CHAPTER 1

INTRODUCTION
Prevention is a classical concept proclaimed from ages for any ailment or disease. This primary and old practice can be used against the second largest killer disease of the world - cancer.

Cancer arises from the abnormal and uncontrolled division of cells that have lost their regulatory mechanism of normal cell cycle. Transformation of normal cell into cancer cells may occur due to alteration in the genetic level or mutation in DNA that results in expression of genetically altered cell (Tomatis, 1990). Some of these cells may migrate through the bloodstream to neighbouring tissues or organs away from the site of origin and form fresh colonies which are called metastasis or secondary growth (Jaggi, 1990).

According to the cancer epidemiological estimate of the year 2000, ten million new cases of cancer has been recorded worldwide and 6 million people have died of cancer (Parkin, 2001). The absolute number of new cancer cases is rising rapidly due to growth in size of the population and increase in the period of life expectancy.

Several cohort studies on the mechanisms involved in the inception, progression and manifestation of cancer suggest that
it is a long time, complex process (Pitot and Dragon, 1994) and its causes are multiple and diverse which varies for different types of cancer (Rusch, 1954).

Cell division is a fundamental and well co-ordinated cyclic process of body. When population of cells acquires the ability to multiply and spread without the regulation of cell cycle, cancer develops. Oncologists studying the process of carcinogenesis have defined it as a multi-stage process ultimately leading to invasive cancer. The most acceptable theory is the three-stage mechanism of carcinogenesis, named initiation, promotion and progression (Wattenberg, 1997, Yuspa, 2000).

According to the report of WHO (1997) 80-90% of human cancers may be attributed to environmental factors, lifestyle and dietary habits. The major environmental factors can be categorized as physical, chemical and biological agents (Pustay, 1991).

The cells in the body undergo changes in the genetic level due to action of carcinogens and transform into initiated cells. They become less responsive to various growth factors and
programmed cell death, eventually forming neoplastic cells with continuous cell differentiation.

Among genetic factors, age, chromosomal aberration, immune deficiency etc. are implicated as causes of cancer (Jaggi, 1990). The long incubation period of cancer or long exposure to a carcinogen is co-related with ageing. Chromosomal damage and deformity from birth is characteristic of some forms of cancer, but a definite inter-relationship has not been established (Duesberg et al., 2001).

Reactive oxygen species (ROS) are generated within the body due to imbalance in the pro-oxidant-antioxidant status of the cells (Flyod, 1990; Henmani and Parihar, 1998). ROS is a collective term used by biologists to include not only oxygen radicals but also oxygen derivatives that contain unpaired electrons, for e.g., H_2O_2, OH. ROS are produced continuously in living cells as by-product of normal metabolism or during high temperature or radiation. Evidences have shown that controlled generations of these highly reactive metabolites are important for various cellular functions. However, their uncontrolled production is considered as an important factor in the etiology of cancer.
Skin is the primary barrier between cellular integrity and external environment. Continuous exposure of the skin to a variety of agents results in generation of reactive oxygen intermediates (ROIs) that leads to alterations in the skin which ultimately results in skin carcinogenesis (Diamond, 1984). Skin carcinogenesis is characterized by skin tumors known as papilloma; hence skin carcinogenesis is also termed as papillomagenesis. Initiation of skin cancer is brought about by a single exposure to carcinogen with a permanent genetic alteration such as mutation, which gives rise to epidermal tumors upon subsequent promotion by inflammatory skin irritants such as phorbol esters (Berenblum and Shubik, 1947; Slaga 1986). The mechanism of tumor is reversible and they activate or inhibit cytosolic enzymes, stimulate cell proliferation and induce cytotoxicity (Yuspa et al., 1996). This two-stage protocol of mouse skin carcinogenesis is widely used for mechanism involved in chemical carcinogenesis and future implications for control of the disease.

The war against cancer began 25 years ago with a hope to develop interventions to reduce incidence, risk, morbidity
and mortality due to cancer (Rimer, 2000). However, the continuing magnitude of the cancer problem and the failure of conventional therapeutic measure to control malignancy indicate that new approaches to the control of cancer are critically needed (Sporn, 1996). Re-evaluation of the basic knowledge about cancer paves the way for adoption of an alternative and rational strategy for the control of cancer. This has given rise to a new branch of cancer control research – ‘Chemoprevention’.

The term ‘Chemoprevention’ was put forwarded by Wattenberg (1985) and is defined as the administration of specific chemicals or constituents to reverse or suppress the process of carcinogenesis and prevent the development of invasive cancer (Hong and Sporn, 1997). The concept of chemoprevention was introduced by Michael B. Sporn (Greenwald, 1996).

Cancer prevention includes primary and secondary preventive measures (Murthy and Mathew, 2004). Primary prevention refers to avoid cancer causing substances in the environment or dietary elements associated risk factors and supplementation of protective agents. Secondary prevention aims at
early detection and removal of benign tumors (Murthy and Mathew, 2004)

Chemoprevention strategies can be employed in the initiation and the promotion stages of the carcinogenic process. Prevention at the initiation stage involves elimination of potential carcinogens, inhibition of metabolic activation of carcinogens, inactivation of ultimate carcinogens and blocking DNA binding site (Wattenberg, 1983, 1985; Wilkinson and Clapper, 1997).

The promotion stage provides greatest potential for intervention. This long term process can be easily modified and also it is reversible. (Wattenberg, 1983, 1985).

The agents capable of protecting cell against neoplastic development are called chemopreventive agents. In recent years, administrations of non-nutrients and nutrients have been extensively studied for possible chemopreventive role (Boone et al., 1999; Rao, 2004). The chemopreventive agents may not only vary in their composition but also in their mechanism of action. An ideal chemopreventive agent may have the following characteristics:-
- Negligible toxic effect
- High efficacy
- Capable of oral administration
- Known mechanism of action
- Low cost

On the basis of mechanism of action, there are three major types of chemopreventive agents, such as

(i) Inhibitors of carcinogen formation:

These agents prevent the formation of active carcinogens from pro-carcinogens (e.g., ascorbic acid)

(ii) Blocking agents:

They enhance the metabolic activation of carcinogen and prevent the active carcinogen from reaching the target tissue by sequestering them (e.g., indole-3-carbinol).
(iii) Suppressing agents:

They inhibit carcinogenesis at the cellular level by interrupting at different steps in their metabolic pathway required for the development of tumor (e.g., retinoids).

Thus, intervention with the help of chemopreventives is either to prevent the formation of ultimate carcinogens from pro-carcinogens or block the binding of carcinogens to cellular targets (Wilkinson and Clapper, 1997). There are two basic facts that contribute to the chemopreventive strategy. Firstly, most of the environmental xenobiotics are hydrophobic in nature and can be easily excreted out from the body. Secondly, the body possesses an extensive system of xenobiotic metabolising enzymes, which help in the detoxification of these harmful chemicals (Miller and Miller, 1979).

Liver is the principal organ of the body where bulk of xenobiotic metabolism takes place since the xenobiotic metabolising enzyme systems are extensively found in liver (Athar et al., 1997). Williams (1959) categorize these enzyme systems into two types according to their mechanism of action.
They are Phase I enzymes, characteristically the most important one being Cyt P$_{450}$ associated with Cyt b$_5$ which participate in hydrolysis, oxidation or reduction of the substrates (xenobiotics). The Phase II enzymes, comprising of GST family acts on the Phase I metabolites and transform them to excretable products. The transformation of substrate by Phase I enzymes may result in both inactive as well as active carcinogens. The abundance of Phase II enzymes determine the fate of ultimate carcinogens, whether they will bind to DNA or they will be excreted. So, a balance between Phase I and Phase II enzymes is essential to mitigate the carcinogenic process (Wilkinson and Clapper, 1997). Chemopreventive agents judged to have potential to induce either or both Phase I and Phase II enzymes are considered as effective in chemopreventive strategy (Wattenberg, 1992a,b; Tanaka et al., 2001).

Our body posses an exclusive amount of cellular scavengers which can protect cells from the effect of free radicals. The enzyme family comprises reduced glutathione (GSH), glutathione peroxidase (GP$_x$), glutathione reductase
(GR), superoxide dismutase (SOD) and catalase (CAT) (Forni and Wilson, 1983).

GSH is an endogenous cellular thiol with diverse functions such as maintenance of intracellular redox state, detoxification of peroxides, free radicals and electrophiles. GPx is regarded as the primary anti-oxidant as it functions directly in the destruction of $\mathrm{H}_2\mathrm{O}_2$ produced in cells. When GPx is saturated with the substrate, CAT acts on it. Chemopreventive agents having anti-oxidant property act by virtue of its induction of specific activities of these anti-oxidant enzymes which can be measured by a concomitant decrease in the LPO level of the cells (Geetami et al., 1989).

Among the potential chemopreventive agents known, a good number of them are present in our diet and also are of plant origin. Food derived products are regarded as highly efficient for development as chemopreventive agent because they are safe and are not perceived as medicine. Numerous diet derived compounds are among 40 promising agents which are
on clinical trials as chemopreventive agents for major cancer targets (Kelloff et al., 2000).

Dietary nutrients and non-nutrients act by modulation of expression of xenobiotic metabolising enzymes, anti-oxidant enzymes or act as an anti-inflammatory or anti-tumor promoting agent. The chemopreventive agents either scavenge pro-carcinogens, act as blocking agents that can decrease the risk of cancer or may exert their role as suppressing agents. It is, therefore, essential to identify dietary agents that are non-toxic, consumable and would form a part of life style for the prevention of cancer.

Numerous naturally occurring phytochemicals are effective against the carcinogenesis process (Hansen, 2000; Greenwald, 2002). These natural compounds are important in the production of chemopreventive agents (Pezzutto, 1997) which acts as anti mutagens and inhibit one or more stages of carcinogenesis (Sultana et al., 2003).

Many scientific evidences from epidemiological studies in the past two decades provide valuable information

Thus, fruits, vegetables and several herbs with diverse pharmacological property are potential chemopreventive agents (Ames, 1983; Alam, 2000) and is a promising area of cancer control research (Rimer, 2000). The present piece of work was undertaken to study the chemopreventive potential of a fruit (Syzygium cumuni, Skeels ), a vegetable (Moringa oleifera, Lam.) and a medicinal plant (Phyllanthus urinaria, Linn.) commonly found in North-Eastern region of India.

_S. cumuni_ is a common citrus fruit containing several anti-oxidant compounds (Korima and Afanea’av, 1997) which are used in Ayurvedic medicines (Nair and Santhakumar, 1986; Warrier et al., 1996).

_M. oleifera_ is a rich source of vitamin A and C (Dhar and Gupta, 1982). The drumsticks are specifically rich in
carotene that is converted into vitamin A in the body which has significant hepatoprotective effect (Geervani and Devi; 1981).

*P. urinaria* is effective in stimulating sluggish liver and is used for regeneration of liver tissue in jaundice (Nadkarni, 1976). It is also employed as a healing agent against skin diseases (Nadkarni, 1954; Chunneker, 1982).

In the present study the chemopreventive property of *S. cumuni, M. oleifera* and *P. urinaria* have been evaluated by virtue of modulation of hepatic bio-transformation enzymes, anti-oxidant enzymes and LPO levels. The two stage skin papillomagenesis study has been used as a model to quantitate the chemopreventive response.

Thus, the objectives of the present investigation are:

**Experiment I**: To study the modulatory influence of *S. cumuni, M. oleifera* and *P. urinaria* on

(i) Phase I enzymes i.e.,
   - Cytochrome P<sub>450</sub> (Cyt P<sub>450</sub>) and Cytochrome b<sub>5</sub> (Cyt b<sub>5</sub>)

(ii) Phase II enzymes i.e., Glutathione-S transferase (GST)

(iii) Glutathione content (GSH)
(iv) Anti-oxidant enzyme profiles i.e.,

Glutathione peroxidase (GPx)
Glutathione reductase (GR)
Superoxide dismutase (SOD)
Catalase (CAT) and

Lipid Peroxidation (LPO) in Swiss albino mice.

**Experiment II:** To study the modulatory influence of *S. cumuni, M. oleifera* and *P. urinaria* on 7, 12-dimethylbenz(a)anthracene (DMBA)-induced two stage model of skin carcinogenesis in Swiss albino mice.