Chapter 1

Review of Literature
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Cancer is heterogeneous, genetic disease, characterized by uncontrolled cell division and growth, with varied morphology, behavior, disregulated molecular features and dynamic changes in the genome. The cancer causing agents are known as carcinogens, which are highly capable of inducing drastic mutations within the genome which will sustain by overcoming the repair mechanisms and ultimately leading to cell transformation. These transformed cells will be subsequently accredited with invasiveness and dissemination to their particular homing metastatic sites depending upon the favourable microenvironment. This metastatic progression, triggered by angiogenesis confer the disease a dreadful, life threatening validity with poor prognosis.

Activation or inactivation of about 25 different genes are involved in carcinogenesis, which are present in every cell as inactive proto oncogenes. Proto-oncogenes are altered by mutagens to oncogenes which are over expressed during carcinogenesis and promote survival signals for cancer cells, stimulating their proliferation. The genomic mutations can be produced by free radicals, ionizing radiation, UV radiation and chemicals as well as these mutations may be inherited or acquired. Similarly viruses such as HPV inhibit P53 and RB gene, that may lead to genomic instability. Numerous tumor suppressor genes and anti-oncogenes are also present in all the cells that act as check points to inhibit or kill cancer cells.

Based on their behavior, tumours may be classified into benign and malignant. Benign tumours usually have slow growth that may compress the surrounding tissue and can be usually removed by surgery. On the other hand, malignant tumors have malignant potential and show rapid and uncontrolled growth. There are different types of cancer, based on the tissue origin. They are carcinoma, sarcoma, lymphoma, leukemia, germ cell cancer and blastoma. More than 90 % of the cancers are generated from epithelial cells, called as carcinoma. Examples of this type are cancers of breast, prostate, lung, pancreas and colon. Mostly these types of cancer may occur in adults. Sarcoma develops from connective tissue, whereas lymphoma and leukemia types of cancer originate from blood cells. Leukemia mostly develops in children. Germ cell cancer may develop from pluripotent cells from testicle (seminoma) or ovary (dysegerminoma). Moreover blastoma
type of cancer develop from embryo and thus especially prominent in children than in adults.

1.1. Epidemiology of cancer

Epidemiologic study of cancer helps to identify the reason and chances of cancer in a population which lead to the development of cancer treatment and prevention strategies. Cancer is the second largest leading cause of death in developing countries. Reports suggested that, every year, about 11 million people are diagnosed with cancer and more than 7 million people are dying from cancer. The global increasing rate of cancer is even faster than the global population growth rate. It is predicted that, by 2020, there will be 20 million new cases and 12 million deaths due to cancer, which seems very higher than the current reports (Malcolm, et al., 2001). In 2014 more than 5 lakhs cancer deaths occurred by tobacco use. Hepatocellular carcinoma (HCC) is rare in Western region, but in China HCC is the main cancer which may be due to hepatitis B infection and aflatoxin toxicity. Similarly, tobacco smoking is another major reason for lung cancer, especially in the third world countries. Thus it can be said that, at least some of the cancers could have been preventable.

Several epidemiology research programs were conducted by the International Agency for Research on Cancer (IARC), which is established in 1965 by the World Health Assembly to improve the treatment and prevention of cancer. Based on Cancer Registry Program of the India (CRPI) under Indian Council of Medical Research (ICMR), more than 1300 people in India died every day due to cancer during the year 2013-14, indicating that the mortality rate is increased about 6% when compared to the previous year. During the year 2014, about 5 lakhs people died out of 30 lakhs cases. Moreover, the incidence and mortality rates of cancer were increased ominously, especially in the North East region of the country.

The majority of the cancers is caused by the physical inactivity, poor nutrition, obesity and lifestyle problems and thus could also be preventable. Tobacco use and diet remain as the main reason for the cancer causation in humans. In poor countries, infection is another reason for cancer incidence. Viruses such as human papilloma virus (HPV),
hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV) and Helicobacter pylori (bacteria) are infectious agents of cancer in humans. So these types of cancer are preventable if we control lifestyle and daily diet (Malcolm, et al., 2001). Nutrition plays a major role in cancer incidence. Consumption of low fiber food, high sugar, red meat and fatty food, all contribute to high rate of cancer risk. Thus the intake of fiber rich food, vegetables, abundant fruits, carotenoids, vitamin B-12 and folic acids can prevent or reduce the risk of cancer in humans.

1.2. Etiology of cancer

Causation of cancer in human is related to different factors. The carcinogenic factors are either external or internal. External factors may be various carcinogens present in air, food, water and diet as well as ionizing radiation such as UV radiation, infectious viruses and chemicals (Malcolm et al., 2001). The internal factors are hormones, immune conditions, genetically inherited mutations and metabolic errors.

1.2.1. External factors

1.2.1.1. Tobacco

About thirty percentage of cancers may be caused by tobacco smoking. Tobacco induces different types of cancer associated with pharynx, mouth, larynx, oesophagus, pancreas and kidney. Smoking adversely affects every organ of the body (Bagnardi et al., 2001). More than 15 different types of cancer will be caused by smoking. Reports suggest that about 4.9 million people die every year as a result of smoking (West et al., 2007). More than 16 million people suffer from smoking related health problems. Precisely, smoking is responsible for nearly 1 in 5 deaths.

Other side effects such as chronic bronchitis, emphysema, chronic obstructive pulmonary disease (COPD), tuberculosis, pneumonia, heart attacks, strokes, macular degeneration and ectopic pregnancy were also caused by tobacco smoking. According to US Centers of Disease Control and Prevention (CDC), adult male or female will lose about 13.2 years or 14.5 years of life respectively, due to smoking. Cigarette enclosed 80 different types of mutagenic chemical carcinogens including alkaloid nicotine,
benzopyrene, arsenic, ammonia, formaldehyde and cadmium. All these carcinogens were metabolically activated to form epoxides in the body, which can form DNA adducts in lung epithelial cells (Nishikawa et al., 2004).

1.2.1.2. Alcohol

Alcoholic beverages contain different types of carcinogens such as nitrosamines, asbestos fibers, phenols and hydrocarbons. Intake of alcoholic beverages has been shown to cause cancers of mouth, pharynx, larynx, liver, colon, rectum, breast and oesophagus. Epidemiological research shows that people who use both alcohol and tobacco have higher risk of developing cancer. Alcohol will be metabolized in the liver into toxic substance known as acetaldehyde. Acetaldehyde induces DNA damage and stimulates liver cell proliferation, which could lead to cancer. Some of the bacteria present in our mouth and gut also convert alcohol into acetaldehyde. Alcohol also induce the formation of highly reactive oxygen species (ROS) in the cell which could damage the genetic material, proteins and lipids. Moreover, studies reported that the presence of unusual level of alcohol in the body stimulates estrogen production and increases the risk of breast cancer.

1.2.1.3. Diet, nutrition and physical activity

Infrequent diet causes different types of cancer. The carcinogenic chemicals present in food as preservatives or coloring agent may cause serious side effects, mutations and cancer in the stomach, intestine and liver. Physical inactivity, obesity and poor diet are closely related with cancer development. Diet with low fiber content and vitamin, fast food habit and red meat are also associated with cancer formation. Consumption of high salt diet and aflatoxin contamination may lead to stomach and liver cancer respectively. In Japan, gastric cancer is common due to their high salt administration. Physically inactive condition may harmfully affect immune system and endocrine system and over-nutrition is also another reason for the cause of cancers (Buell et al., 1965; Kushi et al., and 2006; Park et al., 2008, ).
1.2.1.4. Chemical substances

The substances which cause particular cancers are generally known as carcinogens. Tobacco smoke contains about fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons which can cause cancer in different parts of the body, especially lungs. Thousands of chemicals are used by humans, and more than 1,000 new chemicals are introduced each year. These chemicals are found in foods, cosmetics, packaging, prescription drugs, household, lawn care products etc. Chemicals and cancer have an early link, which was founded 200 years ago. An English physician noted that a large number of chimney sweeps had cancer of the scrotum due to exposure to soot, which contains polycyclic aromatic hydrocarbons. Since then, huge number of chemicals have been identified as carcinogens. Human body has a defense mechanism to protect against all types of dangerous exposures, including carcinogens. The mechanism of this protection may be detoxification or elimination by enzymes. Chemical substances may be direct acting carcinogens or pro-carcinogens or co-carcinogens. Every year, around two lakhs people die worldwide due to occupational related cancer. Exposure of chemicals in workplace such as asbestos, tobacco smoke, benzene induce mutations and are additional risk factors for cancer (Table 1.1).

Food may be contaminated with various natural and man-made carcinogenic toxicants. Aflatoxins are one of the most potent toxic substances that occur naturally, which is produced by the fungi *Aspergillus flavus* or *A. parasiticus*. Aflatoxin poisoning is reported from all parts of the world in humans, almost all domestic and nondomestic animals through diet. Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs and a variety of other foods and feeds. Aflatoxin B1, the most toxic aflatoxin, is the most potent naturally occurring chemical liver carcinogen known (Thusu *et al.*, 1991; Towner *et al.*, 2003 and Bumrela *et al.*, 2012). Fumonisin B, a toxic carcinogen produced by the fungus *Fusarium verticillioides*, may be found in grains. Other carcinogenic compounds such as heterocyclic amines, n-nitroso compounds and polycyclic aromatic hydrocarbons were also formed by cooking meat or fish at high temperatures or over a direct flame. Polynuclear aromatic hydrocarbon, is another potent mutagenic and carcinogenic agent present in nature.
Table 1.1: Cancer associated with various chemicals and human cancer site.

<table>
<thead>
<tr>
<th>Carcinogens</th>
<th>Cancer Site</th>
<th>Source of carcinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>Liver</td>
<td>Toxins produced by <em>Aspergillus sp.</em></td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>Bladder</td>
<td>Former color additive and rubber antioxidant.</td>
</tr>
<tr>
<td>Arsenic and arsenic</td>
<td></td>
<td>By-product of metal smelting. Component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides and animal dips.</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Lung, mesothelioma, intestinal tract</td>
<td>Formerly used for many applications because of fire, heat, and friction resistance; will still be found in existing construction.</td>
</tr>
<tr>
<td>Benzene</td>
<td>Leukemia, Hodgkin’s disease</td>
<td>Principal component of light oil. Although use as solvent is discouraged, many applications in printing and lithography, paint, rubber, dry cleaning, adhesives and coatings and detergents. Formerly widely used as solvent.</td>
</tr>
<tr>
<td>Benzidine</td>
<td>Bladder</td>
<td>Formerly widely used in dye manufacture. Now used in clinical laboratories.</td>
</tr>
<tr>
<td>Beryllium &amp; beryllium</td>
<td>Lungs</td>
<td>Missile fuel and space vehicles. Hardener for lightweight metal alloys. (aerospace applications and nuclear reactors)</td>
</tr>
<tr>
<td>Bis (chloromethyl) ether</td>
<td>Lungs</td>
<td>Experimental chemical. Formerly alkylating agent in production of some polymers. Can be contaminant of processes containing chloride and formaldehyde.</td>
</tr>
<tr>
<td>Chloromethyl methyl ether</td>
<td>Lung</td>
<td>Commonly contaminated with BCME. Alkylating agent and solvent in manufacture of ion-exchange resins, industrial polymers, and water repellents</td>
</tr>
<tr>
<td>Cadmium &amp; cadmium</td>
<td>Prostate</td>
<td>Uses include yellow pigments and phosphors. Found in solders. Used in batteries and as alloy, metal platings and coatings</td>
</tr>
<tr>
<td>Chromium compounds</td>
<td>Lung</td>
<td>Component of metal alloys, paints, pigments, and preservatives.</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>Testis, Vagina</td>
<td>Veterinary drug and growth promoter in cattle and sheep. Formerly used in human drug therapies</td>
</tr>
</tbody>
</table>
1.2.1.4.1. Chemical carcinogen metabolism

The carcinogenic activity of chemical compounds depends upon its metabolic activation to reactive intermediates. The activation and elimination of the chemical carcinogens are done by xenobiotic enzymes. These xenobiotic metabolizing enzymes have been functionally divided into two main classes known as phase I and phase II. Phase I enzymes consist of cytochrome P450s, flavin-containing monooxygenases (FMOs) and epoxide hydrolases (EH). The phase II enzymes include the glutathione S-transferases (GSTs), uridine diphosphate-glucuronosyltransferases (UGTs), N-acetyltransferases (NATs), sulfotransferases (SULTs), methyltransferases and a few

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tissue</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erionite</td>
<td>Lung, mesothelioma, gastrointestinal tract</td>
<td>Formerly used for many applications because of fire, heat, and friction resistance; will still be found in existing construction, as well as fire-resistant textiles, friction materials (i.e., brake linings), underlayment and roofing papers, and floor tiles</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>Leukemia</td>
<td>Ripening agent for fruits and nuts. Used in rocket propellant and chemical syntheses; fumigant for foodstuffs and textiles; sterilant for hospital equipment</td>
</tr>
<tr>
<td>Melphalan</td>
<td>Lung</td>
<td>Antineoplastic and alkylating agent.</td>
</tr>
<tr>
<td>Mustard gas</td>
<td>Lung</td>
<td>Poison war gas</td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>Bladder</td>
<td>Formerly used in rubber manufacture. No longer produced commercially</td>
</tr>
<tr>
<td>Nickel compounds</td>
<td>Nasal, lung</td>
<td>Nickel plating. Component of ferrous alloys, ceramics, batteries. By-product of stainless-steel arc welding</td>
</tr>
<tr>
<td>Radon and its decay product</td>
<td>Lung</td>
<td>From decay of minerals containing uranium. Can be a serious hazard in quarries and underground mines</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>Angiosarcoma, liver</td>
<td>Refrigerant. Monomer for vinyl polymers. Adhesive for plastics. Formerly “inert” aerosol propellant in pressurized containers</td>
</tr>
</tbody>
</table>

Source: Jeanne Mager Stellman et al., 1996.
others. Xenobiotic enzymes generally metabolize, inactivate or eliminate drugs and other foreign chemicals present inside the body. Some chemicals are inactivated or others are activated (toxicity and carcinogenicity) by xenobiotic metabolism.

1.2.1.4.1.1. Phase I enzymes

Cytochrome P450 enzymes (phase I enzymes) are the most extensively studied biological proteins, which are present in liver and plays an important role in the metabolism of drugs as well as endogenous and exogenous chemicals. Phase I enzymes can transform a non-toxic foreign substance (pro-carcinogen) into a harmful, toxic carcinogenic substance (Table 1.2). Humans have less number of functional P450 genes than mice and rats (Gonzalez et al., 1990). CYP3A subfamily (CYP3A4) is the most important and well-characterized cytochrome p450 enzyme subfamily, which metabolize a number of anticancer drugs. It is the most abundant P450 enzyme in human liver and it is also inducible (Gonzalez, 1992 and Karit et al., 1995). Most of the drugs and chemicals are metabolized by three cytochrome P450 families (Family 1, 2 and 3). Cytochrome P450 enzyme family 1 consist of three well-studied monooxygenases namely, 1A1, 1A2 and 1B1. Cytochrome p450 enzymes such as CYP1A1, CYP1A2, CYP2E1, CYP1B1, CYP2A6 and CYP3A4, which are active in the metabolism of pro-carcinogens are known as class I types. Class II cytochrome p450 enzymes (CYP2B6, CYP2C9, CYP2C19, CYP2C8, CYP2C9, and CYP2D6) are active in the metabolism of drugs, but not of procarcinogens (Lamba et al., 2002).

Cytochrome p450 enzymes are crucial enzymes involved in cancer development and treatment, where they covalently bind with cellular genetic materials or proteins. Some of the cytochrome p450 enzymes are selectively expressed in tumours, which might have a role in drug resistance (Rodriguez-Antona et al., 2006). Hence the inhibition or activation of these cytochrome p450 enzymes is much important while concerning potent cancer chemopreventive or therapeutic drugs (Jiawang, 2013). The carcinogenic mechanism of aflatoxin B1 has been extensively studied. It has been shown that aflatoxin B1 is metabolically activated by hepatic cytochrome P450 enzymes to
produce a reactive intermediate, aflatoxin B1-8,9-epoxide, which consequently binds to nucleophilic sites in

**Table 1.2: Cytochrome p450 enzymes and carcinogen metabolism**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Activation of carcinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Polycyclic aromatic hydrocarbons; benzo(a)pyrene, dimethylbenz[a]anthracene, PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine)</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Activation of aryl and heterocyclic amines in industrial N-nitrosodimethyl amine, 4-aminobiphenylamine, PhIP, IQ (2-amin-3-methylimidazo[4,5-f]-quinoline, aflatoxin B1.</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Polycyclic aromatic hydrocarbons: benzo(a)pyrene, dimethylcholanthrene, DMBA, oestradiol</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Activation of tobacco-related N-nitrosamines: NNK, NNAL (4-methylnitrosoamo)-1-(3-pyridyl) -1-butanone, NDEA (N-nitrosodiethylamine), NNN, NATB (n-nitrosoanatabine), Aflatoxin B1, 1,3-butadiene, 2,6-dichlorobenzonitrile</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Aflatoxin B1and 4-(methylnitroamo)-1-(3-pyridyl)-1-butanone.</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Low molecular weight toxicants and cancer suspect agents: benzene, carbontetrachloride, chloroform, styrene, vinylchloride,vinyl bromide,N-nitrosodimethylamine, NNK</td>
</tr>
<tr>
<td>CYP3A4/5/7</td>
<td>Diverse carcinogens; aflatoxin B1, aflatoxin G1, benzo(a)pyrene, naphthalene, NNN (N9-nitrosonornicotine), 1-nitropyrene, 6-amino-chrysene, oestradiol, senecionine, stergma-to-cystine.</td>
</tr>
</tbody>
</table>

Rodriguez-Antona, et al., 2006

DNA and the major adduct 8,9-dihydro-8-(N7 guanyl)- 9-hydroxy aflatoxin B1 will be formed (Hogberg et al., 1974; Schamhart et al., 1979 and Koss et al., 1982). The formation of aflatoxin-DNA adducts is considered as a key step in the initiation of aflatoxin induced hepatocarcinogenesis (Baggetto, 1992) (Fig. 1.1). DMBA is a polycyclic aromatic hydrocarbon and mostly used for studies in carcinogenic animal model experiment (Hamizah et al., 2012). DMBA is metabolized by CYP1A1 and CYP1B1 enzymes, which convert it into the ultimate carcinogen 1, 2-epoxide-3, 4-diol DMBA (Kawajiri and Ikuta, 1999; Anqus et al., 1999; Buters et al., 1999; and Sharma et
al., 2012;), which then forms adducts with DNA and finally leading to carcinogenesis (Cheng et al., 1988). CYP2E1 is the main enzyme present in the liver which is involved in the conversion of NDEA and other pro-carcinogens into active mutagens. Cytochrome P450 1A1 enzyme present in liver plays a main role in the metabolic activation of polycyclic aromatic hydrocarbons like 3-MC which leads to the formation of highly reactive intermediates that can bind with DNA to form adducts, which may be the reason for sarcoma development in mice (Weiwu et al., 2009).

Fig. 1.1: Bioactivation of aflatoxin in liver by cytochrome p450 enzymes

1.2.1.4.1.2. Phase II enzyme detoxification system

Phase II enzymes are involved in detoxification and elimination of carcinogenic substances from the body (Table 1.3). For that several addition and conjugation reactions are taking place in the body, including glucuronidation, sulfation and glutathione/amino
acid conjugation. There are different conjugation categories, including acetylation, acylation, sulphur conjugation, methylations and conjugation with glucuronic acid.

Table 1.3. Phase II enzymes and substrate

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoxide hydrolase</td>
<td>Epoxides</td>
</tr>
<tr>
<td>Glutathione transferases</td>
<td>Electrophiles</td>
</tr>
<tr>
<td>Glucuronyl transferases</td>
<td>Phenols, thiols, amines, carboxylic acid</td>
</tr>
<tr>
<td>Sulfotransferase</td>
<td>Phenols, thiol, amines</td>
</tr>
<tr>
<td>N-and O-methyl transferases</td>
<td>Phenols, amines</td>
</tr>
<tr>
<td>N-acetyl transferases</td>
<td>Amines</td>
</tr>
<tr>
<td>Amino acid transferases</td>
<td>Carboxylic acids</td>
</tr>
</tbody>
</table>

(Lisk, 1998)

1.2.1.5.Radiation

Radiation may be divided into two types, ionizing radiation (IR) and non ionizing radiation (NIR). X-rays, gamma rays, and other forms of ionizing radiation are used to treat different cancers resulting in the death of the cancer cells. But these types of treatment can lead to mutations of other normal cells, which may ultimately lead to the development of a secondary tumor. These ionizing radiations such as UV, X-rays, gamma radiations and cosmic rays may induce genetic damages and that will lead to cancer. Generally ionizing radiation exposed from natural (potassium 40-found in food and uranium-found in soil, cosmic rays-from space/sun) as well as man made ionizing radiation sources such as medical X rays, occupational radiation, nuclear weapons and diagnosis or treatment using medical instruments. Studies reported the increased risk of cancer after diagnosis or treatment using x-rays, fluoroscopy, spine x-rays and CT scans. Ionizing radiation has adequate energy (high energy electromagnetic waves) to produce ions at molecular level (X-rays, gamma rays, alpha or beta particles and neutrons) and can cause damage to DNA/ proteins there by inducing the development of skin cancer,
breast cancer, leukemia, dermatitis, cataracts and other health problems (Frieben, 1902; Rollin, 1904; Scott, 1911 and Von et al., 1911). First reports on radiation induced skin cancer and leukemia were identified in 1902 and 1911 respectively. UV light (10 nm to 125 nm) ionizes air molecules, but mostly ionizing UV does not penetrate the atmosphere as it is prevented by the upper ozone layer (O3). It consists of 3 types, they are UV-A (315-400 nm), UV-B (280-315nm) and UV-C (<280 nm). UV-A and UV-B produce biologically adverse effects and can induce mutation, photokeratitis, conjunctivitis, sunburn, photosensitization reactions and skin cancers. Gamma radiation consists of photons with wavelength less than $3 \times 10^{-3}$ and greater than $10^{19}$ Hz (41.4 keV), which have no mass and electric charge so that it can penetrate more through matter than both alpha and beta radiation. Non-ionizing radiation can cause injury to humans, nevertheless, the injury is generally limited to thermal damage (extremely low frequency, radio frequencies, microwave, lasers, infrared, visible spectrum and ultraviolet).

1.2.1.6. Biological agents

Cancer formation is connected with various internal and external factors. Studies revealed that there has been a relation between microoraganisms (bacteria, fungus and virus) and cancer. Some virus can enter into the cells and can directly integrate with human genome, which leads to tumourgenesis (human papilloma virus (HPV) and herpesvirus) (Chang et al., 1994). Some other bacteria know as Helicobacter pylori, can contribute to gastric cancer (Cover et al., 2009). Nematode parasite (Spiroptera neoplastica) is also associated with cancer in humans.

1.2.1.6.1. Bacteria as causative agent of cancer

Several bacteria have been associated with cancer. Generally known cancer causing bacteria is H. pylori, its strong chronic infection leads to the development of gastric cancer. Recent studies also support that H. pylori is associated with colorectal, gall bladder and pancreatic cancer (Cummins et al., 2013). Moreover, some other bacteria such as Streptococcus mitis, Staphylococcus epidermis, Bacillus sp., Mycoplasma sp. Robinsoniella princess, Pediococcus acidilactici, L.mesenteroides, Leuconostoc lactis
and *Chlamydophila pneumonia* are also associated with different cancers (lung cancer, pancreatic cancer, breast cancer and ovarian cancer) (Cummins et al., 2013). Different mechanisms are available on how bacteria replicate and survive within tumours. Some studies indicated that due to the hypoxic nature of solid tumours, anaerobic growth environment is developed for anaerobic bacterial growth. Another factor is the development of nutrient rich area around the tumour region due to necrosis. Bacteria associated with cancer suppress the immune system, as a result tumour tissues will be subjected to insufficient immune activity (Wei et al., 2007 and Baban et al., 2010).

1.2.1.6.2. Oncovirusus

Any virus, with DNA or RNA genome, that can cause cancer in humans and animals is known as cancer virus. Human T-Cell Leukemia Virus Type 1 (HTLV-1) is the first identified human retrovirus and causes adult T-cell leukemia/lymphoma (ATLL) as well as it is transmitted through blood, semen and breast milk. According to world health organization (WHO), more than 18% human cancers were caused by microbial infection. Virus-induced genomic instability and oxidative stress in cells will lead to accumulation of DNA mutations. This genomic instability elevates viral oncogene expression and promotes cancer progression. Some of these cancer must be prevented by vaccination (*Human papilloma virus*). Two types of tumour virus genomes are present, they are DNA genome (eg: adenovirus) and RNA genome (eg: Hepatitis C virus) (Arbuthnot and Kew, 2001). The retrovirus have both DNA and RNA genomes (Human T-lymphotropic virus and hepatitis B virus). When viral oncogene enters into the cells, it will enhance the expression of proto-oncogenes present in host cells. Moreover, indirect viral oncogene causes chronic inflammation in host organisms and will lead to cancer.

1.2.2. Internal factors

1.2.2.1. Hormones

Hormones are one of the agents that cause cancer, especially gender related cancers of breast, prostrate, ovary, thyroid and testis. (Henderson et al., 1982; Feigelson et al., 1996). Hormones such as estrogens and progesterone are involved in different
types of cancer in men and women. Obese and low exercise people have chances of hormones related cancer. Studies revealed that oestrogen exposure is related with cancers in women, that is early menarche, late menopause and late first pregnancy will increase the chances of causing hormone related cancer. Taking oral contraceptive (long period) have been increased the risk of ovarian and breast cancer (Bosetti et al., 2002) but reduce the risk of bowel cancer (Hannaford and Elliott, 2005). On the other hand, hormone replacement therapy that combines estrogen and progesterone does not increase ovarian cancer risk and may even protect against endometrial cancer (Pike et al., 2004).

1.2.2.2. Inherited germ line mutation

Only a small percentage (5-10%) of cancers are linked to single inherited genes (germ line mutaion) and are trasmitted through egg or sperm. Germ line mutations in a population can contribute to an increased risk of developing cancer compared with other populations. This will lead to mutations in tumour suppressor genes and increase the chance of developing retinoblastoma, lifraumeni syndrome and multiple endocrine neoplasia type 1. Small percentages of cancers such as ovary, prostate, pancreas, and endometrium, may be related to inherited mutations. Acquired mutations through external factors such as radiation or carcinogens, or harmful chemicals are not likely to be passed on to the offsprings.

1.2.2.3. Oxidative stress

 Reactive oxygen species (ROS) generated through normal oxidative metabolism and other sources have the possibility to cause genetic damage, that lead to DNA adduct and cancer. The body has several antioxidant enzyme mechanisms, which can scavenge free radicals to prevent such genetic damages. Antioxidant enzymes can scavenge ROS as well as vitamin C, vitamin E and dietary phenolic compounds can also activate antioxidant responses by upgrading the expression of detoxifying enzymes. Antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase have been long believed to have protective activity by scavenging excess of free radicals.
1.2.2.3.1. Free radicals

Free radicals are mainly derived from oxygen (reactive oxygen species-ROS) and nitrogen (reactive nitrogen species-RNS). They are produced in our body by the induction of various endogenous systems or exposure to different physicochemical and pathophysiological conditions. Radicals derived from oxygen represent the most important class of radical species generated in living systems (Miller et al., 1990 and Valko et al., 2007). Free radicals can undesirably amend DNA, proteins and lipids and have been implicated in aging and a number of human diseases including cancer. Lipids are highly susceptible to free radical damage, resulting in lipid peroxidation that can lead to adverse alterations in cells. Free radicals can also induce damage to proteins, which leads to loss of enzyme activity. Similarly, damage caused to DNA can result in mutagenesis and carcinogenesis.

(a) Superoxide radicals

The addition of one electron to dioxygen (itself a radical) forms the superoxide anion radical (O2 •−) (Miller et al., 1990). Superoxide anion (O2 •−), arising either through metabolic processes or activation by physical irradiation (primary ROS), can further interact with other molecules to generate “secondary” ROS. The production of superoxides occurs mostly within the mitochondria of a cell (Cadenas et al., 1998). The mitochondrial electron transport chain is the main source of ATP in the mammalian cell, during which, a small number of electrons “leak” to oxygen prematurely, forming the oxygen free radical superoxide, which causes a variety of diseases (Kovacic et al., 2005). Superoxide is produced from both Complexes I and III of the electron transport chain (Muller et al., 2004).

(b) Hydroxyl radical (•OH)

The hydroxyl radical (•OH) is the neutral form of the hydroxide ion. Hydroxyl radical is a very dangerous with short in vivo half-life (Pastor et al., 2000). They are released when Fe^{2+} can participate in the Fenton reaction, causing highly reactive hydroxyl radical (Fe^{2+} + H₂O₂ →Fe^{3+} + •OH + OH−). Thus, under stressed conditions,
O2 •− acts as an oxidant of cluster-containing enzymes and facilitates •OH production from H2O2 by making Fe2+ available for the Fenton reaction (Valko et al., 2005)

(c) Peroxyl radicals (ROO•)

Another reactive radicals derived from oxygen that can be formed in living systems are peroxyl radicals (ROO•). The simplest peroxyl radical is HOO•, which is the protonated form of superoxide (O2 •−) and is usually termed either hydroperoxyl radical or perhydroxyl radical. It has been established that hydroperoxyl radical initiates fatty acid peroxidation by two main pathways namely, fatty acid hydroperoxide (LOOH)-independent and LOOH-dependent pathways (Aikens et al., 1991). Xanthine oxidase (XO) and xanthine dehydrogenase (XD) are interconvertible forms of the same enzyme, known as xanthine oxidoreductase (XOR) (Borges et al., 2002). In purine catabolism, XOR catalyzes hypoxanthine to uric acid and uric acid may act as a potent antioxidant and free radical scavenger. Moreover, the XO form can produce large amount of ROS and RNS (Vorbach et al., 2003). Thus we can say that XOR is an important protective regulator of the cellular redox potential.

(d) Hydrogen peroxide (H2O2)

Generally peroxisomes produce H2O2 and this organelle is the major site of oxygen consumption in the cell. Oxygen consumption in the peroxisome leads to the production of H2O2, which in turn, may oxidize a variety of molecules. This organelle also contains catalase, which decomposes and apparently prevents accumulation of this toxic compound. Thus, peroxisome maintains the balance between the activities of this antioxidant enzyme and net production of ROS. Moreover, H2O2 which is released into the cytosol, can significantly contribute to oxidative stress (Vorbach et al., 2003).

(e) Reactive nitrogen species (RNS)

NO• is a molecule that contains one unpaired electron and it is generated in biological tissues by specific nitric oxide synthases (NOSs). These enzymes metabolize arginine to citrulline with the formation of NO• via electron oxidative reaction
Nitric oxide (NO•) is a reactive radical that acts as a vital oxidative biological signaling molecule which can influence different physiological processes such as neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation (Bergendi et al., 1999). NO• has a short stability in an aqueous environment but it has better stability with a lower oxygen concentration. But it is soluble in both aqueous and lipid media, hence it easily diffuses through the cytoplasm and plasma membranes (Chiueh, 1999). Reactive nitrogen species rich condition is known as nitrosative stress which may lead to nitrosylation reactions in proteins thus inhibiting their normal structure and function (Klatt and Lamas, 2000 and Ridnour et al., 2004). During inflammatory processes, both, nitric oxide and the superoxide anion may react together to produce more amount of highly reactive molecule, peroxynitrite anion (ONOO−), which is a powerful oxidising agent that can induce DNA fragmentation and lipid oxidation (Carr et al., 2000).

### 1.2.2.3.2. Lipid peroxidation

Reactive oxygen species induce biochemical changes in nucleic acids and proteins. Lipid peroxidation is a physiological process that takes place in all aerobic cells and is considered as the main molecular mechanisms involved in the oxidative damage of cell structure and integrity that lead to cell death. Lipid peroxidation is a chain reaction mostly induced by free radicals. The cell membrane, which is composed of polyunsaturated fatty acids, is a primary target of reactive oxygen attack leading to cell membrane damage (Figure 1.2). Unsaturated fatty acids, which are structural part of cell membranes, are subjected to lipid peroxidation by a non-enzymatic and free-radical mediated reaction chain. Lipid peroxidation causes disruption in membrane barrier functions and fluidity. The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid and so of starting a chain reaction (Halliwell and Gutteridge, 1984). Oxidative breakdown of biological phospholipids occurs in most cellular membranes, including mitochondria, microsomes, peroxisomes and plasma membrane.
The products and by-products of lipid peroxidation are toxic, which causes oxidative stress, oxidative damage and apoptosis. In a long series of physiological and pathophysiological processes, including aging and neurodegenerative diseases, the rates of mitochondrial O$_2\cdot$- and H$_2$O$_2$ are increased with a parallel increase in the rate of the lipid peroxidation process. Many products of lipid peroxidation such as hydroperoxides or their aldehyde derivatives inhibit protein synthesis, blood macrophage actions and alter signaling pathways and enzyme activity (Fridovich and Porter, 1981). It is expected that supplementation with adequate antioxidants, as for instance, α-tocopherol, will keep sensitive cells and organs in healthy conditions and increase lifespan (Repetto et al., 2012).

1.2.2.3.3. Antioxidant enzymes

Living organism are endowed with protective antioxidant mechanisms such as superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases, glutathione reductase and non-enzymatic antioxidants (vitamin E, vitamin C and carotenoids). There are several research evidences correlating higher intake of antioxidant rich food and significant reduction in the incidence of various free radical-induced damages and diseases. CAT is a very important antioxidant enzyme in living organisms, which decompose hydrogen peroxide to water and oxygen, and studies revealed that CAT activity is decreased in patients with malignant diseases (Craemer et al., 1993 and Ahn et al., 2005). The role of GSH is the defense against dietary xenobiotics and lipid peroxidation (Wakulich et al., 1997). Depletion of GSH may result in the accumulation of free radicals and leads to membrane damage by lipid peroxidation. The decrease in catalase levels leads to an increase in the accumulation of reactive oxygen species (ROS), which can elevate the intensity of lipid peroxidation, tissue damage and increased peroxidase activity. Superoxide dismutase is one of the cellular defenses against oxidative damage, and it is an important enzyme that catalyzes the dismutation of superoxide anions into O$_2$ and H$_2$O$_2$. GPx is selenoenzyme that protects the lipid membranes and other cellular components against oxidative damage. GPx catalyzes the reduction of H$_2$O$_2$ and hydroperoxides to nontoxic products with the help of GSH and
forms (GSSH) oxidized glutathione as well as glutathione reductase (GR), thereby regenerate GSH from reduced glutathione (GSSH). Several studies implicated the association of dysfunctional GPx and GR and cancer risk (Mannervik, 1987, Meister, 1988 and Moscow et al., 1994).

Superoxide dismutase is an enzyme, which catalyse the dismutation of superoxide hydrogen peroxide and oxygen. Moreover SOD has the potential to induce apoptosis through the generation of H$_2$O$_2$. Nevertheless, high levels of SOD and H$_2$O$_2$ are also associated with some cancers (de Haan et al., 1996 and Ho et al., 2001). Recent studies showed that SOD has been found in higher amount in laryngeal carcinoma tissue whereas, CAT and GPx were found lowered (Kacakci et al., 2009 and Khan et al., 2010). Plant derived natural products are known to possess good antioxidant activity. New research area includes gene therapy to produce more antioxidants in the body through genetically engineered plant products with rich antioxidants or synthetic antioxidant enzymes (Valko et al., 2007).

1.3. Inflammation and cancer

Inflammation is a physiological response of the body against infection, foreign bodies, trauma or chemical or other endogenous or exogenous factors. But chronic inflammation can induce DNA damage and causes cancer. Chronically inflamed tissue produce a wide variety of bioactive chemicals such as cytokines, growth factors, reactive oxygen and nitrogen species, cyclooxygenases, and lipoxygenase products. Moreover, chronic inflammation can activate proliferation and differentiation, inhibit apoptosis, and induce angiogenesis. About 5 per cent of patients with ulcerative colitis, a form of irritable bowel disease, will develop colon cancer. Studies revealed that long-term use of non-steroidal anti-inflammatory drugs can inhibit cancer development in a number of tissues including colon, oesophagus, and breast. Antioxidant molecules could suppress the inflammatory process and reduced the cancer risk. Glucocorticoid receptor pathway and the vitamin D receptor mediated signaling are capable of suppressing inflammation. Both the innate and adaptive immune systems constantly survey and eliminate newly
formed cancer cells, and that onset and progression of cancer are kept under control by the immune system.

Several factors have been shown to modify both inflammation and immunity, including vitamins A and E, copper, selenium, zinc, polyunsaturated fats (PUFAs) and epigallocatechin-3-gallate (EGCG) from green tea. Zinc deficiency can lead to abnormalities in adaptive immune responses. Deficiencies of micronutrients such as vitamin A, riboflavin, vitamin B12, folic acid, vitamin C, iron, selenium, and zinc suppresses most immune functions and may fail to control chronic inflammation which then lead to cancer. The cytokine interleukin-6 (IL-6) can act as a pro- or anti-inflammatory molecule. In cancer, IL-6 can either stimulate proliferation or exert anti-tumour effects by enhancing immunity. Dietary phytoestrogens down-regulate IL-6 gene expression and thus potentially influence the development of hormone-related cancers. Circulating levels of IL-6 increases following exercise and this lessens chronic inflammation by reducing pro-inflammatory mediators and elevating anti-inflammatory mediators.

Inflammation is usually initiated by stimulants such as microbial infection, foreign invaders or any irritant. Immune cells such as macrophages, dendritic cells, mast cells, neutrophils and lymphocytes play important roles in inflammatory responses (Akira et al., 2006). Non-immune cells such as epithelial cells, endothelial cells and fibroblasts also involved in inflammation. Bacterial infection activate pathogen-specific receptors, which induces the production of inflammatory mediators such as inflammatory cytokines including Tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6)) and other chemokines. These mediators accelerate vascular endothelial permeability and introduce neutrophils and excess plasma into the site of infection as well as the pathogens are destroyed by immune cells. Inflammatory cytokines alsofacilitate the production of prostaglandins, which are responsible for the symptoms such as pain and fever. Viral infections activate another signaling pathway by the production of cytokines type-1 interferons (IFNs). But, parasitic infections or allergens induce the production of IL-4, IL-5, IL-13 and histamines (Medzhitov, 2010).
Chronic inflammation can lead to different types of diseases including cancer. Research studies and review articles provide strong evidence link between chronic inflammation and cancer. A number of pro-inflammatory gene products such as TNF superfamily members (IL-1α, IL-1β, IL-6, IL-8, IL-18, NF-kB, Cyclooxygenase-2, NO synthase, Chemokines, MMP-9, VEGF, COX-2, and 5-LOX) have a critical role in suppression of apoptosis, proliferation,
angiogenesis, invasion and metastasis (Fig. 1.2) (Aggarwal et al., 2006). These gene expressions are regulated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) which is active in most of the tumor cells and is also induced by carcinogens and oncoviral proteins. Hence, we can conclude that anti-inflammatory agents that suppress NF-kB or NF-kB-regulated products should have a potential in both the prevention and treatment of cancer. Moreover, numerous natural anti-inflammatory agents have been shown to exhibit chemopreventive activities (Aggarwal et al., 2006).

1.4. Genetics of Cancer

Cancer results from a series of molecular changes that fundamentally alter the normal properties of cells. These abnormalities in cells usually result from mutations in genes that regulate cell division and DNA damage repair proteins. These altered cells divide and grow abnormally and develop new characteristics, including changes in cell structure, decreased cell adhesion and production of new proteins. These heritable changes allow the cell cancer cells to spread and invade other tissues resulting in malignancy.

More than 35,000 genes in the human genome have been associated with cancer. Alterations or mutations in these gene often are connected with different types of cancer. These genes can be generally classified into three groups. Among them proto-oncogenes, produce proteins that enhances cell division or inhibit normal cell death. The mutated forms of these genes are known as oncogenes. Another group, known as tumor suppressors genes, synthesize proteins that normally prevent cell division and cause death of altered cells. Last group called as DNA repair genes help to prevent mutations and repair mutated genes (Table 1.4). Proto-oncogenes and tumor suppressor genes regulate normal cell division and cell growth.
Table 1.4. Genes associated with cancer and their functions

<table>
<thead>
<tr>
<th>Name of the Gene</th>
<th>Functions of genes</th>
<th>Cancer types</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC (Tumor suppressor)</td>
<td>Regulates transcription of target genes</td>
<td>Familial Adenomatous Polyposis</td>
</tr>
<tr>
<td>BCL2 (Oncogene)</td>
<td>Involved in apoptosis; stimulates angiogenesis</td>
<td>Leukemia; Lymphoma</td>
</tr>
<tr>
<td>BLM (DNA repair)</td>
<td>DNA repair</td>
<td>Bloom Syndrome</td>
</tr>
<tr>
<td>BRCA1 (Tumor suppressor)</td>
<td>Involved in cell cycle control</td>
<td>Breast, Ovarian, Prostatic, &amp; Colonic Neoplasms</td>
</tr>
<tr>
<td>BRCA2 (Tumor suppressor)</td>
<td>DNA repair</td>
<td>Breast &amp; Pancreatic Neoplasms; Leukemia</td>
</tr>
<tr>
<td>HER2 (Oncogene)</td>
<td>Tyrosine kinase; growth factor receptor</td>
<td>Breast, Ovarian Neoplasms</td>
</tr>
<tr>
<td>MYC (Oncogene)</td>
<td>Involved in protein-protein interactions</td>
<td>Burkitt's Lymphoma</td>
</tr>
<tr>
<td>p16 (Tumor suppressor)</td>
<td>Various cellular factors</td>
<td>Leukemia; Melanoma; Multiple Myeloma;</td>
</tr>
<tr>
<td>p21 (Tumor suppressor)</td>
<td>Cyclin-dependent kinase inhibitor</td>
<td>Pancreatic Neoplasms</td>
</tr>
<tr>
<td>p53 (Tumor suppressor)</td>
<td>Cyclin-dependent kinase inhibitor</td>
<td>Colorectal Neoplasms; Li-Fraumeni Syndrome</td>
</tr>
<tr>
<td>RAS (Oncogene)</td>
<td>Apoptosis; transcription factor</td>
<td>Pancreatic, Colorectal, Bladder Breast, Kidney,</td>
</tr>
<tr>
<td>RB (Tumor suppressor)</td>
<td>GTP-binding protein; important in</td>
<td>&amp; Lung Neoplasms; Leukemia; Melanoma</td>
</tr>
<tr>
<td>SIS (Oncogene)</td>
<td>Signal transduction cascade</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>XP (DNA repair)</td>
<td>Regulation of cell cycle</td>
<td>Dermatofibrosarcoma; Meningioma;</td>
</tr>
</tbody>
</table>

1.4.1. Oncogenes and Signal Transduction

In normal cells, proto-oncogenes code for the proteins that send a signal to the nucleus to stimulate cell division. These signaling proteins act in a series of steps called signal transduction cascade or pathway. Several intermediate proteins are present for carrying signals through cytoplasm and these signals activate transcription factors in the nucleus which regulate the expression of those genes essential for cell division. But,
oncogenes are altered forms of the proto-oncogenes and they activate the signaling cascade continuously so that increased production of factors that stimulate growth will result. The alteration of a proto-oncogenes to an oncogenes may occur by mutation of the proto-oncogene or by virus inserts into the DNA of proto-oncogenes, causing it to become an oncogene which promote the development of cancer. Most oncogenes are results of dominant mutations. **MYC** is a proto-oncogene (code for transcription factor) where mutations in this gene convert it into an oncogene and even seventy percent of cancers are associated with **MYC** oncogene. **RAS** is another type of oncogene (“on-off” switch in the signal cascade) and mutations in **RAS** cause the signaling pathway to remain “on,” leading to uncontrolled cell growth.

### 1.4.1.1. TumorSuppressor Genes

Tumor suppressor gene inhibits cell growth and prevent tumor formation. Mutations in these genes result in cells that no longer show normal inhibition of cell growth and division and these are usually recessive. i.e., The trait is not expressed unless both copies of the normal suppressor genes are mutated. Sometime the first mutation is already done in a germ line cell and all the cells in the individual inherit it. Since it is a recessive mutation, the trait may not be expressed then but, the next mutation occurs in the second copy of the gene in a somatic cell. Thus both copies of the gene are mutated and the cell develops uncontrolled growth (Eg: Retinoblastoma in early childhood).

Some other cancers are also associated with mutation in tumor suppressor genes including familial adenomatous polyposis of the colon (FPC) which occurs as a result of mutations in both copies of the **APC** gene. **APC** gene has been identified in sporadic colorectal cancer, as well as cancer of the stomach, pancreas, thyroid and ovary (Fearnhead et al., 2001). Another kind is hereditary breast cancer, by mutations in both copies of **BRCA2** gene. Tumor-suppressor genes having specific role in breast tumor include p53, Rb, DCC, Brush-1, BRCA-1, BRCA-2. All these reports suggested that heredity is an important factor in cancer.
1.4.1.2. DNA Repair Genes

DNA repair genes are also associated with cancer. They are involved in the repair and maintenance of normal chromosome structure. Different external factors such as ionizing radiation, UV light and chemicals can induce damage in DNA replication which can also lead to mutations. DNA repair genes produce certain proteins that repair damage of chromosomes and DNA, thereby minimizing mutations in the cell. But in the case when a DNA repair gene is mutated, its product is no longer synthesized and this prevents DNA repair resulting in accumulation of DNA mutations in the cell. These mutations can increase the chances of cancer. An example of defect in a DNA repair gene is XP (Xeroderma pigmentosum) that results in individuals who are very sensitive to UV light and have a thousand-fold increase in the incidence of all types of skin cancers. There are seven XP genes available in our body. Second example of this type mutation is associated with Bloom syndrome, an inherited disorder that leads to increased risk of cancer and other health problems. Here, the mutated gene is required for maintaining the stable structure of chromosomes. So mutation in this gene leads to activation of oncogenes.

1.5. Tumor Biology

Tumor development includes a series of different steps. The first step is hyperplasia, where large number of cells will be formed as a result of uncontrolled cell division. The next step is dysplasia, resulting from further growth, accompanied by abnormal changes to the cells. Another step involves the cells to become more abnormal and can now spread over a wider area of tissue. These cells begin to lose their original function and such cells are called as anaplastic. The final step occurs when the cells in the tumor mass will metastasize, which means that they can invade surrounding tissue, including the bloodstream, and spread to other locations. This is the most serious step of cancer progression.

1.6. Cell Cycle and cancer

Different proteins execute controlled signaling pathways in order to regulate the division of cells and hence, the cells will divide only when necessary. The loss of this
regulation is the hallmark of cancer. One of the key regulatory molecules of the cell cycle are cyclin-dependent kinases. There are specific cyclin-dependent kinase/cyclin complexes at the entry points into the G1, S, and M phases of the cell cycle, as well as additional factors that help prepare the cell to enter S phase and M phase. One of the important proteins in the cell cycle is p53, a transcription factor that binds to DNA, activating transcription of a protein called p21. p21 blocks the activity of a cyclin-dependent kinase required for progression through G1. This is the time for the cell to repair the DNA before it is replicated. If the DNA damage is so extensive that it cannot be repaired, p53 triggers the cell to commit suicide. The most common mutation leading to cancer is in the gene that makes p53. Other proteins that stop the cell cycle by inhibiting cyclin dependent kinases are p16 and RB. All of these proteins, including p53, are tumor suppressors. In fact, studies revealed that multiple mechanisms with the involvement of these proteins trigger the development of cancer.

The p53 gene is one of the tumour suppressor genes that normally prevent uncontrolled multiplication of abnormal cells. This gene activates molecular processes that delays the cell cycle progression of proliferating cells and simultaneously stimulating DNA repair processes (Erster et al., 2004 and Harms et al., 2004). If it is not possible, it has been found to activate p53 mediated cell death pathway (apoptosis). But in the case of mutant cells (cancer cells), p53-pathway related molecules are disabled and plays an additional role in tumor progression as well as it can contribute resistance to cancer therapy (Erster et al., 2004). But, 50% of all human cancers carry a mutated p53 gene (Stoklosa et al., 2005). Moreover, p53 is not a typical cancer-specific antigen but wild type p53 gene is essentially required to stimulate programmed cell death (apoptosis) of a cancer cell in response to cancer treatment. Radiation or anticancer drugs damage the DNA of cancer cells by eliciting the action of the p53 leading to apoptosis.

1.7. Apoptosis

‘Apoptosis’ is a word from the Greek (meaning ‘falling off’) and refers to a natural process and programmed cell death which has important roles in the development and health of eukaryotic organisms. It is often referred to as cell suicide. Apoptotic pathways may be useful in the treatment of cancer, infectious diseases, degenerative
syndromes and other pathological conditions. Apoptosis occurs normally during development and aging as a homeostatic mechanism to maintain cell populations in tissues. Sometimes, it may occur as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents (Norbury and Hickson, 2001). Irradiation or drugs used for cancer chemotherapy results in DNA damage in cancer/normal cells, which can lead to apoptotic death through a \( p53 \)-dependent pathway. Some hormones, such as corticosteroids, may lead to apoptotic death in cells (e.g., thymocytes) although other cells are unaffected or even stimulated.

Although there are a wide variety of physiological and pathological stimuli and conditions that can trigger apoptosis, some cells express Fas or TNF receptors that can lead to apoptosis via ligand binding and protein cross-linking. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses. Types and strength of the stimuli is the major factor that determines whether the cells should die by apoptosis or necrosis. Characteristic changes of apoptosis are variation in nuclear morphology, including chromatin condensation and fragmentation, cell shrinkage, blebbing of the plasma membrane and development of apoptotic bodies that contain nuclear or cytoplasmic material.

Apoptosis is an energy-dependent process that involves the activation of a group of cysteine proteases called “caspases”. The upstream caspase for the intrinsic and extrinsic pathway are caspase 9 and caspase 3 respectively (Ghobrial et al., 2005). Caspases have proteolytic effect and it can cleave proteins at aspartic acid residues. Once caspases are initially activated, there seems to be an irreversible commitment towards cell death. Different caspases have been identified, they are initiators (caspase-2,-8,-9,-10), effectors or executioners (caspase-3,-6,-7) and inflammatory caspases (caspase-1,-4,-5) (Cohen, 1997; Rai et al., 2005). The stimuli that initiate the apoptosis may be due to the absence of certain growth factors, hormones and cytokines as well as other factors such as radiation, toxins, hypoxia, hyperthermia, viral infections and free radicals that can also lead to failure of suppression of death programs, thereby triggering apoptosis.
1.7.1. Mechanisms of Apoptosis

The mechanisms of apoptosis are highly complex and sophisticated. Studies indicate that there are two main apoptotic pathways known as extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Fig. 1.3). But recent evidences showed that the two pathways are linked and it can influence each other (Igney and Krammer, 2002). There is one more additional pathway also which is known as T-cell mediated cytotoxicity and perforin-granzyme-dependent (granzyme B) cell death. The apoptotic pathways such as extrinsic, intrinsic and granzyme B pathways are similar executional pathways and are initiated by the cleavage of caspase-3. As a result, DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells take place. The granzyme A pathway activates caspase-independent cell death pathway via single stranded DNA damage (Martinvalet et al., 2005). Another apoptotic pathway is the intrinsic endoplasmic reticulum (ER) pathway and it is mediated by the activation of caspase 12 and is mitochondrial dependent (Szegedzi et al., 2003 and Wong, 2011).

1.7.1.1. Intrinsic Pathway

Intrinsic pathway was stimulated by internal stimuli such as genetic damage, hypoxia, cytosolic Ca\(^{2+}\) concentration and oxidative stress. This pathway closely related to the Bcl-2 family, which include two types of proteins such as pro-apoptotic protein and anti-apoptotic proteins (Karp, 2008 and Reed, 1997). Balance between these two proteins determines whether apoptosis would be initiated. The intrinsic signaling pathways are non-receptor mediated and the signals act directly on targets within the mitochondria. Stimuli can change in the inner mitochondrial membrane and results in an opening of the mitochondrial permeability transition (MPT) pore, and release of two main groups of normally sequestered pro-apoptotic proteins (cytochrome c, mac/DIABLO and the serine protease HtrA2/Omi) from the intermembrane space into the cytosol (Saelens et al., 2004; Du et al., 2000; Loo et al., 2002; Garrido et al., 2005). Cytochrome c activates caspase 3 through the formation of a complex, which is made up of cytochrome c, Apaf-1
and caspase 9 (Kroemer et al., 2007). It will lead to the activation of the caspase-dependent mitochondrial pathway.

1.7.1.2. Extrinsic Pathway

The extrinsic signaling pathway that initiate apoptosis involve transmembrane receptor-mediated interactions. These involve death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily (Locksley et al., 2001). Members of the TNF receptor family consists of cysteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the “death domain” (Ashkenazi and Dixit, 1998). The extrinsic phase of apoptosis are well-characterized with ligands and corresponding death receptors are FasL/FasR and TNF-α/TNFR1 (Chicheportiche et al., 1997; Ashkenazi et al., 1998; Peter and Kramer, 1998; Suliman et al., 2001; Rubio-Moscardo et al., 2005). However, stimuli induce the formation of ligand-receptor-adaptor protein complex (DISC) and it will lead to pro-caspase 8 formation, which initiates apoptosis (22, 244). Another point of potential apoptosis regulation involves a protein called Toso, which has been shown to block Fas-induced apoptosis in T cells via inhibition of caspase-8 processing (Hitoshi et al., 1998).

1.7.1.3. Perforin-granzyme Pathway

This apoptotic pathway is T-cell mediated pathway and intervened by sensitized CD8+ cells that kill antigen-bearing cells. These T-cells are able to kill target cells through the extrinsic pathway as well as the FasL/FasR interaction, which is the key method of T-cell mediated apoptosis (Brunner et al., 2003). The serine proteases, granzyme A and B, are the two main granzyme components (Pardo et al., 2004). Granzyme A activates caspase independent pathways and Granzyme B will activate pro-caspase-10 in apoptosis (Sakahira et al., 1998 and Fan et al., 2003).
Fig. 1.3: Diagrammatic representation of intrinsic and extrinsic pathway of apoptosis

Intrinsic pathway

Extrinsic pathway

Extrinsic Pathway

Intrinsic Pathway

Perforin/Granzyme Pathway

Favaloro et al., 2012
1.8. Carcinogenesis

Cancer development is understood to be a complex process. The multi-stage carcinogenesis concept was first proposed by Berenblum and Schubik in 1948. Studies revealed that carcinogenesis consists of three main phases such as initiation, promotion and progression. Cancers are caused by somatic or inherited mutations in proto-oncogenes, tumor suppressor genes or DNA repair genes. A compound that acts as both an initiator and a promoter is referred to as a 'complete carcinogen' because tumor development can occur without the help of another compound (benzo[a]pyrene and 4-aminobiphenyl). Compounds which react with genetic material of the cell and somehow changes the genetic makeup of the cell is called a mutagen. The mutagens that genetically influence cells to develop tumors are called initiators and the compound which stimulate tumor development are called promoters. Multicellular organisms have signal transduction pathways and gene regulation systems to control the cell growth and multiplication. In case, this regulatory system is interrupted by mutagens, they will become cancer cells. Tumor suppressor genes participate in growth, regulatory or differentiation pathways of cells. Loss of their function changes the phenotype of the cell toward becoming a cancer cell. Actually, both copies of a tumor suppressor gene need to be inactivated for cancer formation. One of the tumour suppressor gene is p53, which is mutated in more than 50% of human cancers. RB1 (Retinoblastoma gene), BRCA1 and BRCA2, which are also involved in breast cancer (Knudson, 2001).

1.8.1. Initiation

Neoplasia initiation is stable and irreversible changes in somatic cells arise spontaneously or induced by the exposure to a carcinogen. This is considered to be the first step in carcinogenesis. Here, the cellular genome undergoes mutations and affect cell components that subsequently leads to neoplastic transformation. Mutations in somatic cells convert proto-oncogenes into oncogenes, that have been isolated by molecular cloning (Eg. human bladder carcinoma, Burkitt’s lymphoma, lung carcinoma, carcinoma of the breast etc.). Moreover, the activation of more than one oncogene appears to be necessary for neoplastic transformation. In the human bladder carcinoma, a single point
mutation converting the +ras proto-oncogene into a potent oncogene was the first identified mutation among human oncogenes (Tabin et al., 1982). Such oncogene activated cells display unusual biochemical signaling pathways, with uncontrolled cell proliferation or disruption of the natural processes of cellular communication, development and differentiation. The transformed cell undergoes continuous division with reliability to the transformed karyotype and, possibly, with further mutations, before a malignant lesion is manifested.

Different mechanisms are considered for the conversion of proto-oncogenes to active oncogenes (Land et al., 1983). The oncogene transcripts are produced at much higher levels than those of the related normal proto-oncogenes and increased gene copies cause corresponding increase in transcripts and gene products. Another mechanism is the deregulation of the oncogene in chromosome. Alteration in the structure of the oncogene protein (ras gene) is the most well-known mechanism. But, studies described that there is a close association between specific chromosomal translocations and certain human neoplasms (Rowley and Mitelman 1993).

1.8.2. Promotion
Promotion is the further proliferation of transformed cell, which involves more than one step and requires repeated and prolonged exposures to promoting stimuli (Upton et al. 1986). Expression of the initial mutation will depend not only on interaction with other oncogenic mutations, but also on factors that may temporarily change the patterns of specific gene expressions, eg. cytokines, lipid metabolites, and certain phorbol esters. This may result in an enhancement of cellular growth potential and an uncoupling of the intercellular communication processes that restrict cellular autonomy and thereby coordinate tissue maintenance and development (Trosko et al. 1992). Once a cell has been mutated by an initiator, promoters induce proliferation of the cell, giving rise to a large number of daughter cells containing the mutation created by the initiator (Yamagiwa and Ichikawa, 1918). Moreover promoters do not covalently bind to genetic material within the cell, but they bind to receptors on the cell surface and influence intracellular pathways that lead to increased cell proliferation. Promoters have tissue or species specific as well as dose dependent action. Croton oil is generally used as tumour
promoter in murine skin carcinogenesis model. The potent constituent, 12-octadecanoylphorbol-13-acetate, present in this oil acts as promoter via protein kinase C activation. Some of the chemicals which have promoter activity are dioxin, benzoyl peroxide, macrocyclic lactones, bromomethylbenzanthracene, anthralin, phenol, saccharin, tryptophan, dichlorodiphenyltrichloroethane (DDT), phenobarbital, cigarette smoke condensate, polychlorinated biphenyls (PCBs), teleocidins and cyclamates.

1.8.3. Progression

Progression is the process through which successive changes in the neoplasm give rise to increasingly malignant sub-populations. This process may be accelerated by repeated exposures to carcinogenic stimuli and mutations or chromosomal aberrations are also thought to be involved. In the first phase of progression, sometimes referred to as neoplastic conversion, the pre-neoplastic cells are transformed to a state in which they are more committed to malignant development. This may involve further gene mutations accumulating within the expanding pre-neoplastic cell clone (UNSCEAR, 2000). The dynamic cellular heterogeneity, a feature of malignant development, may, in many instances, be a consequence of the early acquisition of gene specific mutations that destabilize the genome. Examples are mutations of the p53 gene (Hartwell and Kastan 1994) or DNA mismatch repair genes (Fishel and Kolodner 1995). Many tumor types develop transforming sequences in their DNA during their progression from the normal to the cancerous state. An elevated mutation rate established relatively early in tumor development may, therefore, provide for the high-frequency generation of variant cells within a premalignant cell population. Such variant cells, having the capacity to evade the constraints that act to restrict proliferation of aberrant cells, will tend to be selected during tumorigenesis (UNSCEAR, 2000).

1.8.4. Tumor metastasis

After tumor progression, the cells lose their adherence property and attack the neighboring tissues. The detached cells enter into the circulating system (blood/lymph)
and will be transported to other areas of the body from the primary site and develop secondary tumours at the new site, resulting in widely spread cancers. Cancer metastasis involves different steps and the key steps are common for all types of tumors. They are, invasion of local normal tissues, entry and transit of neoplastic cells in the circulating (blood or lymph) systems, and the following establishment of secondary tumor growth at new sites (Hart and Saini 1992, Takeichi 1993). Malignant cells have reduced adherence ability, so they can easily detach from the primary tumor and enter into the surrounding tissues. Cell adhesion behavior is controlled by some molecules such as cadherins (Takeichi 1991). Downregulation of E-cadherin expression is significantly correlated with metastatic behavior of cells which means that cadherins function as invasion suppressor gene products (Vleminckx et al., 1991). Metastatic process of tumor is the main reason for the lethal effects of many common human tumors.

1.8.5. Tumor angiogenesis

Tumor growth depends on the availability of growth factors, capability of removal of toxic molecules and efficient oxygen diffusion from capillaries. Therefore, adequate blood supply through blood capillaries (angiogenesis) is necessary for tumor growth and development. The term ‘tumor angiogenesis’ was first coined by Shubik (Shubik, 1982). According to Folkman and colleagues, tumor growth beyond about 2mm size could precede only if a vascular supply is established (Folkman, 1985). A number of tumor angiogenesis factors such as, the vascular endothelial growth factor (VEGF), angioproteins - ang-1 and ang - 2, transforming growth factors (TGFs), interleukin - 1 and platelet-derived endothelial cell growth factor (PD-ECGF) have been identified for endothelial cell proliferation (Folkman, 1974; Mahadevan et al., 1979; Leibovich et al., 1987; Dvorak et al., 1995; Davis and Yancopoulos 1999 and Ishikawa et al., 1989). Tumour blood vessels are often dilated, saccular, convoluted with high plasma protein permeability which means that blood flow through tumour vessels may be lethargic (Jain, 1989).
1.9. Cancer Diagnosis

Different methods are available for diagnosing cancer, which include physical examination, cervical screening, mammography, colonoscopy, laboratory tests (Blood, urine test), Imaging- X-ray, CT scans, MRI and biopsy. Now a days, accurate clinical evaluation can be done with advanced diagnostic tools. In some cases, cancer is incidentally detected while a patient is being treated for some other unrelated diseases. Different modalities currently exist for the diagnosis and treatment of cancer. Morphological examinations and microscopic examination of a tissue sample are the earliest methods of cancer diagnosis by imaging techniques. Molecular techniques provide additional stratification for a more accurate cancer prognosis (Bast et al., 2000). Detection of molecular markers in neoplastic tissue can be used to provide accurate diagnosis. These markers are the products of altered genes/DNA by abnormal pathways as well as they have a high specificity and sensitivity. They can be detected by different techniques such as FISH technique, PCR, NA microarray, Immunocytochemistry (IHC), flow cytometry and electron microscopy. Mammography has become the commonest imaging technique used for mass screening for breast cancer.

1.10. Cancer therapy

Appropriately the early detection of any type of the cancer may be cured by treatment. Even though the cancer can’t be cured, the symptoms of the cancer could be greatly suppressed by the treatment. Different types of treatment are also available based on the type and stage of cancer. Some of the oldest treatment modalities for malignancies are surgery, radiation and chemotherapy. According to the modern concept of cancer treatment each patient and each cancer is different, so treatment must be individualized. The different cancer disciplines work together to formulate the best treatment plan for the patient. 

**Surgery:** Surgery may be the best treatment for most type of cancers. It helps prevention, diagnosis, cure and palliation in cancer patients (colon cancer and breast cancer). The tumor having in one place, we can cut and remove that mass without interfering with the body function. Incisional, excisional, needle biopsy and endoscopy techniques are the
different surgical methods that also help cancer diagnosis. But in the case of late stages of cancer, surgery could offer palliation only.

**Radiation therapy:** This type of cancer treatment can be done by hitting high energy radiation towards the tumor area and it will make shrink or disappear the tumor. Radiation treatment is generally applicable to tumors of the head and neck, colorectal and bladder. Radiation with modern instruments increase this therapeutic ratio with radiation to cover all cancer cells with adequate doses of radiation during each fraction, while simultaneously cautious about surrounding normal tissues. But radiation therapy has lots of side effects. Some of them cause fatigue or tiredness, nausea, vomiting, skin inflammation, appetite loss, dry mouth, changes in sense of taste, immune-suppression, and reduced blood cell count.

**Chemotherapy:** Chemotherapy is another method of treatment used for cancer that has metastasized. Chemotherapy is the most effective and widely used treatment method for cancer. Sometimes treatment can also be a combination of surgery, radiation and chemotherapy. The main approach of chemotherapy drugs target not specifically on the tumor cells but also the normal cells. Combination chemotherapy concepts are based on some objectives such as, it provide maximum cell kill within the range of toxicity. More than 132 cancer chemotherapy drugs were approved by the US Food and Drug Administration (FDA) (Chen *et al.*, 2007). Different type of chemotherapy medicine administration are also available, which may be administered orally or intravenously. Most chemotherapy drugs work only on cells that are actively dividing. This means that normal cells are damaged along with the cancer cells, and this causes side effects. It can produce many side effects such as nausea, vomiting, fatigue or tiredness, appetite loss, hair loss, sore mouth taste changes, immune suppression and infection.

### 1.11. Cancer Chemoprevention

Cancer is a growing health problem in our society especially due to unusual changes in lifestyle, diet and environmental condition. According to World Health Organization (WHO), there are about 10 million new cancer cases per year. According to Michael Sporn (1970) the term ‘chemoprevention’ describes the policy of blocking or slackening the onset of premalignant tumours with relatively nontoxic chemical
compounds. Cancer risk may be reduced by avoiding the known carcinogens. It is suggested that more than 35 % cancers could be preventable by modifying human lifestyle and diet. So many dietary constituents can increase the chance of inducing cancer and, at the same time, different dietary materials such as fruits and vegetables can also reduce the risk of specific cancers. Phytochemicals possess substantial antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties. Several cancer organizations established dietary guidelines to help people reduce the cancer risk (Surh, 2003). Those dietary components include turmeric, ginger, garlic, soybeans, tomatoes, and vegetables such as broccoli, cabbage, cauliflower etc. Moreover, a number of cancer preventive studies have been conducted to evaluate the chemopreventive potential of edible plants or plant parts.

Vegetables and fruit are tremendous sources of cancer preventive constituents. The National Cancer Institute (NCI) has recognized about 35 plant-based foods that possess cancer-preventive efficacy as well as they suggest that more than 1,000 different phyto-compounds possess cancer-preventive activity. Investigations under NCI has already conducted more than 65 Phase I, Phase II and Phase III chemoprevention trials and many of which are using food-borne phytochemicals.

1.11.1 Mechanisms of Chemoprevention

Generally carcinogenesis is a multi-step process involvings -tumour initiation, promotion and progression. Also, in experimentally induced carcinogenesis in rodents, cancer development is very closely linked with all these steps. These stages are considered as the possible opportunities for chemoprevention. Several studies have highlighted the ability of plants micronutrients (antioxidants, vitamins and minerals) and macronutrients (carbohydrate, proteins, phenolic compounds and fiber contents) to prevent cancer in humans. According to Lee et al., chemo-preventive agents are divided into 2 groups, called as blocking agents and suppressing agents (Wattenberg, 1985). Blocking agents can prevent carcinogens from reaching the site of metabolism and also protect from interaction with cellular macromolecules (proteins, DNA or RNA). Suppressing agents can act either the promotion or the progression stage and prevent
transformation from initiated cells. Some of the phytochemicals from plants that have been reported as active chemopreventive agents are given in the figure 1.4 along with their chemical structure. These chemopreventive phytochemicals could induce different cellular and molecular events in cancer cells such as carcinogen detoxification by phase II enzymes, DNA repair, cell-cycle, cell proliferation, differentiation, apoptosis, oncogenes, tumour-suppressor genes, angiogenesis, metastasis, hormonal and growth-factor activity. Chemopreventive phytochemicals can block or reverse the premalignant stages of carcinogenesis and also retard the development and progression of cancer (Surh, 2003).

Chemopreventive agents are selective inhibitors of inducible cyclooxygenases (COX-20). This enzyme initiate the production of inflammatory prostaglandins from arachidonic acid (Sporn et al., 1986). Inflammation and carcinogenesis are very closely related; studies revealed that over expression of COX-2 is an early and important event in colon carcinogenesis (Prescott, et al., 1996 and Oshima et al., 1996). Celecoxib is a novel pharmacological agent, which inhibit enzymatic action of COX-2. Another category of new agent is the Selective Estrogen Receptor Modulators (SERMs) (Sporn et al., 2000). Tamoxifen and raloxifene are antiestrogenic agents included under this category (SERMs). This effect was observed in estrogen receptor positive (ER+) tumors but not in estrogen receptor negative (ER-) tumors. Retinoid X receptors (RXRs) are another important category for chemopreventive agents. Rexinoids have been found as potent chemopreventive agents in experimental animals (Targretin (rexinoids) used as chemopreventive agent in mammary carcinogenesis in the rats). Peroxisomal proliferator activated receptor gamma (PPAR-γ) belongs to the family of nuclear hormone receptors (NHRs) and directly regulate transcription of target genes. This receptor also regulates lipid metabolism and insulin sensitization. Activation of PPAR leads to growth inhibition and apoptosis in cancer cells. Studies revealed the over expression of this receptor in some human cancers (colon cancer) (Krishnana et al., 2007).
Fig. 1.4: Structure of chemopreventive phytochemicals and their dietary sources.

(Surh, 2003)
**Curcumin**, the most extensively studied phytochemical has been shown to suppress tumour promotion. Curcumin inhibited TNF-alpha induced COX-2 gene transcription and NF-kappa B activation in cancer cells (Plummer *et al.*, 1999). **Gingerol** is a phenolic phytochemical which was reported to inhibit tumour promotion and inhibit epidermal growth factor (EGF) induced Ap1 activation in mouse epidermal cells (Bode *et al.*, 2001 and Han *et al.*, 2002). Nf-kappaB induced Ap1 activation in mouse and human leukaemia HL-60 cells were also blocked by **capsaicin** (Han *et al.*, 2002). This phytochemical also induce apoptosis in cancer cells by inducing reactive oxygen radicals (Macho *et al.*, 1996). **Epigallocatechin gallate** is a phenolic chemopreventive phytochemical from green tea, which suppress malignant transformation by blocking Ap1 or Nf-kappaB (Nomura *et al.*, 2000). **Genistein** is a soybean derived isoflavone that could inhibit Ap1, c-FOS and ERK activity in human mammary cell lines (Dampier *et al.*, 2001). It could also downregulate c-Jun and c-Fos in UV-stimulated skin of mice (Wang *et al.*, 1998). In prostate cancer cell lines, genistein inhibited TNF-alpha induced activation of NF-kappaB and also it could inactivate NF-kappaB and downregulate AKT in prostate and mammary cancer cells (Li *et al.*, 2002 and Gong *et al.*, 2003). **Resveratrol** treatment inhibited PMA-induced COX-2 expression in human mammary epithelial cells and also inhibited PKC activation and Ap1 transcriptional activity in cancer cells (Subbaramaiah *et al.*, 1998). Resveratrol also suppressed NF-kappaB activation and proliferation in MCF-7 human breast cancer cells (Banerjee *et al.*, 2002). It is also induces apoptosis in fibroblasts through inhibition of NF-kappaB activation by blocking IKK activity (Holmes *et al.*, 2000). Moreover, phytochemicals such as caffeic acid phenethyl, sulphoraphane, apigenin, silymarin, emodin and quercetin have also been showed chemopreventive activity by suppressing the activation of NF-kappaB and AP1 (Bharti *et al.*, 2002).

### 1.12 Essential oils

Essential oils are defined as complex, volatile secondary metabolites which are isolated from the any part of plants by the process of steam distillation or hydro-distillation (Rubiolo *et al.*, 2010). Essential oils have been used traditionally in religion and medicine for thousands of years. In addition, there are nearly 200 references for
essential oils in the Bible. Essential oils were used extensively in Asian and European countries. Moreover, Indian Ayurvedic System of Medicine (the oldest continuous form of medical practice in the world) also describe about aromatic oils and their medicinal properties. A systematic investigation on essential oil constituents was first done by the French chemist Dumas M.J. (1833). The key characteristics of essential oils are volatile, aromatic, liquid, lipophilic, colored or colorless and generally with lower density than water as well as they are enriched with biologically active constituents. They are synthesized by different parts of the plants such as root, stem, bark, wood, leaf, buds, flowers and seeds, as secondary metabolites and are stored in glandular part, secretory cells, cavities, canals and epidermic cells or glandular trichomes (Table 1.5) (Tongnuanchan, et al., 2014). They can be easily separated from other components of plants (Protzen et al., 1993 and Grassmann et al., 2003). Essential oils and their components are now gaining worldwide interest because of their potential multipurpose functional use (Sawamura, 2000).

Essential oils play some role in the attraction of insects which promote pollination, seed dispersal and protect ecological balance. The role of essential oils in plants is mainly protection against pathogens and also it can act as an insect repellent agent. The constituents present in essential oils can vary in quality and quantity based on climate, soil nature, plant part, age and vegetative cycle stage (Masotti et al., 2003 and Angioni et al., 2006). Around 3000 essential oils are known, 300 of which are commercially significant, especially for pharmaceutical purposes, agriculture, food preservative and additives, sanitary products, cosmetic items and perfume industries (Perry et al., 2003). Essential oils contain a number of chemical constituents, and this complex combination of compounds gives them the characteristic fragrance and flavor.

The compounds present in essential oils have also been recognized as antibacterial, antifungal, antiviral, insecticidal and herbicidal with no side effects on humans and animals (Sumonrat et al., 2008 and Sokmen et al., 1999). EOs from many plants have been used traditionally for treatment of infectious diseases (Sokmen et al., 1999 and Janssen et al., 1993). Their potential for cancer prevention and inhibition of cancer progression have been investigated in recent years (Zu et al., 2010). The use of
essential oils is increasing rapidly among most of the countries in food industries, production of soaps, detergents, cosmetics, nonalcoholic beverages, oral care products, aromatherapy and pharmacology (Buchbauer, 2000). Food and Drug administration (FDA) approved the use of essential oils as food additive and is listed as Generally Recognized As Safe (GRAS) (Smith et al., 2005).

**Table 1.5. Essential oils in different parts of the plants.**

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Name of the plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Basil, bay leaf, cinnamon, common sage, eucalyptus, lemon grass, citronella, melaleuca, mint, oregano, patchouli, peppermint, pine, rosemary, spearmint, tea tree, thyme, wintergreen, kaffir lime, laurel, savory, tarragon, cajuput, lantana, lemon myrtle, lemon, teatree, niaouli, may chang, petitgrain, laurel, cypress</td>
</tr>
<tr>
<td>Seeds</td>
<td>Almond, anise, cardamom, caraway, carrot celery, coriander, cumin, nutmeg, parsley, fennel</td>
</tr>
<tr>
<td>Wood</td>
<td>Amyris, atlas cedarwood, himalayan cedarwood, camphor, rosewood, sandalwood, myrtle, guaiac wood</td>
</tr>
<tr>
<td>Bark</td>
<td>Cassia, cinnamon, sassafras, katrafay</td>
</tr>
<tr>
<td>Berries</td>
<td>Allspice, juniper</td>
</tr>
<tr>
<td>Resin</td>
<td>Frankincense, myrrh</td>
</tr>
<tr>
<td>Flowers</td>
<td>Blue tansy, chamomile, clary sage, clove, cumin, geranium, helichrysum hyssop, jasmine, lavender, manuka, marjoram, orange, rose, baccharises, palmarosa, patchouli, rhododendron anthropogon, rosalina, ajowan, ylang-ylang, marjoram sylvestris, tarragon, immortelle, neroli</td>
</tr>
<tr>
<td>Peel</td>
<td>Bergamot, grapefruit, kaffir lime, lemon, lime, orange, tangerine, mandarin</td>
</tr>
<tr>
<td>Root/Rhizome</td>
<td>Ginger, turmeric, plai, valerian, vetiver, spikenard, angelica</td>
</tr>
<tr>
<td>Fruits</td>
<td>Xanthoxyzlum, nutmeg, black pepper</td>
</tr>
</tbody>
</table>

Tongnuanchan, et al., 2014

### 1.12.1 Constituents present in essential oils

Various methods can be used for the extraction of EOs from plants. Methods like liquid carbon dioxide treatment at low temperature and high pressure, or ultrasound-assisted extraction or microwave and hydro-distillation are frequently employed. Concerning hydro-distillation, the essential oil industry has developed three different types such as water distillation, water and steam distillation and direct steam distillation. Oleoresins, which contain volatile oil and nonvolatile flavor components, are extracted from plants using organic solvents, these oleoresins. Mostly the constituents present in essential oils are analyzed by gas chromatography or mass spectrometry. Generally essential oil constituents are mono and sesquiterpenes, carbohydrates, phenols, aldehydes.
and ketones. These compounds are responsible for the biological activity and fragrance. Essential oil consists of mainly three types of components; they are terpenes, terpenoids and aromatic (phenolic) compounds (Fig. 1.5) (Bagora et al., 2014). Rarely aliphatic components are also present in trace and all the components present in essential oils are considered to possess low molecular weight (Bakkali et al., 2008).

**Fig. 1.5: Selected constituents found in essential oils**

(a) Sesquiterpenes

(b) Terpenoids

(c) Aromatic compounds

1.12.2. Biological and industrial applications of essential oils

Generally, about 100 essential oils are used as flavoring substances in food and non-food items. Essential oils gained widespread recognition as a multi-functional agent due to their strong aromatic effects and olfactory senses. Flavors and fragrances present in essential oils are complex mixtures that directly act on gustatory and olfactory receptors in the mouth and nose, giving good taste and smell. Essential oils have been extensively known as natural food additives due to their taste, fragrance, and different biological properties such as antimicrobial and antioxidant activities. Essential oils have a wide spectrum of activities against most of the gram positive and gram negative bacteria. Moreover, they are known for their bactericidal, virucidal, and fungicidal properties, so they can act as an antiseptic agent. They are also known for their analgesic, sedative, anti-inflammatory, and anesthetic agents. Besides, the essential oils were reported to exhibit antinociceptive, appetite stimulant, olfactory stimulant, and insect repellent properties, which strongly indicate their biological activities (Ou et al., 2014; Kapoor et al., 2009; Misharina et al., 2009; George et al., 2009; Irkin and Korukluoglu, 2009; Amer and Mehlhorn, 2009). It was documented that EOs exhibit antifungal activity against different food poisoning fungi groups (Irkin and Korukluoglu, 2009). They could reduce the level of nicotine craving in humans (Cordell and Buckle, 2003) and possess potential antivirulence strategies against persistent Staphylococcus aureus infections in humans (Lee et al., 2014). Some of them could improve reflexive swallowing movement and have relaxant effects on the tracheal smooth muscles in humans (Ebihara et al., 2006). Furthermore, their anticancer activity, chemopreventive effects, and capability to inhibit aflatoxin induced DNA adduct formation have also been reported (Hashim et al., 1994). The essential oils act as antimicrobial agents against food-borne pathogens in the food industry. Essential oil from cinnamon species act against food pathogens such as Staphylococcus aureus and L. monocytogenes. Moreover, EOs showed mortality against third-instar larvae of mosquito species and insects such as Sitophilus oryzae and Corcyra cephalonica (Amer and Mehlhorn, 2009). Essential oils are extensively used in beverages, baked foods, puddings, meat products, detergents, creams, lotions, perfumes, and soups (Fig. 1.6) (UNIDO and FAO, 2005). The biological activities of different components present in essential oils were also been recently reported.
Fig. 1.6: Industrial and pharmaceutical applications of essential oils
1.12.3. Safety of essential oils

Long history of the usage of essential oils in food additives, preservatives and traditional toxicology approaches has been used to demonstrate the safety of the essential oils. Usually the chemical constituents present in essential oils have no significant risk associated with the intake of essential oil. Toxicity studies have been done on different major chemical constituents (menthol, carvone, limonene, citral, cinnamaldehyde, benzaldehyde, benzyl acetate, 2-ethyl-1-hexanol, methyl anthranilate, geranyl acetate, furfural, eugneol, isoeugenol, etc.) present in essential oils. The majority of these toxicity studies were supported by the National Toxicology Program (NTP). Even at these high intake levels, the majority of the constituents showed no carcinogenic potential (Smith et al., 2005a). According to U.S. Food and Drug Administration (FDA) the essential oils are “generally recognized as safe” (GRAS) based on their intended use. Nevertheless, various levels of intensive scientific toxicity evaluations of essential oils are required. It will give many advantages in economic, scientific as well as traditional aspects related to essential oils. Essential oils are lipophilic so it can easily penetrate through the skin and react with the living cells. Even though the essential oils are safe, sometimes it may be harmful if not used carefully. EOs are highly concentrated secondary metabolites of plant parts. The toxicity of essential oils is based on the presence of toxic constituents present in them. Essential oils are highly volatile and lipophilic in nature, it can easily absorb into the body via respiratory or dermal system and causes systemic effects. Some of the essential oils are photosensitisers (phototoxicity), which means that exposure of essential oil to UV light causes toxicity to skin (neroli, rosemary, cassia, calamus, and bitter almond). Mostly these effects of essential oils are caused by the presence of psolarens or furanocoumarins (Klarmann, 1958 and safety issue in aromatheraphy chapter 7). Some of the fragrance constituents present in essential oils may cause allergy related problems (Balsam of Peru) (Ford, 1991). Thujone is another constituent present in essential oils which can cause lesions of the cerebral cortex (Keith et al., 1935). Moreover, it was documented that Wormwood essential oil caused human acute renal failure (Maistro et al., 2010). Furthermore, studies reported that rarely essential oils and their major components created genotoxicity in animals (Maura et al., 1989). Methyleugenol,
estragole, safron and asarone are the essential oil constituents that are generally regarded as carcinogens. As far as the wide use of EOs are concerned, there is a significant point to analyse its toxicity, if any. In this regard, most of the essential oils have no validated scientific information.

1.12.4. Essential oils and cancer treatment

Cancer is the second largest death case of every year in the world (Loizzo et al., 2008). Recently, essential oils from spices have arised more into the focus of phytomedicine. Their widespread use has raised the interest in basic research on essential oils (Buckle, 1999). Antimicrobial, antioxidant and anticancer activities of essential oils have been reported recently (Sylvestre et al., 2005 and Mimica-Dukic et al., 2005). It has been identified that EOs are being used for the treatment of inflammatory and oxidative stress diseases. Oxidative stress could act as a DNA-damage agent, and it will lead to accumulation of mutations in cells which could lead to cancer (Jackson et al., 2001). Moreover free radicals could disrupt signaling pathways and leads to tumor development by the regulation of cell multiplication, angiogenesis and metastasis (Storz, 2005). Chronic inflammation is also closely related to the different steps of cancer progression (Mantovani, 2005). Numerous studies have shown that essential oils and their major components act against various cancer cell lines (Fig. 1.7) (Bagora et al., 2014).

Another important use of essential oils are aromatherapy and it can be used by cancer patient for improving the quality of their life. Researchers developed a number of available drugs from plants, including camptothecin, taxol, vincristine and vinblastine (Heinrich et al., 2006 and Newman et al., 2007). More than 500 articles have been published on anticancer activities of essential oils. The potential of essential oils have been investigated on different cancers such as melanoma, leukemia, glioblastoma and oral cancer. The effect of EOs against cancers of breast, colon, liver, lungs, ovary, pancreas, prostate and kidney was also explored (Bagora et al., 2014).
1.13. *Curcuma longa*

Spices are mostly used for color, flavor, aroma and preservation of food or beverages. Essential oils from spices are gaining greater interest as natural antioxidants, food preservatives and additives. The traditionally used most important spices are turmeric, ginger, chilly peppers, clove and black pepper, which are grown over a much wider range of tropical and nontropical environmental conditions. About 50 spices are very important due to their global, economic and culinary importance. USA and Japan are the two major importers of spices. The global value of spice imports is estimated at 2 to 2.5 billion US$. Among these, turmeric has its own traditional important for its use as food additives and for treating various ailments.

*Curcuma longa* is a well-known traditional spice with a review of more than 1700 scientific articles published. The rhizome of *Curcuma longa* L., belonging to the family Zingiberaceae, is extensively used in many Asian countries traditionally to enhance the food quality, color, flavor and because of its antioxidant properties (Krishnaswamy, 2008; Ruby *et al*., 1995). Different cultures have also used this spice to
treat a myriad of diseases and ailments. It has long been known to play a significant role in Ayurveda, Chinese medicine and traditional household treatments.

Modern scientific research have identified many pharmacologically active compounds in turmeric rhizomes in which curcumin or diferuloylmethane, [1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione], a hydrophobic polyphenolic compound has been characterized as the most bioactive component responsible for numerous pharmacological activities including anticancer activity. Observational studies have already delineated the dietary intake of turmeric with a reduced incidence of chronic diseases such as Cancer and Alzheimer’s in the subcontinent of India. Commercially available natural curcumin contains diferuloylmethane curcumin (70 to 76%), demethoxy curcumin (12 to 18%) and bisdemethoxy curcumin (4 to 8%) together referred to as ‘Curcuminoids’ (Funk et al., 2006). With more than 3000 preclinical investigations in cancer, curcumin stands as one of the best studied natural products till date; a great promising candidate capable of selectively modulating multiple cell signaling pathways (Goel et al., 2008; Hasima and Aggarwal, 2012; Shureiqi and Baron, 2011; Saha et al., 2010). Considering the multi-targeted mechanism of action of this promiscuous natural agent of immense therapeutic value, curcumin has emerged as an ‘yellow gold’. Curcuminoids were also shown to be extremely safe to humans even at doses like 8 to 12 g/day (Lao et al., 2006).

In spite of all the beneficial effects, curcuminoids suffer from poor systemic oral bioavailability which has been regarded as the major limiting factor hampering its development as a therapeutic agent (Anand et al., 2007; Jantarat, 2013). Extremely low aqueous solubility at acidic pH, high hydrolytic instability at physiological pH and rapid enzymatic in vivo degradation makes the hydrophobic curcuminoids a typical BCS class IV molecule (Biopharmaceutics Classification System) of poor absorption and permeability characterized by low plasma levels, limited tissue distribution, rapid metabolism and short half-life (Wahlang et al., 2011; Wang et al., 1997).
1.14. Turmeric essential oil

Spices containing essential oils are important for their medicinal properties. They impart a pleasant aroma and taste, thereby forming major components of many food items, soft drinks and beverages. As they have low molecular weight and lipophilic in nature, this allows its easy transport across cell membranes to induce different biological activities. The compounds derived from spices and their essential oils are fascinating bioactive principles with fewer side effects (Shoeb, 2006). *Curcuma longa* L. belonging to the family Zingiberaceae is widely used in many countries to enhance the food quality, color and flavor. It has long been known to play a significant role in Ayurveda, chinese medicines, and traditional household treatments.

Turmeric essential oil (TEO) is isolated from the rhizome of *Curcuma longa* L. by the process of steam distillation. TEO is different from oleoresin of turmeric, where curcuminoids are the major compounds, while ar-turmerone is the major constituent of TEO (Sacchetti et al., 2005). It is already reported that ar-turmerone and curlone are the compounds present in TEO which exhibited prolonged and systemic bioavailability and having plasma elimination half-life of 7.2 h (12.66%) and 6.8 (6.82%) h respectively (Prakash et al., 2011). Medicinal and pharmacological properties such as antifungal, insect repellent, anti-bacterial, anti-arthritis and anti-platelet activities of TEO have been reported (Negi et al., 1999; Jayaprakasha et al., 2002; Sacchetti et al., 2005; Funk et al., 2010; Prakash et al., 2011). It has shown efficacy in neuroprotective activity against cerebral ischemia and attenuation of delayed neuronal death via a caspase-dependent pathaway (Jain et al., 2007; Dohare et al., 2008; Rathore et al., 2008). The chemopreventive efficacy of TEO has been reported against submucous fibrosis in humans (Deepa Das et al., 2010). It also acts against benzo[a]pyrene induced DNA damage *in vitro* in oral mucosa cells (Hastak et al., 1997). TEO can improve the bioavailability of curcumin after oral administration in humans (Antony et al., 2008). TEO contains ar-turmerone as its main constituent which has the ability to prevent cancer by the induction of apoptosis in cancer cells (Cheng et al., 2012). Food and Drug Administration (FDA) approved TEO usage as food additive and is listed as Generally
Recognized As Safe (GRAS), while the FDAs GRAS list does not include the dosage of TEO.

1.15. Scope of the study

Essential oils are complex phytochemicals which have various biological activities and have long been known and used throughout the world for treatment of different diseases. Another remarkable property of EOs are antioxidant activity and it is very interesting because antioxidant agents can prevent free radical induced diseases including cancer. Even, EOs may tend to have less deleterious side effects than corresponding synthetic drugs. Researchers found out that the EO may preserve foods from the toxic effect of oxidants. EOs can scavenge free radicals in living systems and prevent diseases such as inflammation, cataract, gastric ulcer, cancer, heart disease and immune suppression (Maestri et al., 2006; Kamatou et al., 2010 and Aruoma, 1998). The constituents present in spices and their essential oils are interesting sources of natural products with fewer side effects (Shoeb, 2006). The essential oils isolated from the spices have medicinal properties like antioxidant, anti-inflammatory, anticancer etc and has attracted the attention of many researchers to investigate their uses in various diseases including cancer (Sacchetti, 2005).

*Curcuma longa* L. (Turmeric), a perennial herb belonging to the family of Zingiberaceae, is a popular dietary spice widely used in Indian curries for thousands of years. Curcumin, the active ingredient of turmeric is a potent antioxidant (Sharma, 1976). Turmeric essential oil (TEO) is prepared from the rhizome of turmeric by steam distillation and it consists of a unique set of sesquiterpenoids. Ar-turmerone and curlone are the major constituents present in TEO which are considered to have significant antimicrobial, antioxidant and pharmacological activities (Cheng et al., 2012).

At present, we have evaluated acute and subchronic toxic effects as well as genotoxic effects of TEO on rats, if any. Moreover, the qualitative and quantitative analysis of TEO is done by GC/MS. We have analysed several parameters to evaluate the toxicity of TEO which included body weight changes, food and water consumption, hematological parameters, organ toxicity and histopathology of various tissues. *Salmonella* reverse mutation test (Ames test) was done to evaluate the mutagenicity of
TEO and the genotoxic effect of TEO was studied by observing micronuclei formation, chromosomal aberration and comet assay. The study also aimed to evaluate the antioxidant, anti-inflammatory, antiulcer and antinociceptive properties of TEO. We have also tested whether TEO possess any antimutagenic ability against direct acting mutagens such as sodium azide (NaN₃), 4-nitro-o-phenylenediamine (NPD), MNNG as well as mutagens needing mammalian liver microsomal activation, such as 2-acetamidoflourene (2-AAF) using Salmonella strains TA 98, TA 100, TA 1535 and TA 102. Effect of TEO on tobacco induced mutagenicity was also evaluated. We have also evaluated a short term cytotoxic activity of TEO using DLA and EAC cancer cell lines as well as the antiproliferative effect of TEO on L929, HeLa, HepG2 and Vero cell lines by MTT assay. Thereafter, we have examined the antitumour activity of TEO using DLA induced ascites and solid tumour models in mice. We have also evaluated the protective effects of TEO to analyse its use as a chemopreventive agent against NDEA induced hepatocellular carcinoma in male Wistar rats. The effect of TEO against two-stage mouse skin papilloma development induced by DMBA (as initiator) and croton oil (as promoter) is also being reported in this study. In order to assess the anticarcinogenic efficacy of TEO, we have used 3-methyl cholanethrene (3-MC) induced sarcoma model in mice. To determine the possible mechanism of action of TEO, we have evaluated the inhibition of different cytochrome P450 enzymes (Phase I enzymes) by TEO in vitro and in vivo. Moreover, we have studied the levels of the drug metabolizing phase II enzymes such as glutathione-S-transferase (GST) and UDP-glucuronyl transferase, after TEO administration in rats. We have also investigated the radioprotective activity of TEO by using gamma rays as a DNA damaging agent. Different parameters such as haematopoietic changes, chromosomal aberrations, micronucleus formation and genetic damages in bone marrow and spleen cells were analyzed after exposed to whole body gamma irradiation in mice. Moreover, the antioxidant status of intestine and liver in irradiated mice were also evaluated. Finally we aimed to find out the effects of TEO on the growth of Aspergillus flavus, production of aflatoxin by the organism, its toxicity and consequent histopathological changes in ducklings. Moreover, we studied the ameliorative effects of TEO on aflatoxin induced liver carcinogenesis in rats.