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1.1. INTRODUCTION

Cancer is the uncontrolled proliferation of cells without any differentiation. Cancer cells undergo an uncoordinated mitosis violating the normal homeostatic control of the cell cycles. A cell mass formed by the uncontrolled cell proliferation is known as tumour or neoplasm. It consists of relentlessly growing mass of abnormal cells. If the neoplastic cells remain clustered together in a single mass without migration to abnormal territories, the tumour is said to be benign. Benign tumour can be cured by surgical removal. Generally benign tumours are harmless and remain confined to original locations. The most common example of benign tumour is skin wart. Malignant tumour does not remain confined to its original location. It is capable of invading the surrounding normal tissues and spreading throughout the body via circulatory or lymphatic systems. This capacity of malignant tumour cell to break loose and enter bloodstream or lymphatic vessels and form secondary tumours is known as metastasis. Such metastasizing cancers are dangerous. Malignant tumours are generally classified into four types on the basis of the type of cells from which they originate.

- Carcinomas are cancers arising from epithelial cells of ectoderm or endoderm. Cervical, breast, skin and brain carcinomas are examples. About 85% of human cancers are carcinomas.
- Sarcomas are solid tumours derived from mesodermal connective tissues such as muscle, bone, cartilage and fibrous tissue. These types of malignant tumours are relatively rare in humans accounting for only 2-3% of all cancers.
- Leukemias arise from blood forming cells or hematopoietic cells. These are commonly called blood cancers and are characterised by excessive production of leukocytes. These leukocytes have decreased immune activity. They accumulate in the circulatory system disrupting normal circulation and other normal body functions. This cancer accounts for about 10% of all cancers.
- Lymphomas are cancers in which lymphocytes are produced in excess by lymph nodes and spleen. This cancer is also rare in humans.

1.2. CANCER EPIDEMIOLOGY

Cancer causes 1 in 8 deaths worldwide and is rapidly becoming a global epidemic. According to the International Agency for Research on Cancer, there were 12.7 million new cancer cases and 7.6 million cancer deaths in 2008. If rates don’t
change, the global cancer burden is expected to nearly double to 21.4 million cases and 13.15 million deaths by 2030. Cancer occurs in all countries of the world, although the predominant types of cancer vary widely. In general, cancers caused by chronic infections (e.g. stomach, liver, cervix) predominate in economically developing countries, whereas those related to Western patterns of tobacco use and diet (e.g. lung, breast, prostate, and colon) are most common in high-resource countries. Lung cancer is the most common cancer worldwide in terms of both new cases and deaths. The incidence rate varies from more than 70 per 100,000 among men in Eastern Europe to less than 5 per 100,000 among men in West Africa. Breast cancer is the most common cancer in women worldwide. Prostate cancer is the second most commonly diagnosed cancer in men globally. Cancers of colon and rectum are more common in economically developed countries than in developing countries. In India, around 5,55000 people died of cancer in 2010 (Dikshit et al., 2012). Tobacco-related cancers represented around 42% of male and 18% of female cancer deaths. In men, two of the most common fatal cancers were oral (including lip and pharynx) and lung. Cervical, stomach and breast cancers accounted for 41% of cancer deaths in women in rural and urban areas.

1.3. THE HALLMARKS OF CANCER
Malignant transformation proceeds through six acquired essential alterations in cell physiology: self-sufficiency in growth signaling, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis (Hanahan and Weinberg, 2000) (Figure 1.1).

1.4. GENETIC BASIS OF CANCER
1.4.1. Oncogenes
By definition, an oncogene is a gene whose abnormal expression or altered gene product leads to malignant transformation. Normal cellular genes that can give rise to oncogenes are called proto-oncogenes. A proto-oncogene can be transformed to an oncogene by a genetic process called 'oncogene activation'. Among the proteins encoded by proto-oncogenes are growth-promoting signaling proteins and their receptors, signal-transduction proteins, transcription factors, and apoptotic proteins. An activating mutation of one of the two alleles of a proto-oncogene converts it to an oncogene. This can occur by point mutation, gene amplification and gene translocation. The first human oncogene to be identified encodes a constitutively
Figure 1.1. Hallmarks of cancer

[Adapted from Hanahan et al., 2000]
active form of Ras, a signal-transduction protein. Various factors including chemicals, radiations and mutagens can convert a proto-oncogene to oncogene. Slow-acting retroviruses can cause cancer by integrating near a proto-oncogene in such a way that transcription of the cellular gene is activated continuously and inappropriately (Lodish et al., 2000).

### 1.4.2. Tumour-suppressor Genes

Tumour-suppressor genes generally encode proteins that in one way or another inhibit cell proliferation. Loss-of-function mutations in one or more of these genes contribute to the development of many cancers. RB, the first tumour-suppressor gene, is mutated in retinoblastoma and some other tumours. Inheritance of a single mutant allele of RB greatly increases the probability that a specific kind of cancer will develop, as is the case for many other tumour-suppressor genes (e.g., APC and BRCA1). Five broad classes of proteins are generally recognized as being encoded by tumour-suppressor genes:

(a) Intracellular proteins that regulate or inhibit progression through a specific stage of the cell cycle (e.g., p16 and Rb).
(b) Receptors or signal transducers for secreted hormones or developmental signals that inhibit cell proliferation (e.g., TGFβ, the hedgehog receptor patched).
(c) Checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal (e.g., p53).
(d) Proteins that promote apoptosis.
(e) Enzymes that participate in DNA repair.

Although DNA-repair enzymes do not directly inhibit cell proliferation, cells that have lost the ability to repair errors, gaps, or broken ends in DNA accumulate mutations in many genes, including those that are critical in controlling cell growth and proliferation. Thus loss-of-function mutations in the genes encoding DNA-repair enzymes prevent cells from correcting mutations that inactivate tumour suppressor genes or activate oncogenes. Since generally one copy of a tumour suppressor gene suffices to control cell proliferation, both alleles of a tumour suppressor gene must be lost or inactivated in order to promote tumour development. Thus oncogenic loss-of-function mutations in tumour suppressor genes are genetically recessive. In many cancers, tumour suppressor genes have deletions or point mutations that prevent production of any protein or lead to production of a non functional protein. Another
A mechanism for inactivating tumour-suppressor genes is methylation of cytosine residues in the promoter or other control elements (Robertson, 2005).

1.5. ETIOLOGY OF CANCER

Cancers are primarily an environmental disease with 90-95% of cases attributed to environmental factors and 5-10% due to genetics (Kravchenko et al., 2009). Common environmental factors that contribute to cancer death include tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants.

1.5.1. Exogenous Causes

1.5.1.1. Physical Factors

An carcinogen is anything that causes cancer in humans or animals. Carcinogens can be chemical or physical agents. UV radiation of the sun, X- radiation (when frequently used), gamma rays and similar emissions due to radioactivity are well known physical agents for cancer. Physical carcinogens also include fibers, particulate matter, hard and soft synthetic materials and gels. Some physical carcinogens are naturally occurring, while others are synthetic. Physical carcinogens are highly variable in their chemical structure, and many of them are poorly understood.

1.5.1.1.1. Ionizing Radiation

Up to 10% of total cancer cases may be induced by radiation. Radiation may be ionizing and nonionizing. It can be generated from radioactive substances, ultraviolet light (UV) and pulsed electromagnetic fields. Cancers induced by radiation include some types of leukemia, lymphoma, thyroid cancers, skin cancers, sarcomas, lungs and breast carcinomas. Ionizing Radiation (IR) contains high amount of energy in discrete packets, which is carried as waves or in a particulate nature. It constitutes the high frequency, short wavelength part of the electromagnetic spectrum. These rays have enough energy to dislocate electrons from the atoms of the matter they fall on and to ionize them. Thus IR exposure could lead to varied effects ranging from point mutations to severe damage to the genetic material depending on the dose of radiation exposure. Cancer incidence varies with the type of radiation, with radon radiation being more lethal compared to low linear energy transfer radiation like gamma and X-rays (Nambiar et al., 2011). IR induces the damage either directly by interacting with the macromolecules and ionizing them or indirectly by creation of free radicals which
execute the ultimate process of damage. Radon and radon decay products in the home and/or at workplaces (such as mines) are the most common sources of exposure to ionizing radiation. Another source of radiation exposure is x-rays used in medical settings for diagnostic or therapeutic purposes.

1.5.1.1.2. Nonionizing Radiation

UV rays, one of the nonionizing radiations derived from sunlight, are carcinogenic to humans. UV exposure increases the risk of various types of skin cancers like basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. Besides UV exposure from sunlight, UV exposure from sun beds for cosmetic tanning may account for the growing incidence of melanoma. Stratospheric ozone layer depletion can augment the dose-intensity of UVB and UVC. This can further increase the incidence of skin cancer. The health effects of UV radiation vary according to wavelength. UV radiation is divided into three categories depending on the wavelength: long wave UVA (315–400 nm), medium wave UVB (280–315 nm), and short wave UVC (100–280 nm). UVB and UVA are responsible for cutaneous carcinogenesis as these wavelengths can bypass the earth’s atmosphere including stratospheric ozone layer. UVB is more genotoxic and about 1000 times more capable of causing sunburn than UVA. UVB is less penetrating and acts mainly in the epidermal basal cell layer of the skin. It induces direct damage to DNA [the formation of cyclobutane-pyrimidine dimers (CDPs) and pyrimidine-pyrimidone (6-4) photoproduc}tions (6-4)-PP)] and proteins. UVB also induces indirect damage to macromolecules. It provokes free radical production and induces a significant decrease in skin antioxidants, impairing the skin’s ability to protect itself against the free radicals generated after sunlight exposure. Furthermore, UVB causes photoisomerization of trans- to cis-urocanic acid (UCA), induction of ornithine decarboxylase (ODC) activity and cell cycle arrest or impairment of DNA synthesis in the skin. Both direct and indirect adverse biological effects of UVB may result in photoaging and photocarcinogenesis. Target genes for solar radiation–induced mutations include p53 (SCCs and BCCs), and p16 (melanoma).

1.5.1.2. Chemical Carcinogens

Relationship between chemicals and carcinogenesis has been identified since the initial observations of Hill and Pott in 1700’s. Today, more than 200 different chemical compounds and mixtures are known or anticipated to be human carcinogens (Table 1.1). Environmental pollution has been linked to various cancers. The pollution
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Main sources/Uses</th>
<th>Affected organs/Cancer type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoazodyes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Aminoazotoluene</td>
<td>Pigments; colouring oils; immunosuppressents</td>
<td>Liver, lung, bladder</td>
</tr>
<tr>
<td>N,N-dimethyl-4-aminoazobenzene</td>
<td>Colouring polishes; waxes</td>
<td>Lung, liver</td>
</tr>
<tr>
<td><strong>Anticancer drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>Chemotherapy</td>
<td>Leukaemia</td>
</tr>
<tr>
<td>Thiotepa</td>
<td>Chemotherapy</td>
<td>Leukaemia</td>
</tr>
<tr>
<td><strong>Aromatic amines/amides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Naphylamine</td>
<td>Dyes</td>
<td>Bladder</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>Dyes</td>
<td>Bladder</td>
</tr>
<tr>
<td>2-Acetylaminofluorene</td>
<td>Model compound; tested as a pesticide</td>
<td>Liver, bladder</td>
</tr>
<tr>
<td><strong>Aromatic hydrocarbons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>Coal tar; roofing; cigarette smoke</td>
<td>Skin, lung, stomach</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzo-p-</td>
<td>No commercial use; tested as a pesticide</td>
<td>Lung, lymphoma, liver</td>
</tr>
<tr>
<td>dioxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td>Flame retardants; hydraulic fluids</td>
<td>Liver, skin</td>
</tr>
<tr>
<td><strong>Metals (and compounds)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>Natural ores; alloys; pharmaceutical agent</td>
<td>Skin, lung, liver</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Natural ores; pigments; batteries; ceramics</td>
<td>Lung, prostate, kidney</td>
</tr>
<tr>
<td>Nickel</td>
<td>Natural ores; alloys; electrodes; catalysts</td>
<td>Lung, nasal cavity</td>
</tr>
<tr>
<td><strong>Natural carcinogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>A mycotoxin (found in contaminated food)</td>
<td>Liver</td>
</tr>
<tr>
<td>Asbestos (fibrous silicates)</td>
<td>Thermal insulation; gaskets (declining usage)</td>
<td>Lung, mesothelioma</td>
</tr>
<tr>
<td><strong>N-nitroso compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodimethylamine</td>
<td>Polymers; batteries</td>
<td>Liver, lung, kidney</td>
</tr>
<tr>
<td>4-((methylnitrosamino)-1-(3-pyridyl)-1-butanone</td>
<td>Research tool; cigarette smoke</td>
<td>Lung, liver</td>
</tr>
<tr>
<td><strong>Olefines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>Glycol and polyester production; sterilization</td>
<td>Leukaemia and lymphoma</td>
</tr>
<tr>
<td>Vinyl chloride (VC)</td>
<td>Plastics (PVC); co-polymers</td>
<td>Liver (angiosarcoma)</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Degreasing operations; adhesives; lubricants</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td><strong>Paraffines/ethers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>Vinyl chloride production; solvent</td>
<td>Liver, lung, breast</td>
</tr>
<tr>
<td>Mustard gas (sulphur mustard)</td>
<td>Chemical warfare in first world war; research</td>
<td>Lung</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>Limited application as antineoplastic agent</td>
<td>Lung, skin, lymphoma</td>
</tr>
</tbody>
</table>
includes outdoor air pollution by carbon particles like polycyclic aromatic hydrocarbons (PAHs), indoor air pollution by tobacco smoke, formaldehyde, and volatile organic compounds such as benzene and 1, 3-butadiene, and food pollution by food additives and by carcinogenic contaminants such as nitrates, pesticides, dioxins, and other organo chlorines. Carcinogenic metals and metalloids, pharmaceutical medicines, and cosmetics also cause environmental pollution.

PAHs increase the risk of cancers especially lung cancer. PAHs can adhere to fine carbon particles in the atmosphere and thus penetrate our body primarily through breathing. Another environmental pollutant-nitric oxide was found to increase the risk of lung cancer in non-smokers. The increased risk of childhood leukemia associated with exposure to motor vehicle exhaust was also reported. Indoor air pollutants such as volatile organic compounds and pesticides increase the risk of childhood leukemia and lymphoma. In utero exposure to environmental organic pollutants was found to increase the risk of testicular cancer (Anand et al., 2008).

1.5.1.2.1. Carcinogen Metabolism

Chemical carcinogens are of two distinct types-direct acting and indirect acting carcinogens. Carcinogens that do not require metabolic activation are referred to as direct acting carcinogens (e.g. N-nitroso alkyl urea, β-propiolactone, ethyleneimine etc). Carcinogens that require metabolic activation are called indirect acting carcinogens. The conversion of parent compound (procarcinogen) to a reactive state (an ultimate carcinogen) requires biotransformation by ‘xenobiotic metabolizing enzymes’. Like direct acting carcinogens, ultimate carcinogens are electrophilic and attack nucleophilic groups in DNA to initiate carcinogenesis. Genotoxic carcinogens can transfer simple alkyl or complexed (aryl) alkyl groups to specific sites on DNA bases. These alkylating and arylalkylating agents include N-nitroso compounds, aliphatic epoxides, aflatoxins, mustards, poly aromatic hydrocarbons, and other combustion products of fossil fuels and vegetable matter. Other genotoxic agents like aryl aromatic amines, amino azo dyes and heterocyclic aromatic amines can transfer arylamine residues to DNA.

The reactions catalyzed by xenobiotic metabolizing enzymes have been categorized into phase I and phase II reactions. Phase I reactions include oxidation, reduction, and hydrolysis reactions and generally expose functional groups that enable phase II biotransformation to proceed. Phase I metabolites display minimally increased hydrophilicity while phase II reactions catalyze addition of cofactor
molecules to the parent compound resulting in a significant increase in hydrophilicity. Phase I biotransformation of carcinogens often results in reactive metabolites capable of covalent modification of cellular macromolecules. The phase I xenobiotic metabolizing enzymes, which are called mixed-function oxidases or mono oxygenases, include many enzymes like cytochrome P450, cytochrome b5, and NADPH-cytochrome P450 reductase and other components. The hepatic cytochrome P450s (Cyp) are a multigene family of enzymes that play a critical role in the metabolism of many drugs and xenobiotics with each cytochrome isoenzyme responding differently to exogenous chemicals in terms of its induction and inhibition. For example, Cyp 1A1 is particularly active towards polycyclic aromatic hydrocarbons (PAHs) and activate them into reactive intermediates which covalently bind to DNA, a key event in the initiation of carcinogenesis (Figure 1.2). Likewise, Cyp 1A2 activates a variety of bladder carcinogens such as aromatic amines and amides. Some forms of cytochrome P450 isozymes such as Cyp 3A4 and Cyp 2E1 activate the naturally occurring carcinogens like aflatoxin B1 and N-nitrosamines respectively into highly mutagenic and carcinogenic agents (Table 1.2). The carcinogenic potential of PAHs and other carcinogens and the extent of binding of their ultimate metabolites to DNA and proteins are correlated with the induction of cytochrome P450 isoenzymes. About 66% of bioactivation of carcinogens is mediated by cytochrome P450 enzymes. Within this group, six Cyp P450 enzymes like Cyp 1A1, Cyp 1A2, Cyp 1B1, Cyp 2A6, Cyp 2E1 and Cyp 3A4 are responsible for 77% of cytochrome P450 mediated bioactivation reactions (Rendic and Guengerich, 2012). Phase II biotransformation reactions catalyze glucuronidation, sulfation, acetylation, methylation and glutathione conjugation reactions. Therefore phase II biotransformation reactions ultimately result in metabolites that are less toxic and more readily excreted. Phase II drug-metabolizing enzymes such as glutathione-S-transferase, aryl sulfatase and UDP-glucuronyl transferase inactivate chemical carcinogens into less toxic or inactive metabolites.

### 1.5.1.2.3. Chemical Carcinogenesis

Chemical carcinogenesis is a multi-stage process and can be divided into three steps: tumour initiation, tumour promotion and tumour progression.

#### 1.5.1.2.2.1. Tumour Initiation

Tumour initiation results from irreversible genetic damage. Mutations can accumulate only when they arise in cells that proliferate. Proliferating cells are often
Figure 1.2. Metabolic activation of Benzo(a)pyrene and formation of DNA and protein adducts
Table 1.2. Precarcinogens Metabolised by Cytochrome P450

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activation of carcinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Polycyclic aromatic hydrocarbons: benzo(a)pyrene, dimethylbenz[a]anthracene, PhIP&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Activation of aryl and heterocyclic amines in industrial settings and food mutagens: N-nitrosodimethylamine, 4-aminobiphenyl, 2-acetyl-amino-</td>
</tr>
<tr>
<td></td>
<td>fluorene, N-nitrosodiyethylamine, PhIP, IQ, aflatoxin B1</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Polycyclic aromatic hydrocarbons: benzo(a)pyrene, dimethylbenz[a]anthracene, benz[a]anthracene, 3-methylcholanthrene, DMBA, oestradiol</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Activation of tobacco-related N-nitrosamines: NNK, NNAL, NDEA, NNN, NATB, Aflatoxin B1, 1,3-butadiene, 2,6-dichlorobenzonitrile</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Aflatoxin B1 and 4-(methylamino)-1-(3-pyridyl)-1-butanone</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Low-molecular-weight toxicants and cancer suspect agents: benzene, carbon tetrachloride, chloroform, styrene, vinyl chloride, vinyl bromide, N-nitrosodi-</td>
</tr>
<tr>
<td></td>
<td>methylamine, NNK</td>
</tr>
<tr>
<td>CYP3A4/5/7</td>
<td>Diverse carcinogens: aflatoxin B1, aflatoxin G1, benzo(a)pyrene, naphthalene, NNN, 1-nitropyrene, 6-amino-chrysene, oestradiol, seneconine, stergma-</td>
</tr>
<tr>
<td></td>
<td>to-cystine</td>
</tr>
</tbody>
</table>

<sup>a</sup>DMBA, 7,12-dimethylbenz[a]anthracene; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; NATB, N-nitrosoanatabine; NDEA, N-nitrosodiethylamine; NNAL, 4-(methylamino)-1-(3-pyridyl)-1-butanone; NNK, 4-(methylamino)-1-(3-pyridyl)-1-butanone; NNN, N9-nitrosonornicotine; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine.
presumed to be more mutable than quiescent cells because they have less time to repair DNA damage before DNA replication. A chemical carcinogen causes genetic error by modification of the molecular structure of DNA, often by a single base alteration, that can lead to a mutation during DNA synthesis. Most often, this is brought about by formation of an adduct between the chemical carcinogen or one of its functional groups and a nucleotide in DNA (Yuspa and Poirier, 1988). Carcinogen–DNA adduct formation is central to theories of chemical carcinogenesis and it can be considered to be a necessary, but not a sufficient prerequisite for tumour initiation. DNA adduct formation that results in either the activation of a proto-oncogene or the inactivation of a tumour-suppressor gene can be considered to be a tumour initiating event.

1.5.1.2.2. Tumour Promotion

Tumour promotion comprises the selective clonal expansion of initiated cells via altered gene expression that gives the cell a selective growth advantage (Cairns, 1975). Tumour promoters cause cells to proliferate but not to terminally differentiate, resulting in proliferation of praeoplastic cells and the formation of benign lesions such as papillomas, nodules or polyps. Many of these lesions will regress, but a few cells may acquire additional mutations and progress to a malignant neoplasm. Tumour promoters generally are non mutagenic, not carcinogenic alone. They are often (but not always) able to mediate their biologic effects without metabolic activation. These agents are characterized by their ability to reduce the latency period for tumour formation after exposure of a tissue to a tumour initiator and to increase the number of tumours formed in that tissue. In addition, they induce tumour formation in conjunction with a dose of an initiator that is too low to be carcinogenic alone. Chemicals or agents which are capable of both tumour initiation and promotion are known as complete carcinogens; examples are benzo[a]pyrene and 4-aminobiphenyl. Croton oil (isolated from croton tiglium seeds) has been used widely as a tumour promoter in murine skin carcinogenesis. The mechanism of action of croton oil is due to its most potent constituent called 12-tetradecanoylphorbol-13-acetate, which acts via activation of protein kinase C and is the best understood among tumour promoters (Yuspa et al., 1996). Chemicals, complex mixtures of chemicals and other agents with tumour-promoting properties include dioxin, benzoyl peroxide, bromo methyl benzanthracene, anthralin, saccharin, dichloro diphenyl trichloro ethane (DDT), phenobarbital, polychlorinated biphenyls (PCBs), estrogens and other hormones.
1.5.1.2.2.3. Tumour Progression

The final stage of carcinogenesis is referred to as progression. The original tumour mass increases in size, additional mutations accumulate, and invasion and metastasis occur. Increased genetic instability and karyotypic alterations are hallmarks of progression. The ability of cancer cells to invade the surrounding stroma, enter the bloodstream, and extravasate to colonize in distant sites (i.e. metastasis) is crucial to progression of solid tumours. For cells to break away from the primary tumour, down-regulation of cell adhesion often by repression of E-cadherin expression must occur. Subsequently, the cells must acquire mobility and ability to invade the surrounding stroma and blood vessel basement membranes. Invasion requires the action of enzymes such as the matrix metalloproteinases (MMPs). Once established, the metastatic colony must develop adequate nutrition and oxygen supply via stimulation of angiogenesis. During progression in the two-stage mouse skin carcinogenesis model, premalignant papillomas convert to SCCs (squamous cell carcinomas) (Harper et al., 1986).

1.5.1.4. Biological Factors

1.5.1.4.1. Infectious Agents

About 18% of cancers are related to infectious diseases worldwide. Viruses are usual infectious agents that cause cancer but bacteria and parasites may also have an effect. A virus that can cause cancer is called an oncovirus. These include human papilloma virus (cervical carcinoma), Epstein-Barr virus (B-cell lympho proliferative disease and nasopharyngeal carcinoma), Kaposi's sarcoma herpes virus (Kaposi's Sarcoma), hepatitis B and hepatitis C viruses (hepatocellular carcinoma), and Human T-cell leukemia virus-1 (T-cell leukemias). Viruses increase the risk of cancer through a variety of mechanisms. Human papilloma virus is directly mutagenic by inducing the viral genes E6 and E7 (Mu’nger, et al., 2004), whereas HBV is believed to be indirectly mutagenic by generating reactive oxygen species through chronic inflammation (Gulam and Haseeb, 2006). Human T-lymphotropic virus is directly mutagenic, whereas HCV (like HBV) is believed to produce oxidative stress in infected cells and thus to act indirectly through chronic inflammation (Koike et al., 2002). Bacterial infection may also increase the risk of cancer as seen in Helicobacter pylori-induced gastric carcinoma (Pagano et al., 2004). Parasitic infections strongly associated with cancer include Schistosoma
*haematobium* (squamous cell carcinoma of the bladder) and liver flukes- *Opisthorchis viverrini* and *Clonorchis sinensis* (cholangiocarcinoma).

### 1.5.1.5. Dietary Factors

The extent to which diet contributes to cancer deaths varies greatly according to the type of cancer (Willett, 2000). For example, diet is linked to cancer deaths in as many as 70% of colorectal cancer cases. Most carcinogens that are ingested (such as nitrates, nitrosamines, pesticides, and dioxins) come from food or food additives or from cooking. Heavy consumption of red meat is a risk factor for several cancers like gastrointestinal tract, colorectal, prostate, bladder, breast, gastric, pancreatic, and oral cancers. Heterocyclic amines are formed by cooking meat at high temperatures are carcinogens. Polycyclic aromatic hydrocarbons can be produced in meat and fish that have been grilled (broiled) or barbecued (charbroiled) over a direct flame and have strong cancerous effect. For instance, PhIP (2-amino-1-methyl-6-phenyl-imidazo[4, 5-b]pyridine) is the most abundant mutagen in cooked beef and is responsible for approximately 20% of the total mutagenicity found in fried beef (Lauber and Gooderham, 2007). Some N-nitroso compounds are formed in foods containing added nitrates or nitrites; examples include fish and meat preserved with salt or preservatives. Nitrites and nitrates are used in meat because they bind to myoglobin and inhibit botulinum exotoxin production; however, they are powerful carcinogens (Divisi et al., 2006). Furthermore, bisphenol from plastic food containers can migrate into food and may increase the risk of breast and prostate cancers. Saturated fatty acids, trans fatty acids, and refined sugar and flour present in most foods have also been associated with various cancers. Aflatoxin B is a toxin produced by *Aspergillus* fungus, which is a common contaminant of improperly stored cereals (grains) and peanuts and cause liver cancer.

### 1.5.1.6. Medication

A number of medical treatments increase the risk of some cancers. The most notorious example is diethylstilbestrol. It was once prescribed in pregnancy and now withdrawn as it caused cancers of vagina and cervix of female children born to mothers who received this drug (Troisi et al., 2007). X-rays are carcinogenic as radiation is used in cancer treatment. Chemotherapy as cancer treatment during childhood is followed by an increased risk of lymphoma in adulthood.
1.5.1.7. Habitual Factors
1.5.1.7.1. Tobacco

Tobacco use causes about 25–30% of all deaths from cancer and 87% of deaths from lung cancer. Compared with non-smokers, male smokers are 23 times and female smokers are 17 times more likely to develop lung cancer. Cigarette smoke contains at least 80 known mutagenic carcinogens including arsenic, cadmium, ammonia, formaldehyde and benzo(a)pyrene. Each will have a separate mechanism for causing cancer. For example, following metabolic activation, the activated derivative of benzo(a)pyrene called benzo(a)pyrene diol epoxide can form DNA adducts in lung epithelial cells (Alexandrov et al., 1992). Cigarette smoke is a powerful carcinogen and also a source of oxidative stress. Smokers are more likely to develop several types of cancers such as cancers of mouth, larynx, esophagus, pancreas, bladder, kidney and cervix than nonsmokers. The use of smokeless tobacco (chewing tobacco and snuff) causes cancer of mouth and throat. Exposure to environmental tobacco smoke, which is called involuntary smoking, increases the risk of lung cancer for nonsmokers.

1.5.1.7.2. Alcohol

Approximately 3.6% of cancers worldwide derive from chronic alcohol drinking. They are cancers of upper aerodigestive tract, liver, colorectal and breast. Although the mechanisms of alcohol-associated carcinogenesis are not completely understood, the most recent researches have focused on acetaldehyde—the first and the most toxic ethanol metabolite as a cancer-causing agent. Ethanol may also stimulate carcinogenesis by inhibiting DNA methylation and by interacting with retinoid metabolism. Other mechanism by which alcohol stimulates carcinogenesis include the induction of cytochrome P-4502E1, which is associated with enhanced production of free radicals and enhanced activation of various procarcinogens present in alcoholic beverages (Seitz and Stickel, 2007).

1.5.1.7.3. Obesity

Obesity has been associated with increased mortality from cancers of colon, breast (in postmenopausal women), endometrium, kidneys (renal cell), esophagus (adenocarcinoma), prostate, gallbladder and liver. Increased modernization and westernized diet and lifestyle have been associated with an increased prevalence of overweight people in many developing countries (Drewnowski, 1997). The factors that link between obesity and cancer include neurochemicals, sex steroids, adiposity,
inflammation and hormones such as insulin and insulin like growth factor 1 (IGF-1) (Hursting, 2007).

1.5.2. Endogenous Causes

1.5.2.1. Inherited Germline Mutations

Only a minority (5–10 per cent) of cancers are linked to inherited genes. Such inherited alterations are termed germ line mutations and are passed on from egg or sperm DNA. Individuals with inherited germ line mutations will not definitely get cancer but have an increased risk of developing cancer compared with the general population. Often mutations in tumour suppressor genes increase the chance of developing cancer at a young age. These include retinoblastoma (a rare cancer of the eye), Li-Fraumeni syndrome and kidney cancer in Von Hippel-Lindau disease. Mutations in BRCA1 and BRCA2 (breast cancer susceptibility) genes cause 5–10 per cent of all breast cancer cases. Patients with the syndrome familial adenomatous polyposis coli have a predisposition to colorectal cancer due to mutations in the adenomatosis polyposis coli tumour suppressor gene.

1.5.2.2. Oxidative Stress

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Jones, 2008). Under a sustained environmental stress, ROS are produced over a long time, and thus significant damage may occur to cell structure and functions and may induce somatic mutations and neoplastic transformation. Indeed, cancer initiation and progression have been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation (Visconti and Grieco, 2009).

1.5.2.2.1. Free Radicals

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell and Gutteridge, 1999). This unpaired electron(s) gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems (Mahantesh et al., 2012). Free radicals include reactive oxygen species (ROS) like hydroxyl (OH•), superoxide (O2•−), peroxyl (ROO•) and lipid peroxyl radicals (LOO•) and reactive nitrogen species.
(RNS) like nitric oxide (NO) and nitrogen dioxide radical (NO₂•). Hydrogen peroxide (H₂O₂), ozone (O₃), hypochlorous acid (HOCｌ), nitrous acid (HNO₂), peroxynitrite (ONOO⁻), dinitrogen trioxide (N₂O₃), lipid peroxide (LOOH) are not free radicals, but they can easily lead to free radical reactions in living organisms (Valko et al., 2007).

1.5.2.2.2. Generation of Free Radicals and Oxidants

Free radicals and other reactive species are derived from normal essential metabolic processes in human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals. Formation of ROS and RNS can occur in cells by two ways: enzymatic reactions and non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in respiratory chain, phagocytosis, prostaglandin synthesis and cytochrome P450 system (Kumar, 2011). For example, superoxide anion radical (O₂•⁻) is generated via several cellular oxidase systems such as NADPH oxidase, xanthine oxidase and peroxidases. Once formed, it participates in several reactions yielding various ROS and RNS such as hydrogen peroxide, hydroxyl radical (OH•), peroxynitrite (ONOO⁻), hypochlorous acid (HOCl), etc.

The production of superoxide occurs mostly within the mitochondria of a cell (Han, 2001). The mitochondrial electron transport chain is the main source of ATP in the mammalian cell and thus is essential for life. During energy transduction, a small number of electrons “leak” to oxygen prematurely, forming the oxygen free radical viz superoxide, which has been implicated in the pathophysiology of a variety of diseases. Superoxide is produced from both Complexes I and III of the electron transport chain (Muller et al., 2004).

NADPH oxidase in the inflammatory cells (neutrophils, eosinophils, monocytes and macrophages) produces superoxide anion by a process of respiratoty burst during phagocytosis. The superoxide is converted to hydrogen peroxide and then to hypochlorous acid (HClO) with the help of superoxide dismutase (SOD) and myeloperoxidase (MPO) (Mark et al., 1998). The superoxide and hypochlorous ions are the final effectors of bactericidal action. This is a deliberate production of free radicals by the body. ROS generated by this mechanism play an important role in cellular defense by killing bacteria but have also been involved in the development of tumours. In fact, it is considered that infection and chronic inflammation can contribute to 1 out of 4 of all cancers diagnosed.
Glucose

\[ \text{glucose-6- phosphate dehydrogenase} \]

Macrophage and neutrophil
(reator burn)

NADPH

O₂

NADPH oxidase

NADP⁺

O₂⁻

SOD

H₂O₂

myeloperoxidase

+ Cl₂

HClO

Bacteria killed

Xanthine oxidase (XO) is an enzyme that is widely distributed among species (from bacteria to man) and within various tissues of mammals. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. This enzyme plays an important role in the catabolism of purines. Xanthine oxidase generates reactive oxygen species (Ardan et al., 2004). The following chemical reactions are catalyzed by xanthine oxidase.

Xanthine oxidase:

\[
\text{Hypoxanthine} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{xanthine} + \text{H}_2\text{O}_2
\]

\[
\text{Xanthine} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{uric acid} + \text{H}_2\text{O}_2
\]

Peroxisomes are major sites of oxygen consumption in cell and participate in several metabolic functions that use oxygen. Oxygen consumption in the peroxisome leads to \( \text{H}_2\text{O}_2 \) production, which is then used to oxidize a variety of molecules. The organelle also contains catalase which decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound. Thus, the peroxisome maintains a delicate balance with respect to the relative concentrations or activities of these enzymes to ensure no net production of ROS. When peroxisomes are damaged and their \( \text{H}_2\text{O}_2 \) consuming enzymes are down regulated, \( \text{H}_2\text{O}_2 \) releases into cytosol and significantly contribute to oxidative stress. Cytochrome P450 has also been proposed as a source of ROS. The production of superoxide anion and hydrogen peroxide takes place following the breakdown or uncoupling of the cytochrome P450 cycle.

Hydroxyl radical (\( \text{OH}^\bullet \)), the most reactive free radical \textit{in vivo}, is formed by the decomposition of \( \text{H}_2\text{O}_2 \) in the presence of \( \text{Fe}^{2+} \) or \( \text{Cu}^{2+} \) (catalyst). This reaction is known as the Fenton reaction (Thomas \textit{et al.}, 2009). The hydroxyl radical has high reactivity, making it a very dangerous radical with a very short \textit{in vivo} half-life of
approximately $10^{-9}$ S. Thus when produced in vivo, •OH reacts with any biomolecules close to its site of formation. Hydrogen peroxide (H$_2$O$_2$) is the most stable reactive oxygen metabolite. H$_2$O$_2$ may be generated directly by divalent reduction of O$_2$ or indirectly by univalent reduction of O$_2$•. Hydrogen peroxide is the primary product of the reduction of O$_2$ by numerous oxidases. H$_2$O$_2$ is very sensitive to decomposition by redox-active metal complexes, of which catalase and peroxidase are the most effective exponents. Metal ions have a strong effect on the chemistry of O$_2$ and its reduction products. The well-known Fenton reaction is initiated when Fe$^{2+}$ comes in contact with H$_2$O$_2$ to produce •OH.

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{•OH} + \text{OH}^-$$

H$_2$O$_2$ also reacts with O$_2$• to initiate Haber-Weiss reaction producing •OH in the presence of Fe$^{2+}$ (James, 2000).

$$\text{O}_2\text{•}^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{•OH} + \text{OH}^-$$

Superoxide radical and hydrogen peroxide are less reactive oxidants than OH• but they have a longer life time which allows them to react with molecules in locations far from the site where the free radical has been produced.

Nitric oxide (NO) is a free radical. In mammals including humans, NO is an important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life of a few seconds in the blood. Nitric oxide, known as the 'endothelium-derived relaxing factor', or 'EDRF', is biosynthesized endogenously from L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes (Groves and Wang, 2000). Nitric oxide is also generated by phagocytes (monocytes, macrophages, and neutrophils) as part of the human immune response. Nitric oxide secreted during immune response is toxic to bacteria. Cells of the immune system produce both superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion (ONOO•), which is an oxidising free radical that can cause DNA fragmentation and lipid oxidation (Carr et al., 2000).

$$\text{NO} + \text{O}_2\text{•}^- \rightarrow \text{ONOO}^-$$
1.5.2.2.3. Beneficial Activities of Free Radicals and Oxidants

At low or moderate concentrations, ROS and RNS are necessary for the maturation process of cellular structures and can act as weapons for the host defense system. Indeed, phagocytes (neutrophils, macrophages and monocytes) release free radicals to destroy invading pathogenic microbes as a part of the body’s defense mechanism against disease (Huy et al., 2008). Other beneficial effects of ROS and RNS include their physiological roles in the function of a number of cellular signaling systems. Their production by nonphagocytic NADPH oxidase isoforms plays a key role in the regulation of intracellular signaling cascades in various types of nonphagocytic cells including fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes and thyroid tissue. For example, nitric oxide (NO) is an intercellular messenger for modulating blood flow, thrombosis and neural activity. Another beneficial activity of free radicals is the induction of a mitogenic response. In brief, ROS and RNS at low or moderate levels are vital to human health.

1.5.2.2.4. Deleterious Activities of Free Radicals and Oxidants

When produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress. It is a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) (Flora, 2009). Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed. For example, hydroxyl radical and peroxynitrite in excess can damage cell membranes and lipoproteins by a process called lipid peroxidation. Polyunsaturated fatty acids (PUFAs) are particularly susceptible to peroxidation and once the process is initiated, it proceeds as a free radical-mediated chain reaction involving initiation, propagation and termination (Sarma et al., 2010). Initiation of lipid peroxidation occurs when target polyunsaturated fatty acid (PUFA) is attacked by free radical (R*) and generates fatty acid radical (L*). This carbon centered lipid radical tends to be stabilized by a molecular rearrangement to form a conjugated diene, followed by reaction with oxygen to give a peroxyl radical. Peroxyl radicals are capable of abstracting a hydrogen atom from another adjacent fatty acid side chain to form a lipid hydroperoxide, but can also combine with each other or attack membrane proteins. When the peroxyl radical abstracts a hydrogen atom from a fatty acid, the new carbon-centred radical can react with oxygen to form another peroxyl radical, and so the propagation of the chain reaction of lipid peroxidation can continue. Hence, a
single substrate radical may result in conversion of multiple fatty acid side chains into lipid hydroperoxides. Decomposition of hydroperoxides generates a complex mixture of secondary lipid peroxidation products such as hydrocarbon gases (e.g. ethane and pentane), lipid peroxy, lipid alkoxyl radicals and aldehydes (e.g. malondialdehyde (MDA) and 4-hydroxynonenal) (Figure 1.3). These metabolic by-products can cause direct destruction of structure of membrane or can indirectly damage other structures of cell e.g. DNA and RNA mainly by aldehydes like MDA. A measured level of MDA is used as a direct index of tissue damage associated with lipid peroxidation. Proteins may also be damaged by ROS/RNS leading to structural changes and loss of enzyme activity (Shinde et al., 2012). Oxidative damage to DNA leads to the formation of different oxidative DNA lesions which can cause mutations. Body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants. If not regulated properly, oxidative stress can induce a variety of chronic and degenerative diseases like malignant diseases, diabetes, atherosclerosis, chronic inflammation, viral infection, ischemia-reperfusion injury as well as the aging process (Uttara et al., 2009).

### 1.5.2.2.5 Oxidative Stress and Cancer

Initiation, the first step of carcinogenesis, requires a permanent modification of the genetic material (DNA) in cell. It is estimated that the number of oxidative hits to DNA is about 10,000 per cell per day in human (Ames et al., 1993). DNA damage from oxygen free radicals (OFR) is continuously removed by specific and non-specific repair mechanisms. Nonetheless, a very small part of the oxidative DNA lesions escapes repair and represents an important mutagenic potential that accumulates with age (Lindahl, 1993). When levels of OFR increase drastically, the DNA lesions may not be effectively counteracted by DNA repair. Thus, exposure of mammalian cells to oxidative stress increases mutagenesis. In various cancer tissues, an increase in OFR specific DNA modifications was found (Olinski et al., 1992). The hydroxyl radical reacts with all components of the DNA molecule: the deoxyribose backbone, the purine bases and the pyrimidine bases. The chemical alteration of the deoxyribose elements can cause the release of purine or pyrimidine bases, producing abasic sites which have been shown to be mutagenic in vivo. OH· attack on the duplex DNA results in radical adducts with purine or pyrimidine bases that yield a variety of
Figure 1.3. Steps involved in lipid peroxidation

- Fatty acid with three double bonds
- Hydrogen abstraction by Hydroxyl radical
- Unstable carbon radical
- Molecular Rearrangement
- Conjugated diene
- Oxygen uptake
- Peroxyl radical
- Hydrogen abstraction → Chain reaction
- Lipid hydroperoxide
- malondialdehyde
- 4-hydroxynonenal
- ethane/pentane
- etc.
end products. The OH• adducts of adenine or guanine can result either in ring-fragmented bases such as the 2,6-diamino-4-hydroxy-5-formamido pyrimidine (FaPy), or in hydroxy purines such as 8-hydroxy-guanine (8-OH-Gua). Examples of thymidine and cytosine products are thymine glycol (5, 6-OH-Thy) and 5-hydroxy-cytosine (5-OH-Cyt) respectively. While many of the OFR-related DNA base modifications result in a replicative block, some can induce point mutations by base misreading at replication. The most ubiquitous oxidative DNA base modification is 8-OH-Gua (Rozalski et al., 2002). 8-OH-Gua can produce GC to TA transversions as a result of 8-OH-Guanine-Adenine mispairing. GC to TA transversion is frequently detected in the RAS oncogene and represents a possible mechanism of tumour initiation by OFR. GC to TA transversions in the TP53 tumour suppressor gene has been observed in lung and liver cancer (Hsu et al., 1991).

Role of free radicals in tumour promotion was suggested with the hypothesis that chemical promoters such as phorbol esters act via the stimulation of intracellular oxygen free radical production. ROS can affect a number of cellular processes critical in tumour development such as cell proliferation, senescence, inflammation, metastasis, etc. In terms of cell proliferation, ROS have been shown to modulate cell cycle regulation through modulation of various cell cycle proteins including p53 and ATM (ataxia telangiectasia mutated). ROS can coordinate a variety of redox-sensitive transcription factors such as NFκβ, HIF (hypoxia inducible factor), and p53 (Lara et al., 2010). A moderate increase of ROS often leads to NFκB activation, with subsequent induction of genes encoding for proteins that inhibit apoptosis, including Bcl-xL, cellular inhibitors of apoptosis (cIAPs), FLICE inhibitory protein (FLIP\textsubscript{L}), Gadd45, and TNFR-associated factors (TRAF)-1 and -2 (Karin and Lin, 2002). Other than acting at the transcriptional level, ROS can also act at the signal-transduction level to exert pro-survival functions. Oxidative stress can activate the ERK/MEK and the PI3K/AKT pathways (James et al., 2007). This could result in the inactivation of proapoptotic proteins and in the upregulation of antiapoptotic genes. ROS can also participate in the metastatic process by directly stimulating cell invasiveness and cell migration (Nishikawa, 2008). Both in vitro and in vivo studies report that ROS signalling is crucial for triggering the angiogenic response (Maulik, 2002). Recent
studies indicate that even endogenous ROS are involved in regulating angiogenesis and tumour growth.

1.5.2.3. Inflammation and Cancer

Various factors contribute to inflammation include microbial and viral infections, autoimmune and chronic diseases, obesity, consumption of alcohol, tobacco use, high-calorie diet, and exposure to allergens, radiation and toxic chemicals (Schetter et al., 2010, Aggarwal et al., 2009). The risk of cancer development increases when the inflammation persists for a long time. Two stages of inflammation are acute and chronic inflammation. Acute inflammation is an initial stage of inflammation (innate immunity), which is mediated through the activation of the immune system. This type of inflammation persists only for a short time and is usually beneficial for the host. If the inflammation lasts for a longer period of time, the second stage of inflammation or chronic inflammation sets in and may predispose the host to various chronic illnesses including cancer. During inflammation, mast cells and leukocytes are recruited to the site of damage, which leads to a ‘respiratory burst’ due to an increased uptake of oxygen and thus these is an increased release and accumulation of ROS at the site of damage. Following an inflammatory stimulus, initiation of carcinogenesis mediated by ROS may be direct (oxidation, nitration, halogenation of nuclear DNA, RNA, and lipids), or mediated by the signaling pathways activated by ROS. On the other hand, inflammatory cells also produce soluble mediators such as metabolites of arachidonic acid, cytokines and chemokines, which act by further recruiting inflammatory cells to the site of damage and producing more reactive species. These key mediators can activate signal transduction cascades and induce changes in transcription factors such as nuclear factor kappa B (NF-κβ), signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor-1α (HIF1-α), activator protein-1 (AP-1), and nuclear factor of activated T cells (NFAT), which mediate immediate cellular stress responses. This sustained inflammatory/oxidative environment leads to a vicious circle, which can damage healthy neighbouring epithelial and stromal cells and over a long period of time may lead to carcinogenesis (Costa et al., 2012).

1.5.2.4. Hormonal Factors

Some hormones play a role in the development of cancer by promoting cell proliferation. Hormones are important agents in sex-related cancers such as cancers of breast, endometrial, prostate, ovary and testis, and also in cancers of thyroid and bone.
An individual's hormone levels are mostly determined genetically, so this may at least partly explain the presence of some cancers that run in families even in the absence of any cancer-causing gene. For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene. However, non-genetic factors are also relevant: obese people have higher levels of some hormones associated with cancer and have a higher risk of the cancers. Lifetime exposure to estrogen-increased by early menarche, late menopause, not bearing children, and late first pregnancy—raises the risk of breast, ovarian, and endometrial cancers in women (Yager and Davidson, 2006). Women who take hormone replacement therapy have a higher risk of developing cancers associated with those hormones.

1.6. TREATMENT OF CANCER

Cancer treatment depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and additional personal characteristics. There is no single treatment for cancer. Patients often receive a combination of therapies and palliative care. Treatments usually fall into one of the following categories: surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or gene therapy.

1.6.1. Surgery

Surgery, often the first among the treatments for cancer, is used to remove solid tumours (benign tumour as well as cancer in early stage). Surgical resection is often not advised when size of tumour is large, location of tumour is close to other vital structures and also when there is presence of distant metastasis (high grade tumours) requiring assistance treatment. Surgery has no great effect if the tumour is already spread to other organs.

1.6.2 Radiation Therapy

Radiation therapy is a "local" therapy. It uses high energy x-rays to damage the DNA of cells, thereby killing the cancer cells or at least stopping them from reproducing. Tumours are made up of cells that are reproducing at abnormally high rates. Radiation therapy specifically acts against cells that are reproducing rapidly. It is estimated that more than 50% of cancer patients will receive radiation at some point during their treatment. There are two main types of radiation therapies: external radiation therapy, where a beam of radiation is directed from outside the body, and
internal radiation therapy, where a source of radioactivity is surgically placed inside the body near the tumour. Internal radiation therapy is also called brachytherapy or implant therapy. External radiation therapies include x-ray therapy, cobalt therapy, proton therapy, intensity modulated radiation therapy (IMRT) and image-guided radiation therapy (IGRT). Radiation treatments can be given once or twice a day depending on the treatment protocol being used. Treatments are given 5 days in a week for several weeks depending on the total dose of radiation that is planned. Patients are given a break from treatment to give normal cells some time to heal, thus reducing side effects. Internal radiation therapy places the source of the high-energy rays inside the body as close as possible to the cancer cells. This may be done by implanting "seeds" (small pieces of the radioactive substance) or by using an implanted reservoir, into which a liquid radioactive substance is injected. This delivers very intense radiation to a small area of the body and thereby limits the radiation exposure to normal tissues. Internal radiation therapy allows the doctor to give a higher total dose of radiation in a shorter time than is possible with external treatments. The radioactive substances used (also called radiation source) typically include radium, cesium, iodine, and phosphorus. Depending on the substance, the implant may be temporary or permanent, although the effect wears off over time in all cases. Depending on the type of radiation source, patients with radiation implants may need to be isolated from visitors for a period of time so as not to expose others to radioactivity. The doses of radiation used to destroy cancer cells can also hurt normal cells. Thus the side effects are directly related to the area of the body being treated. Some of the most common side effects of radiation therapy are given in Table 1.3.

1.6.3. Chemotherapy

Cancer chemotherapy is the use of various chemical agents in the treatment of malignancy. Most chemotherapeutic agents interact with enzymes or substrates that are related to DNA synthesis and hence exert anti-tumour effects by inhibiting cells that undergo DNA synthesis. Chemotherapy is generally used to treat cancer that has spread or metastasized because the medicines travel throughout the entire body. It is a necessary treatment for some forms of leukemia and lymphoma. Chemotherapy treatment occurs in cycles so the body has time to heal between doses. Six major classes of drugs are used in chemotherapy. They are:
Table 1.3. Common Adverse Effects of Radiation Therapy

<table>
<thead>
<tr>
<th>System</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Erythema, rash, hair loss</td>
<td>Fibrosis, sclerosis, telangiectasias</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Malnutrition, mucositis, nausea, vomiting</td>
<td>Adhesion, fistulas, strictures</td>
</tr>
<tr>
<td>Cardiac</td>
<td></td>
<td>Conduction defects, pericardial effusion, pericarditis</td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td>Airway fibrosis, pulmonary fibrosis, pneumonitis</td>
</tr>
<tr>
<td>Renal</td>
<td>Glomerulonephritis</td>
<td>Glomerulosclerosis</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Sinusoidal obstruction syndrome</td>
<td></td>
</tr>
<tr>
<td>Hematologic</td>
<td>Bone marrow suppression</td>
<td>Coagulation necrosis</td>
</tr>
</tbody>
</table>
(1) Alkylating agents: - They contain electrophilic alkyl group or a substituted alkyl group. They can form covalent bonds with cellular nucleophilic sites like DNA and thus impeding replication (cyclophosphamide, thiotepa etc).

(2) Platinum complexes: - They include cisplatin and its analogues like carboplatin and oxaliplatin. They inhibit DNA synthesis by forming intra-strand cross linking.

(3) Antimetabolites: - These are structural analogues of normal metabolites that are required for cell function and replication and hence they can block or subvert one or more of the metabolic pathways involved in DNA synthesis. Examples include anti-folates like methotrexate, pyrimidine analogues like fluorouracil, cytarabine, and purine analogues like fludarabine, pentastatin, etc.

(4) Anti-tumour antibiotics: - These compounds are of microbial origin. They intercalate with DNA and stabilize the DNA-topoisomerase-II complex, and in turn cause DNA double strand breaks and inhibition of DNA synthesis. Examples are doxorubicin, epirubicin, etc.

(5) Plant derivatives: - They include anti-microtubule agents like vinca alkaloids (vincristine, vinblastine) and taxanes (paclitaxel and docetaxel).

(6) Hormone antagonatists: - Tumours derived from hormone sensitive tissues are hormone dependent and can be inhibited by hormone antagonatists. Examples include antiestrogen (tamoxifen), antiandrogen (flutamide), etc. These treatments target any rapidly dividing cells, not necessarily just cancer cells. Some common side effects include hair loss, nausea, fatigue, and vomiting. Bone marrow suppression, cardiovascular and pulmonary toxicity, and central and peripheral nervous system damage are among the most serious adverse effects of cancer chemotherapy.

1.6.4. Immunotherapy

Cancer immunotherapy encompasses the use of specific antibodies against defined molecular targets, as well as recruitment and augmentation of the natural anti-tumour immune response. The aim of cancer immunotherapy is to utilise the unique power of the immune system to track down cancer cells and destroy them. Currently, eleven antibodies are approved for use in oncology (Reichert, 2011). By targeting tumours through specific or associated antigens, it is possible to selectively eliminate tumour cells and maintain an acceptable toxicity profile. Therapeutic antibodies that target immune cells are also being developed with the goal of breaking local tolerance and stimulating the patient’s anti-tumour immune response. Role of tumour antigens
(TAs) in eliciting an immune response leads to the development of cancer vaccines. There are two types of cancer vaccines: preventive and therapeutic vaccines. The preventive vaccines target infectious agents known to contribute to cancer development. Two preventive vaccines are currently marketed—one against human papillomavirus (HPV) types 6, 11, 16, 18 and another against hepatitis B virus (HBV). HPV types 16 and 18 are responsible for approximately 70% of cervical cancers. HBV vaccination is now recommended in childhood as part of a strategy to reduce the incidence of hepatocellular cancer. The premise behind therapeutic cancer vaccines is that injection of tumour antigen can be used to stimulate an immune system response against tumour cells. In 2010, the U.S. Food and Drug Administration approved the first therapeutic cancer vaccine, sipuleucel-T (Provenge) for the treatment of some cases of metastatic prostate cancer. As with other treatment modalities, immunotherapy is far from perfect and requires additional study to optimize clinical response and overcome therapeutic resistance.

1.6.5. Hormone Therapy

Some cancers are stimulated by hormones and may rely on them to grow. Blocking the action of these hormones could possibly stop the cancer from growing. Hormone therapy is most often used to treat breast and prostate cancers. Research is ongoing to study the potential efficacy of hormonal manipulation in treating other types of cancer. Hormone therapy can cause a number of side effects. Patients may have nausea and vomiting, swelling or weight gain. In women, hormone therapy also may cause interrupted menstrual periods and sometimes loss of fertility. Hormone therapy in men may cause impotence or loss of fertility.

1.6.6. Gene Therapy

Gene therapy is a technique of introducing the foreign genetic material into cells with a correction of a dysfunctional gene as its final goal. Gene therapy appears as a potential new strategy in cancer therapy and offers unique opportunities for tumour targeting. In cancer gene therapy, different approaches can be used such as mutation correction, enhancement of the immune response against tumour cells, RNA interference, targeted lysis of tumour cells using selective replicative viruses, anti-angiogenic and suicide gene therapies (Anderson et al., 2004). Gene therapy is so new that we do not know what side effects it may have, particularly long-term side effects that may occur years after receiving this therapy. There is a fear that the genes could enter healthy cells causing damage to them, which could then lead to another disease
or another cancer. If genes enter reproductive cells, they could then be passed on to future generations.

1.6.7. Oncolytic Viral Therapy

Viral oncolysis can be defined as the killing of tumour cells by selective viral infection, replication, cell lysis, and spread of progeny viruses in the tumour. Thus, as opposed to viral gene therapy, the virus itself is the therapeutic agent. The aim of oncolytic virotherapy is to achieve a strong cytolytic effect highly restricted to transformed cells. Many clinical trials around the world have had good results with high success rates using oncolytic virotherapy. Significant active research is being done to improve the accessibility, safety and efficacy of oncolytic virotherapy.

1.6.8. Nanoparticles in Cancer Therapy

Nanoparticles are the simplest form of structures with sizes in the nano meter range. In principle, any collection of atoms bonded together with a structural radius of < 100 nm can be considered a nanoparticle. They are particularly attractive for cancer treatment due to their small size, varied composition, surface functionalization and stability which provide unique opportunities to interact and target the tumour microenvironment (Yu et al., 2012). These interactions of nanoparticles with the tumour include aiding the small molecule transport to the intracellular organelles to induce the greatest cytotoxic effect (Paulo et al., 2011). However, rigorous evaluation is still warranted regarding: the short and long term toxicity effects by nano particles, targeting efficacy of nano particles, the off-target effects of radiothermal and magneto-thermal therapies, clearance of nano particles from the body, etc (Gwinn and Vallyathan, 2006).

1.7. CANCER CHEMOPREVENTION

Cancer chemoprevention has emerged as an ideal approach whereby the occurrence and progression of the disease can be prevented, slowed, or reversed by the administration of one or more naturally occurring and/or synthetic compounds (Sporn and Suh, 2000). The importance of cancer chemoprevention continues to grow due to poor prognosis of advanced cancers, inadequate therapeutic responses, and toxicities associated with chemotherapy and radiotherapy. Educational awareness programmes have had limited success in reducing exposure to tobacco products and to workplace and environmental carcinogens. The NIH and other health organizations have been focusing on chemoprevention by recommending guidelines for a ‘healthy diet’ and funding research on mechanisms of dietary supplements. Much of the
information and starting point for these studies originate from traditional medicine systems that for centuries have used plants to treat and prevent a variety of human diseases including cancer. The National Cancer Institute, based on numerous reports describing anticancer activity, identified about 40 edible plants possessing cancer-preventive properties (Aggarwal and Shishodia, 2006). Recent population studies have associated the consumption of fruits and vegetables with a reduced risk of various types of human cancer. This protective effect is thought to be due to high levels and variety of phytonutrients (phytochemicals) particularly found in dark coloured fruits and vegetables.

Chemoprevention is divided into three groups (a) Primary prevention: - This strategy seeks to prevent malignancies (cancers) in an otherwise healthy population. These individuals may have a history that puts them at higher risk, such as a history of smoking or particular genetic mutations predisposing them to cancer development, (b) Secondary prevention:- It involves giving medications or vitamins to patients who have known premalignant (pre-cancerous) lesions in order to prevent the progression of these lesions into cancers, and (c) Tertiary prevention :- It focuses on the prevention of new cancers in patients cured of an initial cancer or individuals who have been treated for premalignant lesions. Oxidative stress, mutagenesis, and inflammation are important processes in carcinogenesis and are possible targets for cancer chemoprevention (Nowsheen et al., 2012).

Compounds with cancer chemopreventive properties have been subdivided into blocking agents and suppressing agents on the basis of the stage of carcinogenesis at which they act. Blocking agents prevent carcinogens from modifying DNA and causing mutations. Diverse classes of compounds such as chemicals that inhibit metabolic activation of carcinogens or enhance their detoxification, antioxidants that scavenge free radicals, and chemicals that trap ultimate electrophilic carcinogens act as blocking agents. The salient features of these cancer-blocking agents have been categorized into mono- and bifunctional inducers of phase I and II drug-metabolizing enzymes. Blocking agents like tert-butylhydroquinone (tBHQ) and sulforaphane induce phase II detoxifying enzymes only and are called monofunctional inducers. Compounds like β-naphthoflavone (β-NF) and indole-3-carbinol (I3C) induce both phase I cytochrome P450 (CYP) enzymes and phase II detoxifying enzymes concomitantly and are classified as bifunctional inducers (Chen and Kong, 2004). As bifunctional inducers could
potentially activate carcinogens via induction of CYP enzymes, it would appear that all bifunctional inducers are not suited for chemoprevention. However, it is also plausible that some bifunctional agents could elicit stronger induction of phase II detoxifying enzymes than the induction of phase I CYP enzymes resulting in an overall detoxification and protection rather than activation of carcinogenic species. Some blocking agents act as dual-acting agents by inhibiting phase I enzymes and at the same time inducing phase II enzymes (Henderson et al., 2000). Compared with blocking agents, suppressing agents mostly interfere with the promotion and progression of carcinogenesis via the influence on cell proliferation, differentiation, and apoptosis, thereby inhibiting the premalignant and malignant transformation. Their actions include antagonism of oncogenes, activation of tumour suppressor genes, inhibition of angiogenesis, stimulation of apoptosis and modulation of arachidonic acid cascades (Figure 1.4).

Natural products have been used in conventional chemotherapy for many decades. Examples of natural chemotherapeutic agents include taxanes, vinca alkaloids and anthracyclines. Some natural compounds are marketed as nutraceuticals - products which are isolated or purified from foods and sold in a different form such as dietary supplements. Many natural products from our daily consumption of fruits, vegetables and tea beverages such as sulforaphane from broccoli, resveratrol from grapes, genistein from soy, curcumin from turmeric, and epigallocatechin-3-gallate (EGCG) from green tea can be considered blocking or suppressing agents (Russo, 2005). The mechanisms that are likely to underlie the effectiveness of these chemopreventive compounds are summarized in Table 1.4. There appear to have several different cellular and molecular mechanisms that underlie the blocking and suppressing effects of the chemopreventive compounds, and many of these compounds appear to possess both blocking effects and suppressing effects. Thus, the anti-carcinogenic function of these compounds might be attributed to a combination of their cytoprotective effect on normal cells and their cytotoxic effect on pre-neoplastic and/or neoplastic cells (Nair et al., 2007).

1.8. CHEMO AND RADIOPROTECTORS

The leading cancer therapies used today are surgery, radiotherapy and chemotherapy. In spite of advances in the field of cancer treatment, each of these known therapies has serious side effects. For example, surgery disfigures the patient or interferes with normal bodily functions. Chemotherapy and radiotherapy cause the
Figure 1.4. Mode of action of chemopreventive agents

A general scheme for interactions occurring between blocking agents and suppressing agents and the various stages in the sequence of events associated with the stepwise development of neoplasia. Blocking agents act immediately before or during the initiation of carcinogenesis by chemical carcinogens, and suppressing agents act after initiation, during the prolonged stages of promotion and progression. (Adapted from Johnson et al. 1994.)
Table 1.4. Potential Mechanisms of Dietary Chemopreventive Compounds

<table>
<thead>
<tr>
<th>Function</th>
<th>Examples</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blocking agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enhance detoxification of carcinogens</td>
<td>Curcumin</td>
<td>Turmeric</td>
</tr>
<tr>
<td></td>
<td>Sulforaphane</td>
<td>Cruciferous vegetables</td>
</tr>
<tr>
<td></td>
<td>Indole-3-carbinol</td>
<td>Cruciferous vegetables</td>
</tr>
<tr>
<td>Inhibit cytochrome P450 mediated activation of carcinogens</td>
<td>Isothiocyanates</td>
<td>Cruciferous vegetables</td>
</tr>
<tr>
<td><strong>Antioxidant activity</strong> (Scavenge free radicals)</td>
<td>Selenium</td>
<td>Nuts and meat</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>Vegetable oil</td>
</tr>
<tr>
<td>Trap carcinogens and prevent their interaction with DNA</td>
<td>Flavonoids</td>
<td>Fruits and vegetables</td>
</tr>
<tr>
<td><strong>Suppressing agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disrupt the cell cycle and/or induce apoptosis</td>
<td>EGCG</td>
<td>Green tea</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>Onions and Tomatoes</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
<td>Grapes</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>Turmeric</td>
</tr>
<tr>
<td></td>
<td>Sulforafane</td>
<td>Cruciferous vegetables</td>
</tr>
<tr>
<td>Modulate hormone activity</td>
<td>Genistein</td>
<td>Soy beans</td>
</tr>
<tr>
<td>Modulate nuclear receptors</td>
<td>Vitamin D</td>
<td>Fish</td>
</tr>
<tr>
<td></td>
<td>Retinoids</td>
<td>Eggs and milk</td>
</tr>
<tr>
<td>Suppress gene expression by DNA methylation</td>
<td>Folic acid</td>
<td>Fruits and vegetables</td>
</tr>
</tbody>
</table>
patients to experience acute debilitating symptoms including nausea, vomiting, diarrhea, hypersensitivity to light, hair loss, etc. The main reason for side effects of chemo and radiation therapies is that these agents are often unable to differentiate between normal healthy cells and tumour cells. Chemoprotective agents are administered concurrently with chemotherapies in order to selectively protect healthy cells from chemotherapeutic drugs, while still allowing cancer cells to be targeted (Schmidt and Fan, 2001). Today chemoprotective drugs are usually administered with a specific type of chemotherapy drug. Sodium-2-mercaptopoethane sulphonate (Mesna) is a thiol-producing compound that is used in clinical oncology to prevent bladder damage from high doses of chemotherapeutic alkylating agents like cyclophosphamide, cisplatin, ifosfamide, carboplatin, doxorubicin and its derivatives, and mitomycin and its derivatives. Mesna is excreted rapidly in the urine which limits its general utility except for bladder protection. Amifostine [S-2-(3-aminopropylamino)ethyl phosphorothioic acid, WR-2721] is a cytoprotective adjuvant used in cancer chemotherapy to reduce the incidence of fever and infection induced by DNA-binding chemotherapeutic agents including alkylating agents (e.g. cyclophosphamide) and platinum-containing agents (e.g. cisplatin). Amifostine was originally used to reduce the cumulative renal toxicity from cisplatin which was used in the treatment of non-small cell lung cancer. Though amifostine showed nephro protection, its ability to protect tumours could not be ignored. So the use of amifostine for the treatment of non-small cell lung cancer was withdrawn in 2005. Dexrazoxane is another chemoprotective drug which is particularly well-suited to prevent anthracycline antibiotics from damaging cardiac tissue. The most often reported side effects of dexrazoxane include pain on or at the injection site, flushing, bruising, rash, numbness, or general body discomfort.

Chemical/biological agents used to alter normal tissue toxicity from radiation are radioprotectors and can be broadly divided into three categories based on the timing of delivery in relation to radiation: chemical radioprotectors, mitigators, and treatment (Citrin et al., 2010). Agents delivered prior to or at the time of irradiation with the intent of preventing or reducing damage to normal tissues are termed radioprotectors. Agents delivered at the time of irradiation or after irradiation but prior to the manifestation of normal tissue toxicity are described as mitigators of normal tissue injury. Finally, agents delivered to ameliorate established normal tissue toxicity are considered treatments. There is a growing body of literature describing
radioprotection with a variety of agents after total body exposures or localized exposures. Best known agent in this class is amifostine. Amifostine is an organic thiophosphate prodrug that is dephosphorylated in vivo by alkaline phosphatase to form active cytoprotective thiol metabolite called WR-1065. Higher alkaline phosphatase activity, higher pH, and vascular permeation of normal tissues enable amifostine to give selective protection to non-malignant tissues. Amifostine dephosphorylation takes place preferentially in normal blood vessels but to a much lesser extent in tumour vessels because tumours are more acidic and the newly formed tumour blood vessels do not significantly express the enzyme alkaline phosphatase.

Some common side effects of amifostine include diarrhea, nausea, vomiting, sneezing, hypocalcemia, somnolence and hiccoughs. Serious side effects include: hypotension (found in 62% of patients), erythema multiforme, toxic epidermal necrolysis, immune hypersensitivity syndrome, erythroderma, anaphylaxis, and loss of consciousness (rare). Other radioprotective agents include N-acetyl-L-cysteine, diethyl dithiocarbamate etc but they are of lower efficacy when compared with amifostine.

The adjuvant role of herbal drugs in cancer treatment is well established. Some of the plants with known immunostimulatory activity are Viscum album (Kuttan and Kuttan, 1992), Tinospora Cordifolia, Withania Somnifera (Davis and Kuttan, 2000) etc. Herbal drugs in the form of rasayana were reported as radioprotectors (Rekha et al., 2000). Brahma rasayana, a poly herbal preparation containing almost 60 herbals, was reported to increase total WBC count in cancer patients undergoing chemo or radiation therapy (Joseph et al., 1999). Crude extract as well as isolated compounds were also reported to have protective effect against chemo and radiation toxicities (Kumar and Kuttan, 2007; Manesh and Kuttan, 2005). Naturally occurring compounds like sulphoraphane (Thejass and Kuttan, 2007) and monoterpenes (Raphael and Kuttan, 2003) were also reported to have immunomodulatory activity.

1.9. CAROTENOIDS

Carotenoids are widespread in nature and found in plants, animals and microorganisms. These intensely coloured molecules are responsible for the yellow, orange and red colours of various fruits, vegetables, flowers, birds, fish and crustaceans (Sugawara et al., 2009). In all, more than 750 carotenoids have been isolated from natural sources and structurally characterized. They are biosynthesized de novo by various plants, algae and photosynthetic bacteria (Okada et al., 2008). Carotenoids
perform critically important functions within photosynthetic systems. They function as accessory pigments harvesting the light energy of wavelengths, which are only weakly absorbed by chlorophyll, and thus increasing the efficiency of photosynthesis. In addition, carotenoids play a protective role by effectively dissipating excess energy, preventing the formation of reactive oxygen species and deactivating singlet oxygen generated during the photosynthetic process (Bonnie and Choo, 1999). Animals including humans cannot synthesize carotenoids, therefore these compounds must be assimilated from their diets (Fujisawa et al., 2008). The typical human diet contains about 40 carotenoids, most of which are obtained from fruits, vegetables, and seafood (Rao and Rao, 2007).

Carotenoids belong to the tetraterpenes family and have a common C_{40}H_{56} isoprenoid backbone structure. Carotenoids are compounds constituted by C5 eight isoprene units joined in a head to tail pattern, most of them have 40 carbon atoms (Mattea et al., 2009). These compounds are derived from phytoene, which is synthesized by a reductive dimerization of geranyl geranyl pyrophosphate (GGPP), after its dehydrogenation, cyclization, hydroxylation, oxidation and epoxidation (Yano et al., 2005). Carotenoids are structurally divided into two major classes: carotenes and xanthophylls. Carotenes are exclusively hydrocarbons without any oxygen molecule. Examples are α-carotene, β-carotene, lycopene etc (Aizawa and Inakuma, 2007). Xanthophylls are oxygenated hydrocarbons containing hydroxyl, methoxy, carboxyl, keto or epoxy groups. Examples are lutein, zeaxanthin, fucoxanthin, astaxanthin etc (Basu et al., 2001). The most characteristic feature of the carotenoid structure is the long system of alternating double and single bonds that forms the central part of the molecule. This constitutes a conjugated system in which the π electrons are effectively delocalised over the entire length of the polyene chain. This feature is responsible for the molecular shape, chemical reactivity and light absorbing properties, and hence colour of carotenoids (Britton, 1995). At least seven conjugated double bonds are needed for the carotenoid to impart colour. Each double bond in the polyene chain of a carotenoid can exist in two configurations, trans or cis geometrical isomers. The presence of a cis double bond creates greater steric hindrance between nearby hydrogen atoms and/or methyl groups, so that cis isomers are generally less stable thermodynamically than the trans form. Most carotenoids occur in nature predominantly or entirely in the all-trans form (Stahl and Sies, 2003).
Carotenoids share many common properties: they are insoluble in water but dissolve in organic solvents, they are bleached upon exposure to light or atmospheric oxygen, and they possess an absorption band near the blue to violet end of the visible spectrum. Common sources of carotenoids include carrots, corn, citrus fruits and dark green leafy vegetables such as spinach and broccoli. Carotenoids are absorbed along with dietary lipids through passive diffusion into intestinal epithelial cells (enterocytes) (Rock, 1997). For dietary carotenoids to be absorbed effectively, they must first be released from the food matrix, and their bioavailability varies from a small percentage for raw whole foods to >50% for some cooked and processed foods (Tanaka et al., 2012). The initial step in carotenoid absorption involves incorporation into mixed micelles composed of bile salts, cholesterol, and other lipids. The mixed micelles are then transferred into the enterocytes, where they are incorporated into triglyceride-rich lipoproteins called chylomicrons and released into the lymphatic system. From the lymphatic system chylomicrons enter systemic circulation through the subclavian vein. Through the action of endothelial enzyme lipoprotein lipase, triglycerides are removed from chylomicrons and distributed to extrahepatic tissues. The resulting chylomicron remnants are taken up by the liver, where the remaining carotenoids are incorporated into lipoproteins and secreted back into the circulation for delivery to the tissues. In mammals, carotenoids can exist in three forms: as solutes dissolved in body fat, as chromoproteins and as lipids found in specialized cells. Specifically they are concentrated in ovaries, ovum, testes, liver, skin, milk and eyes of humans and other primates (Handelman et al., 1992).

1.9.1. Carotenoids and Health Benefits

Of the approximately 700 carotenoids found in nature, only about 50 have provitamin A activity. Among them, only three are the most important precursors of vitamin A in humans: α-carotene, β-carotene and β-cryptoxanthin which are converted into vitamin A or retinol in the body (Zeb and Mehmood, 2004). β-carotene is the major pro-vitamin A component of most carotenoid-containing foods. Vitamin A is involved in vision, cell differentiation, synthesis of glycoprotein, mucus secretions from epithelial tissues, reproduction, overall growth and development of bones (Olson and Krinsky, 1995). Animals and humans cannot synthesize carotenoids de novo, although they are able to convert them into vitamin A. Diet is the only source for these precursors of retinol synthesis. Fruits, vegetables and microalgae are the major suppliers of provitamin A active carotenoids. As a reference value, a
recommended daily intake of 6 mg of carotenoids has been proposed. This value is based on the contribution of compounds with pro-vitamin A activity, especially β-carotene, which has been assigned a pro-vitamin A activity of 100% (Vílchez et al., 2011). Carotenoids increase gap junctional intercellular communication (GJIC) and induce the synthesis of connexion 43, a component of the gap junction structure (Zhang et al., 1991; Zhang et al., 1992). Loss of GJIC may lead to malignant transformation, and its restoration may reverse the malignant process. This effect was independent of provitamin-A and the antioxidant properties of the carotenoids. Carotenoids have been linked with enhancement of the immune system and lowering the risk of degenerative diseases such as cancer, diabetes, inflammatory diseases, cardiovascular disease, age related muscular degeneration and cataract formation (Figure 1.5) (Tanaka et al., 2012). Carotenoids can also influence the cellular differentiation, apoptosis programme and cellular anti-proliferation potential with different molecules as target points (Neuhouser et al., 2003; Aggarwal and Shishodia, 2006). Most of these biological effects are independent of the pro-vitamin A activity and can be attributed to the antioxidant property of carotenoids (Palozza and Krinsky 1992).

1.9.2. Antioxidant Property of Carotenoids

Generally, vitamin C, vitamin E and carotenoids are considered as the major dietary antioxidants. Carotenoids act as antioxidants both by quenching single oxygen ($^{1}\text{O}_2$) and by scavenging free radicals, but pro-oxidant activity may occur in some conditions. The electron rich conjugated double bond structure is primarily responsible for the excellent ability of carotenoids to physically quench singlet oxygen. The maximum ability to quench singlet oxygen is shown by those carotenoids having nine or more double bonds (Terao, 2010).

1.9.2.1. Quenching of Singlet Oxygen

The quenching of singlet oxygen is primarily by a physical mechanism (electron exchange energy transfer), in which the molecule of a carotenoid accepts the excitation energy from singlet oxygen. The added energy causes excitation of the carotenoid molecule, resulting in the generation of a “triplet” state ($^{3}\text{Car}^*$).

$$^{1}\text{O}_2^* + ^{1}\text{Car} \longrightarrow ^{3}\text{O}_2 + ^{3}\text{Car}^*$$

This triplet state of carotenoid dissipates energy harmlessly through rotational and vibrational interactions, and relaxes into its ground state ($^{1}\text{Car}$).

$$^{3}\text{Car}^* \longrightarrow ^{1}\text{Car} + \text{Heat}$$
Figure 1.5. Various functions of carotenoids
Since this is a physical mechanism (as opposed to a chemical reaction), the structure of the carotenoid molecule remains unchanged. Thus, the carotenoid acts as a catalyst for deactivating $^1$O$_2$. As the number of conjugated double bonds increase, the energies of the excited state decrease. This is reflected in the dependence of the $^1$O$_2$ quenching rate constant on carotenoid chain length (Jaswir et al., 2011).

1.9.2.2. Interactions of Carotenoids with Free Radicals

Carotenoids can also react with free radicals in a number of ways: (i) electron transfer to the radical, (ii) radical addition and (iii) allylic hydrogen abstraction.

(i) $\text{ROO}^+ + \text{CAR} \rightarrow \text{ROO}^- + \text{CAR}^+$ \textbf{(Electron transfer)}

(ii) $\text{ROO}^+ + \text{CAR} \rightarrow [\text{ROO-CAR}]^+$ \textbf{(Adduct formation)}

(iii) $\text{ROO}^+ + \text{CAR} \rightarrow \text{ROOH} + \text{CAR}^+$ \textbf{(Allylic Hydrogen abstraction)}

Firstly, the free radical obtains its missing electron by removing an electron from the electron-rich molecule of the carotenoids. Secondly, the free radical adds itself to the carotenoid molecule to pair its single electron, thus forming a covalent bond. In either case, the electron-rich structure of the carotenoid molecule attracts free radicals, thus sparing the cell components such as lipids, proteins and DNA from oxidative damage (Eperjesi and Beatty, 2005). The antioxidative capacity of carotenoids appears to depend on the number of conjugated double bonds and functional groups (Böhm et al., 2002).

1.10. MACULAR CAROTENOIDs AND IMPORTANCE

Lutein and zeaxanthin are xanthophylls (dihydroxy-carotenoids) that can be found in high concentration in primate’s eye (Bone et al., 1985, Khachik et al., 1997). They are concentrated in the macula region of primate’s retina. In the macula, lutein is present as a single stereoisomer (3R,3'R,6'R)-β-□-carotene-3,3'-diol, while zeaxanthin occurs primarily as a mixture of (3R,3'R)-β-β- carotene-3,3'-diol and (3R,3'S)-β-β-carotene-3,3'-diol which are referred to as zeaxanthin and meso-zeaxanthin respectively (Bone et al., 1993). It is thought that lutein and zeaxanthin protect the macula through their role as blue-light filters and also because of their antioxidant and singlet oxygen quenching properties, which reduce oxidative damage. In this way, lutein and zeaxanthin may reduce the risk of developing age-related macular degeneration (AMD), the leading cause of vision loss in older adults of the Western world. The clinical and epidemiologic studies have shown that plasma concentrations (Gale et al., 2003) and dietary intake (Seddon et al., 1994) of these xanthophylls are inversely correlated with the risk of AMD. Furthermore, it is found
that the content of lutein and zeaxanthin was lower in retinas of patients with AMD than in retinas without AMD. Lutein and zeaxanthin have been demonstrated as strong antioxidants and are widely distributed in vegetables and fruits. Epidemiological studies indicated that high intake of lutein/zeaxanthin could reduce the risk of a variety of cancers including lung, liver and colon cancer. Although the clear molecular mechanisms of lutein and zeaxanthin have not been studied well yet, several studies have already revealed their chemopreventive effects in animals. Emerging studies suggest the potential contribution of lutein and zeaxanthin to the prevention of heart disease and stroke (Judy et al., 2004).

1.11. MESO-ZEAXANTHIN

meso-Zeaxanthin [(3R,3’S)-β,β-carotene-3,3’-diol, MZ] is a non-provitamin A xanthophyll carotenoid present in the macula lutea of primate’s retina. The macula houses a yellow pigment that can be attributable to the presence of carotenoids meso-zeaxanthin (MZ), lutein (L), and zeaxanthin (Z) (Figure 1.6). Indeed this pigment lends its name to the macula lutea (Latin for yellow) and has been more recently referred to as macular pigment (MP). In terms of its distribution in the retina, the macular pigment appears to be localized in the Henle’s fibers (the axons of the photoreceptors), an appropriate location for shielding the photoreceptors from blue light (Snodderly et al., 1984). Though more than 700 carotenoids have been identified in nature, only these three carotenoids selectively accumulate at the macula indicating an exquisite degree of biological selectivity in retinal tissue (Bone et al., 2001). MZ was found to be effective in the aging macula to maintain its structural density (Bone et al., 2003). MZ is found to be present in the centre of the macula, where light is focused and where the strongest need for hazardous actinic blue light protection exists (Landrum et al., 1999). The presence of MZ results in the filtration of a wider range of damaging blue light. Moreover, MZ is more closely related to vulnerable photoreceptors at an anatomic level than either L or Z, and is therefore ideally located to afford protection against free radical damage of these important cells of vision. And most importantly, supplementation with MZ will ensure that this component of macular pigment accumulates at the target tissue (i.e. the central macula) in a way that is not dependent on an enzyme to isomerise lutein (Thurnham, 2008). Indeed, such an enzyme may be lacking in some individuals. Humans ingest relatively low levels of MZ, setting the stage for a potential deficiency, especially among individuals who are inefficient in synthesizing MZ, or lack the ability. There has been no exhaustive
Figure 1.6. Macular carotenoids

Zeaxanthin, 3,3'-dihydroxy-β,β-carotene

Meso-Zeaxanthin, 3,3'-dihydroxy-β,β-carotene

Lutein, 3,3'-dihydroxy-β,ε-carotene
assessment of the amounts of MZ in a normal diet. Brightly coloured egg yolks from hens fed with MZ are the richest potential human dietary source (Bone et al., 2007).

In the US, L and Z are abundant in the serum of people, but MZ cannot normally be detected in the serum. This observation led to a hypothesis that MZ is formed in the retina as a conversion product of L (Bone et al., 1993 and Bone et al., 1997). In the central 10° of retina, there is more MZ and less L relative to Z, whereas in the periphery (>35° eccentricity) the situation is reversed. This suggests that the postulated conversion process operates with greater efficiency in the foveal center compared with the peripheral retina. Monkeys raised on a carotenoid-free diet, and then supplemented with L only, subsequently exhibited both L and MZ in the MP. When the animals supplemented with Z only, they did not exhibit MZ in their MP. They exhibited only Z. These data are strong evidence in support of the L-to-MZ conversion hypothesis (Johnson et al., 2005). The absolute configuration of the hydroxyl groups located on the 3 and 3’ carbon atoms of the carotenoid end groups is identical in the L and MZ molecules. Thus the conversion of L into MZ need only involve a shift of one carbon-carbon double bond in the ε-ring of L, thereby increasing the conjugation. An alternative mechanism for the formation of MZ posits that the metabolite, dehydrolutein, gives rise to MZ through an enzymatic reduction pathway (Bone and Landrum, 2004).

1.1.1. Natural Occurrence of MZ

Birds make MZ and concentrate it and other carotenoids in their retinas within in brightly coloured oil-droplets. In chicken retinas, 47% of the total Zeaxanthin is MZ and in turkeys it is 28%. As with humans, MZ is formed in the retina from lutein. The deposition of carotenoids in fish was thoroughly investigated. Analysis of skin from rainbow trout fed with a diet rich in astaxanthin revealed that significant quantities of both MZ and SS-Z were formed from the astaxanthin. Like the rainbow trout, the Atlantic salmon also deposits MZ and SS-Z within its skin. These isomers of zeaxanthin are present in many aquatic species. Research showed that MZ, zeaxanthin and SS-Z are present in 21 species of edible fish, shrimp and sea turtles (Maoka et al., 1986).

1.1.2. Safety of MZ

The safety of MZ has been evaluated in a recent toxicity trial using Hans Wistar rats. The results of this trial verified that the “No-Observed-Adverse-Effect-Level” (NOAEL) was in excess of 200 mg/kg/day (Chang, 2006). It is far greater than
the doses used in dietary supplements, which are typically less than 0.5 mg/kg/day. Absence of mutagenicity was confirmed by Ames test. The mutagenicity was tested using *Salmonella typhimurium* tester strains TA 98, TA 100, TA 1535, and TA 1537 and *Escherichia coli* tester strain WP2uvrA both in the presence and absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9). The doses of MZ in the mutagenicity assay were 10, 33.3, 100, 333, 1000, and 5000 µg per plate. No dose caused a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes (Mecchi, 2006). In 2011, the FDA acknowledged that meso-zeaxanthin has GRAS (Generally Regarded As Safe) status and is therefore eligible for use in dietary supplements. MZ is a regular dietary component in countries like Mexico, where it is a major pigment used by the poultry industry and no adverse effects have been reported (Thurnham, 2007). A recent study of supplemental MZ in human has confirmed by serum analysis that renal and liver function, lipid profile, hematological parameters, and indicators of inflammation are unaffected by such supplementation (Connolly *et al.*, 2011).

1.1.3. Human Studies

A placebo-controlled study to evaluate the effects of a dietary supplement containing predominantly MZ was conducted. The supplement contained all three macular carotenoids in a ratio, MZ:lutein:zeaxanthin, of ~11:4:1 (total 21.8 mg) and was consumed daily over a period of 120 days. MZ was effectively absorbed into the serum, and macular pigment density was increased significantly in many subjects in the supplementation group. No such increases were observed in the placebo group. In another study, 19 subjects consumed a supplement also composed of all three macular carotenoids in a ratio, MZ:lutein:zeaxanthin, of 7:9:1 (total 20 mg) over a period of 22 days. Results demonstrated that MZ was absorbed and reached serum concentrations of approximately 0.24 µmol/L (Thurnham *et al.*, 2008). Interestingly, serum levels of MZ among women were almost three times higher than men. In a case-control study, differences in the amounts of individual macular carotenoids between AMD and healthy donor retinas were investigated. In the central 3 mm, the AMD group had, on average, ~30-percent less MZ and ~30-percent less lutein, but only ~20 percent less zeaxanthin than the healthy group. At the Waterford Institute of Technology, the Meso-zeaxanthin Ocular Supplementation Trial (MOST) was conducted to evaluate macular pigment response and serum carotenoid response in subjects with and
without AMD, following consumption of a supplement containing all three macular carotenoids in which MZ was predominant. This study identified statistically significant increases in serum concentrations of MZ and lutein from baseline. Significant increases in central macular pigment levels were also observed after just two weeks of supplementation. Furthermore, in patients who had an atypical macular pigment spatial profile (i.e., a central dip) at baseline, the more typical macular pigment profile (i.e. highest macular pigment at the center) was observed after eight weeks of supplementation with this MZ-dominant supplement (Bone et al., 2007).

1.11.4. Antioxidant Effect of MZ

In vitro experiments indicate that Z is a more potent antioxidant than L (Bone et al., 2011). It was found that quenching of singlet oxygen by Z was approximately twice as effective as quenching by L (Cantrell et al., 2003). The reason is presumably due, at least in part, to the extended conjugation of Z compared with L. MZ shares this electronic feature with Z and therefore should possess the same antioxidant potential as Z. The singlet oxygen quenching capability of carotenoids depends on the number of conjugated double bonds. L has 10 conjugated double bonds, while the other two macular carotenoids Z and MZ contain 11 such bonds (Conn et al., 1991). The singlet oxygen quenching abilities of MZ, Z, and L were investigated by UV-absorption spectroscopy using DMF as the reporter compound. The order of the ability of MP to quench singlet oxygen was MZ>Z>L (Li et al., 2011). It has also been reported that in association with a zeaxanthin binding protein, the pi isoform of glutathione S transferase, MZ provides slightly better protection against lipid membrane oxidation than Z (Bone et al., 2007).

1.12. SCOPE OF THE PRESENT STUDY

The importance of macular carotenoids like L and Z is well established. Most of the health benefits of these carotenoids come from their antioxidant activities. Recently a third macular carotenoid namely meso-zeaxanthin (MZ) was discovered. It is exclusively formed in the fovea centralis of primate’s retina from ingested L. Fovea centralis is the region of the retina where vision is sharpest and it is the site where the strongest need for protection from harmful blue light exists. Previous studies showed that MZ has profound antioxidant activity due to its extensive conjugated double bonds. Most of the studies on MZ showed importance of this carotenoid against age-related-macular degeneration (AMD). To the best of our knowledge, there is no report on the use of this cutting-edge carotenoid for any other
degenerative conditions. MZ can be easily synthesised from marigold flower extract. So in the present study, we have evaluated antioxidant, anti-inflammatory, anti-mutagenic, cytotoxic, induction of apoptosis in transformed cells, anti-tumour and anti-carcinogenic effects of carotenoid MZ synthesised from marigold flower extract. The anti-carcinogenic effect of MZ was studied against various chemical carcinogens induced cancer models in animals like nitroso diethyl amine (NDEA) induced hepatocellular carcinoma, 3-methylcholanthrene (3-MC) induced sarcoma and DMBA and croton oil induced two-stage skin papillomagenesis. Besides this, the present work aims to elucidate the possible chemopreventive mechanism of action of MZ since a precise assessment of mechanism of action of phytonutrients is necessary before they can be recommended for inclusion in dietary supplements or before they can be tested in human intervention trials. In this study, we further evaluated the adjuvant role of MZ during chemotherapy and radiotherapy. Hepatic diseases are also a leading cause of death today. So hepato-protective potential of MZ was also studied.