuli (Gao Z et al.,2005,Chang HK et al.,2006). These suggest a potential mechanism for induction of apoptosis by Nobiletin. An increased expression of p53 induced by Nobiletin upregulated the ratio between Bax and Bcl-2, which results in the release of cytochrome c,
Caspases activation, and cell apoptosis ultimately. Nobiletin showed significant antitumor effects on human lung adenocarcinoma cell line A549 both in vitro and in vivo. Nobiletin had good perspectives as a potent and selective antitumor agent on lung tumors.