CHAPTER 1

INTRODUCTION
Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullaev et al., 2000). After decades of research, it still remains a major cause of morbidity and mortality. Cancer is one of the major problems of modern medicine; it is also a fascinating biological problem. In biological terms, it is the manifestation of changes in one of the more general properties of the cells, their ability to adjust their growth rate to the architectural requirements of the organism. A cancer arises from a single cell that undergoes permanent hereditary changes and consequently multiplies, giving rise to billions of similarly altered cells (Purohit, 2003).

Skin cancer is the most common type of cancer in the United States, with more than a million reported cases and 9000 deaths per year. Increasing incidence of these cancers due to constant exposure of skin to environmental carcinogens, including both chemical agents and ultraviolet radiation, provides a strong basis for chemoprevention with synthetic and natural, internal and topical remedies (Gupta and Mukhtar, 2002).

Carcinogenesis in mouse skin can be divided into three distinct stages: initiation, promotion and progression. Initiation, induced by a single exposure to a genotoxic carcinogen, can result from a mutation in a single critical
gene, apparently in only a few epidermal cells and the change is irreversible. Promotion, resulting in the development of numerous benign tumors (papillomas), is accomplished by the repeated application of a non mutagenic tumor promoter. The effects of single application of tumor promoters are reversible since papillomas do not develop after insufficient exposure of initiated skin promoters or when the interval between individual promoter applications is increased sufficiently. Promoter treatment provides an environment that allows the selective clonal expansion of foci of initiated cells. The third stage known as progression shows self sustained growth, reduced nutritional requirement and metastasis involving sequential genetic alterations (Hennings et al., 1993).

As carcinogenesis is a long term process and as researches have suggested that cancer is an avoidable disease, different ways have developed to reduce the risk of cancer. One such growing wing is chemoprevention, a process which aims to modulate specific steps in the carcinogenic process. It can be defined as one or several compounds in combination that prevents the initiational and promotional events that occur during neoplastic development (Wattenberg, 1997). Skin cancer chemoprevention is a useful model for cancer chemoprevention in general (Richmond and Viner, 2003).
Particularly, the two stage mouse skin carcinogenesis model is widely used for studies on mechanisms involved in chemical carcinogenesis. It has also been employed to quantitate the chemopreventive response of a wide variety of both naturally occurring and synthetic compounds. One sub minimal dose of carcinogen [7, 12 dimethyl benz (a) anthracene] initiates tumorigenesis and repeated application of the promoter [croton oil] promotes them to visible tumor stage (Berenblum and Shubhik, 1947). The process of promotion may involve the genetic expression of modified regions of DNA in the cells altered critically by initiating agents followed by proliferation of these cells into visible tumors (Boutwell, 1964).

The naturally occurring as well as synthetic compounds employed for chemoprevention are generally termed as ‘chemopreventive agents’ and are divided into three groups depending on whether or not the compound is perceived to act before or after the mutagenic step of carcinogenesis process (Wattenberg, 1985)(Chart I).
**Chart-I** : Classification of chemopreventive agents on the basis of the time at which they exert their protective effects (Wattenberg, 1985)
The first category consists of compounds that prevent the formation of carcinogens from precursor substances. The second category of compounds inhibit the carcinogenesis by preventing carcinogenic compounds from reaching or reacting with critical target sites in the tissues and are referred to as blocking agents. Blocking agents are further subdivided depending on their ability to regulate the expression of phase I and phase II enzymes. Those inducers that are capable of inducing both phase I and phase II enzymes are called bifunctional inducers and those that can increase the expression of only phase II enzymes are called monofunctional inducers. The compounds which are capable of inducing the phase II enzymes and inhibit the expression of phase I enzymes are referred to as dual acting agents (Wattenberg, 1997; Manson et al., 1998). The third category of compounds acts subsequent to the exposure to carcinogenic agents and are termed as suppressing agents. They basically suppress the expression of neoplasia in cells previously exposed to a carcinogenic agent that might cause cancer (Wattenberg, 1997; Haverd et al., 1998).

Results from various epidemiological studies reveal that 80-90% of all cancers are caused by environmental factors and chemicals, either naturally
or synthetically (Higginson and Muir, 1973). Often not correctly appreciated the skin covers an enormous surface area of 1.5-2.0 m² that is constantly exposed to a variety of physical and chemical insults making it the most accessible organ to environmental contaminants (Gallagher et al., 1996; Green et al., 1999). Exposure to various xenobiotics, both exogenous and endogenous have also resulted in increasing episode of skin related occupational health hazards that also results in skin cancer (Rockley et al., 1994). The exogenous factors associated with cancer are physical, chemical and biological agents present in the environment. The various physical agents include natural radiation, chronic sunlight exposure, X-rays, UV rays; ionization radiation etc. Physical agents also include the chemicals which are the predominant form of cancer causing agents. Chemical carcinogens are of extremely diverse structure and include both natural and synthetic products. Besides the chemical carcinogens, diet and nutrition as a lifestyle is thought to play more of a role in the origin and promotion of various cancers (Wallaszeck et al, 2004). Smoking, alcoholic beverages, consumption of tobacco and its products, chewing of betel quid etc are associated with higher incidence of cancer (Rao, 1996). One of the other endogenous agents is the Reactive Oxygen Intermediates (ROI) such as superoxide anions, hydrogen peroxides, hydroxyl
radical etc which are electrophilic in nature and are generated during the cellular processes of the body (Halliwell and Gutteridge, 1989). Formation of free radicals is not limited to normal cellular processes but also occur upon exposure to certain chemicals (polycyclic aromatic hydrocarbons, cadmium, lead etc.), radiation, cigarette smoke, and high fat diet. Exposure of a healthy cell to free radicals is known to damage structures and consequently interfere with functions of enzymes and critical macromolecules. A balance between formation of free radicals and their detoxification is essential for normal cellular function. When such a balance is disrupted as a result of excessive generation of damaging reactive oxygen species or low levels of antioxidants, a cell enter into a state of genetic instability that can lead to chronic diseases including cancer (Frinkel and Holbrook, 2000).

The human body is exposed to a wide array of xenobiotics in one's lifetime, from food components to environmental toxins to pharmaceuticals and has developed complex enzymatic mechanism to detoxify these substances (Liska, 1998). Liver is the main organ responsible for drug metabolism and appears to be a sensitive target site for substances modulating biotransformation (Gram and Gillette, 1971). The endoplasmic reticulum of
liver contains a set of enzymes known as microsomal mixed function oxidase (MFO) which is involved in the metabolism of xenobiotics. These xenobiotic enzymes can be categorized into two types—Phase I enzymes and Phase II enzymes (Williams, 1971). The phase I enzymes (Cytochrome P₄₅₀ and Cytochrome b₅) begin the process of biotransformation by oxidizing, reducing or hydrolyzing toxins, creating biotransformed intermediates and the Phase II enzymes perform conjugation reactions which help to convert the biotransformed intermediates from phase I to less toxic water soluble substances that are easily excreted or eliminated from the body (Percival, 1997).

Cytochrome P₄₅₀ and cytochrome b₅ form an important component of the phase I metabolism. The cyt P₄₅₀s are heme proteins and represent a multigene family of isoenzymes involved in oxidative biotranformation of lipid soluble compounds to polar metabolites (Ramachandran et al., 1999). Cytochrome b₅ is a microsomal enzyme which has been reported to form a complex with cyt P₄₅₀ (Tamburini et al., 1985) and enhances the substrate turnover of cyt P₄₅₀ (Jansson et al., 1985).

Glutathione S-transferase (GST) forms an important component of phase II metabolism and it is thought to play a physiological role in initiating
the detoxification of many alkylating agents (Wood, 1970) and environmental chemicals including mutagens and carcinogens. Their main function is the conjugation of GSH to a variety of electrophilic compounds. GST reduces the covalent binding of epoxides of carcinogens with DNA and other macromolecules and this reduction in DNA binding was found to be effective in decreasing carcinogenesis caused by the carcinogens (Kramer et al., 1988).

The effect of free radicals can be mitigated by its cellular scavengers such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) (Forni and Wilson, 1983).

Glutathione often regarded as the first line of defense against oxidative stress, is the most important cellular thiol that acts as a substrate for several transferases, peroxidases and other enzymes that prevent the deleterious effects of free radicals (Thiele et al., 2001). The multiple physiological and metabolic function of GSH includes thiol transfer reactions that protect cell membranes and proteins. GSH participates in reactions that destroy hydrogen peroxide, organic peroxides, free radicals and certain foreign compounds. The apoptotic processes in cells are often associated with decreased levels of GSH due to increased efflux of this antioxidant from cells (Rana et al., 2002). Glutathione also function as an antioxidant by promoting formation of
reduced forms of other antioxidants such as ascorbic acid and detoxification of xenobiotics, carcinogens and free radicals and maintenance of immune functions (Ormeius and Moldeus, 1994).

SOD is a metalloprotein that is detected in a large number of tissues and organs and is thought to protect cells from damage by superoxide radical (Han et al., 1981). As such, it is an important antioxidant defense in nearly all cells exposed to oxygen (Bandyapadhyay et al., 1999).

Catalase is a major peroxisomal enzyme which is involved in a number of important cellular processes including detoxification of $H_2O_2$ produced by dismutase reaction (Alberts et al., 2002).

Epidemiological studies have indicated that the risk of cancer may be modified by changes in dietary habits (Prochaska, 1997). Epidemiological and animal model studies have indicated that cancer risk may be influenced by dietary factors (Kraemer et al., 1988; Doll, 1992; Wattenberg, 1992). Recent studies have indicated that compounds with antioxidant or anti-inflammatory properties as well as certain phytochemicals can inhibit tumor initiation, promotion and progression in experimental animal models (Perchellet and Perchellet, 1989; Chesson and Collins, 1997). Several experimental evidences for all common cancer sites have indicated that intake of fruits and vegetables
and a number of other dietary items are associated with decreased cancer incidence (Morse and Stoner, 1996). These potential agents can either abolish or delay the development of cancer by interfering with one or more steps in the process of carcinogenesis such as preventing the activation of carcinogen, by increasing detoxification or by blocking the interaction of ultimate carcinogen with cellular macromolecules, or by suppressing the clonal expansion of neoplastic cells (Tanaka, 1994). Many of the flavonoid molecules found in fruits, vegetables and herbs are multifunctional inducers and are shown to induce several phase II enzymes while decreasing phase I activity (Manson et al., 1997). In general the increase in phase II supports better detoxification in an individual and helps to promote and maintain a healthy balance between phase I and phase II activities. The enhancement of phase II activity has been proposed to explain, at least in part, the ability of fruits, vegetables and herbs to protect against many cancers (Guengerich, 1984; Smith and Yang, 1994; Elangovan et al., 1994; Park, 1996; Manson et al., 1997).

Presently, screening of edible plant products which can reduce the risk of cancer has become the frontier area of chemoprevention research (Slaga, 1980). Plant derived products for development as chemopreventive agents may find long term use in population at normal risk because of their
safety. In fact, a number of diet-related compounds as well as synthetic compounds have been identified and evaluated as chemopreventive agents for major cancers including breast, prostate, colon and lungs (Kelloff, 2000; Gusting and Brenner, 2002). The exogenous supplementation of the antioxidants in the form of natural or synthetic source have been successful in providing protection against skin carcinogenesis (Perchellet and Perchellet, 1989; Mukhtar and Agarwal, 1996; Bicker and Athar, 2000). In fact, a great number of epidemiological studies of the relationship between food and cancer, together with the research in experimental models, have demonstrated that daily ingestion of some plants or plant products could undoubtedly contribute to cancer prevention (Murakami et al., 1994). Therefore, a promising area of cancer control research is chemopreventive studies with botanicals and evaluation of chemopreventive properties of locally available plants or plant products that could be easily incorporated in the diet and thereby offer protection from the development of carcinogenesis. Thus, the aim of the present study is to assess the chemopreventive potential of the medicinal herb, *Eclipta alba* Linn. belonging to the family Asteraceae. The plant is found throughout India up to 2000 meters on the hills and is used as tonic and deobstruent in hepatic and spleen enlargements (Kirtikar and Basu, 1981;
The tender leaves and young shoots are cooked and used as a vegetable (Tanaka, 1976; Reid, 1977; Kunkel, 1984; Chevallier, 1996). It is used internally in the treatment of dropsy, liver complaints, anaemia, diphtheria (Brown, 1995), tooth loss and premature greying of hair (Yeung, 1985). Externally it is applied to athlete’s foot, eczema, dermatitis, wounds etc (Brown, 1995; Chevallier, 1996). The leaves are also used for the treatment of scorpion stings and as an antidote for snake bites (Khare, 2004).

So, in the present study, the chemopreventive potential of the herb Eclipta alba have been investigated in the two stage skin carcinogenesis model and in the biotransformation process of carcinogenesis by choosing xenobiotic metabolizing enzymes and the antioxidant enzymes of the liver as biomarkers in female Swiss albino mice.

The objectives of the present study are as follows:

Experiment I (A): To study the modulator influence of Eclipta alba on 7,12-dimethylbenz (a) anthracene (DMBA) induced skin papillomagenesis in female Swiss Albino mice.

The following parameters were observed to study the influence of Eclipta alba on mouse skin carcinogenesis:

1. Tumor incidence (percentage of papilloma bearing mice)
2. Tumor yield (average number of papillomas per mouse)
3. Tumor burden (papillomas per papilloma bearing mouse)
4. Cumulative number of papillomas

5. Percent inhibition of tumor multiplicity

Experiment I (B): To study the effect of *Eclipta alba* extract on the dorsal skin of the control and treated mice in comparison to the normal skin tissue.

Experiment II: To study the modulatory influence of *Eclipta alba* Linn on

1. Phase I enzymes i.e.,
   - Cytochrome P\textsubscript{450} (Cyt P\textsubscript{450})
   - Cytochrome b\textsubscript{5} (Cyt b\textsubscript{5})

2. Phase II enzymes i.e., Glutathione S transferase

3. Glutathione content (GSH)

4. Antioxidant enzyme profiles i.e.,
   - Superoxide Dismutase (SOD)
   - Catalase (CAT) and
   - Lipid Peroxidation (LPO) in female Swiss albino mice.