CHAPTER 2

Review of Literature
1. CARCINOGENESIS

The term ‘Cancer’ is derived from ‘cancrum’, a Greek word meaning crab. In this disease, some cells divide in an uncontrolled manner causing local tissue invasion and/or systemic metastasis. Carcinogenesis is however a multi step process that leads to the formation of cancer. It is divided into three sequential stages namely: initiation, promotion and progression.

*Initiation*: It is a stage where genetic alteration or mutation occurs in the genes of the somatic cells that are responsible for controlling differentiation. Thus the initiated cell acquires growth advantage over other normal cells of the body. This step is irreversible and no morphological changes are apparent (Boutwell 1964).

*Promotion*: In this stage, epigenetic alterations in the already initiated cells occur that undergo clonal expansion to form benign lesions. It is a slower and a partially reversible stage but can be enhanced by tumor promoting agents to form visible skin lesions like papillomas (Reiners and Slaga 1983). The papillomas are benign tumors and often regress but occasionally might also get transformed into squamous cell carcinoma especially in the presence of environmental factors (Campo 1997).

*Progression*: The conversion of the benign papillomas to malignant carcinomas occurs during progression that requires additional insult to the genetic material. The dose of the initiating agent affects the yield of the carcinomas, the more the dose the higher the yield of carcinoma. The cells acquire the tendency to grow faster with time as the operation of the switch-off mechanism for growth control becomes poor. The malignancy has inverse relation with the organelles and the metabolic functions of the specialized cells (DiGiovanni 1992).
1. Carcinogenesis by chemical agents

About 70 years back, originated the multistage model of mouse skin carcinogenesis when tumors were observed in mice that had been treated with tar followed by wounding (Miller 1970). It was shown that neoplastic changes could be produced in rabbit skin with single treatment of 3-methylcholanthrene (MCA), followed by treatments with agents (turpentine, chloroform and wounding) that per se did not cause initiation or cancer (Friedewald and Rous 1944). The two-stage protocol of mouse skin tumorigenesis was developed after the discovery of croton oil and this protocol is used even today for the study of experimental carcinogenesis and also for the chemoprevention.

Tumor initiation by chemicals

Initiation stage is considered to be irreversible as it can be maintained for weeks together (Berenblum and Shubik 1949) due to alterations in the genetic material of the normal cells.
The reactive form of carcinogen interacts with the DNA of the target cell (Slaga 1989) to cause initiation that persists for a lifetime of the animal (Van Duuren et al 1975). Initiation can occur by a single topical application of a sub-carcinogenic dose of a carcinogen like 7, 12-dimethylbenz (a) anthracene (DMBA), a polycyclic aromatic hydrocarbon (PAH) that later causes multiple papillomas and carcinomas in the mouse skin. Besides topical applications, initiation in mice skin can be successfully accomplished by administration through intragastric and intra-peritoneal modes (Ritchie and Saffiotti 1955, Hennings et al 1981). In this stage, apparent morphological changes do not occur in the epidermis (Boutwell 1976). Visible tumors result only with prolonged and repeated topical applications of a tumor promoter to the initiated skin (Boutwell 1974). Chemicals that can cause tumor initiation in mouse skin include: PAHs like 7, 12-dimethylbenz (a) anthracene, benzo (a) pyrene, methylcholanthrene; carbamates like urethane; haloaromatics like 2,3,4,5-trichloronitrobenzene; nitrosamides like N-methyl-N'-nitro-N-nitrosoguanidine, ureas like N-methylnitrosourea and 2-acetylaminofluorene (Miller 1970). Chemical initiators can be classified into two categories- direct acting and indirect acting. The activation of direct acting carcinogens is non-enzymatic and occurs by spontaneous hydrolysis, therefore, they interact with the DNA without being metabolised while indirect acting carcinogens interact with the DNA after being metabolised. Directly acting carcinogens include epoxides and alkylating agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl nitrosourea (MNU) (Lawley 1978). Most of the carcinogens are indirect acting and act as initiators only after their metabolic activation. Chemical carcinogens are metabolized by a variety of cytosolic and microsomal enzymes. Cytochrome P-450 dependent monooxygenase enzymes are basically involved in the oxidative metabolism of the
carcinogen causing activation of pro-carcinogens into carcinogens, which have the ability to attack the macromolecules like DNA, RNA and proteins. The metabolically active form of the carcinogen is called as the ultimate carcinogen while its immediate metabolic precursor is called as the proximate carcinogen. The polycyclic aromatic hydrocarbons (PAHs) are indirectly acting carcinogens (Miller 1978, Miller 1970, Cooper and Grover 1990) that are metabolized or oxidized by the cytochrome P-450 dependent enzyme system to epoxides and dihydrodiols. AHH is a membrane bound cytochrome P450 dependent mixed function oxidase system commonly involved in the oxidation of PAHs. Some of the primary metabolites (epoxides and dihydrodiols) get detoxified by being converted into hydrophilic conjugates like glutathione, glucuronide and sulfate conjugates (Gelboin 1980) and get excreted. On the other hand, some of the products that are not detoxified might get reoxidized or recycled (Pelkonen and Nebert 1982) through this pathway and pose threat to cellular macromolecules. Examples of two extensively studied PAHs are - benzo (a) pyrene and 7,12-dimethylbenz (a) anthracene. B(a)P is a naturally occurring PAH that is present in coal tar (Yuspa et al 1976). It is believed that the PAHs are activated in a way similar to that of B(a)P, the activation of which involves a two-step oxidation mechanism to form the ultimate carcinogen, 7,8-dihydro diol- 9,10-epoxide. This epoxide binds covalently to different positions of DNA bases especially with the deoxyguanosine to form adducts. DMBA, on the other hand, is a synthetic PAH that is used in various studies for skin tumor initiation. Its reactive intermediate or the ultimate carcinogen, 3,4-diol-1,2-epoxide binds to the epidermal DNA mainly at the deoxyadenosine as opposed to deoxyguanosine in case of B(a)P (Dipple et al 1984, DiGiovanni et al 1986). It is believed that different tumor initiators produce different point mutations in the epidermal cells (Balmain and Brown
DMBA is known to cause A→T transversion mutations in the 61\textsuperscript{st} codon of the c-Ha\textit{ras} gene while MNNG and MNU cause G→A transition mutations in the 12\textsuperscript{th} codon of the c-Ha\textit{ras} gene (Bonham et al 1989, Brown et al 1990).

**Tumor promotion by chemicals**

Promotion is the most extensively studied stage of carcinogenesis. Promoters produce cellular changes that are transient and reversible (Yuspa et al 1976). Agents that promote the formation of tumors by the selection and clonal expansion of the previously initiated cells are called tumor promoters. They produce multiple squamous cell papillomas each representing a clone of thousands of initiated cells. Promotion may occur by one or more of the following mechanisms (Boutwell 1974, Imamoto et al 1991, Reiners and Slaga 1983, Slaga 1989):

i) A stimulatory effect of a promoter on initiated cells that leads to their division and expansion in number, and may occur by induction of TGF α, a potent mitogen

ii) Alteration of terminal differentiation

iii) Selective cytotoxicity of the normal cells as against the initiated cells provides the initiated cells the ability of selective growth as exhibited by some anthrones and organic peroxides

iv) Defects in programmed cell death

One common feature of the tumor promoting agents is the ability to induce irritation, inflammation, hyperplasia and cell proliferation (Yuspa et al 1976).

Tumor promotion is divided into two stages. Stage I (conversion stage) causes the following changes: induction of dark basal keratinocytes, synthesis of prostaglandins and activation of some growth factors like TGFα. (Klein-Szanto et al 1982, Slaga et al 1982). The effects of
this stage last for nearly 5-8 weeks. Stage II (propagation stage) involves induction of ODC activity, PKC activity, elevation in polyamines, enhanced DNA synthesis and chronic cellular proliferation. In contrast to Stage I, it is reversible immediately (Slaga 1983, Slaga et al 1982). TPA is a complete tumor promoter while mezerein is a good stage II promoter (Boutwell 1974).

A wide variety of compounds like teleocidin, lyngbyatoxin, palytoxin, thapsigargin, okadaic acid, anthralin, hydrocarbons and benzoyl peroxide (DiGiovanni 1992) have also been shown to possess promoting abilities. Most of the skin tumor promoters do not require metabolic activation. Only some promoters like anthrones, organic peroxides, and hydrocarbons require metabolic activation for their promoting activity.

Mode of action of TPA

TPA is the main component of croton oil (isolated from *Croton tiglium*) and accounts for its tumor promoting ability as well (Boutwell 1976). TPA seems to act via an epigenetic mechanism, which involves the genome secondarily (Kinzel et al 1986). It has been shown to cause inflammation, irritation, leukocytic infiltration, increase in the percentage of the dark cells in the interfollicular epidermis within a few hours of topical application (Klein-Szanto and Slaga 1981) and keratinization of the upper layers of the epidermis owing to mitotic activity in the basal cell layer (Balmain 1976).

Other alterations include: induction of ornithine decarboxylase, tritiated thymidine incorporation into epidermal DNA, increase in the rates of protein synthesis (Baird et al 1971, Hennings and Boutwell 1970) and accumulation of prostaglandins (Verma et al 1980, DiGiovanni 1992). Its main mechanism of action lies in its ability to bind at the same site where DAG binds to PKC and activate it (PKC) leading to enhanced DNA synthesis.
(Castagna et al 1982). It is believed that TPA acts indirectly through ROS generation released by the activated phagocytic cells infiltrating the skin which produces chromosomal aberration (Dzarlieva and Fusenig 1982), sister chromatid exchange (Kinsella and Radman 1978, Nagasawa and Little 1981), DNA strand breakage (Birnboim 1982) and gene amplification (Weinstein 1987). It also causes a decrease in the activities of glutathione reductase, glutathione-S-transferase, glutathione peroxidase, superoxide dismutase and catalase (Solanki et al 1981) while it increases the activity of xanthine oxidase, peroxidation of lipids and generation of hydrogen peroxide in the skin tissue (Saleem et al 2001, Reiners et al 1991).

![Diagram of TPA signaling](image_url)

**Fig.2** Depicting role of TPA in producing unscheduled signals manifesting tumor promotion
Mode of action of BPO

BPO can penetrate inside the membrane bilayer, as it is highly lipophilic in nature. It has been postulated to act via free radical generating mechanism. BPO produces benzoyloxy radicals in the vicinity of membrane associated unsaturated lipids that may cause lipid peroxidation. It also produces phenyl radicals subsequently (Kennedy et al 1995).

It is metabolically activated as given below:

\[
\text{Cu}^{+}/\text{Fe}^{2+} + (\text{BzlCO})\text{OO}(\text{OCBzl}) + \text{H}^+ \rightarrow \text{Cu}^{2+}/\text{Fe}^{3+} + \text{BzlCOO}^- + \text{BzlCOOH}
\]

\[
\text{BzlCOO}^- \rightarrow \text{Phenyl}^- + \text{CO}_2
\]

These radicals induce extensive DNA strand breaks and cytotoxic effects in normal epidermal cells. Thus, they indirectly cause clonal expansion of the initiated epidermal cells that are resistant to BPO induced cytotoxicity (Perchellet and Perchellet 1989). They also lead to oxidative DNA damage in double stranded DNA forming 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) leading to G → T transversion mutation (Kawanishi et al 1999).

Tumor progression by chemicals

Tumor progression is the least studied and the least understood stage of carcinogenesis. However, it is believed to be irreversible and both genetic and epigenetic alterations take place in this stage that lead to the conversion of benign papillomas into malignant carcinomas. Most of the papillomas have a tendency to regress and only about 5-7% progress into carcinoma (Verma and Boutwell 1980) and thus papillomas are regarded to be heterogeneous in nature. It has been reported that termination of TPA treatment leads to 80% regression of the papillomas produced in mice initiated with DMBA (Reddy et al 1987). However, once progression begins, the neoplasms acquire the tendency to grow faster with time as the growth control mechanism becomes weak. Progression depends on the dose.
of the tumor initiator. If the dose of the initiator is high then the yield of the carcinoma will be more. Free radical generating compounds can act as agents capable of tumor progression. BPO, a free radical generating compound is also known to enhance the conversion of papillomas to carcinomas (Athar et al 1989).

1.2 Physical carcinogenesis

Exposure to sunlight, a physical carcinogen, is unavoidable and has been linked with skin tumor development inspite of body’s numerous protective mechanisms including melanin production. Melanin helps in photoprotection by absorbing/scattering ultraviolet rays, by electron transfer or free radical scavenging.

Skin cancers, however arise in those parts of the body, which are mostly exposed to the Sun and are commonly seen in the light-skinned people as opposed to the darker people. Besides, the evidence obtained from the experimental animals also suggests the role of a part of sunlight in skin cancer (Urbach 1991). Ultraviolet radiations of the sunlight are considered to be the major epidemiological risk factor for skin cancer development. They are classified into three regions: UVA (320-400nm), UVB (280-320nm) and UVC (200-280nm). There are three factors that really matter in producing the photocarcinogenic response following the exposure to the radiations: the wavelength of the radiation, dose and the duration of exposure (Setlow 1974, Black et al 1997). The ultraviolet radiations exert their action by inducing mutations, stimulating cell proliferation, generating ROS in the cells and suppressing the immune response (Black et al 1997, Ichihashi et al 2003). UVB and UVC mainly induce DNA damage directly (Freemen et al 1987, Keyse and Tyrrell 1987) while UVA causes photosensitization reactions to generate ROS (Tyrrell and Keyse 1990) that react with target molecules and cause damage.
UVA: Radiations of the UVA region of the sunlight are not absorbed by the DNA (Black et al 1997). Nevertheless, the DNA is damaged by these radiations due to their absorption by molecules other than DNA like lipids in cell membranes (Black et al 1997) to form radicals that can react with DNA (Tyrrell and Keyse 1990) causing oxidative DNA damage (Beehler et al 1992) and lipid peroxidation reactions. Therefore, UVA causes indirect damage such as DNA strand breaks and formation of 8-hydroxy-2-deoxyguanosine lesions (Audic and Giacomoni 1993) that produce G → T transversions. Recently, some studies have shown that UVA can induce CPD but its main mechanism of action remains via the generation of ROS (Tyrrell 1991). Chronic exposure to UVA causes ferritin to release ferrous ions, which probably initiates the Haber/Weiss reaction and Fenton-reaction leading the cell in a state of oxidative stress (de Gruijl 2000). It can augment the biological effects of UVB. Intracellular signaling pathways are also triggered, which in turn lead to gene activation (Simon et al 1994, Caceres-Dittmar et al 1995). The effects of UVA exposure on cells resemble in many ways the effects of 12-O-tetradecanoylphorbol-13-acetate, a classical tumor promoter. Thus, UVA can aid in the development of skin carcinogenesis through epigenetic effects. It has been shown to induce squamous cell carcinomas without the characteristic point mutations in p53 gene (de Gruijl 2000).

UVB: it is responsible for grave consequences in human beings (Urbach 1991). It is a major causative factor in the development of skin cancer. It is easily absorbed by the DNA and causes covalent binding of adjacent pyrimidines of the same polynucleotide chain to form cyclobutane pyrimidine dimers. In addition to CPD, 6-4 pyrimidone photoproducts (Setlow 1966, Cleaver et al 1984, Reid and Loeb 1993) are also formed. The (6-4) adducts of TC, CC and TT sequences have been observed depending on the incident UVR of specific
ranges. The frequency of occurrence of TC lesions is more as compared to CC and TT lesions, which occur only at very high doses of UVR (Black et al 1997). These products are mutagenic if not repaired (Black et al 1997). The biological consequences of cyclobutane pyrimidine dimers have been reviewed by Vink and Roza (2001). In 58% of human squamous cell carcinomas, C → T and CC → TT transitions in the p53 gene have been shown (Black et al 1997). Other investigators have also reported p53 mutations in human squamous cell carcinomas and basal cell carcinomas (Rady et al 1992, Pierceall et al 1991). C → T transition mutations serve as molecular fingerprints for DNA damaged by UVB (Grossman and Leffell 1997). In the patients of xeroderma pigmentosum, who are deficient in the enzyme responsible for the repair of pyrimidine photoproducts, CC → TT transition mutations are normally detected (Cleaver et al 1984). UVB exposure has been shown to damage DNA in epidermal keratinocytes and suppress immune system. The DNA-damaged epidermal keratinocytes produce cytokines (Black et al 1997, Nishigori et al 1996) such as cis-urolonic acid, TNFα and interleukin (IL) - 10. These cytokines affect cutaneous antigen-presenting cells and interfere with the induction of cell-mediated immune responses elicited at distant sites and contribute to the activation of antigen specific suppressor T cells (Boonstra and Savelkoul 1997, Schwarz 1999), leukocyte infiltration and production of leukocyte derived oxidants (Liebler and Burr 2000). In addition to these changes, UVB radiations increase the production of factors that can support tumor growth (Morliere et al 1997), up-regulate a large number of growth factors in different cell populations like in keratinocytes (Prehn 1994), induce cell signaling pathways, activate nuclear factor κB (NFκB) and activator protein 1 (AP-1) transcription factor responsible for tumor promotion.
(Oro et al 1997, Barthelman et al 1998). The DNA-damage caused by UVB radiation also leads to apoptosis in keratinocytes (sunburn cell formation).

**UVC**: In the atmosphere, molecular oxygen and ozone absorb UVC. In human skin, it is absorbed in the overlying epidermis. It results in DNA damage and mutations. UVC causes increase in the activity of tyrosine kinase of Src family causing Ha-ras activation and increase in the phosphorylation of Raf-1, which further activates c-Jun and other AP-1 proteins. ROS is supposed to be implicated in this signaling cascade as the antioxidant, N-acetylcysteine (NAC) suppresses the UVC induced activation of mitogen activated protein kinases (MAPK) that leads to AP-1 and NFkB activation (Devary et al 1992). However, the biological responses induced by UVC are thought to be oxygen independent (Black et al 1997).

**Effects of UV radiations on free radical formation and cellular antioxidant depletion**

UV radiations can directly destroy the skin’s antioxidant defense system by impairing the non-enzymatic and enzymatic antioxidants. It adversely affects the non-enzymatic cellular anti-oxidants like ascorbate and glutathione in skin (Taira et al 1992, Tyrrell 1996). Besides, in cultured keratinocytes UVR depletes the antioxidant enzymes (Taira et al 1992, Shindo et al 1993). Many studies have shown a decreased superoxide dismutase activity after exposure to UVA, UVB or their combination (Hasegawa et al 1992, Shindo et al 1994). The activity of catalase has also been reported to be decreased strongly following exposure to UVA or UVB (Hasegawa et al 1992, Punnonen et al 1991, Fuchs et al 1989) or a combined exposure to both UVA and UVB (Shindo et al 1994). Although reactive species such as H$_2$O$_2$ are generated in vivo owing to metabolic processes and via the action of oxidizing agents their rate of generation becomes extremely high after UV exposure. Such an effect is considered
to be due to the damage of the anti-oxidant enzymes (Pigeolet et al 1990). Reactive oxygen species (ROS) generated on exposure to UV radiations include: singlet oxygen, peroxyl radicals, superoxide anion and hydroxyl radicals that adversely affect the DNA and other cellular targets (Afaq and Mukhtar 2001, Black 1987).

1.3 Carcinogenesis by biological agents

The biological agents that cause carcinogenesis include viruses and are considered as oncogenic. Proto-oncogenes, which have normal functions in the cellular pathways of growth control, are the cellular counterparts of oncogenes that are present in the viruses. The amplification, translocation or inversion in these proto-oncogenes leads to the expression of oncogenes. A number of DNA and RNA viruses are capable of causing tumors in various species.

During the past 20 years, several types of human papillomaviruses (HPVs) have been identified that cause specific types of cancers (zur Hausen 2000). These viruses consist of a heterogenic group of viruses (32 different HPV types are known) (Syrjanen 1986) and consist of a family of double stranded DNA viruses (Fazel et al 1999). Human papilloma viruses have been associated with the etiology of cervical cancer. Viral E6 and E7 genes are believed to code for major oncoproteins of these cancer-associated viruses (Scheffner and Whitaker 2003). The E6 and E7 products interfere with the functions of p53 and pRB, respectively and deregulate the cell cycle. The HPV DNA is integrated into the host's chromosomes by disrupting the E2 gene. This disruption promotes the expression of E6 and E7, leading to the accumulation of DNA damage and the development of cancer (Furumoto and Irahara 2002). HPV DNA has been detected in many bronchial carcinomas and is also implicated in other squamous cell cancers (Syrjanen 2002). HPV oncoprotein causes
chromosomal instability and increases the risk for genetic changes that may ultimately facilitate carcinogenic progression (Duensing and Munger 2002). In addition to cervical and bronchial cancers, a major proportion of anal, perianal and vulvar cancers are thought to have a link with HPV infections. They are also known to induce a variety of squamous cell tumors in the skin and on mucous membranes of respiratory, gastrointestinal and genitourinary tracts (Syrijanen 1986). Modifications in host-cell genes that are most likely engaged in the control of HPV gene expression in proliferating cells seem to be an important event in HPV-mediated carcinogenesis (zur Hausen 2000). Human immunodeficiency virus (HIV) is known to cause Kaposi's sarcoma (Trichopoulos et al 1996). Chronic hepatitis B virus infections are a major risk factor for hepatocarcinogenesis. Ryu (2003) has reviewed molecular aspects of the viral infection and highlighted the findings on the viral contribution to hepatocarcinogenesis.

**Oncogenes, tumor suppressor genes and multistage carcinogenesis**

Oncogenes and tumor suppressor genes play an important role in controlling the cell cycle, cell proliferation and differentiation. Mutations such as deletion, insertion, truncation or point mutation in the genes can lead to the formation of cancer via over expression of oncogenes and under expression or suppression of tumor suppressor genes. Oncogenes activate the transcription of genes involved in cell proliferation, cell survival and angiogenesis. Examples include: myc, fos, jun and ras. It has been shown that in squamous cell papillomas c-Ha ras is invariably mutated. A cause-effect relationship was confirmed when the activated v-Ha ras oncogene was introduced in normal epidermis or cultured
keratinocytes. The activated c-Ha ras gene acts via PKC pathway (Yuspa et al 1994) and mutations in these Ha ras genes ultimately affects the growth mechanisms.

The tumor suppressor gene, p53 protects against DNA damage primarily by inducing arrest in the cell cycle or apoptosis (Finlay et al 1989) in order to repair DNA. This arrest in cell cycle is brought about by activation of p21, which subsequently causes G1/S phase arrest in the cell cycle (Prives and Hall 1999, Deng et al 1995). p53 gene also regulates a number of genes that are involved in the induction of apoptosis for example, bax, Bcl-2, insulin-like growth factor binding protein (IGF-BP) (Cadwell and Zambetti 1998).

2. TYPES OF SKIN CANCER

Depending on the types of cells in which they arise, skin cancers are divided into three types namely; squamous cell carcinoma, basal cell carcinoma and melanoma. Nonmelanoma skin cancers (NMSC) comprise of squamous cell and basal cell carcinomas that arise in the squamous cells and the basal cells respectively of the epidermis. On the other hand, the malignant melanomas arise in the melanocytes.

2.1 Squamous Cell Carcinoma: Squamous cell carcinoma (SCC) is the second most common skin cancer. They can be distributed on all the areas of the body but are common in sun-exposed areas like face, neck, hands and arms. Almost hundred percent of all SCC occur in sun exposed areas of the body. They mainly remain in the epidermis for some time but penetrate the underlying tissues if left untreated. The incidence of local spreading and metastasis is 13-14% and some SCC may metastasize within 5-10 years of origin (McDonald 1993). However, if detected timely they can be cured completely (Urbach 1991). The increase in SCC is more as compared to that in BCC in case of immunosuppression.
Premalignant lesions that may convert to malignant SCC are actinic keratosis, Bowen's disease, keratoacanthomas (Kwa et al 1992). SCC initially appears as sharply defined, red, scaling plaques. The more advanced, invasive lesions are nodular, exhibit variable differentiation ranging from tumors formed by polygonal squamous cells arranged in orderly lobules that exhibit numerous large zones of keratinization, to neoplasms formed by highly anaplastic, rounded cells with necrotic foci.

2.2 Basal Cell Carcinoma: Among skin cancers, it is the most common form. These cancers arise in the basal cells, which lie at the bottom of the epidermis. Chronic exposure to sunlight is the cause of most basal cell carcinomas, which occur frequently on exposed parts of the body and rarely on the non-exposed parts. Sixty percent of all BCC occur in sun-exposed areas of the body (McDonald 1993). Factors contributing to BCC may include contact with chemicals like arsenic, physical trauma like burns or use of tattoos. This type of cancer does not spread or are not invasive for the first 5-10 years of their growth cycle (Urbach 1991, McDonald 1993). Once invasive, BCCs become aggressive and locally destructive. Their incidence of metastasis is 0.0028-0.1% (McDonald 1993). Histologically, BCC has large nuclei and small nucleoli. The tonofilament, mitochondria and rough endoplasmic reticulum are present in variable amounts. There is increased collagen synthesis, reduced desmosomes and increased tetraploid DNA (Miller 1991). Clinically, BCC appear as pearly papules mostly containing prominent dilated sub-epidermal blood vessels (telangiectasias). Mostly, it shows two patterns of growth viz.,

i.) Multifocal growths – originating in the epidermis and extending over several square centimeters or more of skin surface
ii.) Nodular lesions -- growing downward deeply into the dermis as cords and islands of basophilic cells with hyperchromatic nuclei embedded in a mucous matrix that is normally surrounded by lymphocytes.

2.3 Melanoma: Melanoma is the most dreaded form of skin cancer. It accounts for three-fourths of all the deaths from skin cancer. The most important clinical sign of the disease is a change in the colour of a pigmented lesion. Melanomas exhibit striking variation in pigmentation and appear in shades of black, brown, red, dark blue and grey. The borders of melanomas are irregular and mostly notched. It is curable if diagnosed and treated timely but becomes fatal if it advances and metastasizes to other parts of the body (Urbach 1991).

3. OXIDATIVE STRESS

Oxidative stress occurs when the balance between the rate of generation and degradation of reactive oxygen species is disrupted and the excessive levels of free radicals overwhelm the capacity of protective natural antioxidant system in cells. Reactive oxygen species (ROS) are those species that are more reactive than the ground state molecular oxygen. Free radicals are atoms or molecules containing one or more unpaired electrons (Moslen and Smith 1992) that are capable of independent existence and are highly reactive. ROS having unpaired electrons include superoxide anion (O$_2^-$), hydroperoxyl (HO$_2^-$), hydroxyl (OH$^-$), peroxyl (RO$_2^-$) and alkoxyl (RO$^-$) radicals. These free radicals may be endogenous products of cellular metabolism or may be formed in vivo by various autooxidation reactions involving metals or easily oxidizable substrates like hemoglobin, myoglobin, thiols etc (Ames 1989). They may be produced by polymorphonuclear leukocytes. Chronic inflammation also enhances the formation of nitrogen oxides. Enzymatically ROS can be generated by xanthine oxidase, prostaglandin synthase, lipoxygenase and myeloperoxidase (Matsumoto et al 2003, Takami
et al 2000, von Kruedener et al 1995) whereas non-enzymatically it is generated by lipid peroxidation (Taffe and Kensler 1989). Exogenous sources of free radicals are: air, pollutants, pesticides, insecticides, drugs, ionizing radiations and ultraviolet light. ROS has been implicated in the causation of many diseases like atherosclerosis, ethanol mediated liver injury, asbestos mediated lung injury, cataractogenesis, Alzheimer’s disease, diabetes mellitus and rheumatoid arthritis (Floyd 1990, Fridovich 1988, Ames 1983, Ames et al 1993). The free radicals are also believed to be involved in all the three stages of carcinogenesis i.e. initiation, promotion and progression (Perchellet and Perchellet 1989).

3.1 Free radicals in tumor initiation

The macromolecules- proteins, lipids, carbohydrates and DNA present in the cells are the target of the spontaneously formed or metabolically activated electrophiles (Athar et al 1987, Ames 1989). The covalent interaction of these electrophiles with the macromolecules causes initiation of the carcinogenic process. Metal ions like iron and copper are capable of causing DNA damage by formation of reactive oxygen species that may cause lipid peroxidation, chromosomal abnormalities and sister chromatid exchange leading to mutagenesis and the formation of neoplasia (DiGiovanni 1992). Metal chelators, on the other hand, block the formation of hydroxyl radicals inhibiting DNA damage, mutations and malignant transformation induced by active oxygen species (Aruoma et al 1989). Free radicals are also involved in the metabolic activation of procarcinogens. Example, the metabolic activation of DMBA by cytochrome P450-dependent microsomal mixed function oxidases is induced in the epidermis by the reactive electrophilic derivatives (Bowden et al 1974). Free radical generators such as cumene hydroperoxide, t-butyl hydroperoxide and hydrogen peroxide have shown mutagenic effects in bacterial and mammalian cells (Sun
However, Perchellet et al (1995) have reported that free radical generators that are applied topically to mouse skin are unable to initiate carcinogenic response as they do not induce point mutations or cause DNA damage that leads to point mutations. For example, benzoyl peroxide, lauryl peroxide and hydrogen peroxide do not have initiating or complete carcinogenic activities (Gimenez-Conti et al 1991). Therefore, ROS production may indirectly contribute to initiation owing to the persistence of unreppaired DNA damage resulting in initiation of the affected cells (Perchellet and Perchellet 1989) and producing other reactive products. Malondialdehyde that is formed as a result of lipid peroxidation or PGH2 breakdown is shown to be mutagenic causing frame shift and base pair substitution mutations, and formation of adducts with deoxyguanosine, deoxyadenosine and deoxycytidine residues in bacteria (Marnett 1992). Hydrogen peroxide reacts with a metal ion in the vicinity of a DNA molecule to generate HO' (Henle and Linn 1997, Cadet et al 1999). Hydroxyl radical so produced is added to the double bond of the nucleic acid base to form a radical that interacts with molecular oxygen to form RO2'. This RO2' reacts with sugar moiety of another nucleotide to produce sugar radical that ultimately results in the cleavage of sugar causing DNA strand break (Perchellet et al 1995). Halliwell and Aruoma (1981) have shown that ROS causes modification of DNA bases, deletions, strand breaks, DNA-protein cross-links and chromosomal rearrangements.

### 3.2 Free radicals in tumor promotion

Tumor promoters select the mutation carrying initiated cells and transform them into skin tumors by clonal expansion (Pelling et al 1987). Tumor promoters interact with the membranes, stimulate and alter genetic expression and increase the rate of cell proliferation. The fact that free radicals are implicated in tumor promotion is clear from the following
observations: 1.) Tumor promoters increase the generation of free radicals, 2.) Tumor promoters decrease the degradation of free radicals, 3.) Free radical generating compounds exhibit tumor promoting activity and 4.) Various antioxidants and free radical scavengers are effective as anti-tumor promoting agents. Tumor promoters are not mutagenic and do not interact directly with DNA. Most of the tumor promoters including TPA, benzoyl peroxide and other organic peroxides are known to act via free radical generation. Promoters including TPA cause sudden and sustained decrease in cellular antioxidant defenses, including superoxide dismutase, catalase, glutathione peroxidase activities and decrease in the GSH: GSSG ratios (Perchellet et al 1986, Perchellet et al 1987, Perchellet et al 1988). They also induce xanthine oxidase activity and the formation of hydrogen peroxide in the keratinocytes (Frenkel and Chrzan 1987, Wei and Frenkel 1993). It has been shown that hydrogen peroxide can inactivate superoxide dismutase while superoxide anion radical can inactivate catalase and glutathione peroxidase (Taffe and Kensler 1989). The increased level of peroxidation and the decreased efficacy of the GSH protective system are considered to be characteristic of the tumor growth process (Capel and Thornley 1983). PKC induction is also implicated in the formation of ROS by neutrophils, leukocytes and inflammatory cells (Serhan et al 1983, Fujita et al 1984, Fujita et al 1986). Moreover, exogenous diacylglycerols stimulate $O_2^{-*}$ production (Fujita et al 1984, Rider and Niedel 1987). ROS favors the clonal expansion of initiated cells that are resistant to excessive oxidative toxicity (Cerutti and Trump 1991). In mouse skin, the free radical-generating peroxides have been shown to induce dark basal cells that is a characteristic of complete and stage I tumor promoters (Klein-Szanto and Slaga 1982).
3.3 Free radicals in tumor progression

The frequency of malignant conversion of papillomas to carcinomas is low, it can however, be increased by selective modification of gene expression in the initiated cells. Benzoyl peroxide and other free radical generating compounds or the tumor initiators like ethylnitrosourea and MNNG enhance such conversion by eliciting DNA damage (O'Connell 1986). BPO is a tumor promoter in mouse skin but it is neither a tumor initiator nor a complete carcinogen (Slaga et al 1981). Cu (II) - (3,5-diisopropylsalicylate) an SOD mimicking agent, inhibits benzoyl peroxide induced tumor promotion and progression in mouse skin, which suggests a role of ROS in both the stages (Duran et al 1993). Unlike TPA (promoter), BPO produces effects like DMBA (initiator) such as single strand breaks in DNA, induction of high carcinoma-papilloma ratio and resistance to inhibition by retinoic acid (Hartley et al 1987). Moreover, BPO and H₂O₂ (free radical generators) are more potent than TPA in stimulating the malignant progression of papillomas to carcinomas (Rotstein et al 1986, O’Connell et al 1986). Mechanism of tumor promotion by BPO has already been discussed earlier which is extensive DNA strand breaks and cytotoxic effects in normal epidermal cells indirectly favouring the clonal expansion of the initiated epidermal cells that are resistant to BPO induced genetic lesions and cytotoxicity (Perchellet and Perchellet 1989). The fact that in two-stage carcinogenesis protocol the yield of carcinoma: papilloma is less as compared to that in complete carcinogenesis, emphasizes that ROS are involved in progression stage of carcinogenesis.

4. ANTIOXIDANT DEFENSE

Antioxidants are molecules that hinder or reduce the process of oxidation. Thus, they prevent the uncontrolled formation of free radicals and activated oxygen species, scavenge them or
inhibit their reaction with biological molecules like the proteins, lipids, sugar or the interaction with RNA and DNA. Antioxidants can also alter the enzymatic activities involved in metabolic activation, degradation and detoxification of the PAHs and/or scavenge free radical species directly or through the enhancement of the host defense systems thereby preventing the carcinogenic process. A large number of naturally occurring antioxidant compounds have been identified that have other protective activities and have been used against cancer chemoprevention (Saito et al 1987). These antioxidants can be either non-enzymatic or enzymatic.

4.1 Types of Antioxidant defense

**Non-enzymatic scavengers**

Antioxidant scavengers trap the free radicals and activated oxygen species or quench the excited species like singlet oxygen. These can be hydrophilic or hydrophobic in nature. Hydrophilic antioxidants are normally found in the aqueous compartments of the cytosol, mitochondria and nucleus while the hydrophobic antioxidants are found in lipoproteins and membranes. The water-soluble non-enzymatic defense includes: γ-glutamylcysteinylglycine (reduced glutathione), ascorbic acid (Vitamin C) and NADPH. GSH is a tripeptide that is most prevalent intracellular thiol present in tissues including skin. It protects against oxidative damage by donating a hydrogen atom from the protonated sulfhydryl (SH) group thus quenching the radical species. In this process much less reactive thiols and oxidized glutathione are produced (Bendich et al 1986, Ross et al 1987). Ascorbic acid acts by donating one electron to the free radicals. The lipid-soluble non-enzymatic defense includes: α-tocopherol, carotenoids (lycopene and β-carotene) and ubiquinol (reduced form of coenzyme Q) (Gerard-Monnier and Chaudiéré 1996, Chaudiéré and Ferrari-Iliou 1999).
Since at the membrane level the concentration of reduced glutathione is less, therefore, the lipid soluble free radical scavenger, α-tocopherol plays an important role in preventing oxidative processes. It acts by scavenging peroxyl radical through hydrogen atom transfer (Burton and Ingold 1981). Carotenoids quench singlet oxygen and oxidizing free radicals (Palozza and Krinsky 1992). Ubiquinol is 1,4-hydroquinone and its monodeprotonated form can act as one electron donor and is also involved in recycling of α-tocopherol from αTO· (Kagan et al 1994).

Certain metal binding proteins such as ferritin, lactoferrin, albumin and ceruloplasmin help in sequestering iron and copper metal ions. Other non-enzymatic antioxidants include metal ion chelators and phytonutrients.

**Enzymatic defense**

Enzymatic antioxidant defense comes into play to cope with reducing radicals such as superoxide radicals or with hydrogen peroxide that ultimately produce peroxynitrites, hydroxyl or peroxyl radicals. These species are taken care of by enzymes like superoxide dismutase, glutathione peroxidase and catalase.

**Superoxide dismutase (SOD):** was first discovered by McCord and Fridovich in 1969. It catalyzes the reduction of superoxide anion to H₂O₂, which is comparatively less reactive. In skin, it is present in different forms: copper/zinc superoxide dismutase (Cu/Zn-SOD) is found in cytosol while manganese superoxide dismutase (Mn-SOD) is located in the mitochondrial matrix (Vessey 1993). The MnSOD act as a first line of defense in the detoxification of superoxide anion and is thought to be involved in tumor suppression and cellular differentiation (Church et al 1993).
Glutathione peroxidase (GPx): was first described by Mills in 1975. The enzyme catalyses the detoxification of hydrogen peroxide at the expense of the oxidation of GSH to GSSG. It also reduces toxic lipid peroxides to less toxic hydroxy fatty acids utilizing glutathione. There are two forms of GPx one of which is selenium (Se)-dependent and the other is Se-independent. Se-dependent GPx is highly reactive towards both H$_2$O$_2$ and organic hydroperoxides (Flohé 1989).

Catalase: Loew discovered it in 1901. It catalyzes the conversion of H$_2$O$_2$ into water and molecular oxygen thereby reducing the grave damaging effects of H$_2$O$_2$ as it can traverse lipid bilayers. H$_2$O$_2$ can react with transition metal ions to form extremely reactive hydroxyl and alkoxyl radicals. Catalase is present in all major organs of the body especially liver and erythrocytes (Schonbaum and Chance 1976).

Gutathione reductase: was observed by Hopkins and Elliott in 1931. In the cells the ratio of GSH: GSSG is relatively higher in order to cope with the oxidative reactions. Reduced glutathione is normally restored by glutathione reductase that catalyses the reduction of GSSG by utilizing the reducing power of NADPH. It is found in cytosol and mitochondria (Sun 1990).

Glucose-6-phosphate dehydrogenase: catalyses the formation of 6-phosphogluconolactone from glucose-6-phosphate using the co-factor NADP that gets reduced to form NADPH (Sun 1990).
Quinone reductase: is also known as DT diaphorase. It catalyses complete two-electron reduction of quinones to relatively stable hydroquinones that in turn is eliminated or undergoes conjugation to form glucuronate or sulfate (Sies 1988) thus preventing the partial one-electron reduction of quinones to semiquinone free radical intermediates. Thus, the free radicals and other oxidizing species generated are scavenged by the above-mentioned non-enzymatic and enzymatic defenses. In this way the antioxidants play a critical role in preventing the process of carcinogenesis.

4.2 Role of antioxidants in carcinogenesis

Role of antioxidants in prevention of tumor initiation

Reducing agents and electron scavengers can inhibit the processes of skin carcinogenesis and tumor initiation by trapping the reactive electrophilic ultimate forms of chemical carcinogens and the free radicals, thereby preventing their mutagenic interaction with epidermal DNA. The reducing agents, therefore, may act as nucleophiles and may compete for the nucleophilic sites on DNA of the generated radical. Antioxidants exert chemoprotective effects against neoplasia as shown in animal studies that induce phase II detoxification enzymes and clear away the activated metabolites through conjugation reactions (DeLong et al 1983). Vitamin E prevents lipid peroxidation and diminishes the damaging effects of lipid radicals and mutagenic species like malondialdehyde. Catalase and SOD are known to reduce thymine glycols in DNA induced by carcinogens like benzo (a) pyrene (Leadon 1987). Mutations caused by bleomycin can be prevented by SOD (Cunningham et al 1984). CuDIPS that mimics SOD is also a potent inhibitor of initiation in mice skin (Solanki et al 1984).
Role of antioxidants in prevention of tumor promotion

It has been shown that agents, which modulate the biological and biochemical events related to skin tumor promotion, also inhibit the ability of the promoter to induce the generation of ROS (Perchellet and Perchellet 1989). Different antioxidant treatments have shown to decrease the oxidative challenge caused by TPA and also inhibit other biochemical events linked to the skin tumor-promotion (Saleem et al 2001). CuDIPS also inhibits the induction of ODC activity that is enhanced by TPA treatment. Various antioxidants like BHA, BHT, Vitamin C and E inhibit the tumor promotion related effects of TPA (Friedman and Cerutti 1983, Smart et al 1987, Borek and Troll 1983, Saito et al 1987).

Role of antioxidants in prevention of tumor progression

Since ROS are implicated in the progression of papillomas into carcinomas, therefore, the antioxidants are also believed to play a part in the prevention of progression. CuDIPS has been shown to inhibit benzoyl peroxide induced skin tumor promotion and progression (Duran et al 1993).

5. CANCER CHEMOPREVENTION

The term ‘chemoprevention’ was coined by MB Sporn (Sporn et al 1976). It is a pharmacological approach for the intervention, arrest or reversal of the process of carcinogenesis (Sporn and Suh 2000). However, chemoprevention differs from cancer treatment because it aims at reducing the cancer inception and incidence. Chemoprevention might not be effective quickly but it is an optimal way to deal with any disease and can be very useful in the long run. Chemopreventive agents may be naturally occurring or synthetic. Natural agents may include fruits, vegetables, beverages and plants with medicinal values while synthetic agents may include compounds like α-
difluoromethylornithine, celecoxib, indomethacin and iboprufen (Gupta and Mukhtar 2002). Recently, a lot of attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding or reversing the multi-stage carcinogenesis. As a result, many compounds have been identified to possess substantial anticarcinogenic and antimutagenic activities. Most of the naturally occurring phenolics possess anti-oxidant and anti-inflammatory properties that appear to contribute to their chemopreventive activity. For e.g. ginger, curcumin, resveratrol, tea polyphenols, epigallocatechin gallate (Surh 1999). Also, epidemiological research shows a lower incidence of many chronic diseases in populations that are regular consumers of vegetables, fruits, red wine and tea (Block et al 1992).

5.1 Properties of an ideal chemopreventive agent
According to Mukhtar and Agarwal (1996), an ideal agent is supposed to have the following advantages: i) No toxicity at all or very less toxicity, ii) Ability of being administered orally, iii) Acceptability to human beings, iv) Effectiveness against multiple sites, v) A defined mechanism of action and vi) Cost effectiveness.

5.2 Mechanisms of Cancer Chemoprevention based on the Categories of Chemopreventive Agents
Chemopreventive agents have been placed into two major categories: Blocking agents that prevent the carcinogenic agents from reaching or reacting with the critical target sites i.e. they have a barrier-like action. There are three major mechanisms by which the blocking agents act (Wattenberg 1992a and b, Wattenberg 1995, Wattenberg 1993). They include:

1.) Prevention of the activation of carcinogens
2.) Enhancement of detoxification system and

3.) Trapping of the reactive carcinogenic species before they actually reach the target site

Suppressing agents are those that act by suppressing the effects taking place during the neoplastic process that might otherwise lead to malignant condition (Wattenberg 1992a and b, Wattenberg 1993). Suppressing agents act by different mechanisms (Wattenberg 1993). Some of the suppressing agents produce differentiation while others counteract the consequences of genotoxic events specially that of oncogene activation.

<table>
<thead>
<tr>
<th>CLASSES OF INHIBITORS</th>
<th>SEQUENCE LEADING TO NEOPLASIA</th>
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<tbody>
<tr>
<td></td>
<td>Precursor Compounds</td>
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<tr>
<td>Blocking Agents</td>
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<tr>
<td>Ascorbic Acid</td>
<td>Procarcinogen</td>
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<tr>
<td>BHA, BHT</td>
<td>Ultimate Carcinogen</td>
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<tr>
<td>Oltipraz</td>
<td>Interaction with Critical Biomolecules</td>
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<tr>
<td>Ellagic Acid</td>
<td>Promotion</td>
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<td></td>
<td>Progression</td>
</tr>
<tr>
<td>Suppressing Agents</td>
<td>Malignant Transformation</td>
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<tr>
<td>Alpha-Tocopherol</td>
<td></td>
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<tr>
<td>Beta-Carotene</td>
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<td>Ascorbic Acid</td>
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<td>Glutathione</td>
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**FIG 4. MECHANISMS OF CANCER CHEMOPREVENTION**

The understanding of mechanisms involved in the cancer development, has made chemoprevention of cancer relatively easy as various agents can be used to interfere with these mechanisms. Resultantly, various strategies have been developed towards the interception of cancer by the use of such agents that can inhibit or block specific sites, reactive entities, specific enzymes and metabolic pathways that contribute to the process of carcinogenesis.
Based on the above-mentioned categories of chemopreventive agents, different mechanisms of cancer chemoprevention are as follows:

**Blocking carcinogen activities:**

**Inhibition of the uptake of carcinogen** - Calcium has been shown to inhibit promotion of colon tumors and also hyperproliferation response in rats (Lamprecht and Lipkin 2003). The excess bile and free fatty acids irritate colonic lumen and promote tumor formation that is prevented when calcium binds to this excess bile and free fatty acids.

**Inhibition of carcinogen activation / formation** - This category of inhibitors prevent the activation of a procarcinogen to carcinogen. The function of the family of cytochrome P-450 is to form more water soluble non electrophilic products but it can also lead to the metabolic activation of the xenobiotics which can then form adducts with DNA. Therefore, agents which inhibit cytochrome P-450 metabolism or interact with the carcinogen can prevent the carcinogen to reach the target molecules. Diallyl sulfide (constituent of *Allium*), which is known to inhibit cytochrome P-450 enzyme (Hong et al 1992) also prevents skin tumorigenesis in mice (Athar et al 1990). Isothiocyanates, flavonoids and polyphenols also act by this mechanism (Goosen et al 2001). Other agents like, Vitamin E act by scavenging nitrites that produce nitrosamines and thus prevent the formation of tumors (Balansky et al 1986). Mammary gland tumors induced by MNU are inhibited by Vorozole (Lubet et al 1994).

**Deactivation or detoxification of carcinogens** - Agents acting by this mechanism activate phase II enzymes, which are non-toxic and can be easily excreted as glucuronides & glutathione conjugates and sulfate products. Dimethyl fumarate induces the activities of both
GST and QR, and also protects against chemically induced carcinogenesis (Spencer et al 1990). Glutathione conjugation is believed to be a promising way of detoxifying the xenobiotics. Glutathione can react spontaneously or on catalysis by GST. It reacts spontaneously with MNNG and B(a)P metabolite (Sundberg et al 2002, Balansky et al 1986). Agents that enhance the levels of GSH are also believed to be good chemopreventive agents (Huber et al 2002).

Prevention of DNA-carcinogen adduct formation - The formation of DNA-carcinogen adduct is known to initiate the process of carcinogenesis. But there is very little evidence of chemoprevention by this mechanism. Oltipraz and chlorophyllin have been shown to inhibit DNA adduct of dibenzo pyrene in human breast cell lines (Smith et al 2001). Ellagic acid is also known to mediate its effect by this mechanism. It prevents nitroso compound induced carcinogenesis by blocking of methylation of guanine (Balansky et al 1986).

Suppressing carcinogen activities:

Scavenging of reactive electrophiles - Agents that act through this mechanism are potent nucleophiles. They react directly with the carcinogens and other electrophiles before they attack the macromolecules. Examples of this class include GSH and polyphenols that react with the activated carcinogens (Srivastava et al 2002, Cheng et al 2003).

Scavenging of oxygen radicals - Link between the oxygen radicals and the process of carcinogenesis has already been described earlier. N-acetyl cysteine is known to react with hydroxyl radicals (Orzechowski et al 2002) and acts as chemopreventive agent while β-carotene is involved in detoxifying singlet oxygen (Cantrell et al 2003). Phenolic antioxidants, on the other hand, scavenge peroxyl radicals (Fujisawa et al 2002).
Inhibition of arachidonic acid metabolism - Marnett (1992) has reviewed the role of arachidonic acid metabolism in carcinogenesis. Arachidonic acid metabolites participate in reactions that initiate carcinogenesis (Marnett 1987). They are involved in producing free radicals (Marnett 1992). Cyclooxygenase and lipoxygenase associated arachidonic acid metabolism are also associated with the inflammatory response that is related to tumor promotion. Up-regulation of COX-2 is associated with certain types of human cancers (Eberhart et al 1994). Therefore, agents that inhibit these enzymes are also anticipated to inhibit effects mediated by arachidonic acid metabolism. Also, the anti-inflammatory agents are believed to exert chemopreventive effects on carcinogenesis. The process of carcinogenesis can be inhibited by cyclooxygenase inhibitors such as non-steroidal anti-inflammatory drugs like aspirin, ibuprofen and piroxicam and also by the antioxidants like flavonoids (Kelloff et al 1994, Schuller et al 2002, Surh et al 2001, Kokoska et al 2000).

Modulation of PKC activity - Growth factors and the hormones mediate their effects of regulating cell growth, proliferation and differentiation via signal transduction. Chemopreventive agents that exert their effects via this mechanism alter the signal transduction pathways (Brunton and Workman 1993, Powis 1994, Powis and Workman 1994) especially modulating PKC activity. TPA has been shown to act like DAG, a secondary messenger and activate PKC activity. Therefore, agents which inhibit the activity of PKC are expected to act as inhibitors of carcinogenesis. Tamoxifen and glycyrrhetenic acid inhibit PKC activity and are also the inhibitors of carcinogenesis (Sporn and Suh 2000, O'Brian et al 1990).

Modulation of hormonal/growth factor activity - Hormones and growth factors stimulate specific receptor linked cascade of events that finally induces the expression of certain
genes, which may become induced/suppressed during the process of carcinogenesis. The chemopreventive agents may cause inhibition of carcinogenesis by regulation of these molecules. Insulin-like growth factor 1 (IGF-1) causes stimulation of cell replication in many tumors and in case of human breast cancer cells there are receptors for this particular hormone. Tamoxifen, which is used against breast cancer also lowers the concentrations of IGF-1 in blood (Sporn and Suh 2000). Both tamoxifen and raloxifene bind to estrogen receptors and have been shown to prevent breast cancer in experimental animals (Jordan 1976, Anzano et al 1996). Transforming growth factor β (TGF β) has been shown to have antiproliferative activity in both normal and neoplastic cells and promotes differentiation. Chemopreventive agents like tamoxifen and retinoids have been shown to induce TGF β in epithelial cells (Glick et al 1991, Sporn et al 1992).

*Inhibition of oncogene activity* - The products of the oncogenes alter the normal signal transduction activity in carcinogenesis. A membrane receptor - linked enzyme tyrosine kinase is involved in the activation of Ras. The involvement of Ras oncogenes in carcinogenesis has been described earlier. Therefore, agents that inhibit tyrosine kinase will also inhibit Ras activation. D-limonene is shown to inhibit farnesylation of small GTP-binding protein (Crowell et al 1991) and also inhibits the progression of mammary tumors induced MNU or DMBA (Haag et al 1992).

*Restoration of tumor suppressor response* - Tumor suppressor genes prevent the proliferation of cells (described earlier). Mutations or inactivation of the tumor suppressor genes like p53 has been associated with the formation of cancer (Yuspa et al 1994). Patients treated with exogenous functional tumor suppressor genes have been shown to be effective.
Apigenin induces p21 protein (Gupta and Mukhtar 2002) and also inhibits carcinogenic response.

**Inhibition of polyamine metabolism** - ODC is the first step in polyamine biosynthetic pathway (Pegg 1988). It is the only route through which the polyamines are synthesized although they are present in diet and are also produced by intestinal microflora. They are involved in cell proliferation and malignant transformation (Pegg 1988, Verma 1992). In precancerous conditions, the levels of ODC are elevated and a number of tumor promoters have shown such an effect (Pegg 1988, DiGiovanni 1992). In fact, elevation of ODC levels is used for screening tumor promoters (DiGiovanni 1992). Inhibitors of ODC are therefore regarded as candidates for chemopreventive agents like α-difluoromethylornithine (Ohmori et al 1994, Shantz and Pegg 1994).

**Restoration of immune response** - It has been shown that during photocarcinogenesis the immune response is suppressed (Boonstra and Savelkoul 1997, Schwarz 1999). De Flora and Ramel (1988) have documented that producing antibodies against oncogene products and oncoproteins can inhibit the cell transformation and tumor growth. Retinoic acid acts as immunostimulant by enhancing cell-mediated and natural killer cell cytotoxicity. It also causes differentiation in leukemic cells (Hill and Grubbs 1992).

**Enhancement of intercellular communication** - It is through the gap junctional intercellular communication that a cell communicates with other cells and controls growth regulation (Bertram et al 1992). Known tumor promoters, TPA and mezerein inhibit this growth-regulatory mechanism. Carotenoids and retinoids have been found to enhance the
intercellular gap junctional communication (Zhang et al 1991) and also have anti-tumor activities.

*Induction of apoptosis*- Apoptosis is a process by which damaged and excessive cells are programmed for death (Brusch et al 1992). It is inhibited by promoters like TPA (Arita et al 2000). On the other hand, retinoids (Delia et al 1993) and sulindac sulphone (Piazza et al 1995) act as inducers of apoptosis and inhibit the process of tumorigenesis.

*Inhibition of angiogenesis*- Angiogenesis is a process whereby the formation of new blood vessels takes place. It is an important step for the growth and development of tumors. Since PGE1 and PGE2 are angiogenic, therefore, agents that inhibit them will also inhibit carcinogenesis by inhibiting angiogenesis (Kanekura et al 2000). Anti-angiogenic therapy is an effective approach for cancer control (Singh and Agarwal 2003).

*Inhibition of proteases* – Proteases are known to activate an error-prone repair system and are induced by tumor promoters. Protease inhibitors, on the other hand, prevent both the initiation and promotion stages of carcinogenesis (Kennedy and Manzone 1995). For example, Bowman-Birk inhibitor (soybean-derived protease inhibitor) prevents carcinogenesis in different model systems and is an inhibitor of chymotrypsin (Kennedy 1998). Normally the dietary protease inhibitors are not properly absorbed from the GI tract rather they induce synthesis of endogenous protease inhibitors which affect the cell growth. Topically they might also exert anti-inflammatory effects (Clawson 1996). They also modulate the expression of certain oncogenes and the levels of the proteolytic enzymes (Kennedy and Manzone 1995). Their role in chemoprevention has been reviewed by Das
and Mukhopadhyay (1994). However, the results with these inhibitors have not been very encouraging against carcinogenesis in humans (Rubin 2003).

5.3 Cancer Chemoprevention in Different Models Systems

**Breast** - Tamoxifen and retinoids are effective chemopreventive agents against mammary carcinogenesis. Studies in rats show that monoterpenes like perillyl alcohol are also effective against breast cancer (Gould 1995).

**Prostate** - chemopreventive agents that have a hormone dependent role can be used for prostate cancers (Karp et al. 1996).

**Ovary** - The risk of ovarian cancer development is higher in women who have used oral contraceptives as evidenced by epidemiological studies. Therefore, on this basis chemopreventive agents for ovarian cancer can be developed (Henderson et al. 1993).

**Large Bowel** - Chemoprevention of large bowel has been observed in many studies on animals and humans. Nonsteroidal anti-inflammatory compounds (Wattenberg 1992a and b) are effective as chemopreventive agents against large bowel.

**Lung** - Chemoprevention of this organ has mostly been ineffective although certain agents show protective efficacy against adenocarcinomas of the lung (Wattenberg 1992a and b).

**Skin** - It is a classic model for the study of mechanisms of carcinogenesis (Slaga et al. 1982). A large number of synthetic and natural agents are effective in chemoprevention of skin cancer. The agents used for this thesis work have been targeted against skin carcinogenesis.
5.4 Prevention of cancer by already established chemopreventive compounds derived from dietary factors:

*Apigenin* is a flavonoid that is present in the vascular plants, fruits and vegetables like apples, beans, broccoli, cherries, cloves, grapes, onions, barley and tomatoes (Lepley et al 1996). It is also present in tea and wine. It acts by more than one mechanism of cancer chemopreventive action listed earlier. It has anti-inflammatory activity. It is causes reduction in the epidermal ornithine decarboxylase activity as well as cutaneous carcinogenesis in mice (Gupta and Mukhtar 2002) produced chemically as well as by UV light. It also prevents progression i.e. malignant conversion of papillomas to carcinomas. It inhibits the PKC activity and oncogene expression in TPA induced tumor promotion in mice (Birt et al 1997). It induces p21 protein and stabilizes p53 protein and arrests G2/M phase of cell cycle in mouse skin keratinocytes (Gupta and Mukhtar 2002).

*Curcumin*, present in *Curcuma longa*, is used as a spice in Indian food. It also mediates its cancer chemopreventive activity by more than one mechanism of cancer prevention. Curcumin possesses anti-inflammatory activity and is a potent inhibitor of reactive oxygen-generating enzymes such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase and inducible nitric oxide synthase (Subbaramaiah et al 1998, Lin et al 2000), It protects against cancer of different organs (Azuine and Bhide 1992, Limtrakul et al 1997) and is effective against both chemically induced and UV-mediated carcinogenesis. Besides, the anti carcinogenic effects are thought to be a result of its antioxidant nature (Lu et al 1993). Like many other chemopreventive agents, it also raises the levels of reduced glutathione and glutathione -S- transferase and inhibits ODC activity, arachidonic acid metabolism and lipid peroxidation in mouse skin (Lu et al 1993, Iersel et al 1996). It protects against DNA
damage induced by radiations and mediated by scavenging of hydroxyl radical (Prashad et al 1998) and mutagenesis in *Salmonella typhimurium* TA1535 and *Escherichia coli* (Oda 1995). It also induces apoptosis and upregulates p53 (Jee et al 1998).

*Diallyl sulfide* is present in garlic and onion (Singh and Lippman 1998). It is also known to possess inhibitory effects on the induction of skin, colon, liver, esophagus and kidney tumors in animal experimentations (Sadhana et al 1988, Hayes et al 1987, Wargovich 1987, Fukushima et al 1997). The diallyl sulfide has been shown to inhibit benzoyl peroxide-mediated tumor promotion in 7,12-dimethylbenz (a) anthracene-initiated skin of SENCAR mice (Athar et al 1990). It acts by significantly inhibiting TPA-induced ODC activity, increasing glutathione and glutathione peroxidase activity (Perchellet et al 1986).

*Resveratrol* is a polyphenol found in grapes, berries, peanuts and red wine (Jang and Pezzuto 1999, Jang et al 1997). It exerts inhibitory action on all the stages of carcinogenesis i.e. initiation, promotion and progression (Jang et al 1997). Resveratrol suppresses the activation of cytochrome P-450 1A1 and inhibits formation of preneoplastic lesions in mouse mammary organ. It blocks the G1/S transition phase in cell lines (Bhat and Pezzuto 2002). It has strong antioxidant, anti-inflammatory and anti-proliferative properties (Tsai et al 1999, Jang and Pezzuto 1999). It is known to inhibit cell transformation through the induction of apoptosis and p53 activation (Dong 2000). Resveratrol is shown to act as an antioxidant and antimutagenic agent thus acting as an anti-initiation agent. As regards photochemoprotection, it is found to decrease hydrogen peroxide formation, lipid peroxidation, infiltration of leucocytes, cyclooxygenase and ornithine decarboxylase activities (Afaq et al 2001).
Tea Polyphenols Tealeaves are available in the form of black tea (78%), green tea (20%) and oolong tea (2%). It is believed that both the black and green teas exhibit inhibitory effects against carcinogenesis of different organs (Wu et al 2003, Dora et al 2003). (-)-epigallocatechin gallate (EGCG) is the major polyphenol in green tea while theaflavins are the major active components in black tea. Both of them inhibit epidermal growth factor or TPA-induced cell transformation (Dong 2000, Lin and Liang 2000). Tea polyphenols mediate their cancer chemopreventive effects mainly by biochemical mechanisms like: antioxidative activities, modulation of xenobiotic metabolizing enzymes and inhibition of tumor promotion. It has also been proposed that tea polyphenols function as cancer chemopreventive agents through modulation of the mitotic signal transduction (Lin and Liang 2000). Both black tea and green tea show protective action against UVB induced carcinogenesis in DMBA initiated SKH-1 mice skin (Wang et al 1994). Green tea polyphenols act by enhancement of antioxidant (GPx and catalase) and phase II (GST and QR) enzyme activities; inhibition of lipid peroxidation, irradiation- and TPA-induced epidermal ODC, COX and PKC activities, enhancement of gap junction intercellular communication, immunosuppression and hyperplasia (Stoner and Mukhtar 1995, Gupta and Mukhtar 2002).

Vitamin E is soluble in lipids and protects against oxidative stress (Khanduja et al 1999) by preventing unsaturated fatty acids, protein and DNA from oxidation. It also prevents photodamage and carcinogenesis of mice skin (Pinnell 2003, Chen et al 1997, Kuchide et al 2003).

Retinoids (vitamin A, its metabolites and synthetic derivatives) have varying biological properties that may contribute to its cancer-preventive effects. They include: inhibition of
cell growth, induction of differentiation and programmed cell death, inhibition of angiogenesis, decrease in the synthesis of PGE2 and COX-2 expression (Hansen et al 2000, Kanekura et al 2000, Chen et al 1994). They have been shown to inhibit tumor promoter-induced cell transformation and tumor promotion in transgenic mice (Dong 2000).

In the USA, people are recommended to consume five to nine vegetables and fruits a day, and this concept is part of the Federal Government recommendation for health maintenance. Research carried out over the last 20 years provides knowledge of not only what people should eat but also what they should have as a beverage (Weisburger 1999).

5.5 Overview of previously used medicinal plants as cancer chemopreventive agents

*Withania somnifera*, commonly known as Ashwagandha, has multi-medicinal properties. It has anti-stress and anti-oxidant activities (Bhattacharya et al 2001). It is reported to have other activities like immunomodulatory, cognition facilitating and anti-inflammatory, as evidenced in case of animal systems and in clinical findings (Bhattacharya et al 1997). It is used against cancer in a number of studies. It prevents DMBA-induced skin cancer in Swiss albino mice and this chemopreventive activity is attributed to the antioxidant/free radical-scavenging constituents present in the extract (Davis and Kuttan 2001). The anti-inflammatory and immunomodulatory properties are also associated with its preventive effect (Prakash et al 2002). Anti-inflammatory activity has been linked to the biologically active steroids of the plant extract especially the major component, withaferin A (al-Hindawi et al 1992). It is reported to prevent cyclophosphamide-induced immunosuppression in animal model system (Agarwal et al 1999). It causes a stimulatory response in the generation of cytotoxic T lymphocyte that helps in reduction of tumor growth (Davis and Kuttan 2002). The antioxidant and detoxifying properties are considered to be the
mechanism behind the chemopreventive action of *W. somnifera* extract as it modulates reduced glutathione content, lipid peroxidation, GST, SOD and catalase activities in mice injected with 20-methylcholanthrene (Prakash et al 2001). The methanolic extract of the plant also reduces the hydrogen peroxide-induced cytotoxicity and DNA damage in human fibroblasts (Russo et al 2001). It exhibits curative effect against carbendazim-induced hepatic and renal histopathological alterations (Akbarsha et al 2000). Its antitumor activity and other pharmacological effects have also been attributed to the down regulation of p34 cdc2 expression (Singh et al 2001).

*Ocimum sanctum* is commonly called as tulsi. It is a plant that has an array of therapeutic properties. There are numerous studies stressing upon its anti-tumor activity in animal model systems. It has been shown to reduce DMBA-induced skin papillomas at either the perinitiational, post-initiational stages or at peri- and post-initiational stages (Prashar et al 1994). It acts by preventing metabolic activation of the carcinogen (Prashar et al 1998). It possesses antioxidant activity and causes elevation of GSH and GST activity that are considered likely for its protective action (Prashar et al 1994). It is effective against DMBA-induced hamster buccal pouch carcinogenesis (Karthikeyan et al 1999). Its seed oil modulates fibrosarcoma induced by 20-methylcholanthrene in Swiss albino mice (Prakash and Gupta 2000). It also has modulatory effects on humoral and cell-mediated immunity (Mediratta et al 2002). The plant extract also protects against radiation induced lipid peroxidation and enhances the levels of GSH and the antioxidant enzymes (Devi and Ganasoundari 1999). The leaf extract of *O. sanctum* has been shown to contain flavonoids such as orientin and vicenin that possess free radical scavenging activity and are reported to protect against radiation-induced damage (Devi et al 2000). The other compounds of the
plant extract also possess antioxidant and/or anti-inflammatory activities (Kelm et al 2000). Paw edema induced by carrageenan, PGE2, leukotriene and arachidonic acid have been shown to be inhibited by *O. sanctum* and its constituent, linolenic acid (Singh 1998). Thus, it acts basically by the mechanisms listed in the ‘suppressing carcinogen activities’ subheadings.

*Emblica officinalis* is commonly called as amla. The extract of *E. officinalis* possesses anti-tumor activity (Jose et al 2001). It is cytotoxic to cells in culture as it inhibits enzymes involved in the regulation of cell cycle (Jose et al 2001). Tannoids, the active principles of *E. officinalis* have anti-stress activity in the frontal cortex and striatum of the rat brain (Bhattacharya et al 2000). It also shows ulcer protective and healing effects (Sairam et al 2002). *E. officinalis* extract significantly suppresses the genotoxic effects of B(a)P and cyclophosphamide in vivo. Administration of the plant extract induces the levels of GSH, antioxidant and detoxification enzymes such as SOD, GPx, GR, catalase and GST while it decreases the levels of cytochrome P 450 (Sharma et al 2000) i.e. it modulates both phase I and phase II enzymes. Vitamin C, a major constituent of *E. officinalis* has elaborate chemopreventive properties.

*Aloe vera* is commonly called as gheekunvar and is used in a wide variety of health care and cosmetic products (Norikura et al 2002). It has been reported to possess physiological properties such as antioxidant, anticarcinogenic, anti-inflammatory and laxative properties (Norikura et al 2002). *A. vera* L extract is cytotoxic in human tumor cell lines (Lee et al 2000a). The compound isolated from *A. vera* L, diethylhexylphthalate (DEHP), is believed to be the active principle responsible for in vitro anti-leukemic and anti-mutagenic effects (Lee et al 2000a). It has been further shown to induce apoptosis in human leukemic cell lines.
A. vera enhances healing of wounds (Yagi et al. 2002) and its different components have been identified. A glycoprotein fraction of the plant gel also possesses radical scavenging activity against superoxide anion generated by the xanthine-xanthine oxidase system and also inhibits cyclooxygenase-2 and reduce thromboxane A2 synthase level in vitro (Yagi et al. 2003). Besides, the gel of the A. vera leaf also contains a carbohydrate fraction, acemannan, that possesses various therapeutic properties including induction of wound healing, stimulation of immune system, anti-cancer and anti-viral activities (Zhang and Tizard 1996).

Aloe extract also inhibits 1,4-naphthoquinone-induced toxicity in rat hepatocytes probably by the maintenance of cellular thiols (Norikura et al. 2002).

Glycyrrhiza glabra is commonly called as jethi madh. It is anti-inflammatory, anti-viral, anti-ulcerative and anti-carcinogenic (Wang and Nixon 2001) in nature. It acts by most of the mechanisms of carcinogen suppression. Licorice root extract has been shown to induce Bcl-2 phosphorylation and G2/M cell cycle arrest in a way similar to paclitaxel, an anti-microtubule agent. It is believed that 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4′-hydroxyphenyl) 1-propanone (β-hydroxy-DHP), present in the licorice root is responsible for the induction of Bcl-2 phosphorylation, apoptosis, and G2/M cell cycle arrest in breast and prostate tumor cell lines (Rafi et al. 2002). It possesses triterpenoids like glycyrrhizin and glycyrrhizic acid apart from various polyphenols and polysaccharides. It is hypothesized to protect against DNA damage induced by carcinogens. Glycyrrhizic acid inhibits lipoxygenase, cyclooxygenase, protein kinase C and also down regulates the epidermal growth factor receptor (Wang and Nixon 2001). The anti-inflammatory effect of G. glabra is supposed to be due to the anti-thrombin action of glycyrrhizin (Francischetti et al. 1997). It is
also known to act against EBV replication in super infected Raji cells (Lin 2003). Licorice polyphenols are known to induce apoptosis of cancerous cells (Wang and Nixon 2001).

*Zingiber officinale* is commonly called as ginger. Also, topical application of the ginger extract pretreatment results in prevention of DMBA initiated and TPA promoted mouse skin carcinogenesis (Katiyar et al 1996). [6]-gingerol, active principle of ginger is believed to inhibit TPA-induced inflammation, epidermal ODC activity and skin tumor promotion response in ICR mice (Park et al 1998). Like many other plants, the cancer chemopreventive properties of ginger have been attributed to the antioxidant potential of the constituents.

*Thuja standishii* is used for the treatment of various skin related diseases. Out of the six diterpenes contained in the extract of the stem bark of *Thuja standishii*, four strong inhibitory effects against Epstein-Barr virus early antigen (EBV-EA) activation that is induced by TPA (Minami et al 2002). Two of its diterpenoids show stronger protection against EBV-EA activation as compared to that of the known cancer preventive agent, betacarotene (Tanaka et al 2000). Some of its labdane-type diterpenoids also show anti-tumor promoting activity in two-stage mouse skin carcinogenesis (Iwamoto et al 2001).

*Tinospora cordifolia* is commonly called as guruchi. It has immunomodulatory, anti-hepatotoxic, anti-stress and antioxidant properties (Pahadiya and Sharma 2003). *T. cordifolia* has been shown to possess radical scavenging activity in mice (Mathew and Kuttan 1997). Its aqueous extract protects Swiss albino mice against gamma radiations emitted from $^{60}$Co (Pahadiya and Sharma 2003). These radioprotective effects have been attributed to its antioxidant (Goel et al 2002) nature. Its antineoplastic activity in the HeLa cells is supposed to be due to its cytotoxicity (Jagetia et al 1998). The plant extract presents
effects that are comparable to or are better than the effects of drug (doxorubicin) treatment (Jagetia et al 1998).

Similarly, Ram and Kumari (2001) have reviewed a large number of plants that have clinically been proven to possess anticancer activity. These plants include: Catharanthus roseus, Podophyllum peltatum, Taxus brevifolia, Camptothecin acuminata, Cephalotaxus harringtonia, Viscum album, Onchrosia elliptica, Annona bullata, Asmina triloba and Rhizoma zedoariae.

Note: The general trend of the mechanism of action of the plants reviewed here shows that the antioxidant activity and the anti-inflammatory activity of the plant extracts are partly responsible for their antitumor activity.

5.5 Overview of the agents used in the present study for the purpose of cancer chemoprevention

*Balsamodendron mukul* Hook ex. Stocks (Burseraceae) syn with *Commiphora mukul*

**Vernacular names:** Guggul, Gugal

**Origin and Distribution:** The plant is indigenous to India. It grows wild on semi-arid rocky tracks of Rajputana Desert, Bellari, Mysore, Sindh and Baluchistan.

**Chemical constituents:** The chemical constituents present in *B. mukul* include: ellagic acid, ferulic acid, diterpenes like α-comphorene A and cembrane alcohol, allylcebmol, flavones like quercetin, amino acids like arginine, cystine, histidine and serine. The compounds present in gum of guggul include linoleic acid, guggulusterols – I, -II, -III, -IV and –V, and Z & E-gugglosterones (Chatterjee and Prakashi 1994). Guggulusterols have anti-inflammatory activity and are pharmacologically the active principle behind lowering of
blood lipids. The indigenous compound gugullipid is useful in case of heart diseases, spondilitis and gout. The resin of the plant is used in case of ulcers, pyorrhea, tonsilitis and pharyngitis. Inhalation of the fumes of the resin is suggested in hay fever, chronic bronchitis, laryngitis and nasal catarrh.

The hypolipidemic activity of guggul gum has been attributed to more than one mechanism including: inhibition of cholesterol biosynthesis, enhancement of the rate of cholesterol excretion, promotion of faster degradation of cholesterol, stimulation of thyroid and high affinity binding and anion exchange. The initial three mechanisms are related in the sense that their end result is the elimination of cholesterol (Satyavati 1991).

Pharmacological effects: It has anti-inflammatory activity, antiseptic and anti-spasmodic activity. Guggulu is shown to have hypocholesterolemic and hypolipidemic activity in patients of coronary heart disease. It is also reported to inhibit platelet aggregation (Mester et al 1979) and increase serum fibrinolytic activity.

Traditional medicinal uses: B. mukul is used against wide variety of ailments like cardiac disorders, skin diseases, pectoral and hepatic disorders, urinary disorders and nervous diseases. It is used as liver tonic, nerve tonic, rejuvenating and general tonic. It is also beneficial in case of obesity, gout, sciatica, facial paralysis, leprosy, helminthiasis, dyspepsia, cough, asthma, bronchitis, epilepsy, fever, wounds, abscesses and ulcers, anemia, diabetes, aphrodisiac, coronary thrombosis, leucoderma, tumors and in Chinese medicine system also it is used in extra dural haematomas (Kirtikar and Basu 1935, Asolkar et al 1992).
In Ayurvedic system of medicine, it is used for the treatment of several disorders owing to its diverse pharmacological properties. The indigenous drugs containing *B. mukul* include: (1) Septilin for rhinosinal infections, (2) Navakagaggulu for rheumatism, (3) Biliarin for infective hepatitis and (4) Arogya-wardhini for treating acute viral hepatitis. In case of snakebite and scorpion sting, it is given in combination with other drugs (Kirtikar and Basu 1935, Asolkar et al 1992). Other commercially available drugs containing *B. mukul* include: Kama Dudha is useful in case of burning sensation all over body; Anu Taila is excellent for chronic and allergic rhinitis and headaches; Asthi Poshak Vati for hair growth, osteoporosis, pregnancy and lactation. Punarnava Guggul is given for pain and edema in joints, rheumatism, and gout; Kanchnar Guggul for cervical lymphadenitis (swollen lymph glands in neck), benign and other tumors; Triphala Guggul is useful in sciatica and obesity. Yog Raj Guggul for relieving joint pains, paralysis, stiffness of legs, piles, splenomegaly and headache. Kaishore Guggul is beneficial in gout, pain in small joints, skin diseases, tumors, wounds, diabetes, anorexia, cough and anemia; Asanad is excellent for controlling and even eliminating diabetes.

**Hibiscus rosa sinensis** L. (Malvaceae)

**Vernacular names:** Gudhal, Gurhal, China rose, Jabaphool and Sadaphool

**Origin and Distribution:** It is a native of southeastern Asia. It can be cultivated in a wide range of situations and is a common houseplant throughout the world.

**Pharmacological effects:** It possesses various pharmacological effects that include: alkaline phosphatase inhibition activity (Prakash 1979), analgesic activity (Singh et al 1978), anti-
pyretic and anti-inflammatory (Singh et al 1978) activities, anti-spasmodic activity (Bhakuni et al 1969), abortifacient effect (Singh et al 1982), anti-estrogenic activity (Kholkute and Udupa 1976a), anti-implantation effects (Kholkute and Udupa 1976b) antifertility activity (Trivedi and Shukla 1980), antifungal activity (Renu 1983), anti-viral activity (Van Den Berghe et al 1978), hypotensive and hypothermic activities (Bhakuni et al 1969) and radical scavenging effects (Masaki et al 1995).

Chemical constituents: present in *H. rosa sinensis* include: quercetin, β-carotene, apigenidin, β-sitosterol, sterulic acid, stigmasterol, oxalic acid, myristic acid, oxalic acid, tartaric acid, niacin, riboflavin, malvalic acid, gentisic acid, margaric acid and lauric acid (Ross 1999).

Traditional medicinal uses: It is has numerous medicinal properties and is therefore used in a number of countries. In India, it is used differently for different problems. The hot water extract of the plant is used as an agent that causes infertility and is used as contraceptive in Ayurvedic system of medicine. Dried buds are eaten as a treatment for diabetes. Eating one mature bud daily for ten days is considered to be good in the management of diabetes (Alam et al 1990). The juice of flowers and leaves with water is given orally for the cure of constipation and painful motion (Bhandary et al 1995). The extract of the dried stem is used as a diuretic (Maheswari et al 1980); arial parts as aphrodisiaice (Gupta et al 1971) laxative and to expel the placenta after childbirth; flowers for menorrhagia, bronchitis (Jain and Tarafder 1970), as an emmenagogue (Malhi and Trivedi 1972), for the treatment of menarche (Reddy et al 1989) and as a contraceptive (Dixit 1977).

It is used for the cure of other ailments as well, which include treatment of dysentery, venereal diseases, fever, hysteria, gonorrhoea, inflammatory conditions (Burkhill 1966, Verma et al 1967), relieve labor pain, eye problems, for draining abscesses and the paste of
flowerbuds is also applied topically to cancerous swellings (Watt and Breyer-Brandwijk 1962). In Philippines, fresh flowers are applied to tumors and inflammations (Pardo De Tavera 1901).

*Onosma echioides* (Boraginaceae) syn *Onosma hispidum* Wall ex D.Don.

**Vernacular names:** Ratanjyot

**Origin and Distribution:** Distributed widely in the regions of Kashmir, Kumaon and Baluchistan

**Pharmacological effects:** It has antihelmintic and alexipharmic properties

**Chemical constituents:** The main components present in the plant include shikonins and alkannins and their derivatives. Dry callus of the plant yields shikonin acetate (Koul et al 1993). Chemopreventive effects of these two compounds have been examined by various researchers. Shikonin and its derivative acetylshikonin have wound healing and anti-inflammatory effects. Shikonins have been shown to inhibit the biosynthesis of leukotriene B and 5-hydroxyeicosatetraenoic acid. Alkannins, shikonins and their derivatives are known to have bactericidal effect (Papageorgiou et al 1999). Shikonin has been shown to inhibit the early activation of TPA and reduce the incidence of azoxymethane-induced intestinal tumors in rats (Papageorgiou et al 1999). The derivative of shikonin, acetylshikonin is known to inhibit arachidonic acid induced platelet aggregation (Ko et al 1995). The naphthoquinone pigment, shikonin and its derivatives are the active components isolated from the Chinese herbal therapeutic, Zicao. Historically, Zicao root extracts have been used to treat macular eruption, measles, sore throat and burns. Multiple pharmacological actions have been attributed to shikonin. These diverse activities include healing of wounds, suppression of local acute inflammatory reactions, inhibition of the formation of the new blood vessels,
DNA topoisomerase activity, platelet activation, antigonadotropic, antimicrobial and anti-HIV-1 activity. These inhibitory effects account for the broad spectrum of shikonin’s biological and pharmacological activities (Chen et al 2002).

**Traditional medicinal uses:** *O. echioides* var. hispidum has been reported to be used in the treatment of burns and wounds. The leaves of *O. echioides* are used as purgative and are given in powered form to children, the flowers are effective in case of rheumatism and palpitation of heart while the roots are applied in case of bruises and eruptions (Chopra et al 1996). *O. echioides* is used extensively in Tibetan and Spanish systems of medicine for the treatment of fever, wounds, piles, itch, inflammations, rheumatism, palpitations of heart and leucoderma (Chopra et al 1996).

**Soy isoflavones**

Soybean contains soy isoflavones as their active components. The major compounds found in soybean product include: genistein and daidzein (obtained from genistin and diadzin respectively), which are metabolized into their corresponding glycosides by intestinal hydrolysis to 6’-hydroxy-O-desmethyl angolesin and equol respectively (Hwang et al 2000, Guo et al 2002, Setchell et al 2002). Glycitein is another isoflavone obtained from soy foods but is present in relatively small amounts as compared to other isoflavones.
Anticancer effects: Epidemiological studies have shown that soy consumption reduces the risk of breast, colon, and prostate cancers, as observed in the Asians, who have a higher intake of soy foods and a lower risk of these cancers as compared to the Americans and Europeans (Messina and Barnes 1991). Experimental data in animal studies also conveys that soybean fed Swiss mice are protected from bladder carcinogenesis (Mukhtar et al 1998). Both the major components of the soy isoflavones inhibit J82 human cancer cell proliferation and G2/M-phase cell cycle arrest, which is considered to be the possible reason behind their protective action (Zhou et al 1998).

Genistein: constitutes a major part of the soy isoflavones and chemically is 4',5,7-trihydroxyisoflavone. It is known to possess antioxidant and anti-carcinogenic effects in skin (Wei et al 1993, Wei et al 1998). It inhibits TPA–induced proto-oncogene expression in mouse skin significantly in a dose dependent manner. Also, it prolongs tumor latency period and decreases tumor multiplicity in two-stage skin carcinogenesis (Wei et al 1995). It has been shown to inhibit the growth of cancer cells through the modulation of genes that are related to the control of cell cycle and apoptosis. (Sarkar and Li 2002). Genistein treatment also inhibits DMBA induced DNA adduct formation therefore is known to exert anti-initiatational effects (Wei et al 1998). It also exerts anti-promotional effects via inhibition of tyrosine kinase (Akiyama et al 1987) activity that attenuates the growth of cancer cells. It also inhibits hydrogen peroxide production and superoxide formation (Wei et al 1993), and targets estrogen and androgen-mediated signaling pathway in the processes of carcinogenesis. Ingram et al (1997) reported a substantial reduction in breast cancer risk in women with high intake of phytoestrogens. Adlercreutz et al (1995) reviewed the evidence suggesting that isoflavones have hormone-like cancer protection that influences growth
factor action, malignant cell proliferation and cell differentiation. It is also found to be a potent inhibitor of angiogenesis and metastasis (Sarkar and Li 2002). Besides, it has also been shown to inhibit cdc2 kinase (Su et al 2000), DNA topoisomerase II (Yamashita et al 1990) and ribosomal 6S kinase (Linassier et al 1990) that may lead to protein linked DNA strand breaks and tumor cell growth arrest. Genistein is also known to photochemoprevent i.e. prevent against light induced toxicity (Eileen et al 2002). Topical application of genistein prior to low doses of UVB radiation reduces the expression of c-fos and c-jun, dose dependently in SENCAR mouse. It has been proven to be effective against cancer of different organs and its cancer chemoprevention has been reviewed by Sarkar and Li (2002).

Diadzein: is also believed to have inhibitory activity against certain malignant melanoma and may act via different mechanisms as opposed to genistein. It is considered to be potential medical candidate for melanoma cancer therapy (Wang et al 2002). Both daidzein and biochanin-A directly induce apoptosis without causing any alteration in cell cycle unlike genistein (Su et al 2000). Diadzein is converted into equol by intestinal bacteria, binds to estrogen receptors and also acts as antioxidant and anticarcinogenic (Sathyamoorthy and Wang 1997). The clinical significance of equol along with genistein provides an idea about the potential of soy and its isoflavones (Setchell et al 2002).

Antioxidant effects:

Soy isoflavones have antioxidant properties (Wei et al 1995). LDL oxidation initiates a cascade of events that includes the production of cytokines and growth factors. Genistein and daidzein have been shown to inhibit LDL oxidation in the sub endothelium of vessels just like vitamin E (Hodgson et al 1996).
Apart from these effects, soy isoflavones have other beneficial roles like lipid lowering effect (Lovati et al. 1987, Sirtori et al. 1997), cardio protective effect (Honore et al. 1997, Wei et al. 1995) and the ability to treat osteoporosis in humans.

**Gentisic acid**

Gentisic acid (2, 5-dihydroxy benzoic acid) is a phenolic acid and is formed on hydroxylation of salicylic acid. It is generated in vivo also. Phenolic acids, including hydroxybenzoic acids, are secondary plant products and are commonly found in plant-derived foodstuff. Radtke et al. have shown that the total sum of hydroxybenzoic acids amounts to 11 mg/day with 75% of salicylic acid intake by plant derived foodstuff including fruits and juices (Radtke et al. 1998). Most phenolic acids have antioxidant and anticarcinogenic properties. Gentisic acid is also known to possess antioxidant, anti-inflammatory and anti-mutagenic properties (Krizkova et al. 2000, Uhrig et al. 2000). Even salicylic acid and 2, 3-dihydroxy benzoic acid (2,3-DHBA) are known to possess anti-inflammatory properties along with 2, 5-dihydroxy benzoic acid (2,5-DHBA). Since phenolic acids possess antioxidant and anticarcinogenic properties, therefore, one can explain the inverse association between fruit and vegetable intake and the incidence of cancer as found in epidemiological studies.

Gentisic acid is used in cosmetics as a skin-whitening agent for the treatment of skin pigmentation disorders by affecting the synthesis of melanin through the inhibition of tyrosinase activity of the melanocytes (Bian et al. 2003). It is also able to adversely affect LDL peroxidation catalyzed by tyrosyl radical. Salicylate, the precursor molecule of gentisic acid can act like tyrosine via a phenoxyl radical that catalyzes LDL oxidation. However,
gentisic acid effectively counteracts the prooxidant activity of this radical (Hermann et al 1999). Thus, the antioxidative action of DHBA may be attributed to free radical scavenging and/or chelation of transition metal ions catalyzing glucose autooxidation (Exner et al 2000).

Sulfate conjugation by phenolsulfotransferase enzyme is an important process in the detoxification of xenobiotics and endogenous compounds. Gentisic acid like other naturally occurring plant phenolics (p-coumaric acid, caffeic acid, ferulic acid) exhibits protective effects on acridine orange and ofloxacin induced genotoxicity in *Salmonella typhimurium* and the antimutagenic effects of these agents are considered to be due to their ability to scavenge reactive oxygen species produced by ofloxacin (Belicova et al 2001).

A lot of work has been done using skin as a model for understanding the process of carcinogenesis and its prevention. The present work also deals with the chemoprevention of two-stage skin carcinogenesis by the medicinal plants and their compounds. Since it is believed beyond doubt that promotion is the reversible stage and free radicals are implicated in not just this stage but also all the stages of cancer. An attempt has been made to evaluate the effectiveness of above-mentioned, medicinally useful plants and the naturally occurring chemical constituents against cancer formation. From the section 5.3 ‘Overview of cancer chemoprevention by some medicinal plants’, a conclusion was drawn that the plants owed their antitumor activity partially to anti-oxidant, anti-inflammatory and anti-proliferation activities. Therefore, the selection of plants and compounds to assess their potential in the amelioration of the genesis of cancer was based on their anti-oxidant and anti-inflammatory activities.