Chapter 1

Review of literature
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1.9. Scope of the present study
Cancer is a group of diseases in which genetically damaged cells proliferate autonomously. Cancer cells usually invade and destroy normal cells. Depending on the damage they have sustained, abnormal cells may form either benign or malignant tumors. Benign tumors are generally slow-growing expansive masses that compress rather than invade surrounding tissue. Malignant tumors are usually rapidly growing, invading surrounding tissue and, most significantly, colonizing distant organs. Cancers are classified by the type of cell that the tumor resembles and is therefore presumed to be the origin of the tumor.

1.1. Types of cancer

These types include:

- **Carcinoma**: Cancer derived from epithelial cells. This group includes many of the most common cancers including those of the breast, prostate, lung and colon.
- **Sarcoma**: Cancer derived from connective tissue or mesenchymal cells.
- **Lymphoma and leukemia**: Cancer derived from hematopoietic (blood-forming) cells.
- **Germ cell tumor**: Cancer derived from pluripotent cells. In adults these are most often found in the testicle and ovary, but are more common in babies and young children.
- **Blastoma**: Cancer derived from immature "precursor" or embryonic tissue.

The cytological criteria that enable the pathologist to confirm the diagnosis.

1) The morphology of cancer cells is usually different from and more variable than that of their counterpart normal cells from the same tissue.

2) The nucleus of cancer cells is often larger and the chromatin more apparent than the nucleus in normal cells.

3) The number of cells undergoing mitosis is usually greater in a population of cancer cells than in a normal tissue population.

4) Abnormal mitosis and “giant cells” with large, pleomorphic (variable size and shape) or multiple nuclei, are much more common in malignant tissue than in normal tissue.
5) Invasion of normal tissue by a neoplasm may be seen, indicating that the tumor has already become invasive and may have metastasized.

1.2. Epidemiology of cancer

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (WHO, 2008). About 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008 worldwide, with 56% of the cases and 64% of the deaths in the economically developing world (Jemal et al., 2011). In India, according to National Cancer Registry Program (ICMR) data the total cancer cases are likely to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020 (Takiar et al., 2010).

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>In the year 2010</th>
<th>In the year 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco-related cancers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>190,244</td>
<td>225,241</td>
</tr>
<tr>
<td>Females</td>
<td>75,289</td>
<td>93,563</td>
</tr>
<tr>
<td>Digestive system</td>
<td>107,030</td>
<td>86,606</td>
</tr>
<tr>
<td>Gynaecological related cancers</td>
<td>153,850</td>
<td>182,602</td>
</tr>
</tbody>
</table>

The burden of cancer is increasing in economically developing countries as a result of population aging and growth, environmental pollution, infections as well as, adoption of cancer-associated lifestyle choices including smoking and physical inactivity.

1.3. Etiology of cancer

Carcinogenic chemicals and irradiation (ionizing and ultraviolet) are known to affect DNA and to be mutagenic under certain conditions. Thus, one of the long-standing theories of carcinogenesis is that cancer is caused by a genetic mutation; however, it is now known that epigenetic mechanisms are also involved. Current evidence would suggest that all causes of cancer act by generating damage to the genetic material of cells, specifically causing mutations in proto-oncogenes and tumor suppressor genes. In many cases the mutations in such genes can be linked directly to
the types of DNA damage associated with the agents that cause cancer e.g. genetic predisposition, tobacco, infections, dietary-related factors, hormonal factors, radiation, occupational carcinogens, medical carcinogens, and environmental pollution. The causation of cancer can widely classified under the category include physical, chemical, biological, hormone related and due to diet and habits.

1.3.1. Physical factors

The main physical agents capable of inducing carcinogenesis include UV radiation, ionizing radiation, asbestos, erionite, some natural and manmade fibers and nonfiberous particulate materials.

1.3.1.1. Radiations

Radiations are of two major types, electromagnetic waves or ionizing particles. In either case, interaction with orbital electrons results in ionizations and excitations. The ionizing event involves the ejection of an orbital electron from a molecule, producing a positively charged or “ionized” molecule. These molecules are highly unstable and rapidly undergo chemical change. This change results in the production of free radicals, atoms, or molecules containing unpaired electrons. These free radicals are extremely reactive and may lead to permanent damage of the affected molecule, or the energy may be transferred to another molecule. Most of the energy deposited within a cell results in the production of aqueous free radicals, since approximately 80% of the cell is water. Radiation can, by itself, induce a type of genomic instability in cells, which enhances the rate at which mutations and other genetic changes arise in the descendants of the irradiated cell after many generations of replication (Little, 2000).

The hazards of exposure to ionizing radiation were recognized shortly after Roentgen’s discovery of the x-ray in 1895. Later, many experimental and epidemiologic studies have confirmed the oncogenic effects of radiation in many tissues of many species. Ionizing radiation has the ability to penetrate cells and to deposit energy within them in a random fashion. All cells in the body are thus susceptible to damage by ionizing radiation. In radiation carcinogenesis, the damage to DNA, and hence its mutagenic and carcinogenic effect, is due to the generation of free radicals as the radiation passes through tissues. The amount of radical formation and ensuing DNA damage depend on the energy of the radiation. In general, X-rays and gamma rays have a low rate of linear energy transfer, generate ions sparsely along their
tracks, and penetrate deeply into tissue. This profile contrasts with that of charged particles, such as protons and a particles, which have a high linear energy transfer, generate many more radical ions locally, and have low penetration through tissues. The damage to DNA can include single- and double-strand breaks, point mutations due to misrepair deletions, and chromosomal translocations. The underlying cytotoxic and cellular stress responses to radiation are mediated by existing signaling pathways, activation of which may be amplified by intrinsic cellular radical production systems. These signaling responses include the activation of plasma membrane receptors, the stimulation of cytoplasmic protein kinases, transcriptional activation, and/or altered cell cycle regulation (Schmidt-Ullrich et al., 2000). DNA is a target of ionizing radiation, and facilitates activation of cascades of growth factors and chemokines, and of molecules initiating multiple signaling pathways that modulate different cell functions. Nevertheless, under some circumstances, it may promote tumor associated host cells that support invasion and metastasis (Madani et al., 2008). After irradiation of prostate carcinomas and cervix cancer, the risk for rectal and bladder tumors increases, respectively (Dorr & Herrmann, 2008).

Ultraviolet radiation–induced lesions, generated by UV-B (280–320nm wavelength) or UV-A (320–400nm wavelength), result from DNA damage, which is converted to mutations during cellular repair processes. UB-B and UV-A generate different types of DNA damage and DNA repair mechanisms. Skin tumors in man account for about 30% of all new cancers reported annually. Epidemiologic and laboratory studies provide evidence for a direct causal role of sunlight exposure in the induction of cancer, and the high rate of skin carcinogenesis is a direct result of the high dose rate from this causative agent. Irradiation with UV-B produces cyclobutane pyrimidine dimers that are repaired by nucleotide excision repair. If left unrepaired, C→T and CC→TT base transitions occur.

UV-A induced DNA damage produces mostly oxidative lesions via photosensitization mechanisms and is repaired by base excision repair. UV-B and UV-A also produce different effects on the immune system and elicit different transcriptional and inflammatory responses. While the specific mechanisms by which UV radiation induces basal cell or squamous cell carcinomas or melanoma are not clear, a number of signal transduction pathways are affected that can either lead to apoptosis or to increased cell proliferation. UV-B, by inducing lipid and protein oxidation at the plasma membrane, may also cause a loss of membrane fluidity,
inactivation of enzymes, and alteration of the permeability to ions and may cause rupture of cells.

1.3.1.2. Fibrous and Non-fibrous materials

Asbestos, Erionite, Wollastonite, Glasswool, Rockwool, Slagwool etc. are fibrous materials and powdered metallic cobalt as well as nickel and crystalline silica are non fibrous materials. Fibers possess both genotoxic and cytotoxic activities. They have been shown to induce DNA damage, single and double strand breaks, mutations and chromosomal damage. Experimental data indicates that fibers can cause aneuploidy by impairing mitosis and chromosomal segregation. They also induce inflammatory response which results in the production of cytokines which facilitates the growth, selection and expansion of initiated cells. Occupational exposure to asbestos at high levels can cause lung cancer and mesothelioma of the pleura and peritoneum. Mesotheliomas in asbestos workers can be induced by exposure to asbestos alone, where as lung cancers are more likely to be caused by exposure to asbestos in smokers. Non-fibers materials causing Rhabdomyo sarcomas, adenocarcinomas and squamous cell carcinomas of the lung and malignant lymphomas etc.

1.3.2. Chemical factors

The role of chemicals in causation of cancer was first described by Percival Pott in 1775. He related the soot of chimney to be the culprit behind the occurrence of scrotal cancers in chimney sweepers. The experimental evidence for the link between chemicals and cancer was first reported by Yamagiwa and Ichikawa in 1915. They reported the formation of skin tumors in animals by the application of coal tar on the skin. After that a wide variety of chemical compounds were identified to be carcinogens. The mechanism of action of these chemical carcinogens involve the binding of molecules with DNA to form adducts. Some induces cancer directly by binding with DNA while others require enzymatic activation in the liver to form ultimate carcinogen. About 95% of carcinogens fall into one of the three major categories:

(i) Alkylating agents: Chemicals that transfer alkyl groups, often methyl or ethyl groups, to nucleotides to form DNA adducts. Egs: Nitrosamines, aflatoxins etc.

(ii) Aralkylating agents: Chemicals that transfer aromatic or multiringed compounds to a nucleotide to form an adduct. Eg: Polycyclic aromatic hydrocarbons (PAH).
(iii) **Aryl hydroxylamines**: Chemicals that transfer aromatic amines to nucleotides to form adducts (Perantoni, 1998). Egs: Acetamino fluorine, Aniline dyes, 2-napthylamines.

The most common compounds with potential carcinogenicity, their source and cancer type is given in table 1.1.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Main sources/uses</th>
<th>Affected organs/Cancer type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoazo dyes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Aminoazotoluene</td>
<td>Pigments; colouring oils; immunosuppressant</td>
<td>Liver, lung, bladder</td>
</tr>
<tr>
<td>N,N-dimethyl-4-Aminoazobenzene</td>
<td>Colour polishes; waxes</td>
<td>Liver, lung</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-Naphthylamine</td>
<td>Dyes; antioxidant</td>
<td>Bladder</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>Dyes; antioxidant</td>
<td>Bladder</td>
</tr>
<tr>
<td>2-Acetylamino Fluorine</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 Tested as a pesticide</td>
<td>Skin, lung, stomach</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzo-p-dioxin</td>
<td>Flame retardants</td>
<td>Lung, lymphoma, Liver</td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td></td>
<td>Liver, skin</td>
</tr>
<tr>
<td><strong>Metals &amp; 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><strong>N-nitroso compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Nitrosodimethylamine</td>
<td>Polymers, batteries; nematocide</td>
<td>Liver, lung, Kidney</td>
</tr>
<tr>
<td>4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone</td>
<td>Cigarette smoke</td>
<td>Lung, Liver</td>
</tr>
</tbody>
</table>
### Olefines

<table>
<thead>
<tr>
<th>Substance</th>
<th>Uses</th>
<th>Health Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene oxide</td>
<td>Glycol and polyester production; Sterilization Plastics (PVC); co-polymers Degreasing operations; adhesives; lubricants</td>
<td>Leukaemia, lymphoma</td>
</tr>
<tr>
<td>Vinyl chloride (VC)</td>
<td></td>
<td>Liver (angiosarcoma)</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td></td>
<td>Liver, kidney</td>
</tr>
</tbody>
</table>

### Paraffines/ethers

<table>
<thead>
<tr>
<th>Substance</th>
<th>Uses</th>
<th>Health Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-Dichloroethane</td>
<td>VC production; solvent; degreaser Technical applications</td>
<td>Liver, lung, breast</td>
</tr>
<tr>
<td>Bis(chloromethyl) ether Mustard gas (sulphur mustard)</td>
<td>Chemical warfare in First World War Limited application as anti neoplastic agent</td>
<td>Lung</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td></td>
<td>Lung</td>
</tr>
</tbody>
</table>

### Biological agents

#### 1.3.3.1. Bacteria

*Helicobacter pylori* as the most common cause of diffuse superficial gastritis and gastric and duodenal ulcers (McGowan *et al*., 1996). The association of *H pylori* infection with gastric cancer was an important landmark, because it provided the first definitive link between a widespread chronic bacterial infection, chronic inflammation originating within the target tissue, and the ultimate development of a human cancer. *H pylori* infection has been linked epidemiologically to gastric adenocarcinoma; an increased risk of gastric cancer in persons with elevated antibodies to *H pylori* (Nomura *et al*., 1991; Parsonnet *et al*., 1991). *H. pylori* infection is also associated with intestinal type, mucosa-associated lymphoid tissue lymphoma and non-Hodgkin lymphoma. Like *H. pylori* some other bacterial infections are involved in initiation and progression of cancer. A chronic persistent dermatological infection termed astropicalphagedenic ulcer caused by *Fusobacterium fusiforme* and a spirochete, *Borreliavincnetii* can leads to the development of squamous cell carcinoma within the depigmented margins of the ulcerative lesion. *Tropherymawhippelii* causing small intestinal lymphomas and *Vibrio cholera* causing immunoproliferative small intestinal disease and non-Hodgkin lymphoma (Cooper *et al*., 1996; Parsonnet *et al*., 1991).

#### 1.3.3.2. Viruses

Among microorganisms, oncogenic viruses are the most well established procarcinogenic agents. From a universal perspective infectious agents especially
viruses account for several of the most common malignancies up to 20% of all cancers (Pisani et al., 1997). Due to the limited coding capacity of viral genomes that is imposed by packaging limits, viruses have developed strategies to reprogram hosts cellular regulatory structures for their own purposes that regulate cellular proliferation, differentiation, apoptosis and life span. Viruses manipulate the anabolic and proliferative capacity of the host cell in order to maximize virion production and dissemination. Host cells that are driven to replicate and evade apoptosis are at risk of acquiring mutations activating oncogenes and disrupting tumor suppressor genes, suggesting that deregulation of host cell growth and death, two hallmarks of neoplastic transformation (Hanahan & Weinberg, 2000), can be seen as side effects of tumor virus propagation. Viruses that encode such activities can contribute to initiation as well as progression of human cancers. All these groups of viruses have been related with human cancer, meaning that they are able to initiate the carcinogenesis process by mutagenesis induction.

1.3.3.2.1. DNA viruses

**Epstein-Barr virus (EBV)**- Infects over 95% of the adult population worldwide, mainly through oral contact. EBV is also linked to a number of lymphoid neoplasias such as Burkitt’s lymphoma, Hodgkin’s disease, AIDS-related lymphoma and epithelial neoplasias like nasopharyngeal carcinoma, gastric adenocarcinomas, and oral hairy leukoplaikia.

**Kaposi’s Sarcoma-Associated Herpesvirus (KSHV)** (Human herpesvirus 8) - Infection is usually asymptomatic and is most commonly spread via infected saliva or sexual contact (Calabro et al., 2001; Schulz, 1999). In addition to Kaposi’s sarcoma (KS), KSHV is linked to primary effusion B cell lymphoma (PEL), a monoclonal tumor often coinfected with EBV, and multicentric Castelman’s disease (MCD), a polyclonal B-cell lymphoproliferative disorder; all three diseases have an increased incidence in immunosuppressed patients (Hengge et al., 2002a,b). The KSHV genome contains a variety of genes with immune modulatory, anti-apoptotic, and cell cycle regulatory functions (Schulz, 2000) these promote cell proliferation, block apoptosis, and evade immune surveillance.

**Hepatitis B Virus (HBV)** - Infected about 380 million people and is transmitted through sexual contact and transfer of contaminated blood or other body fluids. The infection is asymptomatic or limited to acute liver disease characterized by destruction
and regeneration of hepatocytes and inflammation. Over a period of several decades, this chronic liver damage may evolve to cirrhosis, end-stage liver failure, and hepatocellular carcinoma (HCC), a rapidly progressing tumor with a poor prognosis (Arbuthnot & Kew, 2001). The viral protein most strongly implicated in HBV-associated hepatocarcinogenesis is HBx, whose many activities include important effects on mitochondrial function.

**Human Papillomavirus (HPV)** - Is a ubiquitous member of the Papilloma viridae family of small, nonenveloped DNA viruses (Lowy & Howley, 2001). The direct mutagenic action of HPV causes more than 90% of cervical cancers, which is spread by skin to skin contact during sexual intercourse. In addition to cervical carcinoma, HPV is associated with other anogenital carcinomas, upper airway carcinomas, and cutaneous tumors.

### 1.3.3.2.2. RNA viruses

These retroviruses incorporate their genome with host genome which triggers oncogenesis. Sometimes regulatory proteins of viral genome are expressed which activates several transcriptional pathways and upregulates key molecules involved in tumorigenesis.

**Human T-Cell Leukemia Virus Type 1 (HTLV-1)** - was the first human retrovirus to be identified and is the only one with a direct etiological link to cancer. HTLV-1 is the causative agent of adult T-cell leukemia/lymphoma (ATLL), an aggressive neoplasm of mature CD4⁺ cells, and tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM), a severe demyelinating neuropathy. The virus is transmitted through blood, semen, and breast milk. The immortalizing potential of HTLV-1 is attributable primarily to the viral protein Tax (transcriptional transactivator), which, in addition to transactivating the viral promoter, affects the expression and function of cellular genes controlling signal transduction, cell growth, apoptosis, and chromosomal stability.

**Hepatitis C Virus (HCV)** - is classified in the Hepacivirus genus of Flaviviridae, a family of enveloped, positive-strand RNA viruses. Over 170 million people worldwide are chronically infected with the virus, with infection most often traceable to contact with infected blood. HCV is mainly hepatotropic and is associated with chronic hepatitis, cirrhosis, and hepatocellular carcinoma; the virus may also infect lymphoid cells and is linked with immune system disturbances, lymphoproliferative disorders, B-
cell lymphomas, and other extrahepatic manifestations (Pawlotsky, 2004; Poynard et al., 2003; Zuckerman & Zuckerman, 2002).

The RNA genome of is translated into a single polyprotein, which is then cleaved to produce at least 10 mature proteins comprising virion components, enzymes, and regulatory proteins. The core protein, associates with the viral genome in the nucleocapsid of HCV is able to influence cell proliferation, senescence, apoptosis, and immune modulation (Ray & Ray, 2001). The nonstructural protein NS5A, which forms part of the viral replicase complex of HCV influences cell growth, intracellular calcium homeostasis, apoptosis, and the IFN-γ-mediated antiviral response (Reyes, 2002).

1.3.3.3. Hormonal factors

Hormones are involved in cell multiplication, either favoring the process or inhibiting it, and therefore play a role in the development of cancer. Substantial and convincing bodies of experimental, clinical, and epidemiologic evidence indicate that hormones may implicate in the etiology of several human cancers. These include cancers of the endometrium, breast, ovary prostate, ovary, thyroid, bone, and testis (Henderson et al., 1982; Feigelson et al., 1996). Hormone related cancers like breast, endometrial, ovary, prostate, testis, thyroid and osteosarcoma share a unique mechanism of carcinogenesis. Exogenous and endogenous hormones cause cellular proliferation and thus lead to the accumulation of random genetic errors. The malignancy depends on a series of somatic mutations that occur during cell division and the genes involved include those in endocrine pathway, DNA repair genes, tumor suppressor as well as oncogenes (Sager, 1989; Stanbridge, 1990; Knudson, 1971). Estrogens are the prime suspect for hormone related cancers. The established risk factors for breast cancer include early menarche, late menopause, alcohol consumption, post menopausal obesity, hormone replacement therapy and for endometrial cancer it is late menopause, sequential oral contraceptives, obesity, and estrogen replacement therapy. These factors cause the longer exposure of estrogen to the epithelial cells of respective tissues which predispose them to carcinogenic effect of estrogen (Feigelson & Henderson, 2000).

1.3.3.4. Genetic predisposition

Some cancers are indicated to have a link with familial occurrence. For example, women whose close relatives like grandmother, mother, maternal aunt or
sister has suffered from breast cancer, are found to run about 3 times higher risk of developing breast cancer than those who do not have such a family history. Similarly, cancers of the uterine cervix (females) and of prostate (males) are also thought to have a familial connection.

Certain genetic conditions are known to predispose the individual to cancer. For example, individuals with genetic conditions like xerodermapigmentosum, ataxia telangiectasia, Bloom’s syndrome, and Fanconi’s anaemia are found to be highly susceptible to different types of cancer (Bale & Li, 1997). The most common cancers include tumors of the colon, breast, ovary, endometrium, and endocrine glands (particularly the thyroid). The most prevalent hereditary syndromes associated with these tumors are hereditary nonpolyposis colorectal cancer (HNPCC), familial adenomatous polyposis (FAP), hereditary breast and ovarian cancer (HBOC), and multiple endocrine neoplasia type 2 (MEN 2). Some of these diseases are observed in the presence of particular exposures capable of causing genetic damage and are associated with a failure of DNA repair mechanisms. The inherited susceptibility is then the failure to repair this damage either correctly or completely. Some of these susceptibilities are recessively determined, meaning that a person with such a syndrome has two defective copies of the same gene, one inherited from each parent. The parent has only one copy of the defective gene and may therefore have either complete or marginally defective DNA repair capabilities which translate into only minimal extra cancer risk, if any at all.

1.3.4. Dietary and habitual factors

Doll and Peto (1981) were the first to point out an association between dietary constituents and cancer. A vegetarian diet is considered to be beneficial in reducing cancer incidence. Epidemiological studies have suggested that diets rich in vegetables and fruits reduce the risk of certain cancers. For example, diets rich in fibre, vitamins A, C and E, beta-carotene, retinols, alpha-tocopherol, polyphenols, and flavonoids, and minerals like selenium and zinc, have cancer chemopreventive effect. Fruits and vegetables are rich sources of chemopreventive chemicals. These include inhibitors of carcinogen formation, blocking agents (block conversion of procarcinogens to carcinogens), stimulators of detoxifying system, trapping agents (trap and eliminate potential carcinogens) and suppressing agents (suppress the different steps of the metabolic pathway leading to cancer) (Stavric, 1994). A study in China showed a high
incidence of oesophageal and gastric cancers in a population whose diet is deficient in beta-carotene and vitamins C and E. An interventional program, where the diet was supplemented with beta-carotenes, vitamin E and selenium, produced a 20% reduction in the stomach cancer mortality over a period 5 years (Blot et al., 1993).

1.3.4.1. Natural pesticides

Natural pesticides are produced by plants for protection against fungi, insects, and animal predators. Many plant derived products were found to be either mutagenic or carcinogenic which include pyrrolizidine alkaloids from plants, hydrazines from mushroom etc.

1.3.4.2. Mycotoxins

Mycotoxins are secondary metabolites produced by molds in foods. A classical example of mycotoxin is Aflatoxin B1 (AFB1) and it naturally occurring as mixtures of aflatoxins are known to be genotoxic and carcinogenic in humans. Fumonisin B1 is also a mycotoxin which produced by the corn pathogen, Fusarium moniliforme, is reported to be carcinogenic in rats.

1.3.4.3. Products of food preparation and processing

Smoke condensates derived from grilling fish and meat is rich in heterocyclic amines which are highly carcinogenic. These compounds showed alterations in cancer related genes like H-ras, Kras, p53 and β-catenin. Red meat and processed meat intakes are associated with increased risks of colorectal cancer. Salt-preserved foods and dietary nitrite found in preserved meats are potentially carcinogenic. Sodium chloride acts as a promoter for gastric carcinogenesis. High salt concentration leads the disruption of mucin layer of gastric epithelium and prolonged damage results in atrophic gastritis and intestinal metaplasia, the precursor lesions for gastric cancer. The development of breast and prostate cancer and colon carcinogenesis has direct link with excess of fat intake

1.3.4.4. Habitual factors

1.3.4.4.1. Tobacco chewing and cigarette smoking

The role of cigarette smoking in lung cancer is established. Tobacco smoke contains a chemical, nitrosamine, which can induce neoplastic changes in the lung cells. Non-smoking tobacco habits, like chewing, are found to greatly increase the
cancers of the upper alimentary tract and buccal mucosa. India has the highest incidence of oral cancers in the world, which is correlated with the tobacco chewing habit. Alcoholism is found to increase the risk of liver and bladder cancers. Smoking combined with alcohol consumption posses a higher risk of cancers of the breast, oesophagus, liver, stomach and urinary bladder. Alcoholism along with hepatitis B virus infection is a more serious risk factor in liver cancer.

Smoking accounts for 80–90% of the of lung cancer diagnosed in men and 55–80% in women each year worldwide. Efforts to reduce tobacco consumption are therefore of the utmost importance for preventing premature death, not only from cancer but from other tobacco related diseases (ischaemic heart disease, chronic obstructive lung disease, and cerebrovascular disease). A cigarette smoke contains thousands of chemicals, of which more than 60 are known carcinogens, i.e. polycyclic aromatic hydrocarbons (BaP, dibenz [a,h] anthracene), nitrosamines (Nitrosamines 4-(methyl nitros amino)–1-(3-pyridyl)–1 -butanone (NNK), N-nitrosonornicotine (NNN), aromatic amines (4-Aminobiphenyl, 2-naphthylamine), aldehydes (formaldehyde, acetaldehyde), volatile organic compounds, metals, and others (Hecht, 2003; 2008). Genotoxic carcinogens, present in tobacco smoke are also capable of inducing mutations and potentiate the development of cancer in lungs, larynx, nasal, oral cavity, esophagus, liver, pancreas, stomach, cervix, bladder and can even cause leukemia.

1.3.4.4.2. Pan masala

Pan masala is a mixture of areca nut, slaked lime, spices and tobacco. Areca nut contains alkaloids that give rise to nitrosamines like N- nitrosoguvacoline, 3 (methylnitrosamino) propionitrile, 3-(methylnitrosamino) propionaldehyde and N-nitroguvacine which are reported to be carcinogenic.

1.3.4.4.3. Alcohol

Alcohol consumption is one of the best established dietary risk factor for cancer. Alcohol is classified as a carcinogen by the International Agency for Research on Cancer. Consumption of alcohol increases the risk of numerous cancers, including those of the liver, esophagus, pharynx, oral cavity, larynx, breast, and colorectum in a dose-dependent fashion. Evidence is convincing that excessive alcohol consumption increases the risk of primary liver cancer, probably through cirrhosis and alcoholic hepatitis. At least in the developed world about 75% of cancers of the esophagus,
pharynx, oral cavity, and larynx are attributable to alcohol and tobacco, with a marked increase in risk among drinkers who also smoke, suggesting a multiplicative effect (Salaspuro et al., 2006). Mechanisms may include direct damage to the cells in the upper gastrointestinal tract, modulation of DNA methylation, which affects susceptibility to DNA mutations, and an increase in acetaldehyde, the main metabolite of alcohol, which enhances proliferation of epithelial cells, forms DNA adducts, and is a recognized carcinogen. The association between alcohol consumption and breast cancer is notable because a small but significant risk has been found even with one drink per day. Mechanisms may include an interaction with folate, an increase in endogenous estrogen levels, and elevation of acetaldehyde. Some evidence suggests that the excess risk is mitigated by adequate folate intake probably through an effect on DNA methylation. Although the epidemiologic data on the association between alcohol consumption and cancer of the colon and rectum are not entirely consistent, the majority of evidence suggests an increase in risk for both sites, possibly through inhibition of DNA repair, and deficiencies in nutrients like folate and other antioxidants. Notably, for most cancer sites, no important difference in associations was found with the type of alcoholic beverage, suggesting a critical role of ethanol in carcinogenesis (Longnecker, 1994).

1.3.4.5. Dietary Fat

In recent years, reduction in dietary fat has been at the center of cancer prevention efforts. In the landmark 1982 National Academy of Sciences review of diet, nutrition, and cancer, reduction in fat intake to 30% of calories was the primary recommendation. Interest in dietary fat as a cause of cancer began in the first half of the 20th century, when studies by Tannenbaum indicated that diets high in fat could promote tumor growth in animal models. Dietary fat has a clear effect on tumor incidence in many models, although not in all; however, a central issue has been whether this is independent of the effect of energy intake. Strong associations were seen with cancers of the breast, colon, prostate, and endometrium, which include the most important cancers not due to smoking in affluent countries. These correlations were observed to be limited to animal, not vegetable, fat. Although the range of fat intake that can be studied is restricted to the range of diets in the study population, this typically includes both the levels that have often been recommended (less than 30% of
energy) as well as more traditional U.S. levels (more than 40% of energy) (Hunter & Spiegelman et al., 1996).

1.3.5. Free radicals in cancer

Reactive oxygen species (ROS)

ROS are the more abundant free radicals in nature and have been related with a number of tissue/organ injuries induced by xenobiotics, ischemia, activation of leucocytes, UV exposition, etc. Oxidative stress is caused by an imbalance between ROS production and a biological system’s ability to readily detoxify these reactive intermediates or easily repair the resulting damage. Thus, oxidative stress is accepted as a critical pathophysiological mechanism in different frequent human pathologies, including cancer (DalleDonne et al., 2006; Jenner, 2003; Sayre et al., 2001; Valko et al., 2007). In fact ROS can cause protein, lipid, and DNA damage, and malignant tumors often show increased levels of DNA base oxidation and mutations. It is generally accepted that ROS eventually cause DNA damage, where by insufficient cellular repair mechanisms may contribute to premature aging and apoptosis. Conversely, ROS induced imbalances of the signaling pathways for metabolic protein turnover may also result in opposite effects to recruit malfunctioning aberrant proteins and compounds that trigger tumorigenic processes (Wang, 2008). Consequently, DNA damage plays a role in the development of carcinogenesis, but is also associated with an aging process in cells and organisms (Bertram & Hass, 2008). Hence additional actions of ROS must be important, possibly their effects on p53, cell proliferation, invasiveness and metastasis. Chronic inflammation predisposes to malignancy, but the role of ROS in this is likely to be complex because ROS can sometimes act as anti-inflammatory agents (Halliwell, 2007). The damaged nucleosides accumulate with age in both nuclear and mitochondrial DNA. Mitochondrial DNA (mtDNA) mutations appear involved in tumorigenesis, tumor growth promotion and/or metastatic potential (Shidara et al., 2005). An example is the mutated complex I (NADH dehydrogenase, amtDNA gene), and its reduced activity, which in certain tumors may lead to overproduction of ROS (Wallace, 1999), thus inducing up regulation of different nuclear genes associated with high metastatic potential: antiapoptotic MCL1 (myeloid cell leukemia1), HIF1 a (hypoxia inducible factor1 a) and VEGF (vascular endothelial growth factor). High metastatic potential is regulated by ROS mediated reversible upregulation of nuclear genes but not by ROS mediatedacceleration of genetic
instability, which suggests that ROS scavengers may be therapeutically effective in suppressing metastasis (Ishikawa et al., 2008).

1.3.5.1 Oxygen Radicals

A radical is an atom or group of atoms that have one or more unpaired electrons. Radicals can have positive, negative or neutral charge. They are formed as necessary intermediates in a variety of normal biochemical reactions, but when generated in excess or not appropriately controlled, radicals can wreak havoc on a broad range of macromolecules. A prominent feature of radicals is that they have extremely high chemical reactivity, which explains not only their normal biological activities, but how they inflict damage on cells. There are many types of radicals, but in biological systems most significant are those derived from oxygen. Oxygen has two unpaired electrons in separate orbitals in its outer shell. This electronic structure makes oxygen especially susceptible to radical formation.

1.3.5.2. Superoxide Anion Radical \((\cdot O_2^-)\)

The acceptance of a single electron by \(O_2\) generates cellular \(\cdot O_2^-\). The mitochondrial respiratory chain is the major source of \(\cdot O_2^-\) (Nohl & Hegner, 1978). The superoxide anion radical is abundant and can reach an intracellular concentration of about 10–11 M (Forman & Boveris, 1982). \(\cdot O_2^-\) is not highly reactive with biological molecules however once formed it quickly undergoes dismutation to generate hydrogen peroxide, which is highly reactive. This reaction is markedly accelerated by a family of enzymes, the superoxide dismutases (SODs). \(\cdot O_2^-\) can react with \(H^+\) to form \(HO_2^\cdot\) (hydroperoxy radical) which is much more reactive than \(\cdot O_2^-\). Other enzymes can generate superoxide anions. A notable example is NADPH oxidase, primarily located in phagocytes, neutrophils and monocytes, this enzyme produces large amounts of \(\cdot O_2^-\) and other reactive oxidants that are used for fighting invading microorganisms (Babior, 2000).

1.3.5.3. Hydroxyl Radical \((\cdot OH)\)

It is an extremely reactive oxidant (Halliwell, 1999). It is also a short-lived molecule with an estimated half-life of nanoseconds at 37°C, during which it can travel only a few Ångstroms. Despite its short life span, \(\cdot OH\) is capable of inducing considerable damage to nuclear and mitochondrial DNA. This radical alone can cause over a 100 types DNA modifications (Michalik et al., 1995). In addition, \(\cdot OH\) can lead to lipid peroxidation and oxidation of carbohydrates and proteins. The brain is particularly susceptible to lipid peroxidation since it is rich in lipids and contains high
levels of iron. The hydroxyl radical is a major product of IR due to radiation-induced dissociation of water molecules.

1.3.5.4. The Peroxy Radical (L·)

Lipid peroxidation is a process in which lipids undergo oxidation and peroxy radicals are formed. The hydroxyl radical is not the only radical that can initiate lipid peroxidation: `O2 and the peroxynitrite anion can also do so (Hogg & Kalyanaraman, 1999). Lipid peroxidation, and decomposition of fatty acids can lead to the formation of toxic products, such as lipid hydroperoxides (LOO·) and peroxy radicals. LOO· in turn can attack adjacent polyunsaturated fatty acids (PUFA) and reinitiate the process. Thus, this complex, self-propagating process, can lead to oxidation of most or all of the cellular lipids, making it highly destructive.

1.3.5.5. Hydrogen Peroxide (H2O2)

This is one of the most stable ROS and acts as a messenger in cellular signaling pathways (Kamata & Hirata, 1999). In addition to SOD, there are several other cellular systems that generate H2O2, including monoamine oxidase (MAO), diamine and polyamine oxidase, and glycolate oxidase. H2O2 is quite stable and under normal conditions is not toxic up to a cellular concentration of about 10−8 M. However, H2O2 is highly diffusible through cell membranes and organelles and it is this capacity to travel long distances from its site of generation that makes it hazardous. In the presence of transition metals such as Fe2+ or Cu+, H2O2 can be converted to the highly reactive hydroxyl radical, either by Fenton or Harber–Weiss reactions (Halliwell, 1999). H2O2 is detoxified by a set of enzymes that includes the selenium-dependent glutathione peroxidase (GPx) and catalase.

1.3.5.6. Nitric Oxide (NO) and Peroxinitrite Anion (ONOO−)

NO is synthesized from l-arginine by any of the three NO synthase isoforms. NO is quite stable and benign for a free radical, with a lifetime of several seconds. Under normal conditions NO has many physiological functions as a neuronal messenger and modulator of smooth muscle contraction. However, when its intracellular level is increased it can induce a cascade of events that can eventually lead to cell death. NO can interact with ·O2– to generate the peroxynitrite anion (ONOO−). This molecule accounts for much of the NO toxicity. The reactivity of ONOO− is roughly the same as that of ·OH and NO2·. Its toxicity is derived from its ability to directly nitrate and hydroxylate the aromatic rings of amino acid residues and to react with sulphhydryls (Radi et al., 1991), lipids, proteins and DNA. Peroxynitrite anion can also affect
cellular energy status by inactivating key mitochondrial enzymes and it may trigger calcium release from the mitochondria (Packer & Murphy, 1994). The peroxynitrite anion and perhaps a few other reactive nitrogen species (RNS) with the exception of NO, can nitrate tyrosine residues (Halliwell, 1997) potentially leading to protein dysfunction (Ischiropoulos et al., 1992). This strong ubiquitous activity can have devastating effects on cellular physiology and viability.

1.4. Carcinogenesis

The various etiological factors leads to cancer through a multistep process called carcinogenesis. It involves initiation, promotion, progression and malignant conversion. Several characteristics of tumor, initiation, promotion, and progression provide some insight into the mechanisms involved in these processes.

1.4.1. Initiation

Initiation can occur after a single, brief exposure to a potent initiating agent. The actual initiation events leading to transformation into dormant tumor cell appear to occur within onemiotic cycle, or about 1 day for the mouse skin system. Furthermore, initiation appears to be irreversible; the promoting agent can be given for up to a year later and a high percentage of tumors will still be obtained. Thus, the initiation phase only requires a small amount of time, it is irreversible, and it must be heritable because the initiated cell conveys the malignant alteration to its daughter cells. All these properties are consistent with the idea that the initiation event involves a genetic mutation, although other “epigenetic” explanations are possible. The promotion phase, by contrast, is a slow, gradual process and requires a more prolonged exposure to the promoting agent.

1.4.2. Promotion

Tumor promotion involves activation of cell surface receptors, activation/inhibition of cytosolic enzymes and nuclear transcription factors, stimulation of proliferation, inhibition of apoptotic cell death etc. The promotion stage does not involve direct structural changes in the genome of the cell but is characterized by an altered expression of the genome of the initiated cell. Unlike initiation and progression, the stage of promotion requires extended treatment with the promoting agent. The effects of promoting agents are reversible, and if the promoter is removed,
disappearance of the expanding clones of cells will result. They will reappear if the promoter is reapplied (Pierce, 1998). Therefore, promoters are not considered to be genotoxic. Croton oil (isolated from Croton tiglium seeds) has been widely used as a promoter in mouse skin carcinogenesis. 12-O-tetradecanoylphorbol-13-acetate, the constituent of croton oil phosphorylates receptor protein kinase C (PKC) and thus plays a key role in mouse skin tumour promotion (Ashendel, 1985). However the stage of promotion does not always occur during the process of carcinogenesis. When the dose of carcinogenic agent is sufficiently high or there is involvement of multiple factors, the stage of promotion and at times even the stage of initiation may be bypassed (Pitot et al., 1990).

1.4.3. Progression

Progression is an irreversible process through which successive changes in the neoplasm give rise to increasingly malignant sub-populations. The process may be accelerated by repeated exposures to carcinogenic stimuli or by selection pressures favoring the autonomous clonal derivatives. The initiated cells proliferate causing a fast increase in the tumor size. As the tumor grows in size, the cells may undergo further mutations, leading to increasing heterogeneity of the cell population. In the first phase of progression, sometimes referred to as neoplastic conversion, the pre-neoplastic cells are transformed to a state in which they are more committed to malignant development. This may involve further gene mutations accumulating within the expanding pre-neoplastic cell clone. The dynamic cellular heterogeneity, a feature of malignant development, may, in many instances, be a consequence of the early acquisition of gene specific mutations that destabilize the genome (Hartwell & Kastan, 1994).

1.4.4. Malignant conversion

It involves multifocal change in premalignant lesions. There will be up regulation of transcriptional activity and expression of modified cell surface molecules, gene amplification, alterations in cell-cycle regulatory genes, secreted proteases and methylation of DNA. All these changes facilitate migration and invasion. Malignant transformation of cells in a benign tumor may be detected by pathologic examination of tissues. Often the clinical signs and symptoms are suggestive of a malignant tumor.

1.4.5. Metabolism of carcinogens

Cytochrome P450 enzymes (phase I monooxygenase enzymes) are widely known for their role in the metabolism of drugs and other foreign compounds. Thus, modulation of this enzyme system can influence the metabolism of xenobiotics,
producing effects of pharmacological and toxicological importance. Many carcinogens are metabolized by CYP enzymes to either biologically inactive metabolites or to chemically reactive electrophilic metabolites that covalently bind to DNA producing carcinogenicity. The reactive metabolites may undergo additional metabolism by phase I or II enzymes to inactive products. Therefore, induction of either phase I or phase II enzymes can result in increased detoxification of carcinogens (Conney, 2003). Since many chemical carcinogens are metabolized by CYP enzymes to both inactive, as well as to carcinogenic metabolites, the effects of inducers of these enzymes on the carcinogenicity of a chemical will depend on the inducers effects on the different metabolic pathways. In animal studies, it has been reported that inducers of CYPs usually decrease the carcinogenicity of chemical carcinogens in vivo; this suggests that induction of Phase I and II detoxification pathways may occur to a greater extent than induction of CYPs involved in the formation of carcinogenic metabolites (Conney, 2003). The CYP1 family consists of 1A1, 1A2, and 1B1 members that are capable of activating procarcinogens. CYP1A1 and 1B1 are both involved in the biotransformation of polycyclic aromatic hydrocarbons (PAHs), a class of ubiquitous environmental chemicals, to carcinogenic metabolites (Guengerich & Shimada, 1991). This process is believed to contribute to pulmonary carcinogenesis, because increased lung CYP1A1 expression and activity are associated with a high risk of lung cancer (McLemore et al., 1990). High CYP1A1 activity is also associated with colorectal cancer (Sivaraman et al., 1994).

CYP1A2 mainly metabolizes important drugs such as phenacetin, theophylline, caffeine, imipramine, and propranolol (Brosen, 1995), and also activates some procarcinogens to carcinogens. CYP1A2 plays a role in human tobacco-related cancers (Smith et al., 1996). CYP1A1 is poorly expressed in human liver although its synthesis can be markedly induced in many extrahepatic tissues, notably the lungs (Rendic & Di Carlo, 1997). In contrast, CYP1A2 is expressed principally in the liver (Rendic & Di Carlo, 1997). CYP1B1 is an extra hepatic estradiol 4-hydroxylase that activates procarcinogens and elevated levels have been associated with estrogen carcinogenesis (Jefcoate et al., 2000). Normal human breast and human breast tumor tissues are known to express CYP1B1, producing carcinogenic 4-hydroxy estrogen. Inhibition of CYP1B1 affects the production of mutagenic estrogen 3,4-catechols (Roberts et al., 2004). CYP2E1, an ethanol-inducible enzyme, is important in the field of toxicology and carcinogenesis, and it also has a role in drug metabolism (Guengerich et al., 1991).
For example, following an overdose of acetaminophen CYP2E1 converts acetaminophen to toxic quinones, which is responsible for the initiation of centrilobular liver toxicity (Lindros *et al.*, 1990). CYP3A4 is the largest subfamily of CYP enzymes expressed in the human liver and gastrointestinal tract. It is involved in the metabolism of 50% of therapeutic agents as well as in the activation of toxic and carcinogenic substances (Yee *et al.*, 1995).

Activation of phase II detoxifying enzymes, such as UDP-glucuronosyltransferase (UGT), glutathione S-transferase (GST), and NAD(P)H:quinoneoxidoreductase (QR) results in the detoxification of carcinogens. The importance of induction of Phase II metabolism in cancer prevention has been demonstrated in studies of nrf-2 knockout mice; nrf-2 is a transcription factor necessary for Phase II enzyme induction (Ramos-Gomez *et al.*, 2001). Nrf2 is normally localized in the cytosol, where it is associated through protein-protein interactions with the chaperone Keap1 (Itoh *et al.*, 1999). The presence of an inducer disrupts the Keap1-Nrf2 interactions, allowing Nrf2 to translocate to the nucleus and bind to the antioxidant/electrophile response element (ARE), in conjunction with small Maf proteins, after activation (Hayes & McMahon, 2001).

Glucuronidation, catalyzed by the UDP-glucuronosyl transferase family of enzymes, is a major metabolic pathway of endogenous steroids, bile acids, drugs, and carcinogens. UGTs have been divided into two families, termed UGT1 and UGT2 (Mackenzie *et al.*, 1997). UGT1 enzymes mainly catalyse glucuronidation of exogenous agents (drugs, pesticides, benzo[a]pyrene, etc.), whereas UGT2 enzymes glucuronidate endogenous agents (steroid hormones and bile acids). In humans, glucuronidation capacity is prominently present in the liver, but UGT activity toward bile acids, phenols, and bilirubin is present in human intestinal, kidney, and colon tissue (Tukey & Strassburg, 2000). GSTs catalyze the binding of a large variety of electrophiles to the sulphydryl group of glutathione, are involved in the detoxification of (oxygen) radicals, and have a main function in the binding and transport of a wide variety of harmful compounds. GSTs have a considerably important role in the detoxification of carcinogens (Hayes & Pulford, 1995).

1.5. Oncogenes and tumor suppressor genes

1.5.1. Oncogenes:

They are positive regulators of carcinogenesis. In non-transformed cells, they are inactive (proto-oncogenes). Gene mutations can activate proto-oncogenes, resulting
in a gain of function. They are activated proto-oncogenes which are the normal counter parts of the genome. They get activated by either of various etiologic factors through mutation. When get activated proto-oncogenes are converted to oncogenes and are expressed in high level to produce gene products which are involved in the process of carcinogenesis. The major proto-oncogenes are src, ras, cyclin, c-myc, c-abl, erb-B1 etc. Some cancer cells over express oncogenes which code for growth factors (e.g. sis, PDGFb) to which they are responsive. These signals bind to specific cellsurface receptors that transduce growth stimulatory signals into the cell interior, thereby promoting autonomous cell proliferation. Some of the oncogenes code for growth factor receptors, which when over expressed lead to cancer, as reported for EGF-R/erbB-2 in breast cancer (Lear-Kaul et al, 2003). During mitogenic stimulation, growth factors bind to their receptors and undergo cascade of activation/deactivation leading to cell proliferation. A typical example of ras oncogene activation is given in figure 1.1.

**Figure 1.1 Ras oncogene activation**

Ras activates MAP kinase via a phosphorylation cascade that proceeds from Ras to Raf kinase, to MEK kinase, and finally to MAP kinase. MAP kinase then dimerizes and enters the nucleus.

### 1.5.2. Tumor suppressor genes

Tumor suppressor genes typically control processes fundamental to the maintenance of stable tissue compartments. These processes include the maintenance of
genetic integrity, the progression of the cell cycle, differentiation, cell-cell interactions, and apoptosis. Mutational inactivation of tumor suppressor genes contributes to the loss of tissue homeostasis that may contribute to developing neoplasm. They may act as negative regulators of oncogenes, cell cycle check points, or gene products that supply the appropriate nutrients or components to complete a faithful cell cycle division in the absence of stress. Mutations in tumor suppressor genes are loss-of-function mutations and so occur in both alleles of a gene (the mutations act in a recessive fashion). Deletions, nonsense mutations, frame-shift mutations, insertions, or missense mutations that inactivate functional activity of a protein are all observed in tumor suppressor genes.

The diversity of functions of tumor suppressor gene products include transcription factors (p53), proteins that negatively regulate transcription factors (Rb), proteins that contribute to the degradation of oncogene functions by activating ubiquitin ligases (APC-β-catenin) or inhibiting ubiquitin ligases that degrade tumor suppressor genes (ARF acts on MDM-2, which degrades p53). There are GTPases (NF-1, TSC-1, 2) that inhibit pro-oncogenic G-proteins and a large number of DNA repair functions (BRCA-1, 2, ATM, MSH2 and MLH1, Franconianemia genes, etc.). Protein kinases (LKB1) and inhibitors of protein kinases (PTEN, which degrades PIP3, which is a second messenger for several lipid-activated protein kinase, p16-INK4A, which inhibits cdk-2), histone modifications (Men-1 is a histone methylase that silences transcription), and cytoskeletal and adhesion components (Ecadherin, α-catenin etc) are all represented by tumor suppressor genes. In common the tumour suppressor gene products populate signal transduction pathways that participate directly in the cell cycle or confer fidelity to the events in the cell cycle when stress is encountered to avoid errors in the DNA replication and the chromosome segregation process. They prevent cells from entering into cell cycle division under conditions of stress that would result in errors and the development of cancers. In the extreme, they initiate apoptosis to kill clones of cells that may contain or have the potential to obtain these mutations. Various tumor suppressor genes and their products function have been identified in inherited and sporadic cancers.

1.5.3. Apoptosis
Apoptosis or programmed cell death is the essential process for maintaining normal physiology. It specifically refers to an energy dependent, asynchronous, genetically
controlled process by which unnecessary or damaged single cells self-destruct when the apoptosis genes are activated (Martin, 1993; Earnshaw, 1995). Briefly, the cell shrinks and detaches from neighboring cells and the nucleus is broken down. The nuclear fragments and organelles condense and are ultimately packaged in membrane-bound vesicles, exocytose and ingested by surrounding cells. Apoptosis is controlled genetically, and the genes of Bcl-2 family, caspases and p53 are important in its regulation. Bcl-2, is a family of genes that regulates apoptosis by promoting (Bax, Bad, Bak and Bcl-xS) or inhibiting apoptosis (Bcl-2 and Bcl-xL) (Burlacu, 2003). Cellular disruption results from activation of a family of cysteine proteases called caspases (CASP) (Fan et al, 2005). The actions of the caspases are varied; some are endonucleases that cleave DNA, some cleave cytoskeletal proteins and others cause a loss of cell adhesion. There are two principal pathways for apoptosis which include the death receptor pathway, initiated by the engagement of death receptors, and the mitochondria-mediated pathway, induced by various forms of stress-like intracellular damage, developmental cues, and external stimuli.

**Intrinsic pathway:** The mitochondrial pathway is activated by a variety of extra and intracellular stresses, including oxidative stress and treatment with cytotoxic drugs. The apoptotic signal leads to the release of cytochrome c from the mitochondrial intermembrane space into the cytosol, where it binds to the Apoptotic Protease Activating Factor-1 (Apaf-1), which triggers the formation of the apoptosome. This complex catalyses the activation of caspases. Procaspase-9 is the initiator caspase of the apoptosome (Waterhouse et al, 2002). The apoptosome-bound procaspase-9 is activated and can then activate an effector caspase (e.g. caspase-3), which then can cleave the cellular substrates needed for the orchestration of apoptosis.

**Extrinsic pathway:** This pathway is plasma membrane receptor mediated mechanism that results in the activation of caspase- 8 and/or -10. The receptor-mediated apoptosis involves participation of surface receptors like Fas/Apo-1/CD- 95, tumor necrosis factor-alpha (TNF-α) receptor 1 (TNFR1), and the TRAIL receptors etc. and ligands including Fas-L, TNF, LT-α and -β, CD40L, etc. Upon receptor-ligand association, the receptor undergoes oligomerization, which allows the recruitment of intracellular adaptor proteins, such as FADD and TRADD, for Fas and TNF receptors, respectively. These adaptor proteins recruit and activate the initiator caspase-8 and 10 (Chen & Wang, 2002).
Caspase independent apoptosis: Mitochondria act as a crossover point between caspase dependent and independent apoptotic pathways. Caspase-independent pathways of cell death are also known, initiated by molecules released from mitochondria such as endonuclease G and the apoptosis-inducing factor (AIF) (Cregan et al., 2004). AIF is cleaved by calpains and/or cathepsins (Polster et al., 2005; Yuste et al., 2005) and translocates from mitochondria to cytosol and nucleus. Release of AIF results in generation of apoptotic phenotypes like chromatin condensation and phosphatidyl serine exposure and large scale (~50 kb) DNA fragmentation in a caspase-independent fashion (Collingwood et al., 2007).

1.6. Treatment for Cancer: Principles and Methods

Cancer treatment modalities are highly variable and are dependent on a number of factors such as the type, location and amount of disease and the health status of the patient. The treatments are designed to directly kill or remove the cancer cells or to lead to their eventual death by depriving them of signals needed for cell division or to stimulate the host immune system after kill or removal of these cells. However, some are effectively utilized where as others are still in their infancy. The major modalities utilized are surgery, radiation, chemotherapy, and immunotherapy.

1.6.1. Surgery

Surgery can be a simple, safe method to cure patients with solid tumors when the tumor is confined to the anatomic site of origin. It has an important role in diagnosing and staging of cancer. Although offering the greatest chance to cure many types of cancer by removing the primary tumors, surgery is ineffective for metastasized or disseminated tumors. There are many types of surgery, prophylactic surgery, also called preventive surgery, aims at removing a body tissue likely to become malignant. Diagnostic surgery is used to get a tissue sample to identify a specific cancer and make a diagnosis, which is often confirmed only by cellular microscopy, and staging surgery. Curative surgery is involving the removal of a tumor when it appears to be confined to one area. Palliative surgery refers to the treatment of complications of advanced disease. Restorative reconstructive surgery is used to restore a person’s appearance or the function of an organ or body part after primary surgery, like breast reconstruction after mastectomy or the use of tissue flaps, bone grafts, or prosthetic (metal or plastic) materials after surgery for oral cavity cancers.
1.6.2. Radiation therapy

Radiation, which constitutes the second modality in cancer treatment. Therapeutic radiation can be categorized by how it is produced from high energy machines, such as linear accelerators, that generate electron beams or x-rays or through radioisotope decay. Radiation is administered to cells either in the form of photons (i.e., x-rays and gamma rays) or particles (protons, neutrons, and electrons). When photons or particles interact with biological material they result in ionizations that can either directly interact with subcellular structures or they can interact with water, the major constituent of cells, and generate free radicals that can then interact with subcellular structures such as DNA (Prise et al., 2001).

New technologies have been evolved in the field of radiotherapy like three dimensional conformal radiation therapy (3-D CRT). The goal of 3-D CRT is to confirm the delivered radiation to the tumor while decreasing the dose to the surrounding normal tissues. Intensity-modulated radiation therapy (IMRT) is an even more specialized 3-D CRT technique used to further minimize normal tissue dose. It differs from the standard 3-D CRT approach in that the physician defines the target dose and dose constraints for normal structures, and then an optimal treatment plan is generated via computer algorithms that modulate the intensity of the radiation beams.

1.6.3. Chemotherapy

Chemotherapy confers the use of chemical compounds to treat cancer. Like radiation therapy, chemotherapy is administered in attempts to eliminate and halt the development of secondary tumors. The purpose of treating cancer with chemotherapeutic agent is to prevent cancer cell from multiplying, invading, metastasizing and ultimately killing the host. There are different classes of compounds used as chemotherapeutic drugs which include alkylating agents, platinum compounds, antimetabolites, topoisomerase interactive agents, antimicrotubule agents etc.

Some chemotherapy agents are able to kill a cell during any phase of the cycle (these are called cell-cycle nonspecific), others are only able to kill during a specific phase and are unable to work in the resting phase (called cell-cycle specific). By giving cell-cycle specific agents at multiple time points, they are able to reach the maximum number of cells in the particular phase they affect. Therefore, these are most effective when given in divided doses (over multiple days or time points, for example: once a day for 5 days or every three hours for 4 doses) or by continuous infusion. Cell-cycle nonspecific drugs act against cancer cells at any phase of the cell cycle, including the
resting phase. Mostly these drugs induce apoptosis. Like radiation treatment there is lot of severe side effects to chemotherapeutic drugs too which include myelosuppression, alopecia, stomatitis, dysphagia, nausea and vomiting, diarrhea etc.

1.6.4. Biological therapy

Molecules of biologic origin have been tried in clinical experiments for the control and removal of cancer cells in the system. This includes interferon, interleukin-2, monoclonal antibodies specific to cancer cell type etc. In case of cancers of hormone dependent organs like breast and prostate, hormone therapy using estrogen modulators, aromatase inhibitors etc. are successfully used.

1.6.4.1 Gene therapy

As genetic dysregulation is at the heart of the cancer cell genome, the engineering of cancer cells with genetic information that can reverse the transformed state has simplistic appeal. Tumor suppressor (TS) genes are the key cell genetic regulators in this paradigm, and much effort has been directed to the study of tumor suppressor gene replacement (Bossi & Sacchi, 2007). The vast majority of these studies have focused on p53, but other TS genes such as Rb, PTEN, and BRAC1 have also been investigated.

The p53 protein has been extensively studied as a potential target for cancer gene therapy because it is mutated in approximately half of all human cancers. Gene therapy involves replacing missing or nonfunctioning genes in cancer like p53 or by stopping the function of oncogenes genes which are expressed in cancer condition or use the body's own immune system by inserting genes into cancer cells that then trigger the body to attack the cancer cells as foreign invaders or inserting genes into cancer cells to make them more susceptible to or prevent resistance to chemotherapy, radiation therapy, or hormone therapies or to create "suicide genes" that can enter cancer cells and cause them to self-destruct or to use genes to protect healthy cells from the side effects of therapy, allowing higher doses of chemotherapy and radiation to be given. Given that gene therapy is so new, the side effects are not known particularly long-term side effects that may occur years after receiving this therapy (Levine et al., 1991).
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<th>Classification</th>
<th>Mechanism of action</th>
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<tr>
<td>a) Cyclophosphamide</td>
<td>Alkyl group transfer and alkylation of DNA</td>
<td>Bone marrow suppression, nausea, vomiting, central and peripheral nervous system neuropathies</td>
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<td>b) Mechlorethamine</td>
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<td>c) Melphalan</td>
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</tr>
<tr>
<td>d) Chlorambucil</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other Alkylating Drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Procarbazine</td>
<td>Alkylation of DNA</td>
<td>Nausea, vomiting, central and peripheral nervous system neuropathies.</td>
</tr>
<tr>
<td>b) Dacarbazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) Cisplatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antimetabolites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Methotrexate</td>
<td>Mitotic spindle inhibition and thus enhances tubulin polymerization</td>
<td>Bone marrow suppression, nausea, vomiting, alopecia</td>
</tr>
<tr>
<td>b) Purine Antagonists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) Pyrimidine Antagonists</td>
<td>Block cell cycle: in late S-G2 phase</td>
<td></td>
</tr>
<tr>
<td>d) Plant Alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antitumor antibiotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Actinomycin D</td>
<td>Intercalates DNA</td>
<td>Bone marrow depression, Total, severe alopecia</td>
</tr>
<tr>
<td>b) Bleomycin</td>
<td>Binds DNA, causes single strand breaks, and is G2-phase specific</td>
<td></td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Adrenal corticosteroid</td>
<td>Steroid hormones bind to steroid receptors,</td>
<td>Fluid retention, Androgens-masculinization, strogens-feminization, hypertension.</td>
</tr>
<tr>
<td>b) Estrogen and Androgen Inhibitors</td>
<td>Competitive partial agonist-inhibitor of estrogen, Binds to estrogen-sensitive tissues (receptors present), Suppresses serum levels of insulin-like growth factor-1; and up-regulates local TGF-beta production.</td>
<td>Hot flashes, fluid retention, nausea.</td>
</tr>
<tr>
<td>c) Gonadotropin-Releasing Hormone Agonists</td>
<td>Antagonizes remaining androgenic effects after orchiectomy or leuprolide treatment, GnRH agonists, inhibition of follicle-stimulating hormone and luteinizing hormone. Reduction in estrogen concentration, inhibitor of adrenal steroid synthesis, inhibits extra-adrenal estradiol and estrone synthesis, inhibits aromatase enzyme.</td>
<td>Nausea, vomiting, edema, thromboembolism, painful gynecomastia.</td>
</tr>
<tr>
<td>d) Aromatase Inhibitors</td>
<td></td>
<td>Nausea, vomiting.</td>
</tr>
</tbody>
</table>

| Miscellaneous Anticancer Drugs | DNA intercalation: produces single-and double-strand breaks, interaction with topoisomerase II-DNA complexes | Does-limiting hepatic toxicity, Cardiac arrest has been noted with amsacrine infusion. |
| a) Amsacrine | | Bone marrow suppression, nausea, vomiting, diarrhea. |
| b) Asparaginase | Depletion of serum asparagines, Decreased blood levels of asparagine and glutamine inhibit protein synthesis in those neoplastic cells that express decreased levels of asparagine synthase, Most normal cells express sufficient levels of asparagine synthase to avoid toxicity. | Leukopenia, mild nausea. |
| c) Mitoxantrone | Induces DNA strand breaks | |
| d) Bone Marrow Growth Factors | Inhibits RNA and DNA synthesis | |
1.6.4.2. Vaccines

Vaccines against tumor cells are produced and administered to trigger the immune system to combat the tumor are the principle behind vaccination. For this, tumor specific antigens, gene that encodes tumor antigens, cytokines and other immune stimulatory molecules are administered as vaccines. This type of approach is still under clinical trials.

1.7. Radioprotectors and chemoprotectors

Radioprotectors and chemoprotectors are chemicals that decrease the biologic effects of radiation therapy and chemotherapy. Since both these modalities of cancer treatments induce a wide range of acute and chronic toxicities. Acute side effects include mucositis, dysphagia, aspiration, hoarseness, and dermatitis (Trotti et al., 2003; Garcia-Periset et al., 2007; Rosenthal et al., 2006). Both these therapies also cause non hematologic toxicities, such as nausea, vomiting, neuropathy, nephropathy, ototoxicity, and hematologic toxicities and myelosuppression. In addition to causing acute toxicities, these may also be potentially associated with nonresolving, chronic toxicities or late occurring side effects with an onset of several months after completion of radiation or chemotherapy. Late toxicities include osteoradionecrosis (Zbaren et al., 2006), dental caries (Kielbassa et al., 2006), subcutaneous fibrosis, trismus, thyroid dysfunction (Jereczek- Fossa et al., 2004), pharyngeal or esophageal stenosis (Lee et al., 2006), myelitis, and sensorineural hearing loss (Jereczek-Fossa et al., 2003).

Various chemicals has been extensively studied and used as protectors during radio and chemotherapy. Amifostine has been demonstrated to protect salivary tissue, mucosa, and bone marrow (Brizel et al., 2000). Several plant extracts and plant derived compounds has also been extensively studied in mouse models for their chemo and radioprotective effects. However, studies are being continuing to develop agents that can give complete remission of the toxicities associated with radio and chemotherapy.

1.8. Chemopreventive natural dietary compounds

Cancer being one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this dreadful disease. Extensive preclinical and clinical research has led to substantial progress in understanding the multistep nature of the prolonged tumorigenesis process. Most cancers seem to be potentially preventable because of controllable or removable
causative exogenous factors, such as cigarette smoking, dietary factors, environmental and occupational chemicals, lifestyle and socioeconomic factors, radiation, and specific microorganisms. These exogenous factors offer the most likely opportunities for interventions targeted to primary prevention i.e., elimination of or avoiding exposure to these environmental factors.

In addition, however, as a serious and practical approach to the control of cancer, cancer chemoprevention can play an integral role in the overall strategy geared toward reducing the incidence of cancer. Thus, in addition to cancer therapy, cancer prevention has become an important approach to control cancer (Hail, 2005; Sun et al., 2004). Common prevention strategies include avoiding exposure to known cancer-causing agents, enhancement of host defence mechanisms against cancer, lifestyle modifications, and chemoprevention. Cancer chemoprevention uses agents that slow the progression of, reverse, or inhibit carcinogenesis in healthy subjects, thereby lowering the risk of developing invasive or clinically significant disease (Sporn and Liby, 2005). Consequently, an effective chemopreventive agent should intervene early in the process of carcinogenesis to eliminate premalignant cells before they become malignant, or protect normal cells from undergoing transformation. Since the latter strategy is more difficult to implement since otherwise healthy individuals would perhaps need a lifetime of exposure to a particular chemopreventive agent to achieve efficacy. It could be reasonably argued that the same benefit could be derived from avoiding exposure to known cancer-causing agents and consuming a balanced diet. However, concerns like long-term toxicity and the possibility of developing chemoresistance are formidable obstacles that could limit a chronic application strategy in the chemoprevention for many cancers.

Chemoprevention through the consumption of natural dietary compounds such as resveratrol from grapes, lycopene from tomatoes, and genistein from soy products may reduce both morbidity and mortality from cancer. The foods and herbs that possess anticancer activity include garlic, soybeans, cabbage, ginger, licorice, onions, flax, turmeric, cruciferous vegetables, tomatoes, peppers, brown rice, wheat and the umbelliferous vegetables such as carrots, celery and parsley. Natural products and their isolated constituents have been shown to possess strong chemopreventive activity in animal models. (Ramos, 2008) The effects of nutraceuticals on apoptotic pathways, signaling pathways, and/or different targets in cancer mean that they could be helpful starting points in the design and development of novel cancer preventive agents. Many
natural dietary compounds in fruits and vegetables have been isolated and have demonstrated health-promoting properties. They can be categorized into several classes. They are carotenoids, flavanoids, proanthocyanidins, flavonolignans other polyphenolic compounds, isothiocyanates, and terpenoids and omega-3 fatty acids etc. (Palozza & Krinsky, 1992). Table 1.4 shows chemopreventive agents using in cancer and their mechanism of action (Pana M & Ho C, 2008).

1.8.1. Carotenoids

Carotenoids are natural, fat-soluble pigments that provide bright coloration to plants and animals. They also act as antioxidants, and some of them possess vitamin A activity. One defining characteristic of carotenoids is the chemical structure of their backbone molecule, a 40-carbon polyene chain, derived from isoprene. The polyene backbone consists of conjugated double bonds, which allows the carotenoids to take up excess energy from other molecules through a non radiative energy transfer mechanism. This characteristic may be responsible for the antioxidant activity seen in biological carotenoids, as it has the ability to quench singlet oxygen. Carotenoids are lipid-soluble and can be categorized as follows: (a) vitamin A precursors that do not pigment such as β-carotene; (b) pigments with partial vitamin A activity such as cryptoxanthin, β-apo-8'-carotenoic acid ethyl ester; (c) non-vitamin A precursors that do not pigment or pigment poorly such as violaxanthin and neoxanthin; and (d) non-vitamin A precursors that pigment such as lutein, zeaxanthin and canthaxanthin. Due to the numerous conjugated double bonds and cyclic end groups, carotenoids present a variety of stereo isomers with different chemical and physical properties.

The most important forms commonly found among carotenoids are geometric (E-/Z-). A double bond links the two residual parts of the molecule either in an E-configuration with both parts on opposite sites of the plane, or a Z-configuration with both parts on the same side of the plane. Geometrical isomers of this type are interconvertible in solution. This stereoisomerism exerts a marked influence on the physical properties. Isomers differ not only in their melting points, solubility and stability, but also in respect to absorption affinity, colour and colour intensity. Animals cannot synthesize carotenoids, so their presence in the body is due to dietary intake of foods such as pink salmon flesh. The plumage of many birds owes its colour to carotenoids. Plant, algae, fungal and synthetic (nature-identical) carotenoids are
### Table 1.3 Chemopreventive agents using in cancer and their mechanism of action (Pana M & Ho C, 2008).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dietary source</th>
<th>Chemopreventive mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carotenoids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Red palm oil, carrots, pumpkin and leafy green vegetables</td>
<td>Inhibits NNK-induced lung carcinogenesis, increases antioxidative ability in the skin and liver of UVB-irradiated, improves the cell viability, and increases catalase activities and GSH levels in hepatocytes from chronically ethanol-fed rat.</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Tomatoes, watermelon, papaya and orange</td>
<td>Against g-radiation induced DNA damage, lipid peroxidation, induces apoptosis.</td>
</tr>
<tr>
<td>Lutein</td>
<td>Spinach and kale</td>
<td>Protects against UV light irradiation-induced lipid peroxidation, inhibits mouse mammary tumor growth by regulating angiogenesis and apoptosis. Reduces oxidative stress-induced apoptosis in photoreceptors and protects against UV light.</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>Squash, peas, cabbage and orange</td>
<td></td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonols</td>
<td>Onion, broccoli, apples and berries.</td>
<td>Modulates phase I and phase II enzyme. Inhibits proinflammatory mediator.</td>
</tr>
<tr>
<td>Flavones</td>
<td>Parsley, celery, citrus peels. Tea</td>
<td>Induces apoptosis in a human breast cancer cell xenograft model, induces cell cycle arrest by modulation of MAPK and PI3K. Induces cell cycle arrest and apoptosis in various cancer cells and inhibits ROS.</td>
</tr>
<tr>
<td>Flavanols (catechins)</td>
<td>Orange peel</td>
<td>Activation of phase II detoxifying enzymes, against UVB-induced apoptosis.</td>
</tr>
<tr>
<td>Epicatechin (EC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavanones</td>
<td>Cherries and strawberries</td>
<td>Decreases CCl₄-induced lipid peroxidation, induces G₂/M arrest and apoptosis in U937 leukemia cells.</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td>Soybean</td>
<td>Induces apoptosis by activation of calpain-caspase.</td>
</tr>
<tr>
<td>Isoflavonoids</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Proanthocyanidins
- **Proanthocyanidins A2**
  - Fruits, berries, beans, nuts, cocoa and wine
  - Modulates the expression of the antioxidant enzyme genes, inhibits lipid oxidation and protects the membrane against the attack of oxidants.
- **Proanthocyanidins B1**
  - Fruits, berries, beans, nuts, cocoa and wine
  - Inhibits the tumor growth by suppressing PI3K/Akt survival signaling, inhibits lipid peroxidation and nitric oxide.
- **Proanthocyanidins C1**
  - Fruits, berries, beans
  - Inhibits DMN-induced liver carcinogenesis and tumorigenesis.

### Flavonolignans
- **Silibinin**
  - Milk thistle
  - Inhibits activation of STAT3 and promotes cell cycle arrest and MAPK and Akt signaling.

### Other polyphenolic compounds
- **Curcumin**
  - Turmeric
  - Prevents peroxynitrite-induced oxidation and nitration reactions, inhibits growth and modulates secretion of angiogenic factors in ovarian cancer cells.
- **6-Gingerol**
  - Ginger
  - Inhibits proliferation and induces apoptosis by down-regulation of STAT3.
- **Resveratrol**
  - Grapes, red wine
  - Induces apoptosis through mitochondrial pathways in DMBA/TPA induced mouse skin tumorigenesis.

### Isothiocyanates
- **Phenethylisothiocyanate**
  - Cabbage, turnips, broccoli, kale, cauliflower, Brussels sprouts
  - Reduces the number of polyps and inhibits growth of adenomas through an increase of apoptosis.
- **Sulforaphane**
  - Cabbage, turnips, kale Brussels sprouts
  - Induces expression of phase II detoxification genes through the Nrf2/ARE pathway.

### Terpenoids
- **Monoterpenes**
  - Citrus fruits, cherries, spearmint, dill, caraway, apricots and grapes.
  - Induces apoptosis through activation of the ERK and caspase dependent mitochondrial death pathways.
- **Diterpenes**
  - Citrus fruits, cherries.
  - Induces G1 arrest through hyperphosphorylation of Rb2/p130, Up-regulation of MnSOD by activation of the NF-kB pathway.
- **Omega-3 fatty acids**
  - Fish oils, golden algae oil.
  - Inhibits TNF-a-induced MMP-9 expression, inhibits colorectal cancer growth by p53 dependent and independent pathways *in vitro* and *in vivo.*
permitted as colorants in food products, but not animal carotenoids (Takuji Tanaka et al., 2012).

β-Carotene is the most common carotenoid in food and the most potent of the provitamin A carotenoids. β-Carotene is known for its many health promoting characteristics such as enhancement of the immune system by improved activity and changes in immune cell numbers, and decreased risk of degenerative diseases such as cancer, cardiovascular diseases, age-related macular degeneration and cataract formation (Johnson & Russell, 2005). β-Carotene is primarily found in red palm oil, palm fruits, leafy green vegetables, carrots, sweet potatoes, mature squashes, pumpkin, mangoes and papayas. Epidemiological and animal studies support the hypothesis that β-carotene can prevent cancer in humans (Wang & Russell, 1999). In contrast, chemoprevention trials with β-carotene either alone or in combination with vitamin A or vitamin E actually increased the incidence of lung cancer in high risk groups of humans (Paolini et al., 1999). Other in vitro and in vivo studies have shown that while β-carotene itself may be anticarcinogenic, its oxidized products may facilitate carcinogenesis, thus providing an explanation for the chemoprevention trial results. Additionally, β-carotene is believed to have antioxidant activity, inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1;butanone (NNK) - induced lung carcinogenesis, (Kim et al., 2006) and improve cell viability through induced Phase 2 enzyme (Yang et al., 2004). It has been shown to exhibit radical-trapping behaviour only at partial pressures of oxygen substantially less than that of normal air. Such low oxygen partial pressures are found in most tissues under physiological conditions. At higher oxygen pressure, it loses the antioxidant activity and shows a pro-oxidant effect (Burton & Ingold, 1984) Lycopene, a carotenoid found in tomato, watermelon, papaya, apricot, and orange and pink grapefruit, has antioxidant and anticancer activities. About 80% of dietary lycopene is from tomatoes and tomato-related products. The bioavailability of lycopene is rather poor, but it is improved by thermal processing (Stahl & Sies, 1996). Numerous studies have suggested reduced risk of prostate cancer from the consumption of processed tomato products. Consistently, a lower risk of a variety of cancers and cardiovascular disease has been associated with the higher consumption of tomato-based products. Although, the beneficial health effects of lycopene are thought to be due to its antioxidant properties (Srinivasan et al., 2007) evidence is accumulating to suggest other mechanisms of action, such as the induction of apoptosis in smoke-induced lung carcinogenesis (Velmurugan et al., 2005) and the inhibition of
azoxy methane (AOM)-induced colon carcinogenesis (Sengupta et al., 2006) Lycopene is the most abundant carotenoid in human plasma, which may imply its elevated level of importance in the human body compared with other carotenoids, such as β-carotene and lutein.

1.8.2. Lutein

Lutein and its isomer, zeaxanthin, are yellow pigments that belong to the classes of non-provitamin A carotenoids. Unlike other carotenoids, hydroxyl groups are substituted on the ring structures at the end of the conjugated double bond chains of lutein and zeaxanthin; therefore, they are also called oxycarotenoids or xanthophylls. Lutein is naturally occurring and found predominantly in dark green, leafy vegetables such as spinach and kale. Zeaxanthin gives corn its yellow color. Lutein and zeaxanthin protect from UV light irradiation-induced lipid peroxidation (Palombo et al., 2007) inhibit tumor growth in mouse mammary tumor cells (Chew et al., 2003) and reduce oxidative stress-induced apoptosis in rat photoreceptor cells (Chucair et al., 2007).

Lutein is found in humans and may have protective effects against disease. Lutein and its structural isomer, zeaxanthin, have been identified as the pigments in the macula densa of the retina (Bone et al., 1985, Bone et al., 1993). The carotenoid lutein (3,3′-dihydroxy-b,e-carotene) (Figure 1.2) is widely present in many green leafy vegetables, fruits and flowers, (Park et al., 1998) and is commercially prepared from the marigold flower (Tagetes erecta L) (Figure 1.3) in which it occurs at 1.5–1.8%.

**Figure 1.2 Structure of lutein**

![Structure of Lutein](image)

Lutein and zeaxanthin cannot be synthesized by humans and must be obtained through diet. Foods that are rich in lutein and zeaxanthin include egg yolk, corn, orange juice, honeydew melon, and orange pepper (Sommerburg et al., 1998) and dark green leafy vegetables such as kale, spinach, collards, turnip greens, and broccoli (Holden et al., 1998).
Figure 1.3 *Tagetes erecta* L (Marigold flower)
Lutein, zeaxanthin and mesozeaxanthin are the only macular pigments and due to their extended conjugated structure have been shown to have significant antioxidant potential and a protective effect against the oxidative damage to macula induced by singlet oxygen produced by ultraviolet light. In the eye, lutein appears mainly in the photoreceptor axon layer; it has also been found in the rod outer segments and retinal pigment epithelium of the retina (Landrum & Bone, 2001, Snodderly et al., 1984) where it may act as a blue light filter to protect underlying structures from phototoxic damage (Snodderly, 1995). Lutein, zeaxanthin, and meso-zeaxanthin represent about 36%, 18%, and 18% of the total carotenoid content of the retina (Landrum & Bone, 2001). Crystalline lutein is readily absorbed from foods and from dietary supplements whereas, to enter the blood stream, lutein esters require prior de-esterification by intestinal enzymes. Lutein can be obtained from the diet in several different ways, including via supplements, and most recently in functional foods. Animal toxicology studies have been performed to established lutein’s safety as a nutrient (Hari et al., 2008). The recommended daily intake of lutein has been reported to be 6 mg, which has to be derived from either food or supplements (Chung H et al., 2004) Intake of foods rich in lutein and zeaxanthin is related to an increased level of carotenoids in the serum as well as in the macula. Lutein is one of the ten phytochemicals identified by the US Food and Drug Adminstration as GRAS (generally regarded as safe) for nutritional supplements.

Historical back ground of lutein is started in 1782. A yellow pigment was described in the macula in 1782 by the Milanese ophthalmologist Francesco Buzzi (1751–1805). Over a decade later, similar observations were made by Everard Home (1756-1832). After the development of the ophthalmoscope in the mid-nineteenth century, there were inconsistent observations of yellow pigmentation in the macula, and this variability was probably related to the wavelength of light that was used in the ophthalmoscope, as the yellow color was more readily visible with the use of red-free light. In 1869, Johann Ludwig Wilhelm Thudichum (1829–1901), a chemist at St. Thomas’s Hospital in London, found that parts of plants and animals contain a yellow crystallizable substance, which he named ‘luteine’ (Thudichum, 1869).

It was initially believed that lutein esters were less bioavailable than the free form because of their characteristics (Bowen et al., 2002). Lutein having hydroxyl group (s) that makes them significantly more hydrophilic than othercarotenes. Because
of this hydrophilic property, a substantial proportion of free lutein is expected to be situated on the surface of dietary emulsion lipid droplets in the intestinal lumen. On the other hand, hydrophobic carotenoids such as β-carotene and lycopene are more likely to be situated in the hydrophobic core of the lipid droplets (Borel et al., 1996). In relation to β-carotene, lutein is more readily absorbed possibly due to the relative ease of transfer from dietary emulsion lipid droplets to micelles (Tyssandier et al., 2001). To date, no reliable data exist on the hydrophobicity of lutein esters, but lutein esters contain fatty acids, mainly palmitate, esterified to the hydroxyl groups. These large hydrophobic molecules are more likely to be situated in the core of lipid droplets. Furthermore, lutein esters must go through hydrolysis by lipases in the intestinal lumen or in the enterocytes before absorption. In the human bloodstream, high-density lipoprotein (HDL) is the major carrier of lutein and zeaxanthin because of high polarity of these carotenoids than others, while carotenes are preferentially carried by low density lipoprotein (LDL) (Clevidence & Bieri 1993).

Lutein absorbs visible light and plays a role in singlet–singlet energy transfer and the quenching of singlet oxygen (Krinsky & Rock, 1999). The absorption of light energy produces a transition $\pi \rightarrow \pi^*$ in which one of bonding $\pi$-electrons of the polyene chain is promoted to a previously unoccupied $\pi^*$ antibonding orbital. The $\pi$-electrons are delocalized over the polyene chain, and the energy that is needed to produce the transition of $\pi \rightarrow \pi^*$ is small and corresponds to light in the visible spectrum of 400–500nm (Britton, 1995). Carotenoids have two low-lying electronic excited singlet states (Frank, 1993). The strong absorption of light in the visible region has been attributed to the transition from the ground state $S_0$ to the second singlet excited state $S_2$. Carotenoids can also accept excitation energy from highly reactive singlet oxygen, $^1O_2$, and this property of carotenoids may protect against damage caused by a combination of light and oxygen (Foote & Denny, 1968, Stahl & Sies, 1993). Singlet oxygen is highly reactive and can damage DNA and lipids. The reaction with singlet oxygen generates a triplet excited carotenoid:

$$^1O_2 + \text{carotenoid} \rightarrow ^3O_2 \rightarrow ^3\text{carotenoid}.$$  

The triplet excited carotenoid then dissipates the energy harmlessly through rotational and vibrational interactionsto recover the ground state:

$$^3\text{carotenoid} \rightarrow \text{carotenoid} + \text{thermal energy}$$
Lutein was protected from secondary oxidative breakdown in the presence of melanin, glutathione, \( \alpha \)-tocopherol, and ascorbate due to the antioxidant activity (Semba & Dagnelie, 2003).

The biological activities of lutein are well established. Lutein could significantly increase the immune responses in animals (Dietary lutein stimulates immune response in the canine, Kima et al., 2000). Lutein has been shown to inhibit mammary tumor growth in mice (Chew et al., 1996, Park et al., 1998) and prevent lipid peroxidation (Stahl et al., 1998). Lutein is also known to possess immune regulatory activity. Mice fed lutein had increased phytohemagglutinin (PHA) stimulated lymphocyte proliferation response (Chew et al., 1996) and enhanced antibody production in response to a T cell dependent antigen (Jyonouchi et al., 1994). Lutein has protective effect on retinal ischemia and the inhibitory effect of nNOS and COX-2 expressions and this result suggests that a supplement with lutein may have the potential to inhibit ischemic cell death by this mechanism in the neural retina (Choi et al., 2006). Recent studies suggest that lutein and zeaxanthin, may help maintain heart health by reducing the risk of atherosclerosis (Dwyer et al., 2001; Kritchevsky et al., 2000; Mares-Perlman et al., 2002). Ames test showed that purified lutein does not induce mutagenicity in \textit{S. typhimurium} strains using both the plate incorporation and the preincubation assay. These recent findings are consistent with a number of previous studies demonstrating the absence of any mutagenic effect of lutein using the Ames test in \textit{S. typhimurium} strains (Gonzalez de Mejia et al., 1997; Okai et al., 1996; Rauscher et al., 1998). \textit{In vivo} studies examining the effect of dietary lutein on DNA have revealed that lutein does not result in oxidative damage. Supplementation of human subjects with 15 mg lutein per day for 12 weeks did not increase oxidative damage to lymphocyte DNA (Collins et al., 1998).

1.9. Scope of the present study

In humans, as in plants, oxycarotenoid lutein is believed to function in two important ways: first as a filter of high energy blue light, and second as an antioxidant that quenches and scavenges photo induced reactive oxygen species (ROS). Evidence suggests that lutein consumption is inversely related to eye diseases such as age-related macular degeneration (AMD) and cataract. As lutein is an eye protective compound most of the studies on lutein were based on vision as well as its protective actions on macular degeneration. \textit{In vitro}, animal and human intervention studies continue to
suggest that lutein may protect against a number of chronic disease states. Lutein is non-toxic and is considered as GRAS and these properties leads us to search the anticancer potential as well as other pharmacological activities of lutein. Lutein is safe for use in both food and dietary supplement applications.

The link between free radicals and disease process has lead to considerable research with the aim to discover the nontoxic drugs that can scavenge the free radicals and thereby halt the causation and progression of the diseases. There is an urgent need for developing an effective chemo preventive dietary compound to reduce the incidence of cancer. Presently carotenoid lutein was evaluated for its antioxidant potential both in vitro and in vivo. Ames test used to evaluate the antimutagenic property of lutein using Salmonella typhimurium strains TA 98, TA 100, TA 102 and TA 1535. The mechanism of anticancer as well as antitumor activity was evaluated by the inhibition of cytochrome P450 enzymes both in vitro and in vivo. Chemoprotective and radioprotective activity of lutein was evaluated to confirm the chemopreventive ability of lutein. Nephroprotective activity of lutein was done for determine the nephroprotective activity of lutein against a known chemotherapeutic agent cisplatin caused renal toxicity in mice. The effect of lutein to induce phase 2 detoxifying enzymes was also studied. Apart from these properties lutein was evaluated for its other pharmacological actions such as gastroprotective and hepatoprotective activity.