

Review of Literature

The review of literature pertaining to the study “**Antioxidant and Antitumor Potential of Roots of *Coleus forskohlii* in Balb/c mice With DLA Tumor**” is discussed under the following headings:

- 2.1 Cancer and medicinal plants
- 2.2 Medicinal plants in modern medicine
- 2.3 *Coleus forskohlii* –Species status
- 2.4 Mechanism of action of Forskolin
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Three decades ago, only a few had any appreciation of the number of remedies that had their origin from herbal medicine, and most had vague knowledge of herbal medicine, traditional medicine or other forms of complementary and alternative medical practices (Lipp, 1996). For a variety of reasons, more individuals nowadays prefer to take personal control over their

health with the use of herbal medicines, not only to prevent diseases but also to treat them. This is particularly true of a wide variety of illnesses readily treated at home. Herbal products are also commonly used by patients with certain chronic medical conditions, including breast cancer (12%) (Burstein, 1999), liver disease (21%) (Strader, 2002), human immunodeficiency virus (22%), (Kassler, 1991), asthma (24%) (Blanc, 2001) and rheumatological disorders (26%) (Rao, 1999).

The acceptance and the recognition of herbal medicine has been in part due to the acknowledgement of the value of traditional and indigenous pharmacopoeias, the incorporation of some medicines derived from these sources into pharmaceuticals (Desmet *et al.*, 1992; Winslow and Kroll, 1998), the need to make health care affordable for all and the perception that pharmaceutical drugs are increasingly overprescribed, expensive and even dangerous. Another important perception fomenting this interest is that natural remedies are somehow safer and more efficacious than remedies that are pharmaceutically derived (Bateman *et al.*, 1998; Murphy, 1999).

2.1 Cancer and medicinal plants

Cancer is a disease that is principally characterized by the loss of genetic control over cell growth and proliferation. In this sense, it is a genetic disease. Tumors of hereditary origin, however are in a vast minority and the factors that cause the process of tumoral genesis are mainly environmental largely related to lifestyle (Carlos *et al.*, 2006).

Cancer is a major public health burden in both the developed and the developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths and 24.6 million persons living with cancer around the world in 2002. Cancer is the second leading cause of death. The increasing magnitude of cancer mortality throughout the world, claiming over six million lives each

year and the failure of conventional chemotherapy of advanced invasive disease to effect major reductions in cancer mortality rates indicate that new approaches are critically needed for the control of cancer. In this context, it is essential to adopt a more intensive and imaginative approach towards prevention of this disease (Sengupta, 2004).

Chemotherapy is now employed in the primary treatment or as an adjuvant or important modality in the management of neoplasm. However, the effective doses of most of the chemotherapeutic agents fall in the range of toxic dose. They are highly reactive and are capable of inducing varying degrees of cell destruction and this leads to unpleasant side effects while undergoing treatment. Hence, search for new antitumor agents with high chemotherapeutic value to fight against cancer is a medical priority. The antitumor agents must be able to kill or inactivate tumor cells without damaging normal tissues. It has been well recognized that allopathic drugs are not without dangers as they exhibit severe toxicity on normal tissues. Therefore worldwide research is going on to find out the best effective antitumor agents from different sources. Recent pharmacological researches revolve around the urgency to evolve suitable chemotherapeutic agents for the treatment of tumors (benign and malignant) without having toxic effects (Pandey and Madhuri, 2006).

Going through the evolution of the cancer chemotherapeutic agents, one could expect to obtain a lead molecule through any one of the drug design methods. One of the potential sources of leads could be the plant kingdom. Plant products have been a source of medicinal agents since time immemorial. From the dawn of civilization, man has been utilizing the important biological properties of various plants for treatment of different diseases. Even today, the plants are the most exclusive source of drugs for the majority of world's population and plant products constitute 25% of prescribed medicines.

The WHO has defined traditional medicine as “the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or writing”. All traditional medicines have their roots in folk medicines and household remedies. WHO has listed 20,000 medicinal plants used in different parts of the world (Arjun *et al.*, 2010). The indigenous system of medicine has several medicinal plants with versatile antitumor properties that need detailed research for the development of antitumor herbal drugs. The contribution of ethnomedicinal plants in discovering new drugs has been enormous for treating disease like cancer.

Initially, the materials employed in these traditional medicines were almost of botanical origin. Several chemotherapeutic agents have been developed in the modern system of medicine as a result of the screening of the medicinal plants in various parts of the world. The isolation of biologically active alkaloids such as atropine, quinine, serpentine, reserpine, narcotine, caffeine, nicotine etc., is the result of the initial leads obtained from the traditional system of medicine (Radha *et al.*, 2008).

2.2 Medicinal plants in modern medicine

India is the largest producer of medicinal plants and is rightly called the “Botanical garden of the World”. Medical information referred in the old Indian literature includes several medicinal herbs, which have been in use for thousands of years, in one form or the other. Under the indigenous system of medicine in India, 45,000 plant species have been identified, out of which about 15-20 thousand plants are of good medicinal value. However, traditional communities use only about 7000-7500 plants for medicinal purposes. Only a few medicinal

plants have attracted the interest of scientists, for investigation so as to find a remedy for tumor (Madhuri and Pandey, 2008).

The existence of traditional medicine depends on plant diversity and the related knowledge of their use as herbal medicine. India is one of the twelve mega diversity hot spot regions of the world and one fifth of all the plants found in India are used for medicinal purpose (Schippmann *et al.*, 2002). Both plant species and traditional knowledge are important for the herbal medicine trade and the pharmaceutical industry whereby plants provide raw materials and the traditional knowledge, prerequisite information (Tabuti *et al.*, 2003). Encompassing concepts and methods for the protection and restoration of health, traditional medicine has served as source of alternative medicine, new pharmaceuticals and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic drugs or as models for pharmacologically active compounds (Mukerjee, 2003). The world market for plant derived chemicals *viz.*, pharmaceuticals, fragrances, flavours and colour ingredients exceeds several billion dollars per year. Classic examples of phytochemicals in biology and medicine include taxol, vincristine, vinblastine, colchicine as well as the chinese antimalarial artemisinin and the Indian Ayurvedic drug – forskolin. More than 50% of all the modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to induce apoptosis in various tumor cells (Rosangkima and Prasad, 2004).

Many drugs of modern medicine have had their origin in traditional medicine. Some common examples include the discovery of the alkaloid diosgenin in *Dioscorea deltoidea* used as source for the partial synthesis of cortisone and steroid hormones in the forties. The discovery of the hypotensive alkaloid ephedrine from *Ephdria sinica* and the anticancer alkaloid

podophyllotoxin in *Podophyllum hexandrum* in the sixties etc. are of great importance.

About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health organization, 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of natural drugs obtained from plants are digoxin from *Digitalis* species, quinine and quinidine from *Chinchona* species, vincristin, vinblastine, morphine and codeine from *Papaver somniferum*. It is estimated that 60% of antitumour and antiinfectious drugs are already in the market or under clinical trial. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds allowing the design and the rational planning of new drugs. Biomimetic synthesis, development and the discovery of new therapeutic properties have not yet been attributed to known compounds. In addition, compounds obtained from plants such as muscrine, physostigmine, cannabinoids, forskolin, colchicines and phorbol ester are important tools used in pharmacological, physiological and biochemical studies (Williamson *et al.*, 1996).

2.3 *Coleus forskohlii* –Species status

Coleus forskohlii Briq. is a member of the mint family, Lamiaceae. It is indigenous to India and is recorded in Ayurvedic Materia Medica under the Sanskrit name “Makandi” and ‘Mayani’. The taxonomic position of *Coleus forskohlii* is as follows:

Kingdom - Plantae

Division - Magnolophytoa

Class - Magnolipsida

Order - Lamiales

Family - Lamiaceae

Genus - Coleus

Species - forskohlii

The genus Coleus was first described by Loureiro in 1790 and the generic name was derived from the Greek word 'COLEOS' meaning sheath. The species name Forskohlii was given to commemorate the Finnish botanist, Forskel. The genus Coleus consists of 150 species and the following species viz., *Coleus amboinicus*, *Coleus forskohlii*, *Coleus spicatus* and *Coleus malabaricus* occur naturally.

The root is typically golden brown, thick, fibrous and radially spreading. Roots are tuberous, fasciculated, 20cm long and 0.5 to 2.5 cm in diameter, conical fusiform, straight orangish within and strongly aromatic. The leaves and tubers have quite different odours. However, the growth habit of *Coleus forskohlii* is strikingly variable, being erect, procumbent or decumbent. Similarly, the root morphology in different populations is also fascinatingly diverse, being tuberous, semi tuberous or fibrous.

Medicinal uses of *Coleus forskohlii*

In India, the major medicinal species of Coleus is the tuberous *C.forskohlii*. *C.amboinicus*, *C. blumei*, *C.malabaricus* and *C.scutellaroides* are the other species and are mainly used to treat dysentery and digestive disorders. *C. forskohlii* is widely used in different countries for various ailments. In Egypt and Africa, the leaf is used as an expectorant, emmenagogue and diuretic. In Brazil, it is used as a stomach aid and in treating intestinal disorders. It is used as

a condiment in India and the tubers are prepared as pickle and eaten. In traditional ayurvedic systems of medicine, *C.forskohlii* has been used for treating heart diseases, abdominal colic, respiratory disorder, insomnia, convulsions, asthma, bronchitis, intestinal disorders, burning sensations, constipation, epilepsy and angina. The roots are also used in the treatment of worms and to alleviate burning in festering boils (Kavitha *et al.*, 2010).

2.4 Mechanism of action of Forskolin

Forskolin being the major chemical constituent of the roots and the tubers, herbal preparations of it act on various multiple pharmacological mechanisms. The blood pressure lowering and antispasmodic effects of extracts of *Coleus forskohlii* roots were reported by Dubey *et al.* (1974) based on the extensive screening of Indian plants for biological activity at the Central Drug Research Institute, Lucknow. De Souza (1977) found that the methanol extract from the root tuber was helpful in lowering blood pressure and positive inotropic activities in animal models. Singh and Tandon (1982) compared physico-chemical properties of coleonol, forskolin and their derivatives and reported that the two compounds did not have the same structure and were stereoisomer that is they differed only in the configuration of acetate group at Carbon 7. In forskolin, it was β while in coleonol it was α . The pharmacological studies of forskolin and coleonol indicated that they had identical properties (Seamon and Daly, 1981).

The principle mechanism by which forskolin exerts its hypotensive activity is by stimulation of adenylate cyclase and thereby increasing cellular concentrations of the second messenger cyclic AMP (Seamon *et al.*, 1981). Forskolin directly activates almost all hormone sensitive adenylate cyclases in intact cells, tissues even solubilized preparations of adenylate cyclase (Metzger and Lidner, 1981). The unique feature of this activation is that the site of action for forskolin is the catalytic subunit of the enzyme or a closely associated protein

(Seamon and Daly, 1981). Of the 9 types of adenylate cyclase in humans, forskolin can activate all except type 1X which is found in spermatozoa (Iwatsubo *et al.*, 2003). Stimulation of adenylate cyclase is thought to be the mechanism by which forskolin relaxes a variety of smooth muscles. This action of forskolin has proved the potential use of the molecule, not only as an invaluable research tool for understanding cyclic-AMP dependent physiological processes, but also as a potential therapeutic agent for diseases like cardiac insufficiency, hypertension, glaucoma, thrombosis, asthma and metastatic conditions.

Forskolin, by increasing cAMP level in turn, inhibits basophil and mast cell degranulation and histamine release (Marone *et al.*, 1987), lowers blood pressure (Dubey *et al.*, 1981) and intraocular pressure (Caprioli *et al.*, 1984), inhibits platelet aggregation (Agarwal and Parks, 1983; Wong, 1993), promotes vasodilation (Dubey *et al.*, 1981; Wysham *et al.*, 1986), bronchodilation (Lichey *et al.*, 1984) and thyroid hormone secretion and stimulates lipolysis in fat cells (Haye *et al.*, 1985; Roger *et al.*, 1987).

Forskolin has a unique property of activating almost all the hormone sensitive adenylate cyclase enzymes in a biological system. Forskolin is reported to be useful in the treatment of congestive heart failure, glaucoma, asthma and certain types of cancers. It is an important compound used against various disorders in indigenous systems of medicine such as anti-aging, antioxidant as a remedy for heart, abdominal and respiratory disorders. In addition it has been shown to have anti-inflammatory property. The total synthesis of forskolin has been reported to be uneconomical because of structural complexity of the molecule and the synthetic forskolin is not as effective as that which has been produced from natural source.

2.5 Plant derived anticancer agents

The antitumor or antineoplastic activities of several medicinal plants have been reported by various authors.

Many of these plants include *Abrus precatorious*, *Aglaia roxburghiana*, *Cassia fistula*, *Catharanthus roseus* (Vinca rosea, sadabahar), *Crocus sativus* (Saffron), *Ervatamia heyneana*, *Hygropilia spinosa* (Talmakhana), *Hippocratea murcantha*, *Indigofera mysorensis*, *Ocimum sanctum* (Tulsi), *Olea polygama*, *Plumbago rosea* (Chitra), *Podophyllum hexandrum*, *Semecarpus anacardium* (Bhela), *Solanum dulcamara*, *S. indicum* (Brahanta), *S.khasianum*, *S,surattense* (Kateli), *Terminalia arjuna* (Arjuna), *Trigonella foenumgraecum*(Methi), *Vanda parviflora*, *Wedelia calendulacea* (Pila bhanra), *Withania somnifera* (Ashwagandha) and *Zingiber capitatum*.

Anticancer drug is one area of modern therapeutics, which has drawn profoundly on compounds of nature, both for use and as platforms for developing more efficacious molecules. Even at present, there is considerable activity in the search for new leads and scaffolds.

Pacitaxel, a diterpenoid was first isolated as anticancer compound from the bark of the tree *Taxus brevifolia* Nutt. in 1996, and after several studies and trials, the compound was marketed as an anticancer drug in 1993 by Bristol-Myers Squibb. This lead compound was followed by the marketing of docetaxel, a taxane based compound in 1995. Both the compounds are being used for treating cancers of ovary, breast and lungs besides some other malignancies (Geffen and Man, 2002). Another important contribution in this area involves drugs developed on camptothecin framewok. Camptothecine, a quinoline alkaloid with anticancer activity was first isolated from the Chinese tree *Camptotheca acuminata*, but now it is mostly obtained from the Indian tree *Nothapodytes*

nimmoniana. Irenotecan (Garcia, 2002; Zamboni, 2009) is the first camptothecin-based drug targeting solid tumors. Since then, two more drugs of this class have been launched in the market. Topotecan, a water soluble derivative of camptothecin with decreased toxicity, is being used as second - line treatment of ovarian cancer. Belotecan has been specially evaluated against small cell lung cancer and recurrent ovarian cancer. At least, six other compounds of this class are being clinically evaluated.

2.6 Acute toxicity studies

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products especially those derived from the plants. This interest in drugs of plant origin is due to several reasons; namely conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and or incorrect use of synthetic drugs resulting in side effects and other problems. A large percentage of the world's population does not have access to conventional pharmacological treatment and folk medicine and ecological awareness suggest that natural products are harmless. However, the use of these substances is not always authorized by legal authorities dealing with efficacy and safety procedures and many published papers point to the lack of quality in the production trade and prescription of phytomedicinal products.

Thus in the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs have led to an increase in the number of investigations in this field (Rates, 2001) .

Acute toxicity is usually defined as the adverse changes occurring immediately or in a short time following a single or short period of exposure to a

substance or substances or as adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hr. An adverse effect is “any effect that results in function impairment and /or biochemical lesions that may affect the performance of the whole organism or that reduces the organ’s ability to respond to an additional challenge.” Consequently, a chemical that enters the organism *via* the oral route during a restricted time and produces any adverse effect with little delay is orally and acutely toxic. However, the term acute oral toxicity is most often used in connection to lethality and LD₅₀ determinations.

Studies of acute systemic toxicity attempt to determine the dose dependent adverse effect that may occur and various appropriate data may be collected when determining the comprehensive acute toxicity profile of a substance. This may include the incidence of lethality. It has been claimed that when properly performed and closely observed, an acute toxicity test can give more information about the biologic properties of chemical compound than any other single test (Paget, 1983).

In screening drugs, determination of LD₅₀ (the dose which has proved to be lethal, causing death to 50% of the tested group of animals) is usually an initial step in the assessment and the evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations (providing information on health hazards likely to arise from short term exposure to drugs) and is one of the initial screening experiments performed with all compounds.

If the dose dependent lethality indices are determined in a precise manner, it is usually expressed as LD₅₀. This is defined as the statistically derived dose when administered in an acute toxicity test is expected to cause death in 50% of the treated animals in a given period (Oliver, 1986).

The test substance may be given at one of the four fixed dose levels (5, 50, 500 and 2000mg/kg) to animals. The objective is to find the dose that produces clear signs of toxicity but no mortality (Tamborini, 1990).

The absolute LD₅₀ value for a compound varies among different laboratories and these variations have been attributed to differences in protocol details, animal strains caging and test chemical source.

The acute toxicity of the aqueous extract of the roots of *Hygrophilia auriculata* was evaluated by administering the extract orally to different groups at the dose level of 250, 500, 1000, 2000 mg/kg body weight. All animals were observed for toxic symptoms and mortality for 72 hours. No mortality was observed upto dose level of 2000 mg/kg body weight. As per the ranking system of the European Economic Community (EEC) for acute oral toxicity, the LD₅₀ dose of 2000mg/kg and above is categorized as unclassified (Shanmugasundaram and Venkataraman, 2005).

2.7 Oxidative stress and associated diseases

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals.

Free radicals may play an important role in the causation and the complication of cancer. Oxidants or free radicals are atoms or molecules capable of independent existence that contain one or more unpaired electrons, making these species highly reactive. Exogenous source of free radicals includes tobacco smoke, ionizing radiations, toxic gases like ozone, certain pollutants, organic solvents and pesticides. While the main sources of endogenous oxidants are

inflammatory cells, the generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are susceptible to attack by free radicals. These agents may cause direct tissue oxidation, release of endogenous oxidants and inactivation of antioxidant defence mechanisms (Ragunath *et al.*, 2006).

Numerous epidemiological studies have shown that free radicals are the leading cause of oxidative stress related diseases like cancer, cardiovascular diseases and neurological degenerative disorders. Free radicals and other reactive oxygen species cause the oxidation of biomolecules which leads to cell injury and death. The increased oxidative stress and the decrease in antioxidants may be related to the causation of cancer (Iniaghe *et al.*, 2008).

Antioxidants protect against free radicals and they are therefore essential in obtaining and preserving good health. Much attention has been given to polyphenols with strong antioxidant activities which are ubiquitously present in a broad range of medicinal plants and dietary products. Furthermore, as reported by many investigators, polyphenol from medicinal and aromatic plants possess a high anti-oxidant potential due to their hydroxyl groups and protect more efficiently against free radical related diseases such as atherosclerosis (Harnafi, 2008).

Free radicals are potentially important in a number of ailment states that can have severe effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction (Lachance, 2001). Although the antioxidant defence system includes both endogenously and exogenously derived compounds, dietary plants based antioxidants have recently received great attention (Bravo, 1998). Many studies have been undertaken to identify antioxidant compounds with pharmacological activity and a limited toxicity from medicinal plants. In this context, ethno pharmacology plays a significant part in the search for therapeutically useful plants.

Reactive oxygen species such as super oxide, hydroxyl radical, iron-oxygen complexes, hydrogen peroxide and lipid peroxides are generated by several reactions. These are metabolism of triplet oxygen molecule; one electron reduction of oxygen; catalytic decomposition of hydrogen peroxide and lipid peroxides by metal ions; attack of metal and or metal oxygen complex, irradiation of visible light and X ray and intake of exogenous radicals. These radicals react with biological molecules such as DNA, proteins and phospholipids and eventually destroy the structure of these in membranes and tissues (Vuillaume, 1987, Meneghine, 1988).

2.8. The three stage model and the mechanisms of carcinogenesis

Carcinogenesis is a complex multi-sequence process leading a cell from a healthy to a precancerous state and finally to an early stage of cancer. There are several theories of carcinogenesis. The old theories describe cancer as a “disease of cell differentiation” or “stem cell disease”. These theories seem to point to a “single cell origin” of the cancer (Trueba *et al.*, 2004).

Two key mechanisms have been proposed for the induction of cancer. In one, an increased DNA synthesis and mitosis by nongenotoxic carcinogens may induce mutations in dividing cells through misrepair. Mutations may then clonally expand from an initiated preneoplastic cell state to a neoplastic cell state. Another mechanism accounts for an equilibrium between cell proliferation and cell death. If the damage to DNA is too great, there exists an important process that eliminates altered cells selectively. This process is called apoptosis. During apoptosis, which is a normal physiological process, cells initiate a programmed suicide mechanism leading to many morphological changes (Hengartner, 2000). During cell proliferation, protein p53 plays a primordial role, checking the integrity of the DNA (Oren, 2003). It triggers mechanisms that eliminate, for instance, the oxidized DNA bases that cause mutations. When cell damage is too

great, p53 triggers cell death by apoptosis. Uncontrolled apoptosis can be harmful to an organism, leading to destruction of healthy cells (Hussain Ayoub and Babikar, 2003). Thus there exists a subtle regulatory system consisting of pro-apoptotic factors (e.g. p53) and anti-apoptotic factors. More than half of cancers have defects in upstream or downstream genes of p53 function. The carcinogenic process can be described as an imbalance between cell proliferation and cell death shifted towards cell proliferation.

In addition to these mechanisms, gap junctional intercellular communication (GJIC) or gap Junctions has been proposed to play an important role in the regulation of cell growth control, differentiation and apoptosis of progenitor cells (Trosko, 2003). The results of molecular biology have shown that there are genes, which when “activated” (oncogenes) or “inactivated” (tumour suppressor genes) contribute to the clonal expansion of an initiated stem cell. Many experiments have shown that tumor cells with activated oncogenes have dysfunctional GJIC. It points to the fact that activated oncogenes and GJIC are functionally linked by the signalling pathways affected by oncogenes. These findings suggest the role of gap junctional intercellular communication in the molecular biology of cancer and provide promising target in chemoprevention/chemotherapy of cancer.

Epidemiological clues and animal experiments have shown that the process of carcinogenesis consists of multiple and distinct stages, each characterized by different underlying mechanisms, i.e. the “initiation–promotion–progression” model of carcinogenesis (Klaunig, 2004).

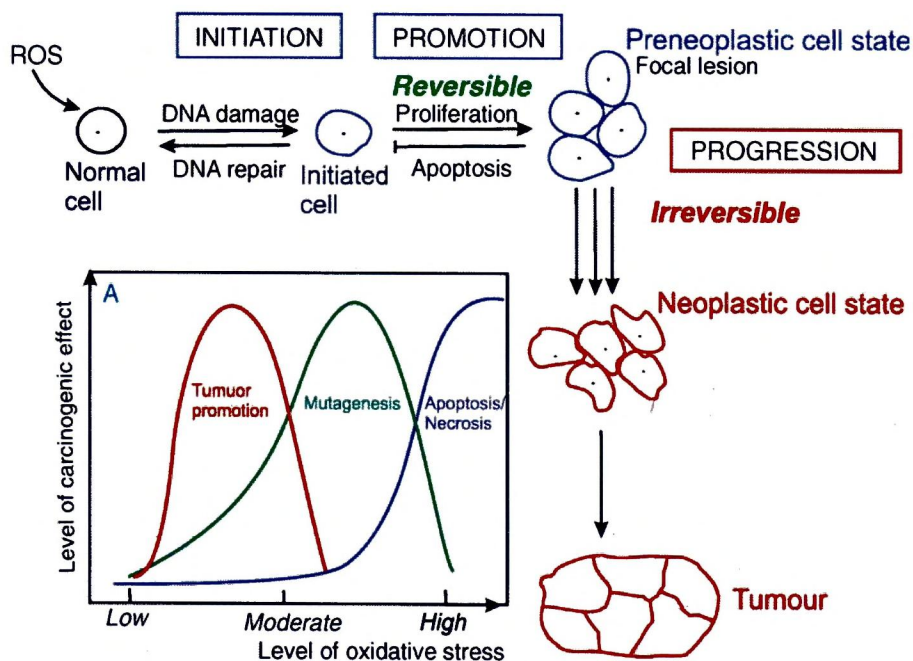
As mentioned above, cancer development is a multistage process. Chemical carcinogens interfere with various stages of this process and function through modifications of cellular and molecular events. Chemicals, participating in the process of carcinogenesis may function differently. To help to better

understand the mechanism through which a carcinogen acts, terms such as “genotoxic” and “epigenetic” (non-genotoxic) have been introduced. Genotoxic agents are usually chemicals that directly damage DNA, which, in turn leads to mutation and/or clastogenic changes. A second category of carcinogenic compounds (non-genotoxic) function through non-DNA or indirect-DNA reaction mechanisms. These compounds modulate cell growth and cell death; however, their mode of action is not yet fully understood.

A multi-stage process of cancer development is characterised by cumulative action of multiple events occurring in a single cell and can be described by three stages: initiation, promotion and progression. ROS can act in all these stages of carcinogenesis (Klaunig, 2004).

The three stage model of carcinogenesis is shown in Figure. 1.

Figure 1. Three stage model of carcinogenesis and the level of carcinogenic effect vs. level of free radicals at various stages of carcinogenic process



Initiation involves a non-lethal mutation in DNA that produces an altered cell followed by at least one round of DNA synthesis to fix the damage (e.g. 8-Hydroxy Guanine-8-OH-G) produced during the initiation (Figure 1). If dividing cells are damaged for whatever reason, they are able to interrupt temporarily their cell cycle at stage G1, S, or G2 (“checkpoints”), repair the damage and resume division (Loft, 1996).

Oxidative DNA damage can occur *via* action of ROS, e.g. hydroxyl radicals, formed through the Fenton-type mechanism, along with other species. Several studies on benign tumors revealed an interesting correlation between the size of tumor and the amount of 8-OH-G adduct formation; the level of 8-OH-G may thus determine the transformation from benign to malignant tumor. The process of initiation further proceeds through oxidative stress-induced Ca (II) changes leading to increase in intracellular free calcium as a result of its release from intracellular Ca (II) stores and through the influx of extracellular Ca (II) (Dreher and Junod, 1996).

The promotion stage is characterized by the clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis). This process results in the formation of an identifiable focal lesion. This stage dose-dependently requires the continuous presence of the tumor promotion stimulus and therefore it is a reversible process. Many tumor promoters have a strong inhibiting effect on cellular antioxidant defence systems such as SOD, catalase, glutathione, etc. While a high level of oxidative stress is cytotoxic to the cell and halts proliferation by inducing apoptosis or even necrosis, a low level of oxidative stress can in fact stimulate the cell division in the promotion stage and thus stimulate the promotion of tumour growth. This implies that production of ROS during this stage of carcinogenesis is the main line of ROS-related tumor promotion.

Progression is the third and the final stage of the carcinogenic process (Klaunig, 2004). This stage involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state. This stage is irreversible and is characterized by accumulation of additional genetic damage, leading to the transition of the cell from benign to malignant. This stage is characterised by genetic instability and disruption of chromosome integrity (Klaunig, 2004).

An important step in the growth of any tumor beyond a few millimeters is the generation of new blood supplies that feed the malignant cells (Carmeliet, 2000). Angiogenesis is a multi-step process, involving degradation of the endothelial cell basement membrane, endothelial cell migration to the perivascular stroma and capillary sprouting. Previously, the tumour suppressor p53 was understood to regulate the process of angiogenesis through the activation of genes that inhibit neovascularization and the repression of genes that promote vessel growth. With the identification of p63 and p73, p53 family regulation of angiogenesis has become broadened and more complex.

2.9. Antioxidant defence mechanisms in carcinogenesis

The effect of reactive oxygen and nitrogen species is balanced by the antioxidant action of non-enzymatic antioxidants, as well as by antioxidant enzymes. Such antioxidant defences are extremely important as they represent the direct removal of free radicals (prooxidants), thus providing maximal protection for biological sites. A good antioxidant should specifically quench free radicals, chelate redox metals, interact with (regenerate) other antioxidants within the “antioxidant network”, have a positive effect on gene expression, be readily absorbed, have a concentration in tissues and biofluids at a physiologically relevant level and work in both the aqueous and/or membrane domains. The most efficient enzymatic antioxidants involve superoxide dismutase, catalase and glutathione peroxidase. Non-enzymatic antioxidants

involve vitamin C, vitamin E, carotenoids, thiol antioxidants (glutathione, thioredoxin and lipoic acid), natural flavonoids, a hormonal product of the pineal gland, melatonin and other compounds. Some antioxidants act in a hydrophilic environment, others in a hydrophobic environment and some act in both environments of the cell. For example, vitamin C reacts with superoxide in the aqueous phase while vitamin E does so in the lipophilic phase. In contrast, lipoic acid is both water and fat soluble and therefore can operate both in cellular membranes and in cytosol. Certain antioxidants are able to regenerate other antioxidants and thus restore their original function. This process is called an “antioxidant network” (Sies *et al.*, 2005). The redox cycles of vitamins E and C form such an antioxidant network. The capacity to regenerate one antioxidant by another is driven by the redox potentials of the [Red/Ox] couple. There is a link between the increased levels of ROS and the disturbed activities of enzymatic and non-enzymatic antioxidants in tumor cells.

2.10 Antioxidants

Antioxidant principles from natural resources possess multifacetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance (Shirwaiker *et al.*, 2006). An antioxidant is any substance when present at low concentrations significantly delays or prevents oxidation of cell content like proteins, lipids, carbohydrates and DNA (Gupta and Sharma, 2006).

2.10.1 Enzymic antioxidants

Three groups of enzymes namely superoxide dismutase (SOD), catalase(CAT) and glutathione reductase play significant role in protecting the cells from oxidant stress (Ahmed *et al.*, 2006).

Superoxide anion ($O_2^{\cdot-}$) is the most abundantly produced free radicals. The biologically generated superoxide anion dismutases into molecular oxygen

and hydrogen peroxide in the presence of protons and these reactions are highly favoured in the presence of superoxide dismutase. Catalase is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water, thus protecting the cell from oxidative damage by H_2O_2 and OH^{\bullet} . It has one of the highest turnover rates of all enzymes. One molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Venukumar and Latha, 2002).

Glutathione reductase also plays a key role in the antioxidant defence processes, by reducing oxidized glutathione, which consumes NADPH, thus allowing a high GSH/GSSG ratio to be maintained. Glutathione-S-transferase and glutathione peroxidase are the most abundant and ubiquitous detoxification enzyme families in the plant system. These enzymes play a pivotal role in inhibiting the cellular damage produced by a wide variety of stresses (Kretschmer *et al.*, 2004).

A decrease in SOD and CAT activities described in tumors is regarded as a marker of malignant transformation. Lowered activities of SOD and CAT were reported in several cancers (Subapriya *et al.*, 2002)

2.10.2 Non – enzymic antioxidants

Non enzymic antioxidants such as reduced glutathione, vitamin C, vitamin E, flavonoids and carotenoids play a significant role in protecting the cells from oxidative damage (Ramprasath *et al.*, 2006).

Vitamin C (ascorbic acid) is a very important and powerful, antioxidant that works in aqueous environments of the body which are present in the lungs and in the lens of the eye. Its primary antioxidant partners are vitamin E and the carotenoids and they work along with the antioxidant enzymes.

Glutathione is the most abundant low molecular weight thiol substance with sulfhydryl group. The sulfhydryl group, which gives the molecule its electron donating character, comes from the cysteine (generated from methionine) residue. It is a significant component of collective antioxidant defences and a highly potent antioxidant and antitoxin in its own right. The –SH group of GSH is important for many facets of cell function. Observations from hereditary GSH synthesis deficiencies confirm that GSH is essential both to the functionality and the structural integrity of the tissues and the organ systems. The glutathione status of a cell (that is excess of reduced over oxidized glutathione) will perhaps turn out to be the most accurate single indicator of the health of the cell (Agarwal, 2007).

The main protective roles of glutathione against oxidative stress are as follows; (i) glutathione is a cofactor of several detoxifying enzymes against oxidative stress, e.g. glutathione peroxidase (GPx), glutathione S- transferase and others; (ii) GSH participates in amino acid transport through the plasma membrane; (iii) GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase; (iv) glutathione is able to regenerate the most important antioxidants like vitamins C and E back to their active forms; glutathione can reduce the tocopherol radical of vitamin E directly, or indirectly, *via* reduction of semidehydroascorbate to ascorbate (Masella *et al.*, 2005).

Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell. Malondialdehyde (MDA), the end product of lipid peroxidation has been reported to be higher in cancer tissues than in non diseased organ. Elevated lipid peroxidation and poor antioxidant system have been reported in cancer patients.

Altered activities of enzymatic antioxidants are reported during carcinogenesis or after tumor formation. Hence, the elevated lipid peroxidation in the circulation of cancer animals is due to a poor antioxidant defence mechanism (Kavitha and Manoharan, 2006).

Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress or a diseased condition. The rate of plasma enzyme activity depends on the concentration of an enzyme in cells, the rate of leakage caused by injury and the rate of clearance of the enzyme from plasma. There are a number of possible mechanisms for the appearance of abnormal activities of enzymes in serum. These include over production of enzyme by the tumor tissues, tumor blockage of the duct system through which enzyme passes into the blood, induction of enzyme by the presence of tumor, change in permeability of the cell allowing leakage of soluble enzymes into the circulation (Siakpere *et al.*, 2010).

The methanolic extract of roots of *Clerodendrum serratum* was screened for *in vivo* anticancer activity using DLA cell model at the dose 100mg and 200mg/kg body weight. The mean survival time, percentage increase in life span, body weight, hematological and biochemical parameters were analysed and the analysis revealed a significant anticancer activity of *Clerodendrum serratum* (John, 2008).

The ethanol extract of *Symplocos racemosa* increased the life span of EAC tumor bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. All these parameters suggest that the ethanolic extract of *Symplocos racemosa* exhibit potential antitumor and antioxidant activities (Vijayabhaskaran *et al.*, 2010).

2.11 Oxidative stress and redox environment of a cell

Oxidation and reduction reactions in biological systems are called redox reactions and represent the basis for numerous biochemical mechanisms. When discussing redox reactions in biological systems, it is more appropriate to use the terms antioxidant and pro-oxidant respectively instead of the terms reductant and oxidant (Nyska, 2002). A reductant or reducing agent, is a substance which donates electrons; an oxidant, or oxidizing agent, is a substance that accepts electrons. A chemical process during which a loss of electrons occurs is called an oxidation process (oxidation reaction). Conversely, a reduction process (reduction reaction) is characterized by the gain of electrons. The theory describing this phenomenon is called the redox (reduction/oxidation) theory of cellular functioning.

The cellular redox environment plays an important role in signal transduction, enzyme activation, DNA and RNA synthesis, cell proliferation, differentiation and apoptosis (Moron, 2001). Generally, while cell death is initiated by an oxidizing environment of the cell, a reducing environment is an important factor for increased cell proliferation. An example of increased cell proliferation involves stimulated proliferation of some tumor cells exposed to high concentrations of thiols. In accord with this feature, antioxidants have been shown to prevent apoptosis.

Extensive evidence has shown that redox balance is impaired in cancer cells compared to normal cells, which may be related to oncogenic stimulation. Altered levels of antioxidant enzymes (SOD, catalase, glutathione peroxidase) and non-enzymatic antioxidants (GSH, Vitamin C, thioredoxin) as well as changes in the related signal pathways are evident in many human cancers. The cumulative production of ROS, typical for many cancer cells is linked with altered redox regulation of signalling cascades. The reducing intracellular

environment in the nucleus and in mitochondria (maintained by elevated levels of glutathione and thioredoxin) not only facilitates escape from apoptosis but also produces a proliferation potential through activation of cell survival signals mediated by redox-sensitive nuclear transcription factors .

A large number of plants are known to possess anticancer and antioxidant properties (Kavitha and Manoharan, 2007). *In vivo* antitumor activity of methanolic extract of *Hypericum hookerianum* against the Daltons's lymphoma ascitic model was determined at 100 and 200mg/kg body weight given orally for 10 days. The results indicated that administration of the extract not only increased the survival of animals with ascitis tumor, decreased the body weight induced by the tumor burden and reduced the packed cell volume and viable tissue cell count, but also altered many hematological parameters during tumor progression, indicating the potent antitumor nature of the extract.

The methanolic extract of the bark of *Careya arborea* given orally to mice at the dose of 250 or 500mg/kg body weight for 10 days caused significant reduction in percent increase in body weight, packed cell volume and viable tumor cell count, compared to the mice of the DLA control group. Restoration of hematological and biochemical parameters towards normal was also observed. Histological observation of liver and kidney also indicated repair of tissue damage caused by tumor inoculation. The extract at the dose of 5 or 25mg/kg body weight given intraperitoneally daily for 14 days significantly reduced the solid tumor volume induced by DLA cells (Natesan *et al.*, 2007).

Sivanesan and Hazina (2007) have confirmed the chemopreventive efficacy of *Gynandropsis gynandra* L., extract against aflatoxin B₁. The extract prevented the rate of lipid peroxidation and influenced the enzymatic and the non- enzymatic antioxidants in AFB₁ induced male albino rats.

Bhaskar Rao *et al.*(2009) showed that plant extracts such as *Clitoria ternata* L. and *Eclipta prostrata* L exhibited strong antioxidant activity and possession of enzymatic and non enzymatic antioxidants.

Pleurotus florida possess antioxidant property, lipid peroxidation inhibition activity and hydroxyl radical scavenging activity. It was confirmed by screening its antioxidant role in Erlisch's Ascites Carcinoma cells induced mice (Nayana and Janardhanan, 2000).

The methanolic extract of *Bauhinia racemosa* showed haematological profile and liver enzyme alteration in EAC bearing mice. Haematological profile is reverted to normal. The level of glutathione, SOD and catalase activity was increased, which shows antioxidant defence mechanism, when treated with the plant extract (Gupta *et al.*, 2004).

The protective effect of Ganoderma, a medicinal mushroom against liver injuries shows its hepatoprotective function which possesses both antioxidant and free radical - scavenging activity (Yihuai *et al.*, 2005).

The abnormal hematological parameters were treated by ethanolic extract of root of *Withania somnifera* against DLA induced swiss albino mice (Christina *et al.*, 2004).

2.12 Free radical scavenging assays

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons usually confers a considerable degree of reactivity upon a free radical. Those radicals derived from oxygen represent the most important class of such species generated in living systems. At high concentrations, ROS can be important mediators of damage to cell structures including lipids and membranes, proteins and nucleic acids (termed oxidative stress).

Oxidative stress, induced by oxygen radicals is believed to be a primary factor in various degenerative diseases, such as cancer. Many antioxidant compounds naturally occurring from plant sources have been identified as free radical or active oxygen scavengers (Zheng and Wang, 2001). Recently, interest has increased considerably in finding naturally occurring antioxidants. In addition, natural antioxidants have the capacity to improve food quality and stability and can also act as nutraceuticals to terminate free radical chain reactions in biological systems / and thus may provide additional health benefits to consumers. Increasing experimental evidence has suggested that these compounds can affect a wide range of cell biological functions by virtue of their radical scavenging properties. In the search for plants as the natural antioxidants, some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging in the last few decades (Singh *et al.*, 2002).

In vitro studies are simple, inexpensive to perform, provide a significant amount of information, can be conducted under controlled conditions and may elucidate the mechanism of cellular toxicity. The results obtained from *in vitro* assays might be indicative of the *in vivo* effects (Geursten, 1998).

Since the mitochondria are the major sites of free radical generation, they are highly enriched with antioxidants including GSH enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), which are present on both the sides of their membranes in order to minimize oxidative stress in the organelle.

Superoxide anion, arising either through metabolic processes or following oxygen activation is considered to be the primary ROS and can further interact with other molecules to generate secondary ROS, either directly or prevalently, through enzyme or metal catalyzed processes. Superoxide radicals formed on both sides of mitochondrial inner membranes are efficiently detoxified initially to

hydrogen peroxide and then to water by Cu, Zn-SOD (SOD-1 localized in their intermembrane space) and Mn-SOD (SOD-2 localized in the matrix).

In addition, microsomes and peroxisomes are sources of ROS. Microsomes are responsible for 80%^{of} H_2O_2 produced *in vivo* at hyperoxia sites. Peroxisomes are known to produce H_2O_2 but not O_2^- , under physiological conditions. The liver is the primary organ where peroxisomal contribution to the overall production is significant over other organs that contain peroxisomes which are exposed to these $H_2O_2^-$ generating mechanisms. Peroxisomal oxidation of fatty acids has recently been recognized as a potentially important source of H_2O_2 production as a result of prolonged starvation.

The hydroxyl radical is highly reactive with a half life in aqueous solution of less than 1 nanosecond (Pastor *et al.*, 2000). Thus when produced *in vivo*, it reacts close to its site of formation. It can be generated through a variety of mechanisms. Production of OH^\bullet close to DNA could lead to this radical reacting with DNA bases or the deoxy ribosyl backbone of DNA to produce damaged bases or strand breaks.

Nitric oxide free radical (NO^\bullet) is generated from the nitrite by non enzymatic method and O^\bullet formation is found in acidic environments such as the stomach and the oral cavity. Peroxy nitrite ($ONOO^-$), the reaction product formed between nitric oxide (NO^\bullet) and superoxide, plays a critical role in the induction of inflammatory reaction and apoptosis. O^\bullet is also associated with tumor promotion and/or progression. High level of NO inhibits hepatocyte mitochondrial respiration *invitro*, DNA damage by inhibiting DNA synthesis and cell cycle arrest (Krishnamoorthy and Sangeetha, 2008).

The ethanolic extract of *Sphaeranthus indicus* (Linn.) at 1000 μ g/ml showed maximum scavenging of the ABTS observed upto 41.99%, followed by the scavenging of stable radical DPPH

(33.27%), superoxide dismutase (25.14%) and nitric oxide radical (22.36%) at the same concentration (Shirwaiker *et al.*, 2006).

Megashri *et al.* (2010) have observed the strong superoxide and DPPH radical scavenging activities compared to other polarity based extracted fractions in crude methanolic extract of *Leucas aspera* leaves.

Rao *et al.* (2010) showed the highest antioxidant and cell cytotoxic properties of methanolic extract from Njavara rice bran. Total antioxidant activity and reducing power were increased with increasing amounts of the extract. IC₅₀ values of cytotoxic assay (MTT assay) were 17.53-57.78 µg/ml.

Methanol extract along with its ethyl acetate and butanol fractions of *Butea monosperma* showed potent free radical scavenging activity, whereas aqueous fraction was found to be devoid of any radical scavenging properties (Lavhale and Mishra, 2007).

The free radical scavenging capacity and antioxidant activities of the methanolic extract of *Cinnamomum verum* leaf were studied and compared to antioxidant compounds like Trolox, butylated hydroxyl anisole, gallic acid and ascorbic acid. The extract exhibited free radical scavenging activity, especially against DPPH radical and ABTS radical cation. They also exhibited reducing power and metal ion chelating activity, along with hydroxyl radical scavenging activity (Mathew and Abraham, 2006).

The methanol extracts of *Phyllanthus* showed strong antioxidant activity, DPPH radical, superoxide anion scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, reducing power and metal chelating activities when compared to standards such as BHT and ascorbic acid (Kumaran and Karunakaran, 2008).

Hepatoprotective and antioxidant role of *Caesalopinia bonducella* were reported by Gupta *et al.* (2003). They have demonstrated that the methanolic extract of *Caesalopinia bonducella* increased the life span of EAC tumor bearing mice and decreased the lipid peroxidation and thereby augmented the endogenous enzymes in the liver.

Dongre *et al.* (2008) observed the cytotoxic and antioxidant properties of methanolic extract of *Hypericum hookerianum*. They observed the significant restoration of antioxidant enzyme levels towards the normal by the methanolic extract treatment which indicates the protection of vital organs from damage induced by DLA.

The reliable criteria for judging the value of an anticancer drug is the prolongation of the life span of the animals. The ascitic tumor implantation induces *per se* local inflammatory reactions with an increase in vascular permeability which results in intense edema formation, cellular migration and a progressive ascitic fluid formation. The ascitic fluid is essential to tumor growth, since it constitutes the direct nutritional sources for tumor cells (Gupta *et al.*, 2004). Badami *et al.* (2003) observed a regular rapid increase in ascetic tumor volume in DLA tumor bearing mice.

2.13 Phytochemical screening of medicinal plants

To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to have a deep study of medicinal plants. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. Saponins are a special class of glycosides which have soapy characteristics. It has also been shown that saponins are active antifungal agents. Phenols, the aromatic compounds with hydroxyl group are

widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Tannins are also known antimicrobial agents. Tannins are water – soluble polyphenols that are present in many plant foods and precipitate proteins. Tannins have been reported to prevent the development of micro organisms by precipitating microbial protein and making nutritional proteins unavailable to them. The growth of many fungi, yeasts, bacteria and viruses are inhibited by tannins. Tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic. Presence of tannins suggests the ability of these plants to play a major role in the treatment of some diseases. Flavonoides are also shown to inhibit microbes which are resistant to antibiotics.

The extracts of root, stem, bark and seeds of *Strychnos potatorum* have revealed the presence of alkaloids, flavanoids, glycosides, lignins, phenols, saponins, sterols and tannins. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Malligarjuna *et al.*, 2007). Patra *et al.* (2009) have found that *Hygrophila spinosa* contains various flavonoids, terpenoids, mucilage etc. which may be responsible for its different pharmacological activities.

2.14 Cytotoxic activity of medicinal plants

The medicinal plants act as cytotoxic agents to tumor cells for cancer treatment. Inhibition of cell division through means of DNA repair mechanism or by distinguishing the malignant information can eventually help in killing the cancerous cell (Donald *et al.*, 2000).

The aqueous extracts of *Viscum album* of different origin showed a cytotoxic effect against B cell lines (Duong *et al.*, 2003). Cytotoxic property of *Nigella sativa* was evaluated using human cancer cell lines and fibroblast cell

line. MTT assay was performed and the result indicated it to be a potential cytotoxic agent (Nazrul *et al.*, 2004).

The cytotoxic assay of methanolic extract of *Emila sonchifolia* was analysed against DLA induced mice by performing trypan blue exclusion method and MTT assay (*In vitro* toxicity). The cell specific cytotoxicity was found by Shylesh *et al.*, 2005.

Andrographis paniculata leaf showed a cytotoxic activity to human HeLA cells by apoptosis (Senthil, 2005). *Andrographis nee's* leaf extract showed a cytotoxic effect towards DLA cells, which was performed by trypan blue and MTT methods (Sheeja and Kuttan, 2005).

Cytotoxicity of extracts of *Solanum trilobatum* was performed *in vitro* against Daltons Lymphoma Ascites and it indicated that petroleum ether extract produced remarkable cytotoxicity (Mohanani *et al.*, 1998).

The antitumor activity of ethanolic extract of *Indigofera aspalathoides* was assessed by *in vitro* cytotoxicity against Erlich Ascites Carcinoma, using trypan blue exclusion method (Rajkumar *et al.*, 2004).

The cytotoxic and the tumor sensitizing action of *Withania somnifera* present itself as a novel complementary therapy for integrative oncology care (Winter, 2006).

Most cancer therapeutics that are in routine clinical use have not been developed using *in vitro* biochemical screening, but have been discovered as having activity in biological assays. Cell based assays have two major advantages over *in vitro* biochemical screens.

Apoptosis or programmed cell death has become of interest as an intervening target in cancer chemoprevention. In the setting of carcinogenesis, inhibition of apoptosis has been correlated with tumor promotion. There is abundant evidence that administration of naturally occurring compounds with antitumor activities triggers the apoptotic death of cancer cells. Strong cytotoxic and anticancer properties have been observed in *H.perforatum* (Roscetti *et al.*, 2004), *H. mysorence*, *H. patulum* (Vijayan *et al.*, 2003), *H. polyanthemum* (Ferraz *et al.*, 2005) and many of their phytoconstituents.

Withania somnifera leaves exhibited growth inhibition of human tumor cell lines by using its alkaloid. 50% inhibition of these cell lines by the leaves extract is detected by MTT assay (Jayaprakasam *et al.*, 2003). Mohanan *et al.* (1998) had observed the remarkable cytotoxic activity of petroleum ether extracts of *Solanum trilobatum* against DLA cells.

Mahakunakorn *et al.* (2003) reported the powerful cytotoxic effect of *Curcuma longa*. Husseinayoub *et al.* (1984) studied the anticancer activity of ethanolic extract of the fruits of *Asteracantha longifolia* (L) Nees using the KB test system and the ED₅₀ found, was more than 1 µg /ml in the KB cell culture.

Further, the antitumor activity in Erlisch ascites carcinoma and sarcoma 180 bearing mice of the petroleum ether extract of the roots of *Hygrophila spinosa* T, Anders was also studied by Mazumdar *et al.*, (1997). The extract showed decrease in packed cell volume, increased life span of EAC/S-180 bearing mice in a day dependent manner and also inhibited the rapid increase of body weight of tumor bearing mice. Sub- acute toxicity study of the hydroalcoholic extract of the whole plant of *H. spinosa* showed no significant change in body weight, organ weight and serum biochemical parameters. The LD₅₀ was found to be 3020mg/kg body weight. The tumor reducing potency

of the extract in DMBA induced mammary tumor in female rats was assessed by recording the reduction in tumor weight (Pattanayak and Sunitha, 2008).

The crude petroleum ether extract of the *H.spinosa* was found to possess low toxicity (LD₅₀ 1gm/ml in mice) and effectively arrest neoplastic growth in swiss mice. The associated pathologic changes in blood cell counts and hemoglobin content due to oncogenesis in the host returned to almost normal by drug treatment. Treatment of the test animals with the drug, previously inoculated with 3 different strains of tumor cells in mice resulted in the inhibition of tumor growth in all three cases. The drug significantly increases the lifespan in Daltons lymphoma treated mice (Maiti, 1995).

During the investigator's evaluation of Indian medicinal plant extracts for various bioactivities, several extracts have shown potent (MIC 2 ppm) anticancer activities against HL-60 (human promyelocytic leukemia cells) cell line. However, morellin, first isolated and characterized in India from the seeds of the Indian plant *Garcinia morella*, has been found to inhibit several human cancer cell lines and *in vivo* exhibited anticancer activity against p38 leukemia in CDF 1 mice.

Aqueous extract of fruits of *Momordica charantia* has potent cancer preventive activity. The extract afforded protection from the development of skin tumor in a two step skin carcinogenesis model in mice (Ganguly *et al.*, 2000). A protein MAP 30 isolated from the fruit and seeds of this plant, showed antitumor activity against breast cancer MDA-MB-231 *in vitro* and *in vivo* (Lee Huang *et al.*, 2000). Two other proteins obtained from the seeds also possess antitumor activity (Ng *et al.*, 1992). These proteins have now been found to be ribosome-inactivating proteins, induce apoptosis and inhibit histone deacetylase-1 selectively in premalignant and malignant prostate cancer cells (Xiong *et al.*, 2009).

The root extract of *C.procera* has been found to produce a strong cytotoxic effect on COLO 320 cells (Smit *et al.*, 1995). Antiproliferative activity of the extracts from the medicinal plants *Hemidesmus indicus*, *P.longifolia*, *Aphanamixis polyustachya*, *Moringa oleifera*, *Lagerstroemia speciosa*, *Paederia foetida*, *Cassia sophera*, *Hygrophila auriculata*, and *Ocimum sanctum* were analysed on different human cell lines, including eruthroleukemia k562, B-lymphoid, T-lymphoid and erythrolukemia cell lines by Lambronti *et al.* (2005).

2.15 Extraction and separation of Forskolin

The range of biological activities associated with the diterpenoids has continued to increase (James, 2003). Different chromatographic methods are employed for quantification of forskolin and the gas liquid chromatography method is the first developed method (Inamder *et al.*, 1980). Later, thin layer and high performance liquid chromatographic methods are employed. HPLC method is found to be more rapid and less sensitive than GLC and used to monitor variation in forskolin content in different germplasm (Inamder *et al.*, 1984). Nuclear magnetic resonance data and gas chromatography-mass spectrometric method are also used to monitor variation in forskolin quantification (Demetzos *et al.*, 2002).

Reversed-phase liquid chromatography with a photodiode array detector at 210nm is found to be successful in the qualitative and quantitative evaluation of forskolin in plant material and in market products claiming to contain forskolin (Schanebera and Khan, 2003). A simple, safe, rapid and economical reverse phase high performance liquid chromatography method using activated charcoal as an adsorbent in column is developed for the isolation of high purity forskolin (Saleem *et al.*, 2006). Wu *et al.*, (2007) reported that HPLC-ELSD fingerprint method can be used in quality control of *Coleus forskohlii*.