STUDIES ON ANTITUMOR AND ANTIMUTAGENIC POTENTIALS OF SOME PLANTS OF MEGHALAYA AND MIZORAM

Forwarded

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DECLARATION

I, G. Rosangkima, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other university/institute.

This is being submitted to the North Eastern Hill University for the degree of ***Doctor of Philosophy*** *in Zoology.*

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(G. Rosangkima)

CONTENTS

ABBREVIATIONS

• B[a]P	Benzo[a]pyrene
• BSA	Bovine serum albumin
• BSO	Buthionine sulfoximine
• CA	Chromosomal aberration
• CDNB	1-chloro-2,4-Dinitrobenzene
• CIS	Cisplatin
• DL	Dalton's lymphoma
• DLC	Differential leukocyte counts
• DPE	*Dillenia pentagyna* extract
• DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
• EDTA	ethylenediaminetetra-acetic acid
• FBS	Fetal bovine serum
• GPx	Glutathione peroxidase
• GR	Glutathione reductase
• GSH	Reduced glutathione
• GSSG	Oxidised glutathione
• GST	Glutathione S-transferase
• ILS	Increase in life span
• i.p.	Intraperitoneal
• LPO	Lipid peroxidation
• MDA	Malondialdehyde
• MN	Micronucleus

- O.D. Optical density
- PBS Phosphate buffer saline
- PCV Packed cell volume
- RBC Red blood cells
- ROIs Reactive oxygen intermediates
- ROS Reactive oxygen species
- TSH Total sulfhydryl
- WBC White blood cells

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INTRODUCTION

INTRODUCTION

CANCER:

The ability to multiply is a fundamental property of cells. In multicellular species many rounds of cell division are required to make a new individual and in adult body cell division is needed to replace cells that are lost by wear and tear or by programmed cell death. The multiplication of different types of cells in the body follows definite sequential stages, referred to as cell cycle.

Cell division cycle comprises four phases in eukaryotes: gap 1 or G1 synthesis, S phase during which DNA synthesis occurs, gap 2 or G2 phase and mitosis or M phase. After passing through mitosis and entering into G1 phase, a cell either continues through another division or ceases to divide, entering a quiescent or resting phase (G_0) of low metabolic activity that may last for hours, days or the lifetime of the cell. Cells in G_0 may either re-enter the cell cycle or proceed down a pathway leading to terminal differentiation. The decision to proceed into S phase or enter G_0 is made in G1 and this 'restriction point' is under strict genetic control and it is carefully regulated. The cell cycle is controlled by a family of protein kinases that are the heterodimers with a regulatory subunit, cyclin and a catalytic subunit, cyclin-dependent protein kinase (Cdk). Cyclical changes in cyclin levels result in the cyclic assembly and activation of the cyclin-Cdk complexes. In vertebrates there are four classes of cyclins involved at the specific stages of cell cycle at which they bind Cdks. For example, at G1, cyclin D binds with Cdk4 and Cdk6; at G1/S cyclin E binds with Cdk2; at S, cyclin A binds with Cdk2 and at M, cyclin B binds Cdk1. The full activation of the cyclin-Cdk complex occurs when another kinase, the Cdk-activating kinase (CAK) phosphorylates an amino acid near the entrance of the Cdk active site.

The activity of a cyclin-Cdk complex can be inhibited by a protein kinase, Wee1 which phosphorylates a pair of amino acids in the roof of the Cdk active site while dephosphorylation of these sites by Cdc25 restores Cdk activity. If the cyclins are overproduced in a cell or made at the wrong time, they stimulate inappropriate cell division by keeping their partner kinases 'on' when they should be turned off, resulting a malfunction that could lead to cancer. For example, the overexpression of the gene for cyclin D1, D2 and D3 has been shown to contribute in many common cancers. P16 proteins function as Cdk/cyclin kinase inhibitors (CKIs) and play an important role in regulating cell cycle. Mutations (deletions) in *p16* gene leads to its inability to inhibit cyclin D-dependent kinase activity causing cyclin D1 overexpression in several human cancers. P27 is another well known CKI.

This control system ensures that cells divide only when needed, so as to maintain the correct shape, size of organs and tissues. Under the effect of cancer causing agents, known as carcinogens, the exquisite control mechanisms of regulating cell multiplication by Cdks and the growth factors break down and a cell begins to grow and divide in an uncontrolled manner. Descendants of such cells inherit the propensity to proliferate without responding to regulation and expand indefinitely to develop as a lump, which is commonly referred to as a tumor. The rate of cell division in tumors exceeds the rate of cell loss. Defects in the synthesis, regulation or recognition of various growth factors (EGF, epidermal growth factor; PDGF, platelet derived growth factor; FGF, fibroblast growth factor etc) may also be involved in developing a tumor.Thus, failures in any one of the safety mechanisms will lead the cell to grow and divide in an uncontrolled manner. Descendants of such cells inherit the tendency to proliferate without responding to regulation and expand indefinitely to develop tumor. Tumors are defined as neoplasm, although the term tumor may be

applied to any swelling (Vincent, 1985). The terms neoplasm and tumor are commonly used interchangeably (Friedberg, 1986). Tumors are of two types, the slowly growing 'benign' and the rapidly growing 'malignant' forms (Vincent, 1985). Benign tumors may kill their host by progressive growth, but they are as a rule easily cured. In contrast, malignant tumors frequently kill their host because invasion and metastasis cause therapeutic failure (Marc, 1986). Malignant tumors have the potential to invade the surrounding tissues including blood vessels and lymphatic channels and metastasize to distant sites of the body (Abercrombie and Ambrose, 1962). Malignant tumors are commonly referred to as '**cancer**' (Latin 'crab') suggesting its tendency to cling and reach out to adjacent tissues. Cancers are generally classified into three broad groups: carcinomas, sarcomas, and leukaemia/lymphomas (Cairns, 1986). Each organ in the body is composed of different types of tissue, and most cancers arise in one of three main types: epithelial, connective, or blood-forming tissue. Carcinomas are cancers that occur in epithelial tissues; the skin and inner membrane surfaces of the body, such as those of the lungs, stomach, intestines, and blood vessels. Carcinomas account for approximately 80-85 percent of human cancers. Sarcomas originate in connective tissues such as muscle, bone, cartilage, and fat that support and connect other parts of the body. Much rarer than carcinomas, sarcomas account for about 2-3 percent of all cancers. Leukemias develop in blood cells, and lymphomas originate in the lymphatic system. These cancers of the blood-forming tissues account for about 8-12 percent of all human cancers.

The transformation of normal into malignant cells requires a number of genetic changes, and some of the genes (tumor suppressor genes and oncogenes) involved in this process have been characterized. The genes involved in the

expression of malignancy are now referred to as oncogenes (Greek; onkos, a tumor) and the normal genes from which they are derived as proto-oncogenes (Land et al., 1983). The changes of proto-oncogenes to oncogenes are in all cases associated with changes in the structure or regulation of the normal genes, and a multiple genetic changes are needed to change proto-oncogenes into highly oncogenic oncogenes. The oncogenes encode proteins that are intimately involved in the basic control mechanisms of one of the most fundamental of all cellular functions, the drive to proliferate. Tumor suppressor genes (about 20 in human) normally act as cell's brakes. In contrast to oncogenes, tumor suppressor genes encode proteins that restrain cell growth and prevent cells from becoming malignant. The transformation of normal cell to cancer cell is accompanied by the loss or decrease of function of one or more tumor suppressor genes. Most of the proteins encoded by tumor suppression genes act as negative regulators of cell proliferation which may be as transcription factors (p53 and WTI), cell cycle regulators (RB and p16), components regulating signalling pathways (NFI), regulating RNA polymerase II elongation (VHL). Thus, their elimination contributes and promotes uncontrolled cell growth (Haber and Harlow, 1997).

Most cancers result from genetic damage by cancer causing agents, or **carcinogens**. Carcinogenesis is a multistep process. The fundamentals of the carcinogenesis model include an initiation step, involving changes at genetic levels, which is followed by promotion, conversion, and progression steps to clinical malignancies (Tanaka, 1992). People who inherit a defective gene are at increased risk that additional environmental-induced genetic damage will cause cancer. The proteins produced in a human cell determine the function of each cell, and ultimately, the function of the entire body. In a cancerous cell, permanent gene alterations, or

mutations, cause the cell to malfunction. For a cell to become cancerous, usually two to seven different oncogenes are involved in a cell.

CANCER THERAPY:

The fundamental goal of cancer research is to understand how normal cells undergo neoplastic transformation and develop into cancer. A thorough understanding of the cancer cells and its interaction with its microenvironment still remains one of the foremost challenges to researchers. Oncologists select from a number of options when treating cancer, depending on the type and stage of the tumor involved. The major treatments currently available are surgery, radiotherapy, chemotherapy and immunotherapy. Often, for better treatment results, combination of more than one type of cancer therapy is commonly used. Surgery is the most effective and fastest treatment for tumors and can lead to a recovery, but undetected malignant cells may metastasize to other organs. Often surgery is combined with chemotherapy. Laser surgery uses a powerful beam of high-energy light to vaporize certain tumors of the cervix, larynx, and skin. Therapeutic radiology utilizes heat energy to literally burn off malignant cells, inflicting genetic damage that kills cancerous cells. Radiation therapy damages rapidly dividing cells, mostly cancer cells but also healthy cells that reproduce quickly. This leads to side effects such as fatigue, skin changes, and loss of appetite. Chemotherapy is an effective treatment against cancers either singly or in combination with surgery and/or radiotherapy. In chemotherapy, drugs like cisplatin, carboplatin, cyclophosphamide, doxo-rubicin, melphalan, mitomycin-C, gemcitabine, etc. have been used for the treatment of cancers (Black and Livingston, 1990a; Black and Livingston, 1990b). However, therapeutic efficacy of most of them is limited due

to the development of various side effects in the host and/or the acquired drug resistance by the cancer cells (Black and Livingston, 1990a). Oncologists used different chemotherapeutic drugs to combat cancer, generally administering more than one drug at the time because some drugs are more powerful in combination. Chemotherapeutic drugs interfere with the cancer cells ability to make new DNA or to undergo division. In some cases, the drugs cause programmed cell death (Eastman, 1990). A combination of drugs with different actions can be more effective to kill cancer cells and reduce the chance that the host may become resistant to a particular chemotherapeutic drug. However, chemotherapy often causes severe side effects, particularly leading to internal bleeding, diarrhea, nausea, vomiting, hair loss and anemia (Black and Livingston, 1990b). Bone marrow suppression and depletion circulating leucocytes are major effects of cancer chemotherapy, and some chemotherapeutic drugs may lead to the development of drug resistance by cancer cells (Deborah and Stephen, 1995).

Alkylating agents affect the mammalian genome by forming DNA lesions and thus, causing base substitution mutations, or preventing DNA replication. It is well known that apoptotic cell death is induced by DNA- damaging agents. 'Apoptosis' (programmed cell death) is accepted as an active and predominant process of cell death observed during chemotherapy by some drugs. Apoptosis is characterized by DNA fragmentation caused by activation of endonuclease. Cyclophosphamide (CP), bleomycin (BL), doxorubicin (DOX) and cisplatin (CIS) are potent antitumor drugs used worldwide against many forms of cancer. As with most such agents, there can be physiological side effects and the possible induction of mutations and other genotoxic effects in non-tumor cells.

NATURAL HERBAL MEDICINES:

Nature has provided many things for humankind over the years, and the use of medicinal plants for health reasons started thousands of years ago and is still continuing. Furthermore, an increasing reliance on the use of medicinal plants has helped to the extraction and development of several drugs.

Chinese herb guides document the use of herbaceous plants as far back in time as 2000 BC. (Holt and Chandra, 2002). In fact, *The Chinese Materia Medica* has been repeatedly documented over centuries starting at about 1100 BC. Egyptians have been found to have documented uses of various herbs in 1500 BC (Cragg and Newman, 2001a; Cragg and Newman, 2001b). The best known of these documents is the Ebers Papyrus, which documents nearly 1000 different substances and formulations, most of which are plant-based medicines (Nakanishi, 1999). A collection of ayurvedic hymns in India from 1000 BC and earlier describes the uses of over 1000 different herbs. For a variety of different reasons, the interest in natural products continues to this very day (Barron and Vanscoy, 1993; Bhattaram et al., 2002; Kaul and Loshi, 2001; Kroll, 2001; Marriott, 2001). In China about 40% of the total medicinal consumption is attributed to traditional tribal medicines. In Japan, herbal medicinal preparations are more in demand than mainstream pharmaceutical products. Africa is also a rich source of medicinal plants, and in Europe, some 1500 species of medicinal and aromatic plants are widely used in Albania, Bulgaria, Croatia, France, Germany, Hungary, Poland, Spain, Turkey, and the United Kingdom. Developed countries, in recent times, are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies. Herbal preparations are popular and are of significance in primary healthcare in Belgium, France, Germany and the Netherlands.

Such popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being.

Higher plants continue to play a dominant role in the primary health care, and the World Health Organization estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care (Farnsworth et al., 1985). Over 100 chemical substances that are considered to be important drugs that are either currently in use or have been widely used in one or more countries in the world have been derived from a little under 100 different plants. Approximately 75 percent of these substances were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional medicine (Cragg and Newman, 2001a; Cragg and Newman, 2001b). More current statistics based on prescription data from 1993 in the United States show that over 50 percent of the most prescribed drugs had a natural product either as the drug or as the starting point in the synthesis or design of the actual end chemical substance (Newman et al., 2000). Thirty-nine percent of the 520 new drugs approved during the period 1983 through 1994 were either natural products or derivatives of natural products (Harvey, 2001; Huang et al., 1992) and natural products play an important role in drug discovery programs of the pharmaceutical industry.

In an attempt to cure various malignancies many plants have also been used. Natural-products-based anticancer drug discovery continues to be an active area of research throughout the world (Da Rocha et al., 2001; Mehta and Pezzuto, 2002; Schwartsmann et al., 2002). While cancer incidences and the frequencies of types of cancer may vary from country to country, the most common sites for the development of neoplasia are generally considered to be the breast, colon/rectum, prostate,

cervix/uterus, oesophagus/ stomach, pancreas, liver, lung, urinary bladder, kidney, ovary, oral cavity, and blood (leukaemia and non-Hodgkin lymphoma) (Schwartsmann et al., 2002). Currently, the chemotherapeutic management of these tumors involves a variety of different plant-based chemicals that are either currently in use or in clinical trials and include such drug classes as the vinca alkaloids, lignans, taxanes, stilbenes, flavones, cephalotaxanes, camptothecins, and taxanes. Plant-derived natural products with documented anticancer properties were classified into 14 chemical groups such as, aldehydes, alkaloids, annonaceous acetogenins, flavonoids, glycosides, lignans, lipids, lipids (unsaponified), Nucleic acids, phenols and derivatives, polysaccharides, proteins terpenoides and unidentified compounds (Kintzios and Barberaki, 2004). Pioneering studies of the active constituents of some plants and the discovery of some anticancer agents prompted the National Cancer Institute (NCI) in collaboration with the United States Department of Agriculture (USDA) to establish a program for the collection and screening of plants for antitumor activity (Suffness and Douros, 1982). Since 1961, over nine plant-derived compounds have been approved for use as anticancer drugs in the US. Some of these compounds are: vinblastine, vincristine, Navelbine, etoposide, teniposide, Taxol (paclitaxel), Taxotere (docetaxel), topotecan and irinotecan (Kuo-Hsiung, 1999). Podophyllin extracted from *Podophyllum peltatum* has also been found to inhibit mitosis *in vitro* and its derivatives are found to be capable of arresting cells in either late S phase or early G2 phase, without inhibiting microtubule assembly. Taxol and docetaxel derived from *Taxus brevifolia* and *Taxus baccata* respectively are also active in preclinical animal screening systems for anticancer drugs (Sllchenmyer and Von Hoff, 1991; Tanaka et al., 1996). Turmeric extract and curcumin isolated from it is effective in reducing animal tumors, indicating its potential for use in cancer

treatment (Kuttan et al., 1985; Krishnaswamy et al., 1998). The root extract of *Camellia sinensis var. assamica* significantly inhibited Ehrlich Ascites carcinoma growth and 3-methylcholanthrene-induced solid tumors in mice (Chaudhuri et al., 1998). Genistein, an isoflavonoid found in soybeans significantly decreases the incidence of cancer metastasis in rats (Iishi et al., 2000). The introduction of active agents derived from natural sources into the anticancer weaponry has already significantly changed the futures of many individuals afflicted with cancer of many different types. Continued research into natural sources will continue to deliver newer and more promising chemicals and chemical classes of anticancer agents with novel mechanisms of action that will improve survival rates to even higher degrees.

In Asia, North-East India is one of the biodiversity hotspots. The region is endowed with varied flora to its diversified topography and climatic conditions marked by high rainfall, moderate temperature and high humidity and the region abounds in dense forests, marshes, swamps etc. with their characteristic and diversified species where a wide spectrum of vegetation ranging from the Tropical to the Alpine forests types occur. Different tribes living in this area mostly rely on traditional herbal medicine for their primary healthcare practices. Meghalaya and Mizoram are small hilly North-Eastern states of India. Meghalaya lies between 25°.00′ and 26°.10′N latitude and 89°.45′ and 92°.45′E longitude (Maikhuri and Gangwar, 1993). Mizoram lies between 21°.58′ and 24°.35′N latitude and 92°.15′ and 93°.26′E longitude (Lalramnghinglova, 1996). Meghalaya is inhabited by three distinct tribes, such as the Khasi, Garo and Jaintia. The state is endowed with rich natural vegetation ranging from tropical to sub-tropical type of vegetation or from an evergreen to mixed deciduous types of forests (Cajee, 1999). Mizoram state has humid tropical, sub-tropical and sub-temperate climates with high rainfall and it is

also endowed with a variety of vegetations (Lalramnghinglova and Jha, 1998). The state is inhibited by fifteen ethnic groups of the Mizo tribes (Dutta, 1992). Majority of the tribes are settled in rural areas where there are no good modern medical facilities. The tribes living in these two states were depending mostly on surrounding plant resources for their food, shelter, medicare, and other cultural purposes. Generally, majority of the tribes in these states follow the indigenous way of medical techniques. They believe in traditional system of medicines prepared by using herbs, roots, animal extraction etc. The treatment given for these diseases by the tribal traditional healers generally do not cost much as they make use of locally available herbs, whereas, in allopathic system the medicines are not only expensive but also cause side effects.

Medicinal plants are used either alone or in combination with another. The people of these states generally use three common preparations of medicinal plants, such as, infusion, decoction and rubbing on grindstone. Other uses involve poultice or paste employed for external applications, massages or dabbing onto the affected part of the body (Lalramnghinglova and Jha, 1998).

Our preliminary investigation through literature search, personal interview with elders and consultation of some herbal practitioners shows that for the treatment of cancer suspected diseases, various plants like *Claoxylon hassianum, Celerodendrum wallichii, Mussaenda macrophylla, Phlogacanthus thyrsiformis, Curcuma longa, Asclepias curassavica, Lonicera macaranda, Youngia japonica, Blumea lanceolaria, Dillenia pentagyna, Ageratum conyzoides,* etc., are traditionally used by the tribal people of Mizoram (Lalramnghinglova, 1999; 1996; Rozika, 2001), and *Taxus baccata, Potentilla fulgens, Panax pseudoginseng,* etc., are used by the tribal people of Meghalaya (Syiem et al., 1999; Thamar and Syngai, 1999). However, only few of these plants were reported to have better activity against cancer suspected

diseases. Most of these plants have not received scientific scrutiny with reference to their antitumor activity.

CHEMOPREVENTION:

One of the most effective strategies in cancer control is chemoprevention, where chemoprevention is defined as the prevention, delay, or reversal of carcinogenesis/mutagenesis (Mehta and Pezzuto, 2002). A few of the more promising cancer chemopreventive agents are: brusatol from *Brucea javanica*; zapotin from *Casimiroa edulis*; apigenin from *Mezoneuron cacullatum*; deguelin from *Mundelea sericea;* brassinin from *Brassica* spp.; and resveratrol from *Cassia quinquangulata* (Mehta and Pezzuto, 2002).

Chemopreventives have different mechanisms of action, which can block or suppress the effect of mutation. Most antimutagens act by inducing enzymes, which mediate reactions that enhance the elimination of carcinogens/mutagens. A growing field of cancer preventing research is chemoprevention, or the use of natural or synthetic compounds to decrease the number of mutations that may lead to cancer. Chemoprevention research seeks to identify those compounds that reduce risk and use them in pills or food additives as a prevention measure for those who are at high risk for cancer. Anticarcinogenic and chemopreventive activities by a variety of agents that have shown promising chemopreventive activity include antioxidants, anti-inflammatory, anti-oestrogens and anti-androgens.

Phenolic compounds, fibre, chlorophyll, b-carotene, and vitamins such as C and E, a component of fresh fruits and vegetables were suggested to have antimutagenic and/or anticarcinogenic properties (Starvic, 1994; Ho, 1992; Kuo, et

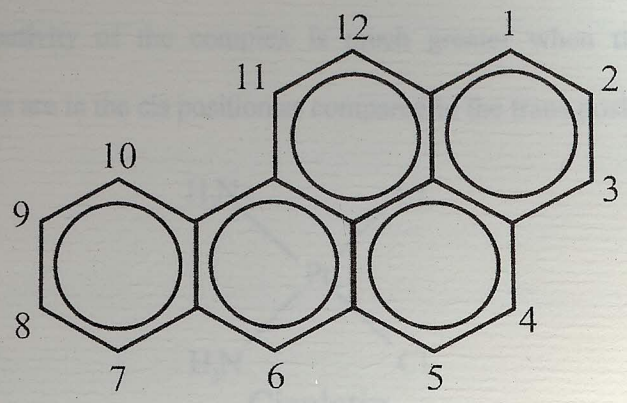
al., 1992), and a negative association between the incidence of cancer and consumption of diet rich in fibres, fresh vegetables, vitamins and minerals was also reported (Archer, 1999; Steinmetz and Potter, 1991). Some of the food ingredients including vitamins, flavonoids and organosulphur compounds possess antimutagenic and anticarcinogenic activities (Starvic, 1994), and extracts of certain plants were reported to have the ability to inhibit the mutagenic activity of well established genotoxins (Ito et al., 1986; Khanduja and Majid, 1993; Abraham et al., 1986). Phenolic compounds, extracted from common beans (*Phaseolus vulgaris*) were shown to have antimutagenic activity against BaP in *Salmonella typhimurium* tester strain YG1024 (Mejia et al., 1999). The fruit extract of *Emblica officinalis* (Family: Euphorbiaceae) has been shown to significantly reduce the mutagenic effects of metals and some environmental carcinogens such as benzo[a]pyrene and cyclophosphamide (Sharma et al., 2000).

MODEL MUTAGENS:

i) BENZO[a]PYRENE:

Benzo[*a*]pyrene is one of the many hundreds of polycyclic aromatic hydrocarbons (PAH) and it's carcinogenic and genotoxic potential has attracted most attention. It causes various toxicological effects, such as haematological effects, reproductive and developmental toxicity and immunotoxicity also. Benzo[*a*]pyrene (CAS Reg. No. 50-32-8), also known as 1,4-benzo[*a*]pyrene (B[a]P), is a polycyclic aromatic hydrocarbon (PAH) with a chemical formula of $C_{20}H_{12}$ and a molecular weight of 252.3. It exists as yellowish plates and needles, has a boiling point of 310-312°C at 10 mm Hg (Budavari et al., 1989), a melting point of 178°C, and a density

of 1.35 (U.S. EPA, 1991). B[a]P is practically insoluble in water, but is soluble in benzene, toluene, xylene, and is sparingly soluble in alcohol and methanol (Budavari et al., 1989). It occurs ubiquitously in products of incomplete combustion and in fossil fuels. It has been identified in surface water, tap water, rain water, ground water, waste water, and sewage sludge (U.S. EPA, 1991). B[a]P is primarily released to the air and removed from the atmosphere by photochemical oxidation and dry deposition to land or water. Biodegradation is the primary transformation process in soil or sediment (ATSDR, 1990).



Benzo[a]pyrene ($C_{20}H_{12}$)

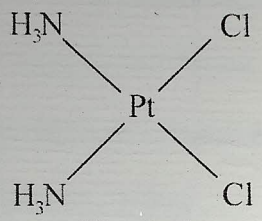
Administration of B[a]P through different routes like gavages, diet, topical, inhalation, subcutaneous, intravenous and intraperitoneal injections etc. induced development of malignant tumors in different tissues of mice and rats. Some of these tumors include malignant and benign forestomach tumors, lung adenomas, skin carcinomas and papillomas, tracheal papillomas and carcinomas, abdominal fibrosarcomas, mammary and uterine carcinomas, etc. (IARC, 1973).

B[a]P can bind to the aryl hydrocarbon receptor (AHR), which then induces the expression of many genes, including members of the cytochrome P450 family of enzymes. B[a]P is then metabolized to an array of reactive species that form covalent

bonds with nucleic acids and proteins within target cells, generate reactive oxygen species (Xie and Herschman, 1995; Balinsky and Jaiswal, 1993), and cause genetic mutations and cancer (Conney, 1982; Shields et al., 1993). The mutagenic potential of B[a]P in the strain YG1024 has been reported (Watanabe et al., 1990).

ii) CISPLATIN:

Cis-diamminedichloroplatinum-II, commonly known as cisplatin (CIS) is a water soluble, square planar coordination complex containing a central platinum atom surrounded by two chloride atoms and two ammonia moieties in the *cis*-configuration. The antitumor activity of the complex is much greater when the chloride and ammonia moieties are in the cis position as compared to the trans position.



Cisplatin

Cisplatin is widely used as a chemotherapeutic agent either alone or in combination with other drugs, radiotherapy and/or surgery (Prasad and Giri, 1994; Go and Adjei, 1999) against a variety of cancers (Carter, 1984). Now, cisplatin has been established to be a potent antitumor drug against a wide spectrum of experimental tumors such as leukemia L1210, DMBA mammary carcinoma, Rous sarcoma, Dunning ascites leukemia, Walker 256 carcinoma (Kociba et al., 1970; Rosenberg, 1985) and also in human malignancies such as ovarian and testicular tumors, bladder carcinoma, head and neck cancer (Pil and Lippard, 1997; Lebwohl and Canetta, 1998). The ability of cisplatin to react with nucleophilic bases in DNA and form intrastrand and interstrand cross-links has been suggested to be the main mechanism

behind its anticancer activity (Coste et al., 1999). Besides DNA, cisplatin has been shown to affect tissue calcium, potassium (Prasad and Giri, 1999) and sialic acid concentrations (Nicol and Prasad, 2002), various enzymes (Prasad et al., 1999) and mitochondrial functions (Kharbangar, et al., 2000). Although cisplatin is one of the most widely used chemotherapeutic agents, its therapeutic efficacy is limited due to the side effects which include nephrotoxicity, neurotoxicity, gastrointestinal toxicity, ototoxicity, embryotoxicity and also its mutagenic potential (Giri et al, 1998). Its mutagenic effects reported in bacteria (Overbeck et al., 1996) as well as in mammalian cells (Cross et al., 1996) raises concern with the development of secondary malignancies (Greene, 1992). An increased carcinogenic risk with the development of secondary tumors in patients/animals treated with cisplatin has also been reported (Krakoff, 1979; Khynriam and Prasad, 2001; Cross et al., 1996). It has been reported to cause genotoxic effects in cultured mammalian cells (Zwelling et al., 1979) and bone marrow cells (Giri et al., 1998).

Carcinogens bind to the cell macromolecules namely, DNA, RNA and proteins and result in mutagenic events leading to cell transformation and neoplastic changes. Some phytochemicals prevent these changes from occurring either by directly binding to the carcinogens/their metabolites or by metabolising and eliminating toxic xenobiotics. These are known as antimutagens/anticarcinogens. Antimutagenicity effect of turmeric was evaluated in human smokers who are known to excrete mutagens. A clinical trial in reverse smokers who are at a high risk of palatal cancers showed that turmeric administration (1g/day for 9 months) had a significant impact on the regression of precancerous lesions such as red and white patches over the palatal regions and decreased the micronuclei and DNA adducts in oral epithelial cells which are markers for genomic damage (Krishnaswamy, 1996).

Thus, considering their mutagenic potential, cisplatin and benzo[a]pyrene were selected as model mutagens in the evaluation of plant extract antimutagenicity.

The recent surge of interest in the use of medicinal plants has generated a great deal of research on major constituents and their effects on human health. However, more research is needed to extend the search for potentially beneficial herbs from natural sources and determine their use in modern medicine. Thus, considering the importance of some of the medicinal plants from Meghalaya and Mizoram with the probable anticancer medicinal value, particularly in the life of the people of these states and other people in general, the present study was undertaken. This may be helpful to derive a comparative therapeutic value of the plants and to establish their antitumor activity particularly against murine Dalton's lymphoma and other cancers in general.

In view of the various findings described above on the importance of natural plant products in malignancy and mutagenicity, the present investigation on the evaluation of anticancer activity by some medicinal plants from Meghalaya and Mizoram were undertaken in murine tumor model in an attempt to:

i. *Establish the therapeutic efficacy of plant crude extracts against murine ascites Dalton's lymphoma*
ii. *Understand the possible mechanism of action of the plant extract against malignancy*
iii. *Explore the antimutagenic potential of the same plant*