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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Dedicated with love

to

my parents

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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

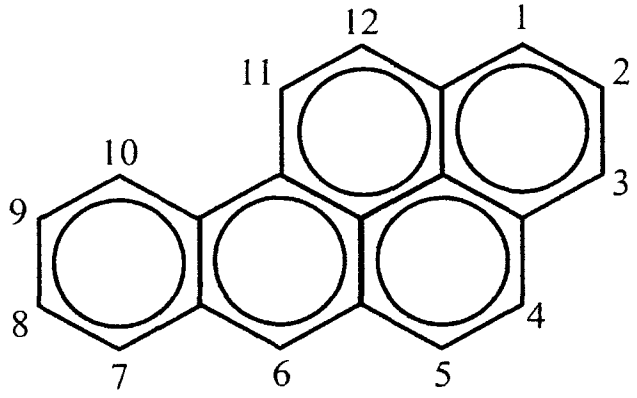
al., 1992), and a negative association between the incidence of cancer and consumption of diet rich in fibres, fresh vegetables, vitamins and minerals was also reported (Archer, 1999; Steinmetz and Potter, 1991). Some of the food ingredients including vitamins, flavonoids and organosulphur compounds 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 ($C_{20}H_{12}$)

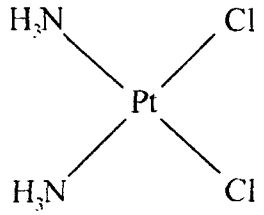
Administration of B[a]P through different routes like gavages, diet, topical, inhalation, subcutaneous, intravenous and intraperitoneal injections etc. induced development of malignant tumors in different tissues of mice and rats. Some of these tumors include malignant and benign forestomach tumors, 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*

MATERIALS AND METHODS

MATERIALS AND METHODS

A. CHEMICALS:

Reduced glutathione (γ-glu-cys-gly; GSH), oxidised glutathione (GSSG), Glutathione reductase (GR), [5,5'-dithiobis-(2-nitrobenzoic acid)] (DTNB), Fetal bovine serum, Benzo[a]pyrene, were purchased from Sigma Chemical Company St. Louis, MO, U.S.A. 1-chloro-2,4-dinitrobenzene (CDNB), nicotinamide adenine dinucleotide phosphate reduced (NADPH), hydrogen peroxide (H_2O_2), sodium azide (NaN_3), colchicines, sodium nitrate, ethylenediaminetetra-acetic acid (EDTA), 2-thiobarbituric acid and other chemicals used in the experiments were of analytical grade and purchased from SRL Pvt. Ltd., Mumbai, India.

B. ANIMALS AND TUMOR MAINTENANCE:

Inbreed Swiss albino mice in the age group of about 10-12 weeks old of both sexes were used for the experiments. All mice were maintained in the laboratory under conventional conditions at room temperature of 20 ± 2^0C with free access to food pellets (Amrut Laboratory, New Delhi) and water *ad libitum*.

Ascites Dalton's lymphoma was initially obtained from Gauhati University in the year 1989, and it is being maintained in mice by serial intraperitoneal (i.p.) transplantations of approximately $1x10^7$ viable tumor cells per animal (0.25 ml in phosphate-buffered saline (PBS), pH 7.4). Early sign of tumor development was visible after 3 to 4 days of tumor transplantation. Tumor transplanted hosts usually survived for 19-21 days.

C. ANTITUMOR STUDIES:

a) Selection of medicinal plants:

Preliminary investigation for selection of antitumor medicinal plant was carried out through literature survey, personal interview and consultation of local herbal practitioners and elders. The following questions were asked in the interview:

- Name of the plant(s) used for the treatment of cancer suspected diseases.
- Suspected cancer type(s) for which the particular plant was used.
- Part(s) of the plant(s) used and methods of preparation.
- Treatment schedule.
- Other type(s) of diseases for which the particular plant was used.
- Comments about the effectiveness of the plant(s).

The selected medicinal plants specimens were taken to the herbaria of Department of Botany, NEHU, Shillong, for identification. The herbaria and voucher specimen numbers of the plants were preserved. The crude extract of various parts i.e. bark, leaves, roots, etc., which are traditionally practiced, were prepared and used for the study of antitumor activity.

b) Plant extracts preparations:

The roots of *A. conyzoides* and *P. fulgens*, leaves of *B. lanceolaria* and *T. baccata* and stem bark of *D. pentagyna* were collected, washed in distilled water followed by 70% alcohol and shade dried in sterilized container at about 40^0C in an oven. The plant tissues were cut into small pieces and ground with a sterile mortar and pestle, and processed further for aqueous and methanol extraction following the method of Alasbahi *et al.*, (1999).

For the preparation of aqueous extracts, 500 g of ground tissue samples were extracted with 500 ml of sterile water in a 2 litres boiling flask, setting the flask in a water bath at 100^0C for 2 hr. After removal of insoluble materials by filtration through Whitman No. 1 filter paper, the filtrates were centrifuged at 800xg for 15 min and the clear supernatants were collected. The supernatants were evaporated to dryness in an oven under the temperature of 50^0-55^0C. The extracts were collected and stored at 5^0C until used for screening of antitumor activity.

Methanol extract of all the plant tissues were also prepared using a slight modification of the method described by Alasbahi *et al.*, (1999). 500 g of ground plant tissue samples were extracted with 500 ml absolute methanol in a 2 litres Erlenmeyer flask for 24 hr at ambient laboratory temperature (20^0-24^0C). The tissue-solvent mixtures were filtered through Whitman No. 1 filter paper to remove the tissue residue. The filtrates were centrifuged at 800xg for 15 min and the supernatants were collected. The supernatants were then evaporated to dryness in an oven under the temperature of 50^0 - 55^0C. All the extracts were stored at 5^0C until used for screening of antitumor activity.

The plant extracts were tried to dissolve preferentially in double distilled water, phosphate-buffered saline (PBS), ethanol, methanol and sodium hydroxide solution. Depending on the solubility criterion the various extracts were dissolved in different solvents for the treatment vehicle as follows: aqueous extract of *A. conyzoides* and methanol extract of *B. lanceolaria* were dissolved in phosphate-buffered saline (PBS) and 2% methanol respectively while all other extracts were dissolved in 0.05% sodium hydroxide solution.

c) Percentage yield of the plants extract:

For the evaluation of percentage yield of different plant extracts, 100g each of the dried and powdered plant tissues were used. The percentage yield was determined using the formula:

$$\% \text{ yield} = \frac{\text{wt. of the extract (mg)}}{\text{wt. of the plant tissue (mg)}} \times 100$$

d) Evaluation of antitumor activity:

Preliminary antitumor activity of the plant extracts was determined following the method of Sakagami *et al.*, (1987). Dalton's lymphoma cells ($1x10^7$) were transplanted intraperitonealy into 10 to 12 weeks old Inbreed Swiss albino mice. Tumor transplantation day was designated as day '0' (Figure 2 and 3). Tumor-transplanted animals were divided into four groups consisting of 10 mice per group. Group I (control) animals received 0.25 ml of the respective extract vehicle, once daily for five days. Group II, III and IV were treated with 50, 100 and 200 mg/kg body wt/day of the extract respectively. Plant extracts were administered by intraperitoneal injection beginning day one of tumor transplantation, once daily for five days. The deaths, if any, of the hosts were recorded daily and the survival pattern of the hosts was determined for different groups. The antitumor efficacy of different extracts was reported in percentage of average increase in life span (ILS), and was calculated using the formula: (T/C x 100) – 100,

where, T and C are the mean survival days of treated and control groups of mice respectively.

In order to determine the most potent dose of the extracts, animals were given higher (above 200 mg/kg body wt/day) or lower (below 50 mg/kg body weight/day)

doses depending on the preliminary screening results for antitumor activity. Extracts/drugs that prolong survival by more than 20% were considered to have antitumor activity.

A single plant extract, methanol extract of *D. Pentagyna,* showing the highest antitumor activity was selected for further dose determination using two routes of treatment i.e. intraperitoneal (i.p.) injection and through diet. In the treatment through i.p. injection, animals were administered with the methanol extract of *D. Pentagyna* (10, 15, 20, 25, 30, 50, 100 and 200 mg/kg body wt/day) beginning 1st day after tumor transplantation, once daily for 5 days. For the treatment through diet, the animals were fed with the diet supplemented with different concentrations of plant extract (70, 175, 350 and 525 mg/kg of diet) beginning 7 days before tumor inoculation till the end of the experiment. As the daily intake of diet per animal is about 6.8-7.5 g, the daily intakes of plant extract from different diet with different extract concentration are approximately 20, 50, 100 and 150 mg/kg body wt. Control mice were fed with the diet mixed with extract vehicle only. The most potent dose of the selected plant extract was used in the biochemical, haematological, microscopical and antimutagenic studies.

D. HAEMATOLOGICAL STUDIES:

Based on the result of preliminary antitumor activity test, *Dillenia pentagyna* (20 mg/kg body weight) was selected and administered to the tumor-bearing hosts on the 10th day post-tumor transplantation, i.e. the mid phase of tumor growth. The plant extract was dissolved in 0.05% NaOH and the pH of the extract solution becomes

6.13. The experimental mice were divided into 3 groups consisting of five mice each as follows;

Group 'I'- Normal mice, without tumor

Group 'II'- Tumor-bearing Control receiving extract vehicle only (0.25ml of 0.05% sodium hydroxide)

Group 'III'- Tumor-bearing mice treated with a single dose of *Dillenia pentagyna* extract (20 mg/kg body wt) on the 10^{th} day of post-tumor transplantation.

The treatment schedule was carried out for 4 days i.e. 24, 48, 72 and 96 hours of time interval. The animals in different groups were sacrificed by cervical dislocation and liver, kidney, spleen, testes, DL cells and blood samples were collected and used for haematological, biochemical, enzymatic and microscopical studies (Figure 9).

a) Collection of blood samples:

Blood samples from different treatment groups of mice were collected from the tail vein into a sterilized tube containing heparin (15-20 IU per ml of blood) and used for the study.

b) Red blood cell counts:

Freshly collected blood was diluted (200 times) with RBC diluting fluid contained in a plastic tube. After sealing the tube, the diluted blood was mixed in a mechanical mixture. The counting chamber, with its cover-glass already in position, was filled with the diluted blood sample. As many cells as possible were counted in a Neubauer chamber using the 4 mm objective and 10x eyepiece, and the erythrocytes absolute values were calculated according to Dacie and Lewis (1975) as follows:

$$\text{Red-cell count per } \mu l = \frac{N \times 1 \times 200 \text{ (dilution)}}{0.02}$$
$$= N \times 10000$$
$$= N \times 10^4 \times 10^6/L.$$

c) *White blood cell counts:*

Freshly collected blood was diluted (20 times) with white blood cells (WBC) diluting fluid (2% acetic acid) contained in a plastic tube. The diluted blood sample was mixed for about 1 minute and counted in a Neubauer chamber using the 16 mm objective and 10x eyepieces. The number of white blood cell was calculated according to Dacie and Lewis (1975).

White blood cell count per μl = N x 10 x 20 (dilution)
= N x 200
= N x 200 x 10^6/L.

d) Differential leukocyte counts:

Differential leukocyte count was carried out according to Dacie and Lewis (1975). A drop of fresh blood was taken on a clean slide and a thin and uniform blood film was prepared with the help of another clean slide. The blood film was air dried for overnight, stained with Leishman's stain the following day and mounted in DPX. Counting was done under microscope in a narrow longitudinal strip of the blood film starting from one end of the film to the other end. The number of different types of white blood cells (neutrophils, basophils, monocytes, lymphocytes and eosinophils) were noted and expressed in percentage.

e) Packed cell volume (PCV) determination:

Packed cell volume was measured according to Dacie and Lewis (1975). Blood was collected in a wintrobe tube and centrifuged for 30 min (2000xg, at 4^0C). The height of the column of red cells was taken as the PCV (the volume occupied by the red cells expressed as a fraction of the total volume of the blood).

f) Haemoglobin estimation:

Haemoglobin content of blood was determined according to Dacie and Lewis (1975). Blood was diluted (200 times) with cyanide-ferricyanide reagent (200 mg potassium ferricyanide and 50 mg potassium cyanide in 1 litre of distilled water) and allowed to stand for 10 min at room temparature. The absorbance was read at 540 nm in a Beckman DU-640 spectrophotometer. The haemoglobin content was calculated as follows:

$$\text{Concentration (g/dl)} = \frac{A^{540} \times 64500 \times \text{dilution factor}}{44.0 \times d \times 1000 \times 10}$$

where, A^{540} = Absorbance at 540 nm, 64500 = molecular weight of haemoglobin, dilution factor = 200, 44.0 = milimolar extinction coefficient, d = layer thickness in cm, 1000 = conversion factor for mg to g and 10 = conversion factor for g/litre to g/dl.

E. BIOCHEMICAL STUDIES:

The plant extract treatment protocol was followed as per details mentioned above in 'haematological studies'.

a) Protein estimation:

The protein content in the different tissue samples was determined following the method of Lowry *et al* (1951) using bovine serum albumin (BSA) as standard. Tissue homogenate (2%) was prepared in 0.25M sucrose solution, while 20 times dilution was done in case of ascites supernatant.

To 1 ml of the homogenate taken in duplicate, 5 ml of freshly prepared protein reagent (49 ml of 2% Na_2Co_3 in 0.1M NaOH + 1 ml of Copper sulphate, Sodium-Potassium tartarate solution (1% $CuSo_4.5H_2O$ in 1% Na-K tartarate, freshly prepared) was added, thoroughly mixed and allowed to stand at room temperature exactly for 10

min. After 10 min, 0.5 ml of 1N Folin-Ciocalteau reagent was added and mixed instantly. The reaction mixture was then kept at room temperature for 30 min to complete the reaction and the optical density was read at 750 nm against the reagent blank. The protein concentration was determined from the standard curve obtained by using bovine serum albumin (BSA) as standard.

b) Glutathione estimation:

Glutathione level was determined as total (TSH) sulfhydryl contents using the method of Sedlak and Lindsay (1968). Briefly, 5% tissue homogenates were prepared in 0.02M EDTA (pH 4.7) in a motor-driven teflon–pestle homogenizer. Total glutathione (TSH) was determined by adding the homogenate (0.1 ml) to 1 ml of 0.2 M Tris–EDTA buffer (pH 8.2) and 0.9 ml of 0.02 M Tris–EDTA buffer (pH 4.7) followed by 20 µl of Ellman's reagent. After 30 min of incubation at room temperature, the reaction mixture was centrifuged at 3000xg for 20 min and the absorbency of the clear supernatant was read against a reagent blank at 412 nm in a Beckman DU-640 spectrophotometer.

c) Glutathione-s-transferase assay:

Tissues collected from animals were washed in ice-cold physiological saline and stored at -70^{0}C until used for enzyme analysis.

Glutathione-s-transferase activity was assayed following the method of Habig et. al. (1974). A 0.1% tissue homogenate was prepared in 0.1M potassium phosphate-1mM Na_2 EDTA buffer (pH 6.5). The tissue homogenate was centrifuged at 27,000g for 20 min at 4^{0}C and the supernatant was collected as the enzyme source.

To a 1.0ml cuvette; 500µl of 0.1M potassium phosphate-1mM Na_2 EDTA buffer (pH 7.0), 50µl of 30mM CDNB in 95% ethanol and 25µl enzyme were added.

The overall reaction (maintained at 30^0C) was started by adding of 50µl of 30mM reduced glutathione and the increase in absorbance at 340 nm was monitored for 5 min. The enzyme activity was calculated using the extinction coefficient (E_{340} = $9.6mM^{-1}cm^{-1}$). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the conjugation of 1µM of CDNB per minute. Specific activity was expressed in units per mg of protein.

d) Glutathione reductase assay:

Glutathione reductase activity was assayed according to Smith et. al., (1988). A 0.4% (w/v) tissue homogenate was prepared in 0.2M potassium phosphate-2mM Na_2 EDTA buffer (pH 7.0). The tissue homogenate was centrifuged at 27000xg for 20 minutes at 4^0C and the supernatant was collected as the enzyme source.

To a 1.0ml cuvette; 500µl of 0.2M potassium phosphate-2mM Na_2 EDTA buffer (pH 7.0), 50µl of 2mM NADPH in 10mM tris-HCL (pH 7.0) and 50µl of 20mM GSSG were added. The reaction was initiated by the addition of 50µl of the enzyme to the reaction mixture and the decrease in absorbance at 340 nm was monitored at 37 °C for 5 min. The enzyme activity was calculated using the extinction coefficient ($E_{340} = 6.22mM^{-1}cm^{-1}$). One unit of glutathione reductase activity is defined as the amount of enzyme that catalyzes the reduction of 1µM of NADPH per minute. Specific activity was expressed in units per mg of protein.

e) Glutathione peroxidase assay:

Glutathione peroxidase activity was assayed according to Flohe and Gunzler (1984). A 0.4% (liver, kidney and spleen) and 1% (testes and DL cells) tissue homogenate was prepared in 0.1M potassium phosphate-1mM Na_2 EDTA buffer (pH

7.0). The tissue homogenate was centrifuged at 27,000g for 20 min at 4^0C and the supernatant was collected as the enzyme source.

To a 1.0ml cuvette; 500µl of 0.1M potassium phosphate-1mM Na_2 EDTA buffer (pH 7.0), 100µl of 1mM NaN_3, 100µl enzyme, 100µl glutathione reductase (0.24 U) and 100µl of 10mM reduced glutathione were added and incubated for 10 min at 37^0C. Thereafter, 100µl of 1.5mM NADPH in 0.1% NaHCO3 was added. The overall reaction was started by the addition of 100µl of prewarmed 1.5mM H_2O_2 and the decrease in absorbance at 340 nm was monitored at 37°C for 5 min. The enzyme activity was calculated using the extinction coefficient (E_{340} = 6.22mM^{-1}cm^{-1}). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1µM of NADPH per minute. Specific activity was expressed in units per mg of protein.

f) Sialic acid estimation:

The sialic acid concentration was determined using the method of Warren L (1959). A 5% tissue homogenate was prepared in 0.1 N H_2SO_4. Tissue homogenate was incubation for 1 hour in a water bath maintained at 80°C to release the bound sialic acid. The homogenate was then centrifuged at 8000 r.p.m. for 15 min and the clear supernatant was used for sialic acid estimation.

0.2ml of the supernatant was taken in a test tube and 0.1ml of periodate solution (sodium periodate, 0.2M in 9 M phosphoric acid) was added, mixed thoroughly and allowed to stand at room temperature for 20 min. 1ml of arsenite solution (sodium arsenite, 10% in a solution of 0.5M sodium sulphate in 0.1N H_2SO_4) was added and mixed until a yellow brown colour disappeared. Then, 3ml of thiobarbituric acid was added, mixed thoroughly and kept in a boiling water bath for

15 min. After cooling, the chromophore was extracted in an equal volume of cyclohexanone by vigorous shaking and then centrifuged at 2000rpm for 10 min. The clear upper cyclohexanone phase containing the chromophore was removed carefully to another test tube and the optical density was recorded at 532 nm and 549 nm against blank. The sialic acid concentration was determined from the extinction coefficient using the formula;

Sialic acid (μmoles) $=0.090 \times O.D_{549} - 0.033 \times O.D_{532}$

g) Lipid peroxidation:

Lipid peroxidation in different tissue sample was carried out following the method of Buege and Aust (1978). A 10% tissue homogenate was prepared in 0.15M KCl. To 1 ml of the tissue homogenate, 2 ml of the trichloro acetic acid (15%)-thiobarbituric acid (0.375%)-HCl (0.25N) reagent was added and mixed thoroughly. The sample was then heated in a boiling water bath for 15 min, and then cooled at room temperature. The precipitate was removed by centrifugation at 1000xg for 10 min. The absorbance of the clear supernatant was then read at 535nm against the blank. The malondialdehyde (MDA) concentration of the tissue sample was calculated using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and expressed as nmol of MDA/mg protein.

F. LIGHT MICROSCOPICAL STUDIES:

The plant extract treatment protocol was followed as per details mentioned above in 'haematological studies'.

Animals in different treatment groups were sacrificed by cervical dislocation. The ascites tumor was collected and centrifuged at 1000 rpm for 5 min. at 4°C, washed in PBS (0.15M NaCl, 0.01 M sodium phosphate buffer, pH 7.4) . The cell pellet was resuspended in PBS (1:4) and a drop of the cell suspension was taken on a clean slide and a thin smear was prepared. The smear was air-dried, fixed in absolute methanol for 15 min. and stained the following day with Leishman's stain. The cells in different slides were studied and photographed.

G. MUTAGENIC STUDIES:

Plant extract was suspended in 0.05% NaOH, cisplatin in 0.89% sodium chloride and benzo[a]pyrene in corn oil. The animals were divided into 3 groups as follows:

Group 'I'- Mutagen control: Animals were treated with a single dose of cisplatin (10 mg/kg body wt) or benzo[a]pyrene (125 mg/kg body wt). Cisplatin and benzo[a]pyrene were inoculated through i.p injection and gavages respectively.

Group 'II'- Animals received a combination treatment of mutagen and *Dillenia pentagyna* extract (20 mg/kg body wt) on the same day.

Group 'III'- Animals were pre-treated with different doses of *Dillenia pentagyna* extract (20, 50 and 100 mg/kg body wt/day) for 7 consecutive days prior to a single dose of mutagen.

The time schedule after mutagen treatment was followed for 4 days i.e. 24, 48, 72 and 96 hours of time interval. Bone marrow cells collected from different groups of treatment, consisting of 5 mice in each treatment group, were used for chromosomal analysis and micronucleus assays.

a) Chromosomal analysis:

Chromosomal analysis was carried out following the method of Sharma and Sharma (1994). Mice in different groups were subjected to mitotic arrest, initiated 2 h prior to sacrifice by intraperitoneal injection of colchicine (4 mg/kg body wt). Bone marrow was collected from humerus and femur by flushing in phosphate buffered saline (PBS, pH 7.4) using a syringe. Bone marrow cells were collected by centrifugation (1000 rpm, for 5 min, at 4^0C). 5 ml of pre-warmed hypotonic solution (0.075M KCl, pH 7.0) was added to the cell pellet and incubated for 25 min at 37^0C. After incubation, 5 drops of Carnoy's fixative (methanol : glacial acetic acid (3 : 1)) was added and mixed gently, followed by centrifugation at 1000 rpm for 5 min. The supernatant was discarded and 5 ml of chilled Carnoy's fixative was added to the cell pellet and incubated at 4^0C for 30 min. Fixation was repeated twice with 30 minutes intervals. Finally the cells were resuspended in a small volume of fixative. A few drops of this suspension was dropped on a cleaned, grease-free chilled slide, burnt on a flame, air-dried and stained on the following day for 5 min with freshly prepared working Giemsa stain (1 ml of 1% stock giemsa (in 50 ml glycerol + 50 ml methanol) + 0.25 ml methanol + 2.8 ml sorensen's buffer, pH 6.8), washed and mounted in DPX. Three hundreds good metaphase spreads were examined per animal and chromosome aberrations were classified into the general categories of chromatid breaks, gaps, exchanges, chromosomal fragments and sister chromatid unions. Gaps

have not been considered for statistical analysis of the data due to their controversial genetic significance (Preston et al., 1981).

b) Micronucleus assay:

Micronuclei were assayed in different groups of mice following the method of Fenech, M. (2000). Ascites tumour was collected from the peritoneal cavity with hypodermic syringe and bone marrow was collected from humerus and femur by flushing in phosphate buffered saline (PBS, pH 7.4) using a syringe. Bone marrow and DL cells were collected by centrifugation (1000 rpm, for 5 min, at 4^0C). The cell pellet was treated with a weak hypotonic solution (0.075M KCl/saline, 1:9, v/v) for 5 minutes. After centrifugation, cells were fixed in Carnoy's fixative (methanol/glacial acetic acid, 3:1, v/v) and repeated twice. Cells were resuspended in a small volume of fixative solution. A drop of this suspension was smeared onto wet chilled slide, air-dried and stained with giemsa in Sorensen's buffer (pH 6.8). Two thousand binucleated cells were analysed under the microscope for the presence of micronuclei. Micronucleated cells were scored at 100x (oil immersion lens) according to Fenech, M. et al., (2003).

c) Sperm abnormality assay:

The male mice in different groups were sacrificed after 10^{th} day of mutagen/mutagen and DPE treatment. The cauda epididymis were removed and placed in physiological saline. It was then minced into pieces and kept undisturbed for 20 min. The spermatozoa were spread on a clean slide, air-dried, fixed in absolute methanol for 15 min and then stained with 1% aqueous eosin-Y on the following day. Five hundred sperms from each mouse were examined for the abnormalities in sperm

head and tail shapes following the criteria as close as possible to those established by Wyrobeck and Bruce (1975).

STATISTICAL ANALYSIS: The significance of change between two groups at the corresponding time point of treatment was determined by Student's t-test. The comparison between different groups as a whole was made using ANOVA. The P value ≤ 0.05 was considered as significant.

RESULTS

RESULTS

A. ANTITUMOR STUDIES:

a) Selection of medicinal plants:

Based on the literatures and information received from the elders and local herbal practitioners, the anticancer medicinal plants were selected for the present study. From this information from the tribal people of Meghalaya and Mizoram for the treatment of cancer suspected diseases, five plants were selected for the comparative evaluation of their antitumor activity. These were *Potentilla fulgens* Wall., Rosaceae (shrubby cinquefoil herb) and *Taxus baccata* Linn., Taxaceae (common yew tree) from Meghalaya, and *Ageratum conyzoides* Linn., Asteraceae (goat weed herb), *Blumea lanceolaria* Linn., Asteraceae (locally called as buarze) and *Dillenia pentagyna* Roxb., Dilleniaceae (simpoh tree) from Mizoram (Figure 1). The details of plant, plant parts screened, families, local names and their possible therapeutic uses are given in Table 1.

b) Percentage yield of the plants extract:

The percentage yield of extract from different plants studied was found between 9.5% and 14.5%. The lowest yield (9.67%) was observed with the methanol extract of *Ageratum conyzoides*, while the highest yield (14.27%) was found with the aqueous extract of *Dillenia pentagyna* (Table 2; Figure 4).

c) Evaluation of antitumor activity:

Aqueous extract:

The test parts of the plants, doses of extracts and their effects on the survivability of the hosts in different experimental groups have been described in

Table 3. The death of mice, if any, was recorded daily and the survival pattern of mice in different experimental groups was determined. Out of five plants used in the study, *Ageratum conyzoides* and *Potentilla fulgens* showed comparatively better antitumor activity against ascites Dalton's lymphoma. The extract of root of *Ageratum conyzoides* showed 27% ILS at a dose of 100mg/kg body wt/day, and root of *Potentilla fulgens* showed 36% ILS at a dose of 50mg/kg body wt/day. However, aqueous extract of *Taxus baccata, Blumea lanceolaria* and *Dillenia pentagyna* did not exhibit significant antitumor activity (Table 3; Figure 5).

Methanol extract:

Among the methanol extracts of five plants studied, stem bark extract of *Dillenia pentagyna* significantly increased the survival time of the hosts (ILS ~ 49%) at a dose of 50 mg/kg body wt/day. However, the extract of other plants did not exhibit antitumor activity (Table 4; Figure 6).

Thus, the comparison of aqueous and methanol extracts of these five plants showed that methanol extract of stem bark of *Dillenia pentagyna* exhibited the highest antitumor activity (Figure 7). Considering the observed better antitumor efficacy, methanol extract of stem bark of *Dillenia pentagyna* was selected for further dose determination. The maximum increase in the survival time of the hosts (ILS ~71%) was observed with intraperitoneal treatment at a dose of 20 mg/kg body wt/day (Figure 8). Inoculation of plant extract through the diet did not show significant increase in the survival time of tumor-bearing mice except at a dose of 100 mg/kg body wt/day (Table 5). Thus, the most potent dose (i.p., 20 mg/kg body wt/day) of *D. pentagyna* extract showing maximum survival of tumor-bearing host was selected for further hematological, biochemical, enzymatic and microscopical studies.

B. HAEMATOLOGICAL STUDIES:

a) Red blood cell (RBC) counts:

The red blood cells or erythrocytes (RBCs) count in normal mice was 7.57±0.46 ($x10^{12}$/l). In tumor-bearing control, the number of erythrocyes decreases significantly showing 2.26±0.22 ($x10^{12}$/l) on the 14^{th} day of tumor growth. Treatment of tumor-bearing mice with the plant extract significantly increased the RBC counts during 48 to 96 h of treatment (Table 6; Figure 10 and 11).

b) White blood cell counts:

The number of white blood cells or leukocytes (WBCs) in normal mice was 01.03±0.12 ($x10^{9}$/l). In tumor-bearing control, there was a significant increase in the number of leukocytes showing more than two folds increase on the 14^{th} day of tumor growth (Table 6). Treatment of tumor-bearing mice with the plant extract significantly increased the number of leukocytes during 48 to 96 h of treatment (Table 6; Figure 10 and 11).

c) Differential leucocytes counts:

As compared to normal mice an increase in the number of neutrophils, eosinophils, basophils and a decrease in lymphocytes and monocytes were observed in tumor-bearing control (Table 7).

As compared to control, the extract treatment caused a significant increase in the number of lymphocytes and eosinophils during 48 to 96 h and 24 to 96 h of treatment respectively. In contrast, a significant decrease in the number of neutrophils during 48 to 96 h and basophils during 24 to 96 h of treatment with the extract was observed. There was no significant change in the number of monocytes after treatment with the extract (Table 7; Figure 12).

d) Packed cell volume (PCV):

Packed cell volume in normal mice was observed to be 42.58 ± 3.32 (%), whereas, in tumor-bearing control, a significant decrease in the packed cell volume (PCV) was noted. As compared to the control, treatments with the plant extract significantly increased packed cell volume after 72 h of treatment (Table 6; Figure 10 and 11).

e) Haemoglobin (Hb):

Hb content of normal mice was 14.36±0.73g/dl. As compared to normal mice, in tumor-bearing control, a significant decrease in Hb content was observed (8.1±0.71g/dl) on the 14th day of tumor growth. However, after the treatment (48 to 96 h) of tumor-bearing hosts with the plant extract, the Hb content increased significantly (Table 6; Figure 10 and 11).

C. BIOCHEMICAL STUDIES:

a) Protein:

Among the different tissues of normal mice studied, the highest protein concentration was observed in liver (314.19 mg/g tissue wet wt) followed by spleen (241.52 mg/g tissue wet wt), kidney (215.84 mg/g tissue wet wt) and testes (175.38 mg/g tissue wet wt) (Table 8; Figure 13). As compared to normal mice, protein content of liver and testes in tumor-bearing control decreased significantly, while in kidney and spleen significant increase was noted. There was no significant change in the protein content of DL cells during 10 to 14 day of tumor growth, while a significant increase was noted in ascites supernatant on the 14th day of tumor growth (Table 8).

As compared to the corresponding tumor-bearing control values, a significant increase in the protein content was noted in liver, testes, DL cells and ascites supernatant after treatment with the plant extract, while a significant decrease was observed in kidney and spleen (Table 8; Figure 13). In liver, the maximum increase in protein content was observed during 48 h of treatment (~106.11%), and in testes, DL cells and ascites supernatant, the highest level of protein content was observed during 72 h (~117.38%), 48 h (~112.81%) and 96 h (~111.79%) of treatment respectively (Figure 14). In kidney and spleen, predominant decrease in protein content was noted during 48 h (~89.05%) and 24 h (~94.2%) respectively (Figure 14).

b) Glutathione:

Glutathione (GSH) content of normal and tumor-bearing mice:

Comparison of GSH level in the tissues of normal mice showed the highest glutathione concentration in liver (13.52 μmoles/g) followed by testes (10.52 μmoles/g), spleen (9.75 μmoles/g) and kidney (8.19 μmoles/g) (Table 9). As compared to the corresponding tissue of normal mice, the glutathione concentration in liver, kidney, spleen and testes of tumor-bearing control significantly decreased (Table 9; Figure 15).

GSH levels decreased in liver, kidney, spleen and testes during tumor growth, and a significant decrease was noticed on the 15^{th} day of tumor growth. In DL cells, a significant increase of GSH (5.96 ± 0.28 μmoles/g wet wt.) was observed on the 10^{th} day of tumor growth as compared to day 5 and decreased slightly over the next 4-5 days (Figure 17).

Glutathione content after DPE treatment:

DPE treatment resulted in a significant decrease in the total GSH concentration in liver and DL cells, and an increase in kidney and testes (Table 9;

Figure 15). A significant decrease in the total GSH concentration was noted in liver and DL cells during 24 to 96 h and 24 to 48 h of treatment respectively. A significant increase in the GSH concentration was also noted in kidney and spleen during 48 to 72 h of treatment, and in testes during 72 to 96 h of treatment. Percentage decrease in the level of GSH in liver (~78.27%) and DL cells (~53.78%) was predominant during 24 h of treatment. In kidney (~137.34%), spleen (~111.12%) and testes (~135.78%), percentage increase in the level of GSH content was predominant during 48, 48 and 96 h of treatment respectively (Figure 16).

c) Glutathione S-transferase:

Among the tissue of normal mice studied, the highest glutathione s-transferase (GST) activity was observed in liver (0.464 μmoles/min/mg protein) followed by testes (0.312 μmoles/min/mg protein), kidney (0.262 μmoles/min/mg protein) and spleen (0.183 μmoles/min/mg protein). In tumor-bearing control, GST activity increased in all the tissues (Table 10, Figure 18). After treatment with DPE, predominant increase in GST activity in kidney (~122%), spleen (~119%) and testes (~125%) were noted during 24 h of treatment, and in DL cells the increase was predominant during 72 h of treatment (~115%). A significant decrease in GST activity was also observed in liver during 24 - 72 h of treatment (Figure 19).

d) Glutathione reductase:

In normal mice the highest level of glutathione reductase (GR) activity was observed in kidney (0.48 μmoles/min/mg protein) followed by liver (0.33 μmoles/min/mg protein), testes (0.25 μmoles/min/mg protein) and spleen (0.20 μmoles/min/mg protein) (Table 11). As compared to the respective tissue of normal mice, GR activity increased significantly in liver, kidney, spleen and testes of tumor-

bearing control (Table 11). After DPE treatment, a significant decrease in GR activity was noted in liver, spleen and DL cells while a significant increase was observed in kidney (Figure 20). DPE treatment did not show significant change in GR activity in testes. Highest percentage increase in GR activity was observed in kidney during 72 h of treatment, while the highest percentage decrease was noted in the spleen during 24 h of treatment (Figure 21).

e) Glutathione peroxidase:

In the tissue of normal mice, glutathione peroxidase (GPx) activity was highest in liver (0.331 μmoles/min/mg protein). In tumor-bearing control, GPx activity decreased significantly in all the tissues (Table 12). As compared to the corresponding control, DPE treatment significantly decreased GPx activity in liver, spleen and testes during 24 to 96 h of treatment, and in kidney and DL cells during 24 to 72 h of treatment (Table 12; Figure 22). Among the tissues studied, percentage increase in GPx activity was predominant in kidney during 24 h of treatment (Figure 23).

f) Sialic acid:

Sialic acid content of normal and tumor-bearing mice:

Determination of sialic acid in the tissues of normal mice revealed that sialic acid content was highest in spleen (~1.36 μmoles/g tissue wet wt) followed by kidney (~1.09 μmoles/g tissue wet wt), liver (~0.86 μmoles/g tissue wet wt) and testes (~0.62 μmoles/g tissue wet wt) (Table 13; Figure 24). During tumor progression i.e. 5^{th}, 10^{th} and 15^{th} day, sialic acid content in liver, kidney and spleen increased significantly while a significant decrease was observed in testes. In DL cells and ascites supernatant also a significant increase was noted on the 15^{th} day of tumor growth as compared to the 5^{th} day (Table 13).

Sialic acid content after DPE treatment:

As compared to the corresponding tissue of tumor-bearing control, the sialic acid level decreased significantly in liver, kidney, spleen and DL cells after DPE treatment, while a significant increase was observed in ascites supernatant (Table 14; Figure 24). Testes did not show significant changes in sialic acid content. The predominant percentage increase was noted in ascites supernatant during 72 h of treatment (~123.65%). Liver showed a predominant percentage decrease during 48 h of treatment (~72.48%) (Figure 25).

g) Lipid peroxidation (LPO):

LPO concentration of normal and tumor- bearing mice:

The LPO was measured in terms of malondialdehyde concentrations in different tissues of mice. In the normal mice, the highest level of LPO was noted in the spleen (0.252 nmol/mg protein) followed by testes (0.170 nmol/mg protein), kidney (0.160 nmol/mg protein) and liver (0.122 nmol/mg protein) (Table 15). As compared to the corresponding normal tissues, a significant increase in the level of LPO in liver, kidney and testes was observed during tumor progression while a significant decrease was observed in Spleen. In DL cells, as compared to the 5^{th} day, a significant decrease in the level of LPO was noted on the 10^{th} day of tumor growth (Table 15).

LPO concentration after DPE treatment:

As compared to their corresponding control tissues, DPE treatment caused to lower the concentration of LPO in liver, kidney, testes and DL cells, while a significant increase was noted in the spleen (Table 16; Figure 26). The predominant percentage decrease in liver (~61.3%), kidney (~45.08%), testes (~27.6%) and DL cells (~78.57%) were noted during 96, 96, 48 and 48 h of treatment respectively,

while predominant percentage increase was also noted in DL cells (~193.61%) during 96 h of treatment (Figure 27).

D. LIGHT MICROSCOPICAL STUDIES:

The light microscopical observation showed rounded shape of control tumor cells. They were surrounded by a very few leukocytes (Figure 28A). After DPE treatment, more leukocyte infiltration towards the tumor cell was noticed (Figure 28B and 28C). DPE treatment for 96 h resulted in the appearance of membrane vacuoles and gradual disintegration of plasma membrane leading to lysis of the tumor cells (Figure 28D, 28E).

E. MUTAGENIC STUDIES:

a) Chromosomal aberrations:

Benzo[a]pyrene (125 mg/kg body wt) significantly increased the total chromosomal aberrations with chromatid breaks more frequently. Various types of aberrations such as chromatid breaks, isochromatid breaks, chromosomal fragments, exchanges and sister chromatid union were observed (Figure 29). Maximum frequency of various aberrations was seen at 24 h of treatment followed by a gradual decrease thereafter (Table 17). After single treatment (20 mg/kg body wt), a significant decrease in total aberrations was seen during 48 to 96 h of treatment (Figure 30A). DPE pre-treatment for 7 consecutive days with three different doses (20, 50 and 100 mg/kg body wt/day) prior to benzo[a]pyrene dose-dependently decreased the frequency of total aberrations induced by benzo[a]pyrene (Table 18, Figure 30B).

Chromosomal aberrations in the bone marrow cells of mice significantly increased after cisplatin treatment (Table 19). Maximum frequency of aberration was seen during 24 h of treatment (8 mg/kg body wt) and a slight decrease was noticed thereafter. Chromatid breaks and exchanges occurred more frequently. A single DPE treatment did not show significant changes in the frequency of chromosomal aberrations induced by CIS (Figure 30C). After pre-treatment with DPE (20, 50 and 100 mg/kg body wt/day) for 7 consecutive days prior to cisplatin, significant reduction in the total aberration was observed during 24 h of treatment (Table 20; Figure 30D).

b) Micronucleus:

Benzo[a]pyrene (125 mg/kg body wt) significantly increased the incidence of micronuclei in bone marrow cells. Single and multiple micronuclei were observed after B[a]P and CIS treatment (Figure 31A, B and C). The frequency of micronuclei was maximally observed during 24 h of treatment followed by a gradual decrease thereafter (Table 21). A single treatment (20 mg/kg body wt) did not show significant changes in the frequency of benzo[a]pyrene-induced micronucleus (Table 21; Figure 32A). DPE pre-treatment for 7 consecutive days with three different doses (20, 50 and 100 mg/kg body wt/day) prior to benzo[a]pyrene significantly decreased the frequency of benzo[a]pyrene-induced micronucleus during 24 to 72 h of treatment (Table 22, Figure 32B).

The incidence of micronuclei in bone marrow cells of mice significantly increase after cisplatin treatment (Table 23). Maximum frequency of micronuclei was seen during 24 h of cisplatin treatment (8 mg/kg body weight) followed by a slight decrease thereafter. A single DPE treatment did not show significant changes in the frequency of micronuclei induced by CIS (Table 23; Figure 32C). Among three

different doses used in pre-treatment, only 100 mg/kg body wt/day significantly reduced the incidence of micronuclei during 24 to 72 h of treatment (Table 24; Figure 32D).

c) Sperm abnormality:

B[a]P and CIS induces different types of abnormalities in the sperm after 10 days of treatment. Different types of abnormalities induced by B[a]P and CIS (double tail, hookless head, microhead, amorphous head and banana head) were as shown in the Figure 33. A single treatment with DPE did not show significant changes in the incidence of sperm abnormalities induced by both B[a]P and CIS (Table 25 and 26; Figure 34A). However, pre-treatment with all the three different doses (20, 50 and 100 mg/kg body wt/day) significantly decreases the frequency of sperm abnormalities induced by both B[a]P and CIS (Table 25 and 26; Figure 34B and 34C).

TABLES

Table 1: Ethnobotanical information of some anticancer medicinal plants selected from Meghalaya and Mizoram states, India.

Plant species	Family	Local name	Part used	Therapeutic use
Ageratum conizoides Linn.	Asteraceae	Vailenhlo*	Root	Treatment of tumor and in cuts and wounds as haemostatic.
Blumea lanceolaria Linn.	Asteraceae	Buarze*	Leaves	Treatment of tumor, asthma, stomachache, wounds and scabies.
Dillenia pentagyna Roxb.	Dilleniaceae	Kaihzawl*	Stem bark	Treatment of tumor, stomachache and diarrhoea.
Potentilla fulgens Wall.	Rosaceae	Langniang**	Root	Treatment of colic pain, spasmodic trouble, pyorrhea and tumor.
Taxus baccata Linn.	Taxaceae	Soh Blei**	Leaves	Treatment of aphrodisiac, epilepsies, irregular menstruation and tumor.

* Local name of the plants in Mizoram. ** Local name of the plants in Meghalaya.

Table 2: Percentage yield (wt/wt) of aqueous and methanol extract of the plants.

Extraction solvent	*D. Pentagyna* (Stem bark)	*T. Buccata* (Leaf)	*A. Conyzoides* (Root)	*B. Lanceolaria* (Leaf)	*P. Fulgens* (Root)
Aqueous extract (%)	14.27	13.84	11.16	14.22	12.30
Methanol extract (%)	11.34	14.21	09.67	13.62	10.87

Table 3: Antitumor activity of aqueous extract of plants against murine ascites Dalton's lymphoma.

Plant	Test part	Dose (mg/kg body wt/day)	Life span (days) (mean ± SD)	ILS (%)	Survival on day 30 (Survival/total)
Blumea lanceolaria	Leaf	TB control*	19.5 ± 0.42	-	0/10
		50	19.0 ± 0.48	-3	0/10
		100	17.0 ± 0.65	-12	0/10
		200	15.2 ± 1.13	-22	0/10
Ageratum conyzoides	Root	TB control	19.8 ± 0.19	-	0/10
		50	22.4 ± 0.60	3	0/10
		100	25.3 ± 0.59	27 *	0/10
		200	19.3 ± 0.57	-3	0/10
Dillenia pentagyna	Stem bark	TB control	19.0 ± 0.89	-	0/10
		50	18.2 ± 0.57	-4	0/10
		100	18.0 ± 0.48	-5	0/10
		200	18.6 ± 1.01	-2	0/10
Potentilla fulgens	Root	TB control	19.8 ± 0.42	-	0/10
		50	27.2 ± 0.67	36 *	2/10
		100	27.0 ± 0.33	35 *	0/10
		200	22.8 ± 1.29	14	0/10
Taxus baccata	Leaf	TB control	20.0 ± 0.31	-	0/10
		50	20.4 ± 0.79	2	0/10
		100	19.8 ± 0.36	-1	0/10
		200	20.0 ± 0.72	0	0/10

Plant extract treatment was given intraperitoneally (i.p) for 5 consecutive days starting from day 1 of tumor growth. ILS = Increase in life span. ILS (%) = [T/C x 100]-100, where T and C are mean survival days of treated and control mice respectively. n = 10. *ILS (%) $\geq$ 20%.

Table 4: Antitumor activity of methanol extract of plants against murine ascites Dalton's lymphoma.

Plant	Test part	Dose (mg/kg body wt/day)	Life span (days) (mean ± SD)	ILS (%)	Survival on day 30 (Survival/total)
Blumea lanceolaria	Leaf	TB control*	19.0 ± 0.36	-	0/10
		50	19.5 ± 0.98	3	0/10
		100	18.4 ± 0.74	-3	0/10
		200	20.0 ± 0.66	5	0/10
Ageratum conyzoides	Root	TB control	19.2 ± 0.94	-	0/10
		50	19.6 ± 0.66	0	0/10
		100	17.6 ± 0.58	-8	0/10
		200	14.8 ± 1.21	-33	0/10
Dillenia pentagyna	Stem bark	TB control	20.0 ± 0.49	-	0/10
		50	29.8 ± 0.62	49*	3/10
		100	19.6 ± 1.34	-2	0/10
		200	13.5 ± 0.66	-32	0/10
Potentilla fulgens	Root	TB control	19.7 ± 0.47	-	0/10
		50	18.0 ± 0.92	-9	2/10
		100	16.6 ± 0.40	-16	0/10
		200	14.4 ± 0.91	-27	0/10
Taxus baccata	Leaf	TB control	20.2 ± 0.51	-	0/10
		50	21.0 ± 0.63	4	0/10
		100	20.4 ± 0.46	1	0/10
		200	18.8 ± 0.57	-7	0/10

Plant extract treatment was given intraperitoneally (i.p) for 5 consecutive days starting from day 1 of tumor growth. ILS = Increase in life span. ILS (%) = [T/C x 100]-100, where T and C are mean survival days of treated and control mice respectively. n = 10. *ILS (%) $\geq$ 20%.

Table 5: Antitumor activity of methanol extract of stem bark of *D. pentagyna* administered through different routes.

Treatment through i.p injection			Treatment through the diet		
Dose[a] (mg/kg body wt/day)	Survival days (Mean ± S.D)	ILS (%)	Dose (mg/kg body wt/day)	Survival days (Mean ± S.D)	ILS (%)
Control[b]	20.0 ± 0.49	-	Control[c]	20.0 ± 2.16	-
10	26.3 ± 0.57	31*	20	19.6 ± 1.83	0
15	27.1 ± 1.08	35*	50	20.6 ± 2.54	3
20	34.2 ± 0.95	71*	100	24.6 ± 1.71	23*
25	32.7 ± 0.81	63*	150	21.8 ± 1.98	9
30	32.4 ± 0.60	62*			
50	29.8 ± 0.62	49*			
100	19.6 ± 1.34	-2			
200	13.5 ± 0.66	-32			

Results are mean ± S.D. [a] 0.25ml volume of the extract solution was administered daily for 5 consecutive days. [b]Control animals received the same volume of the extract vehicle. [c]Control animals were fed with commercial food pellets without the plant extract. *ILS (%) ≥ 20%.

Table 6: Changes in the haematological values of normal and tumor-bearing mice under different treatment conditions.

Treatment	RBC ($x10^{12}/l$)†	WBC ($x10^{9}/l$)†	PCV (%)†	Hb (g/dl)†
Normal	7.57 ± 0.46	04.95 ± 0.35	42.58 ± 3.32	14.36 ± 0.73
Control - 24 h	6.71 ± 0.67	10.41 ± 0.81	37.05 ± 3.91	10.13 ± 0.76
48 h	5.55 ± 0.58	11.82 ± 0.80	34.12 ± 3.65	09.49 ± 0.82
72 h	3.76 ± 0.37	12.42 ± 0.95	30.88 ± 3.37	09.07 ± 0.66
96 h	2.26 ± 0.22	13.36 ± 0.91	26.92 ± 2.53	08.10 ± 0.71
DPE Treated - 24 h	6.56 ± 0.54	11.70 ± 0.90*	38.45 ± 3.25	10.66 ± 0.60
48 h	6.53 ± 0.66*	13.86 ± 0.77*	36.61 ± 2.64	12.58 ± 0.90*
72 h	6.52 ± 0.61*	15.16 ± 1.23*	35.89 ± 2.69*	12.50 ± 1.29*
96 h	6.45 ± 0.70*	16.70 ± 1.12*	35.49 ± 2.29*	12.13 ± 1.24*

Results were mean ± S.D. DPE = *D. pentagyna* extract, (20 mg/kg body wt.) was given on the 10^{th} day of tumor growth. RBC = Red blood cells, WBC = White blood cells, PCV = Packed cell volume, Hb = Haemoglobin. Student's t-test, n = 5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, $^{\dagger}P \leq 0.05$.

Table 7: Differential leukocyte counts in the blood of normal and tumor-bearing mice under different treatment conditions.

Treatment	Lymphocytes	Monocytes	Neutrophils†	Eosinophils†	Basophils†
Normal	63.95 ± 3.35	3.27 ± 0.35	30.36 ± 2.48	1.66 ± 0.16	0.80 ± 0.01
Control - 24 h	30.07 ± 2.12	2.31 ± 0.16	62.85 ± 3.61	3.65 ± 0.29	1.19 ± 0.07
48 h	26.84 ± 2.54	2.21 ± 0.20	65.84 ± 2.55	3.74 ± 0.21	1.18 ± 0.06
72 h	24.46 ± 2.26	2.07 ± 0.17	68.74 ± 2.35	3.90 ± 0.23	1.16 ± 0.08
96 h	20.83 ± 1.96	2.02 ± 0.18	72.49 ± 3.13	3.48 ± 0.19	1.15 ± 0.10
DPE treated – 24 h	31.23 ± 2.50	2.30 ± 0.18	62.53 ± 3.09	4.71 ± 0.35*	0.46 ± 0.04*
48 h	46.62 ± 2.76*	2.09 ± 0.07	46.47 ± 3.72*	4.30 ± 0.20*	0.32 ± 0.02*
72 h	44.31 ± 2.74*	2.06 ± 0.16	48.33 ± 2.59*	4.53 ± 0.32*	0.68 ± 0.04*
96 h	33.15 ± 2.32*	2.16 ± 0.19	59.89 ± 2.90*	4.20 ± 0.26*	0.72 ± 0.07*

Results were mean ± S.D. Normal = Blood from the mice without tumor or DPE treatment. Control = Blood from the tumor-bearing mice without DPE treatment. DPE = *D. pentagyna* extract, (20 mg/kg body wt.) was given on the 10^{th} day of tumor growth. Student's t-test, n = 5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 8: Total protein concentrations in the tissues (mg/g wet wt) and supernatant (mg/ml) of normal and tumor-bearing mice under different treatment conditions.

Treatment	Liver	Kidney†	Spleen†	Testes†	DL cells	SN†
Normal	314.19 ± 15.1	215.84 ± 6.1	241.52 ± 7.0	175.38 ± 5.0	-	-
Control – 24 h	274.67 ± 7.1	228.07 ± 4.9	249.74 ± 5.7	157.85 ± 6.2	157.05 ± 5.8	68.14 ± 7.3
48 h	272.57 ± 7.4	233.79 ± 5.7	251.29 ± 6.6	150.78 ± 6.9	156.20 ± 5.3	69.71 ± 6.8
72 h	265.93 ± 5.7	238.80 ± 5.0	256.04 ± 7.8	145.19 ± 7.0	157.67 ± 4.6	72.43 ± 6.5
96 h	242.18 ± 7.6	243.85 ± 4.7	262.10 ± 10.2	143.09 ± 6.8	156.58 ± 5.2	75.20 ± 3.9
DPE treated – 24 h	280.89 ± 3.1	212.32 ± 6.1*	235.27 ± 7.1*	158.24 ± 7.6	158.38 ± 5.2	69.58 ± 4.8
48 h	289.23 ± 6.9*	208.20 ± 6.3*	240.95 ± 6.7*	173.00 ± 6.9*	176.21 ± 5.3*	73.24 ± 6.3
72 h	264.15 ± 3.7	216.26 ± 7.7*	245.11 ± 5.3*	170.43 ± 6.6*	155.67 ± 9.0	79.29 ± 4.5
96 h	238.04 ± 5.5	226.22 ± 7.5*	259.85 ± 8.5	152.61 ± 7.8	151.13 ± 4.7	84.07 ± 5.3*

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma; SN = supernatant. The results are mean ± S.D. Student's t- test, n=5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 9: Changes in the total GSH content (μmole/g wet wt) in the tissues of normal and tumor-bearing mice under different treatment conditions.

Treatment conditions	Liver†	Kidney	Spleen	Testes	DL cells†
Normal	13.52 ± 0.37	8.19 ± 0.95	9.75 ± 0.44	10.52 ± 0.28	-
Control – 24 h	11.51 ± 0.25	7.86 ± 0.26	8.75 ± 0.16	9.37 ± 0.09	5.82 ± 0.35
48 h	10.62 ± 0.41	7.47 ± 0.58	7.73 ± 0.19	8.16 ± 0.10	5.73 ± 0.24
72 h	09.31 ± 0.48	7.32 ± 0.50	7.21 ± 0.11	7.08 ± 0.11	5.31 ± 0.12
96 h	08.56 ± 0.39	6.96 ± 0.24	7.04 ± 0.14	6.65 ± 0.22	4.68 ± 0.05
DPE treated – 24 h	9.01 ± 0.51*	8.12 ± 0.40	8.82 ± 0.18	9.30 ± 0.11	3.13 ± 0.20*
48 h	8.61 ± 0.27*	10.26 ± 0.38*	8.59 ± 0.15*	8.09 ± 0.11	4.52 ± 0.17*
72 h	7.89 ± 0.41*	8.22 ± 0.30*	7.96 ± 0.16*	8.61 ± 0.13*	4.67 ± 0.52
96 h	7.01 ± 0.25*	6.69 ± 0.28	7.11 ± 0.12*	9.03 ± 0.22*	4.77 ± 0.31

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are expressed as mean ± S.D. Student's t- test, n=5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 10: Glutathione s-transferase activity (μmoles/min/mg protein) in the tissues of normal and tumor-bearing mice under different treatment conditions.

Animal groups	Liver†	Kidney†	Spleen†	Testes	DL cells†
Normal	0.464 ± 0.022	0.262 ± 0.018	0.183 ± 0.014	0.312 ± 0.021	---
Control-24 h	0.697 ± 0.024	0.269 ± 0.015	0.185 ± 0.015	0.372 ± 0.015	0.234 ± 0.008
48 h	0.627 ± 0.016	0.272 ± 0.017	0.191 ± 0.012	0.393 ± 0.020	0.260 ± 0.008
72 h	0.581 ± 0.027	0.278 ± 0.014	0.205 ± 0.013	0.453 ± 0.019	0.283 ± 0.007
96 h	0.546 ± 0.025	0.293 ± 0.017	0.220 ± 0.015	0.484 ± 0.028	0.311 ± 0.004
DPE treated-24 h	0.522 ± 0.017*	0.329 ± 0.026*	0.220 ± 0.015*	0.468 ± 0.026*	0.249 ± 0.009*
48 h	0.352 ± 0.020*	0.331 ± 0.014*	0.219 ± 0.011*	0.460 ± 0.020*	0.284 ± 0.006*
72 h	0.417 ± 0.022*	0.286 ± 0.013	0.214 ± 0.014	0.455 ± 0.025	0.328 ± 0.005*
96 h	0.549 ± 0.018	0.268 ± 0.013*	0.207 ± 0.017	0.475 ± 0.020*	0.333 ± 0.011*

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are mean ± S.D. Student's t-test, n=5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 11: Glutathione reductase activity (μmoles/min/mg protein) in the tissues of normal and tumor-bearing mice under different treatment conditions.

Treatment	Liver†	Kidney†	Spleen†	Testes	DL cells†
Normal	0.333 ± 0.022	0.483 ± 0.042	0.212 ± 0.030	0.252 ± 0.024	-
Control - 24h	0.433 ± 0.032	0.737 ± 0.069	0.415 ± 0.043	0.290 ± 0.023	0.218 ± 0.015
Control - 48h	0.412 ± 0.032	0.712 ± 0.055	0.324 ± 0.029	0.340 ± 0.023	0.186 ± 0.016
Control - 72h	0.383 ± 0.030	0.677 ± 0.087	0.235 ± 0.029	0.396 ± 0.041	0.164 ± 0.014
Control - 96h	0.364 ± 0.036	0.625 ± 0.064	0.217 ± 0.033	0.478 ± 0.035	0.157 ± 0.014
DPE treated – 24h	0.304 ± 0.027*	0.787 ± 0.068	0.178 ± 0.026*	0.265 ± 0.034	0.143 ± 0.016*
DPE treated – 48h	0.269 ± 0.021*	0.842 ± 0.055*	0.216 ± 0.022*	0.310 ± 0.034	0.154 ± 0.011*
DPE treated – 72h	0.222 ± 0.021*	0.887 ± 0.045*	0.250 ± 0.029	0.362 ± 0.021	0.160 ± 0.015
DPE treated – 96h	0.200 ± 0.031*	0.907 ± 0.079*	0.205 ± 0.033	0.444 ± 0.033	0.159 ± 0.012

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are mean ± S.D. Student's t- test, n=5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 12: Glutathione peroxidase activity (μmoles/min/mg protein) in the tissues of normal and tumor-bearing mice under different treatment conditions.

Treatment	Liver†	Kidney†	Spleen†	Testes†	DL cells†
Normal	0.331 ± 0.012	0.135 ± 0.010	0.224 ± 0.004	0.223 ± 0.004	-
Control – 24 h	0.226 ± 0.007	0.115 ± 0.003	0.186 ± 0.009	0.192 ± 0.005	0.202 ± 0.07
48 h	0.183 ± 0.013	0.112 ± 0.013	0.163 ± 0.008	0.174 ± 0.016	0.185 ± 0.012
72 h	0.142 ± 0.010	0.093 ± 0.010	0.151 ± 0.014	0.166 ± 0.019	0.173 ± 0.007
96 h	0.094 ± 0.007	0.085 ± 0.003	0.143 ± 0.009	0.154 ± 0.011	0.165 ± 0.015
DPE treated – 24 h	0.144 ± 0.016*	0.032 ± 0.003*	0.143 ± 0.020*	0.084 ± 0.012*	0.114 ± 0.015*
48 h	0.114 ± 0.009*	0.067 ± 0.011*	0.095 ± 0.020*	0.084 ± 0.055*	0.132 ± 0.019*
72 h	0.096 ± 0.014*	0.074 ± 0.006*	0.080 ± 0.009*	0.064 ± 0.013*	0.144 ± 0.017*
96 h	0.077 ± 0.004*	0.086 ± 0.010	0.050 ± 0.006*	0.073 ± 0.004*	0.161 ± 0.015

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are mean ± S.D. Student's t-test, n=5, as compared to the corresponding control, *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 13. Changes in the sialic acid (μmoles/g tissue wet wt.) in various tissues of mice at different stages of tumor growth.

Day of tumor growth	Liver	Kidney	Spleen	Testes	DL cells	SN[a]
Day 0[b]	0.86±0.04	1.09±0.03	1.36±0.04	0.62±0.04	-	-
Day 5	1.11±0.06*	1.14±0.03	1.51±0.02*	0.60±0.02	1.01±0.06	0.82±0.01
Day 10	1.37±0.06*	1.20±0.02*	1.64±0.01*	0.55±0.02*	1.27±0.11*	0.84±0.02
Day 15	1.75±0.02*	1.27±0.02*	1.79±0.03*	0.45±0.02*	1.34±0.08*	0.97±0.02*

Results are mean ± standard deviation (S.D). Student's t-test; as compared to the corresponding normal tissue, n = 5, *P ≤ 0.05. [a]Sialic acid content was expressed as μmoles/ml SN. [b]Normal mice.

Table 14: Quantitative changes in the total sialic acid content (μmoles/g wet wt) in the tissues of normal and tumor-bearing mice under different treatment conditions.

Treatment	Liver	Kidney†	Spleen†	Testes†	DL cells†	SN†
Normal	0.86 ± 0.04	1.09 ± 0.03	1.36 ± 0.04	0.62 ± 0.04	-	-
Control – 24 h	1.43 ± 0.04	1.17 ± 0.05	1.62 ± 0.05	0.56 ± 0.04	1.26 ± 0.02	0.83 ± 0.05
48 h	1.49 ± 0.05	1.20 ± 0.01	1.70 ± 0.01	0.53 ± 0.01	1.29 ± 0.01	0.86 ± 0.04
72 h	1.64 ± 0.02	1.22 ± 0.02	1.74 ± 0.01	0.46 ± 0.01	1.31 ± 0.01	0.93 ± 0.02
96 h	1.74 ± 0.07	1.28 ± 0.02	1.74 ± 0.04	0.46 ± 0.03	1.32 ± 0.02	1.04 ± 0.08
DPE treated – 24 h	1.12 ± 0.05*	1.04 ± 0.03*	1.64 ± 0.05	0.57 ± 0.05	1.27 ± 0.04	0.87 ± 0.06
48 h	1.08 ± 0.03*	1.01 ± 0.03*	1.65 ± 0.05*	0.51 ± 0.05	1.26 ± 0.02	0.91 ± 0.03
72 h	1.33 ± 0.04*	1.12 ± 0.03*	1.63 ± 0.03*	0.47 ± 0.04	1.13 ± 0.02*	1.15 ± 0.09*
96 h	1.42 ± 0.05*	1.15 ± 0.07*	1.63 ± 0.05*	0.48 ± 0.04	1.14 ± 0.03*	1.20 ± 0.08*

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma; SN = supernatant. The results are mean ± S.D. Student's t- test, n=5, as compared to the corresponding control, *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA, †P ≤ 0.05.

Table 15. The pattern of changes in lipid peroxidation (nmoles/mg protein) in the tissue of tumor-bearing mice during different stages of tumor growth.

Day of tumor growth	Liver	Kidney	Spleen	Testes	DL cells
Day 0[a]	0.122±0.016	0.160±0.010	0.252±0.023	0.170±0.018	--
Day 5	0.162±0.011*	0.184±0.017	0.207±0.016*	0.210±0.055	0.110±0.017
Day 10	0.167±0.011*	0.211±0.013*	0.121±0.015*	0.361±0.012*	0.104±0.012
Day 15	0.193±0.019*	0.245±0.016*	0.100±0.016*	0.403±0.018*	0.080±0.012*

Results are mean ± standard deviation (S.D.). Student's t-test; as compared to the corresponding normal tissue, n = 5, *P ≤ 0.05. [a]Normal mice.

Table 16: Quantitative changes in the level of lipid peroxidation (nmol/mg protein) in the tissues of normal and tumor-bearing mice under different treatment conditions.

Treatment	Liver†	Kidney†	Spleen†	Testes†	DL cells†
Normal	0.122 ± 0.016	0.160 ± 0.010	0.252 ± 0.023	0.170 ± 0.018	-
Control – 24 h	0.171 ± 0.018	0.198 ± 0.032	0.115 ± 0.020	0.364 ± 0.015	0.099 ± 0.010
48 h	0.178 ± 0.010	0.201 ± 0.028	0.104 ± 0.015	0.384 ± 0.016	0.098 ± 0.012
72 h	0.186 ± 0.011	0.213 ± 0.032	0.098 ± 0.012	0.398 ± 0.016	0.086 ± 0.012
96 h	0.199 ± 0.015	0.244 ± 0.023	0.094 ± 0.017	0.389 ± 0.030	0.081 ± 0.010
DPE treated – 24 h	0.156 ± 0.017	0.207 ± 0.020	0.151 ± 0.024*	0.119 ± 0.018*	0.083 ± 0.013
48 h	0.133 ± 0.012*	0.167 ± 0.014*	0.162 ± 0.016*	0.106 ± 0.019*	0.077 ± 0.011*
72 h	0.124 ± 0.012*	0.136 ± 0.017*	0.171 ± 0.014*	0.156 ± 0.016*	0.072 ± 0.012
96 h	0.122 ± 0.016*	0.110 ± 0.018*	0.182 ± 0.026*	0.249 ± 0.020*	0.073 ± 0.014

Normal = tissues from hosts without tumor or DPE treatment; Control = tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg/kg body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are mean ± S.D. Student's t- test, n=5, as compared to the corresponding control, *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 17: Frequency of chromosomal aberrations in the bone marrow cells of mice under single combination treatment of B[a]P and DPE *in vivo*.

Treatment	No of metaphases scored	Mean aberrant metaphases (%)	Average chromosomal aberrations per 100 cells					Total aberrations per 100 cells (Mean±SD)
			CB	ICB	CF	Exch	SCU	
Normal	500	1	1.4	0.2	0.4	0	0	1.8 ± 0.44
B[a]P control 24 h	500	7.2	9.2	0.2	0.4	0	0	9.8 ± 0.83
48 h	500	5	6.4	0	0.6	0	0	7.0 ± 0.70
72 h	500	4.4	5.6	0	0.2	0	0	5.8 ± 0.44
96 h	500	3.8	4	0	0.6	0	0	4.6 ± 0.54
B[a]P+DPE† 24 h	500	6.6	7.6	0.4	0.8	0	0	8.6 ± 1.14
48 h	500	5	4	0.4	0.6	0.2	0.2	5.0 ±0.70*
72 h	500	4.4	2.8	0.6	0.4	0.2	0	3.4 ± 1.14*
96 h	500	3.8	2.8	0.4	0.4	0	0	3.2 ± 1.30*

Normal = Untreated normal mice; B[a]P = benzo[a]pyrene was given through gavages as a single dose (125 mg/kg body wt.); DPE= *D. pentagyna* (20 mg/kg body wt) was given through i.p. injection as a single dose. CB = chromatid breaks; ICB = isochromatid breaks; CF = chromosomal fragments; Exch = exchanges; SCU = sister chromatid unions. Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the corresponding B[a]P control. *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA, $^{\dagger}P \leq 0.05$.

Table 18: Frequency.of chromosomal aberrations induced by B[a]P in the bone marrow cells of mice after pre-treatment with different doses of DPE *in vivo*.

Extract dose	Treatment	No of metaphases scored	Mean aberrant metaphases (%)	Average chromosomal aberrations per 100 cells					Total aberrations per 100 cells (Mean±SD)
				CB	ICB	CF	Exch	SCU	
	B[a]P control –24 h	500	7.2	9.2	0.2	0.4	0	0	9.8 ± 0.83
	48 h	500	5	6.4	0	0.6	0	0	7.0 ± 0.70
	72 h	500	4.4	5.6	0	0.2	0	0	5.8 ± 0.44
	96 h	500	3.8	4	0	0.6	0	0	4.6 ± 0.54
20 mg/kg/day	B[a]P+DPE –24 h	500	7	6.2	0.8	0.4	0	0	6.6 ± 1.14*
	48 h	500	7.4	5.2	1	0.2	0	0	5.4 ± 0.89*
	72 h	500	7	3.6	1.4	0.6	0	0	4.2 ± 0.83*
	96 h	500	6.4	2.8	1.2	0.4	0	0	3.2 ± 0.83*
50 mg/kg/day†	B[a]P+DPE –24 h	500	5	5	0	0.2	0	0	5.2 ± 0.83*
	48 h	500	4.6	4.4	0	0.4	0	0	4.6 ± 0.89*
	72 h	500	4.4	3.6	0.2	0.2	0	0	3.8 ± 1.09*
	96 h	500	3.4	2.6	0	0.8	0	0	3.4 ± 0.54*
100 mg/kg/day†	B[a]P+DPE –24 h	500	4.2	4	0	0.6	0	0	4.6 ± 0.54*
	48 h	500	4.2	3.6	0.4	0.2	0	0	3.8 ± 0.44*
	72 h	500	3.6	3.2	0.2	0.2	0	0	3.6 ± 0.54*
	96 h	500	3.2	2.4	0	0.8	0	0	3.2 ±0.44*

B[a]P control = B[a]P-treated hosts without DPE. B[a]P = Benzo[a]pyrene was given through gavages as a single dose (125 mg/kg body wt.); DPE= *D. pentagyna* treatment was given through the diet for 7 consecutive days prior to B[a]P. CB = chromatid breaks; ICB = isochromatid breaks; CF = chromosomal fragments; Exch = exchanges; SCU = sister chromatid unions. Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the corresponding B[a]P control. *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 19: Frequency of chromosomal aberrations in the bone marrow cells of mice under single combination treatment of CIS and DPE *in vivo*.

Treatment		No of metaphases scored	Mean aberrant metaphases (%)	Average chromosomal aberrations per 100cells					Total aberrations per 100 cells (Mean±SD)
				CB	ICB	CF	Exch	SCU	
Normal		500	1	1.4	0.2	0.4	0	0	1.8 ± 0.44
CIS control	24 h	500	50.8	85.0	39.0	36.8	48.4	11.8	181.8 ± 19.13
	48 h	500	37.8	26.2	18.2	7.8	13.2	7.6	73.0 ± 5.09
	72 h	500	16.8	16.8	6	2.8	5.4	3.6	34.6 ± 3.50
	96 h	500	8.4	7.6	1.8	1.4	1.6	1.2	13.2 ± 0.83
CIS+DPE	24 h	500	42.6	69.4	6.8	40.2	35.8	15	161.8 ± 12.27
	48 h	500	40	44.4	6.2	7	7.2	6	68.8 ± 7.62
	72 h	500	28	24	5.2	3	3.2	2.8	38.2 ± 3.34
	96 h	500	11	10.2	2.2	1.4	1	1	15.8 ± 1.92

Normal = Untreated normal mice; CIS = Cisplatin was given through i.p. injection as a single dose (10 mg/kg body wt.); DPE= *D. pentagyna* (20 mg/kg body wt) was given through i.p. injection as a single dose. CB = chromatid breaks; ICB = isochromatid breaks; CF = chromosomal fragments; Exch = exchanges; SCU = sister chromatid unions. Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the corresponding CIS control. *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA.

Table 20: Chromosomal aberrations induced by CIS in the bone marrow cells of mice after pre-treatment with different doses of DPE *in vivo*

DPE dose	Treatment	No of metaphases scored	Mean aberrant metaphases (%)	Average chromosomal aberrations per 100 cells					Total aberrations per 100 cells (Mean±SD)
				CB	ICB	CF	Exch	SCU	
	CIS control – 24	500	50.8	85.0	39.0	36.8	48.4	11.8	181.8 ± 19.13
	h	500	37.8	26.2	18.2	7.8	13.2	7.6	73.0 ± 5.09
	48 h	500	16.8	16.8	6	2.8	5.4	3.6	34.6 ± 3.50
	72 h	500	8.4	7.6	1.8	1.4	1.6	1.2	13.2 ± 0.83
	96 h								
20 mg/kg/day	CIS+DPE - 24 h	500	60.4	79.6	51	15.6	43.6	17.4	156.2 ± 5.31*
	48 h	500	49	34.6	29.4	12.4	20.4	6.4	73.8 ± 3.89
	72 h	500	24.8	19	3.6	5	6	3.8	33.8 ± 4.32
	96 h	500	10.4	5.8	1.2	2.6	0.8	0.6	9.8 ± 2.86
50 mg/kg/day	CIS+DPE - 24 h	500	59.8	65.8	35.8	14.4	15.2	13	128.4 ± 13.22*
	48 h	500	45.8	43	22.8	12.4	27	8	90.4 ± 10.73
	72 h	500	25.6	10.4	9.2	4	5.4	2.8	22.6 ± 1.67
	96 h	500	21.8	5.8	1.2	2.6	0.8	0.6	8.8 ± 1.92
100 mg/kg/day	CIS+DPE - 24 h	500	60.4	58.4	37.4	11.4	31.2	11	112.0 ± 7.31*
	48 h	500	41.4	24	34.6	6.2	12.4	20.4	70.6 ± 3.57
	72 h	500	40.6	19	3.6	5	6	3.8	27.8 ± 2.16
	96 h	500	36	5.8	1.2	2.6	0.8	0.6	9.8 ± 2.86

CIS control = CIS-treated hosts without DPE; CIS = Cisplatin was given through i.p. injection as a single dose (10 mg/kg body wt.); DPE= *D. pentagyna* treatment was given through the diet for 7 consecutive days prior to CIS. CB = chromatid breaks; ICB = isochromatid breaks; CF = chromosomal fragments; Exch = exchanges; SCU = sister chromatid unions. Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the respective treatment with CIS alone. *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA.

Table 21: Effect of single treatment with DPE (20 mg/kg body wt.) on the frequency of micronucleus induced by B[a]P in the bone marrow cells of mice.

Treatment		Total No of cells examined	MnPCEs (%)
Normal		2500	0.08 ± 0.01
B[a]P control	24 h	2500	1.29 ± 0.13
	48 h	2500	1.02 ± 0.08
	2 h	2500	0.49 ± 0.02
	96 h	2500	0.23 ± 0.01
B[a]P + DPE	24 h	2500	1.20 ± 0.08
	48 h	2500	1.01 ± 0.07
	72 h	2500	0.51 ± 0.02
	96 h	2500	0.21 ± 0.02

PCEs = Polychromatic erythrocytes. MnPCEs = Micronucleated polychromatic erythrocytes. Normal = Untreated normal mice; B[a]P = Benzo[a]pyrene was given through gavages as a single dose (125 mg/kg body wt.); DPE= *D. pentagyna* treatment was given through i.p. injection as a single dose (20 mg/kg body wt). Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the respective treatment with B[a]P alone. *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA.

Table 22: Effect of DPE pre-treatment for 7 days on the frequency of micronucleus induced by B[a]P in the bone marrow cells of mice.

Dose of DPE	Treatment	Total No of cells examined	MnPCEs (%)
	B[a]P control – 24 h	2500	1.29 ± 0.13
	48 h	2500	1.02 ± 0.08
	2 h	2500	0.49 ± 0.02
	96 h	2500	0.23 ± 0.01
20 mg/kg/day	B[a]P+CIS – 24 h	2500	1.12 ± 0.21
	48 h	2500	0.95 ± 0.08
	72 h	2500	0.45 ± 0.03
	96 h	2500	0.21 ± 0.01
50 mg/kg/day	B[a]P+CIS – 24 h	2500	1.12 ± 0.02*
	48 h	2500	0.88 ± 0.04*
	72 h	2500	0.36 ± 0.04*
	96 h	2500	0.20 ± 0.01
100 mg/kg/day	B[a]P+CIS – 24 h	2500	1.10 ± 0.08*
	48 h	2500	0.84 ± 0.05*
	72 h	2500	0.31 ± 0.01*
	96 h	2500	0.19 ± 0.02

PCEs = Polychromatic erythrocytes. MnPCEs = Micronucleated polychromatic erythrocytes. DPE= *D. pentagyna* pre-treatment was given through the diet for 7 consecutive days prior to B[a]P. Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the respective treatment with B[a]P alone. *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA.

Table 23: Effect of DPE single treatment (20 mg/kg body wt.) on the frequency of micronucleus induced by CIS in the bone marrow cells of mice.

Treatment		Total No of PCEs examined	MnPCEs (%)
Normal		2500	0.08 ± 0.01
CIS control	24 h	2500	1.60 ± 0.11
	48 h	2500	1.19 ± 0.13
	72 h	2500	0.65 ± 0.03
	96 h	2500	0.34 ± 0.01
CIS + DPE	24 h	2500	1.52 ± 0.22
	48 h	2500	1.22 ± 0.08
	72 h	2500	0.61 ± 0.02
	96 h	2500	0.28 ± 0.02

PCEs = Polychromatic erythrocytes. MnPCEs = Micronucleated polychromatic erythrocytes. Normal = Untreated normal mice; CIS = Cisplatin was given through i.p. injection as a single dose (10 mg/kg body wt.); DPE= *D. pentagyna* treatment was given through i.p. injection as a single dose (20 mg/kg body wt). Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the corresponding CIS control. *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA.

Table 24: Effect of DPE pre-treatment for 7 days on the frequency of micronucleus induced by CIS in the bone marrow cells of mice.

Dose of DPE	Treatment	Total No of PCEs examined	MnPCEs (%)
	CIS control – 24 h	2500	1.60 ± 0.11
	48 h	2500	1.19 ± 0.13
	72 h	2500	0.65 ± 0.03
	96 h	2500	0.34 ± 0.01
20 mg/kg/day	CIS+DPE - 24 h	2500	1.52 ± 0.11
	48 h	2500	1.13 ± 0.08
	72 h	2500	0.65 ± 0.02
	96 h	2500	0.32 ± 0.01
50 mg/kg/day	CIS+DPE - 24 h	2500	1.41 ± 0.07
	48 h	2500	1.08 ± 0.04
	72 h	2500	0.56 ± 0.02
	96 h	2500	0.29 ± 0.01
100 mg/kg/day	CIS+DPE - 24 h	2500	1.18 ± 0.14*
	48 h	2500	0.98 ± 0.03*
	72 h	2500	0.50 ± 0.02*
	96 h	2500	0.30 ± 0.02

PCEs = Polychromatic erythrocytes. MnPCEs = Micronucleated polychromatic erythrocytes. DPE = *D. pentagyna* pre-treatment was given through the diet for 7 consecutive days prior to CIS. Results are expressed as mean ± SD. Student's t-test, n = 5; as compared to the corresponding CIS control. *$P \leq 0.05$. The significance of the total changes between control and treated groups was also tested by ANOVA.

Table 25: Effect of different doses of DPE on the frequency of sperm abnormality induced by B[a]P in mice.

Treatment	No. of sperms observed	No. of abnormal sperms	Amorphous head	Banana head	Hookless head	Microhead head	Double tail	Mean % of abnormal sperms±S.D.
Normal	3000	49	31	4	13	1	0	1.63 ± 0.27
B[a]P control	3000	130	77	19	18	14	2	4.33 ± 0.35
DPE (20 mg/kg body wt) single treatment								
B[a]P+DPE (20 mg/kg)	3000	144	70	18	22	13	4	4.23 ± 0.19
DPE pre-treatment for 7 days								
B[a]P+DPE(20 mg/kg/day)†	3000	109	72	15	13	8	1	3.62 ± 0.18*
B[a]P+DPE(50 mg/kg/day)†	3000	69	66	7	10	3	1	2.89 ± 0.15*
B[a]P+DPE(100 mg/kg/day)†	3000	85	59	11	10	5	0	2.83 ± 0.31*

Normal = Normal mice without DPE treatment; B[a]P control = Normal mice treated with a single dose of B[a]P; DPE = *D. pentagyna* extract. Results are expressed as mean ± S.D. Student's t- test, n=5 as compared to B[a]P control, *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA, †$P \leq 0.05$.

Table 26: Effect of different doses of DPE on the frequency of sperm abnormality induced by CIS in mice.

Treatment	No. of sperms observed	No. of abnormal sperms	Amorphous head	Banana head	Hookless head	Microhead head	Double tail	Mean % of abnormal sperms±S.D.
Normal	3000	49	31	4	13	1	0	1.63 ± 0.27
CIS control	3000	260	132	28	83	11	6	8.66 ± 0.89
DPE (20 mg/kg body wt) single treatment								
CIS+DPE (20 mg/kg)	3000	238	123	23	80	9	3	7.93 ± 0.38
DPE pre-treatment for 7 days								
CIS+DPE (20 mg/kg/day)†	3000	186	84	20	74	6	2	6.19 ± 0.32*
CIS+DPE (50 mg/kg/day)†	3000	177	78	19	74	5	1	5.85 ± 0.55*
CIS+DPE (100 mg/kg/day)†	3000	108	77	9	70	2	1	5.30 ± 0.24*

Normal = Normal mice without DPE treatment; CIS control = Normal mice treated with a single dose of CIS; DPE = *D. pentagyna* extract. Results are expressed as mean ± S.D. Student's t- test, n=5 as compared to CIS control, *P≤0.05. The significance of the total changes between control and treated groups was also tested by ANOVA, †P ≤ 0.05.

FIGURES

A *(Ageratum conyzoides)*

B *(Blumea lanceolaria)*

C *(Dillenia pentagyna)*

D *(Potentilla fulgens)*

E *(Taxus baccata)*

Figure 1.
Photographs showing different anticancer medicinal plants selected from Mizoram (A-C) and Meghalaya (D,E).

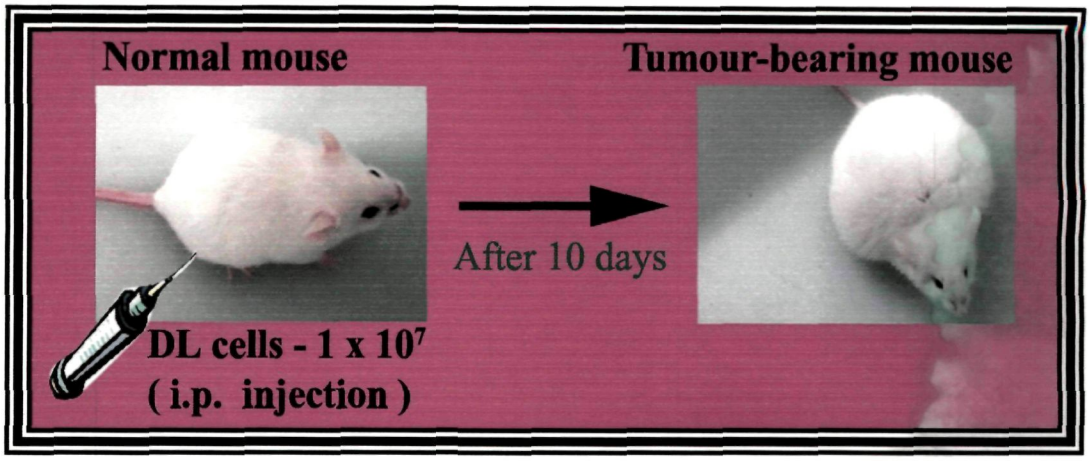


Figure 2.
Photographs showing normal mice and tumor-bearing mice after 10 days of tumor transplantation.

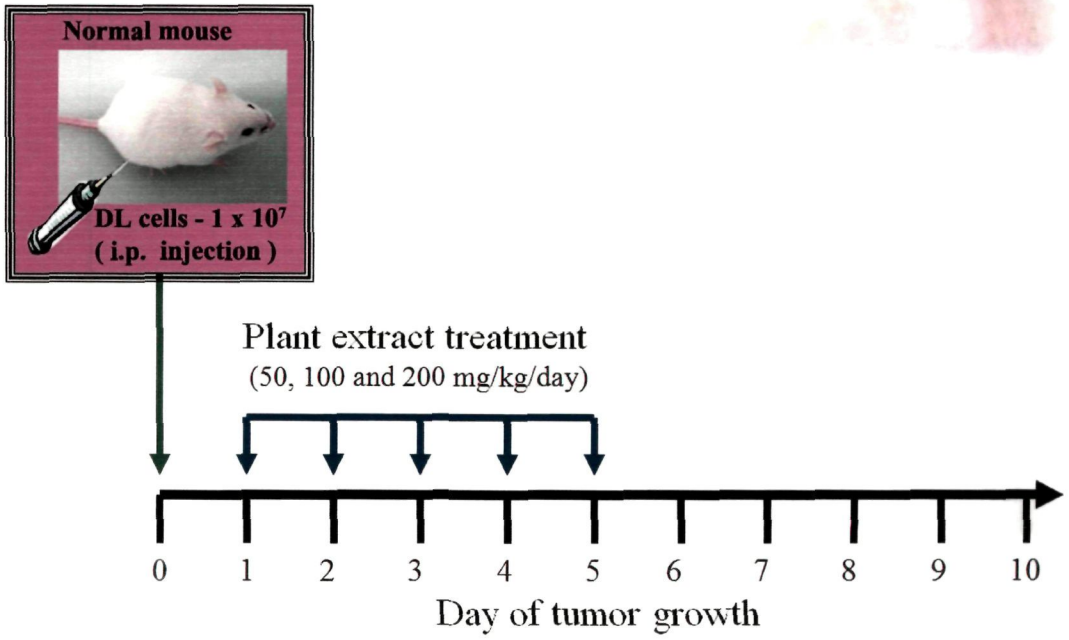


Figure 3.
Photograph showing treatment schedule in the antitumor activity study of different plant extracts.

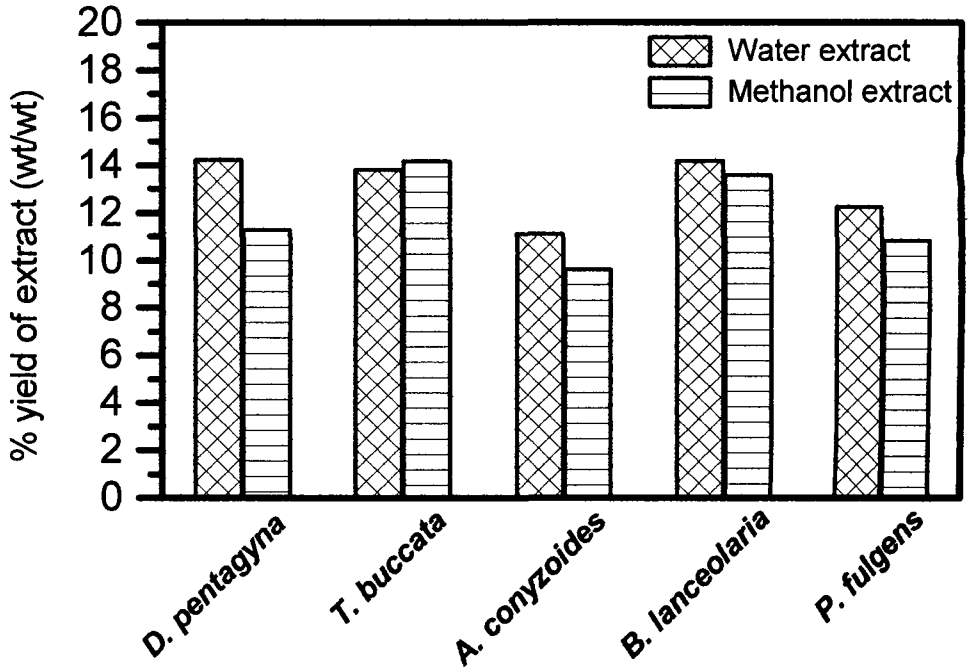


Figure 4.
Histogram showing the percentage yield (wt./wt.) of water and methanol extract of different plants.

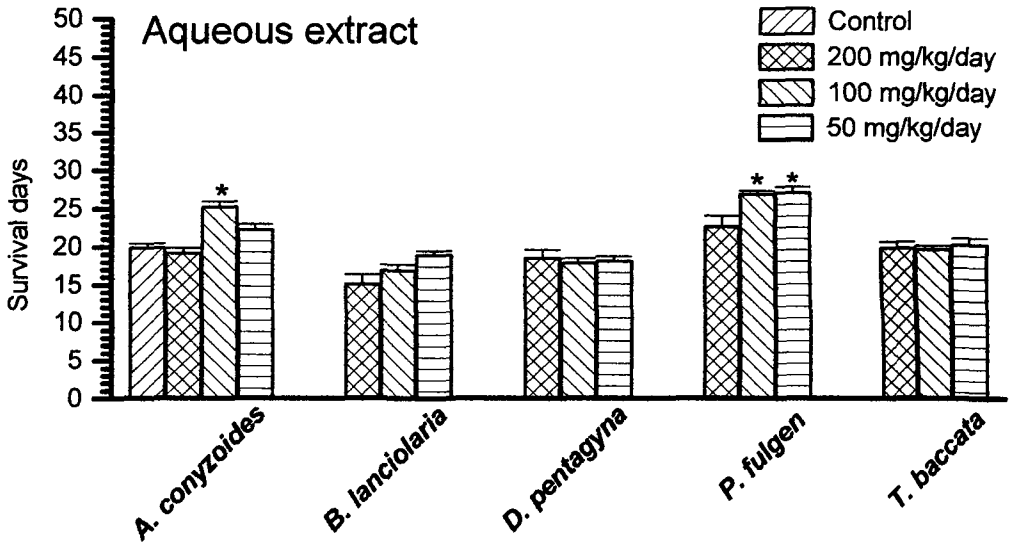


Figure 5.
*Histogram showing the antitumor activity of aqueous extract of different plants. Control animals were tumor-bearing without plant extract treatment. Treatment with the plant extract was given intraperitoneally for 5 consecutive days beginning from day 1 of tumor transplantation. *%ILS ≥ 20%.*

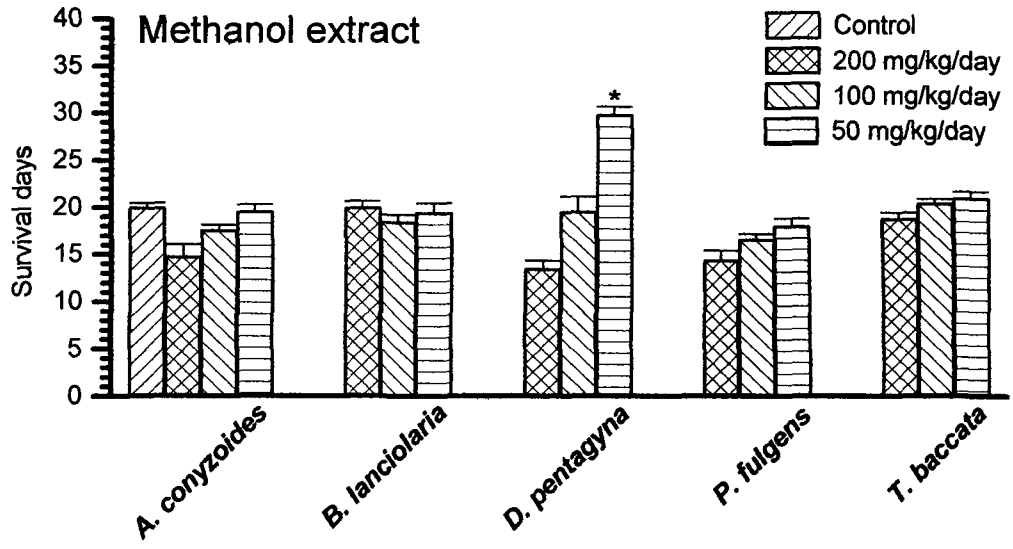


Figure 6.
*Histogram showing the antitumor activity of methanol extract of different plants. Control animals were tumor-bearing without plant extract treatment. Treatment with the plant extract was given intraperitoneally for 5 consecutive days beginning from day 1 of tumor transplantation. *%ILS ≥ 20%.*

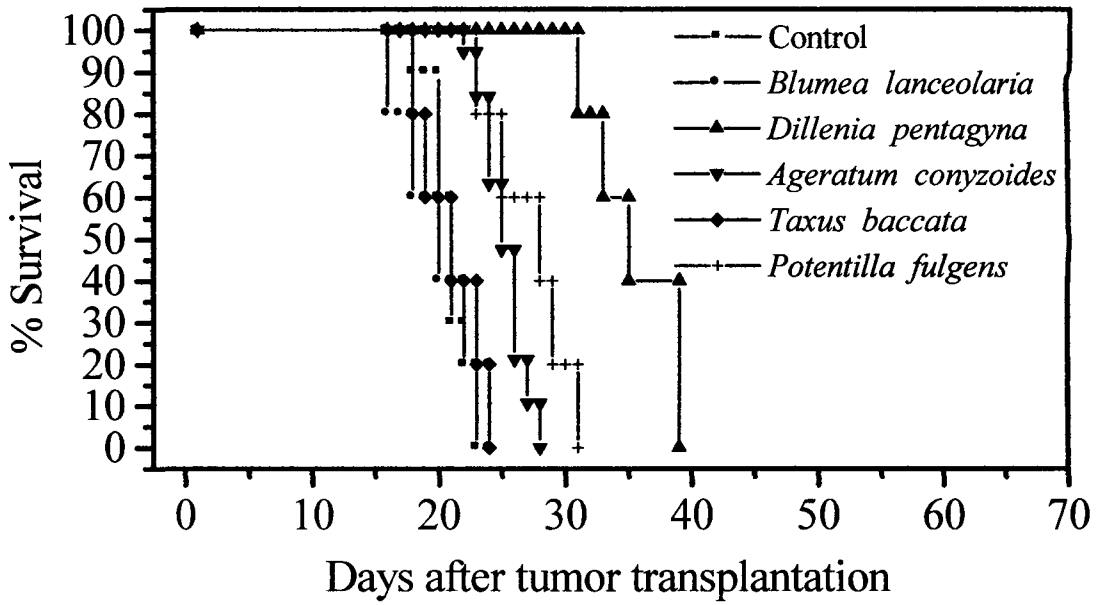


Figure 7.
Graph showing the survival pattern of tumor-bearing mice after treatment with the respective most potent dose of different plant extracts. Results were expressed as mean of 5 independent experimental sets.

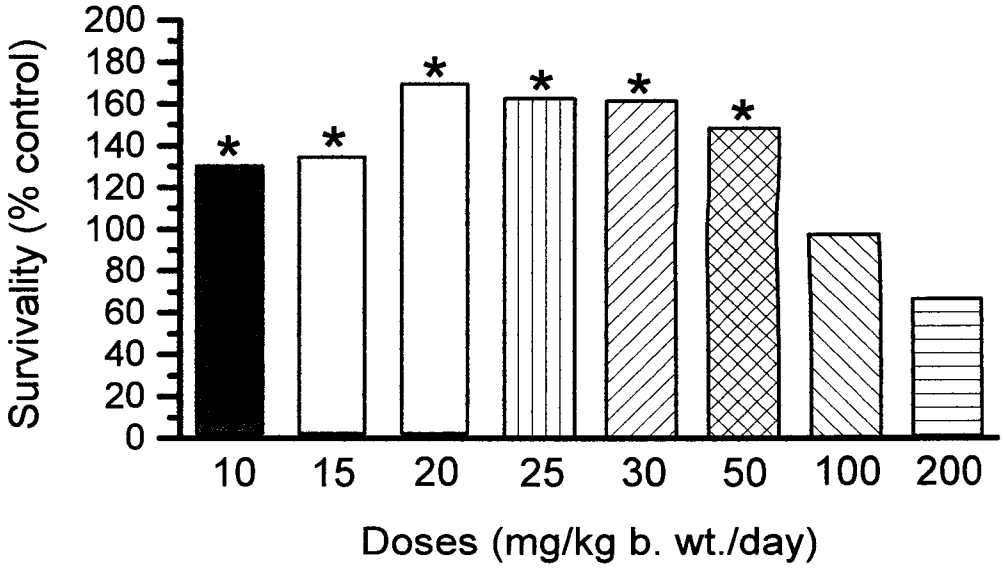


Figure 8.
*Histogram showing the survivality (% control) of different doses of methanol extract of Dillenia pentagyna stem bark. *%ILS ≥ 20%.*

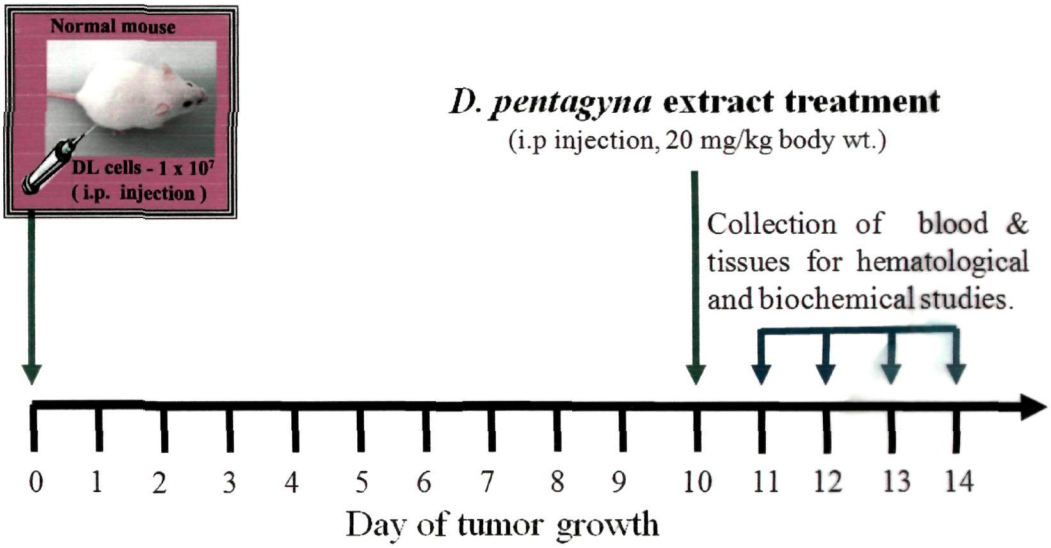


Figure 9.
Figure showing Dillenia pentagyna extract treatment schedule of tumor-bearing mice in hematological and biochemical studies.

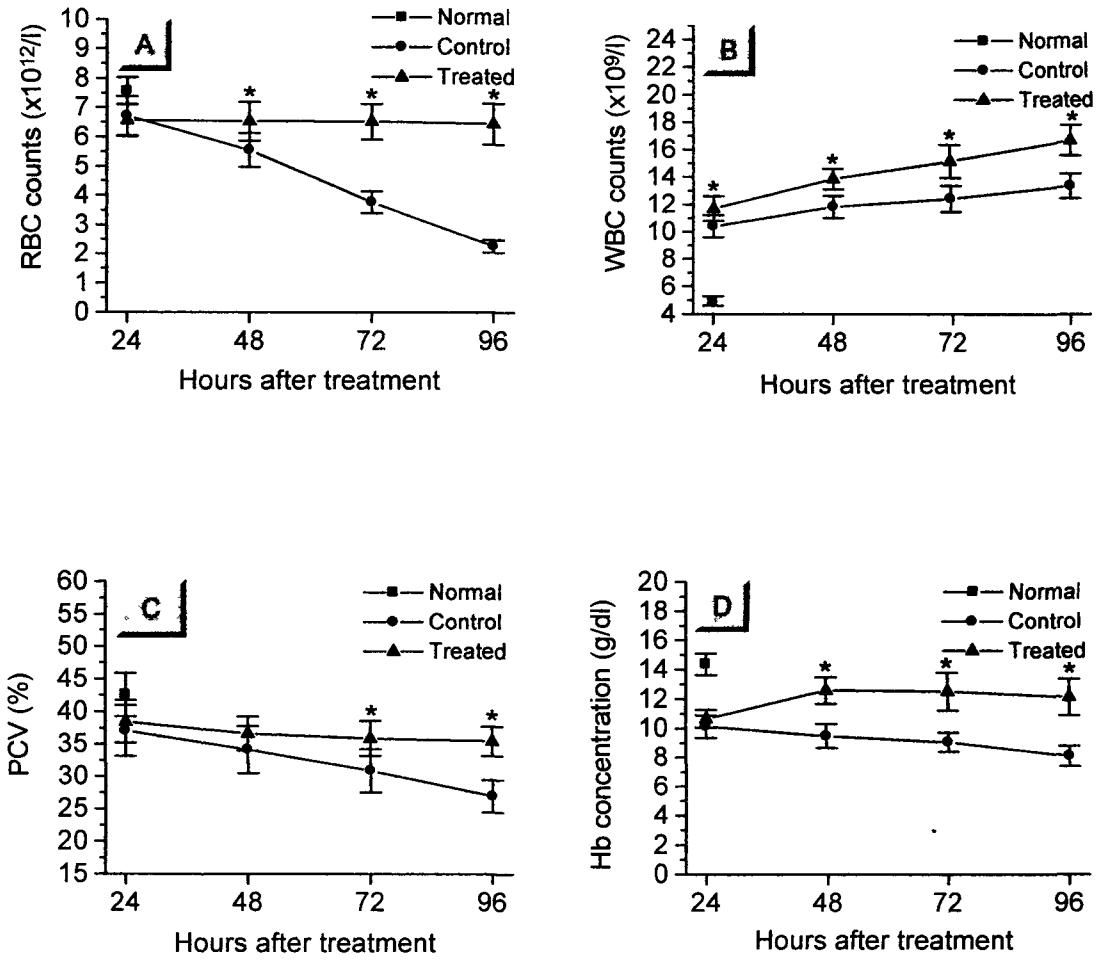


Figure 10.

*Graphs showing the changes in RBC count (A), WBC count (B), PCV (C) and Hb (D) concentration in normal, tumor-bearing control and treated group of mice. Results are expressed as mean±S.D., Student's t-test, n = 6, as compared to the corresponding control, * $p \leq 0.05$.*

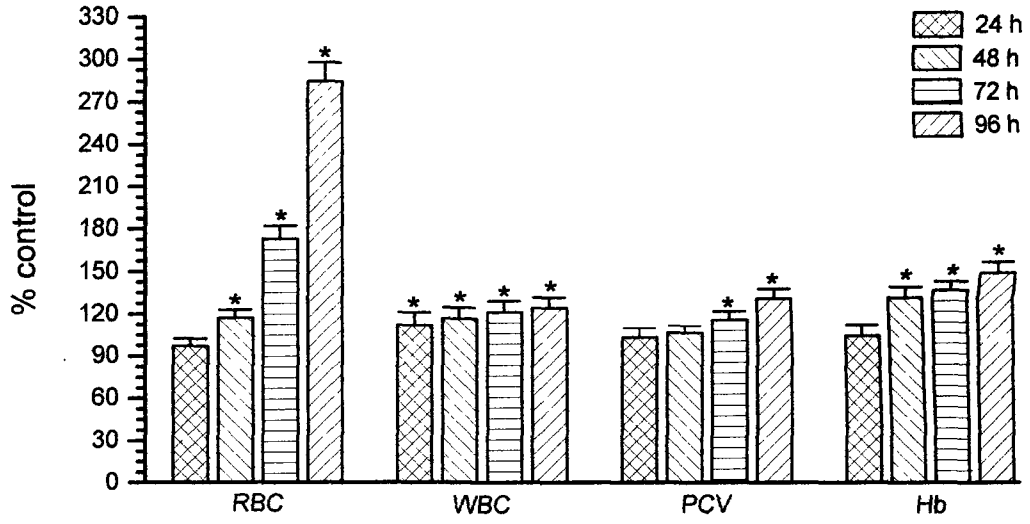


Figure 11.
*Histogram showing the percent changes in RBC count, WBC count, PCV and Hb concentration of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

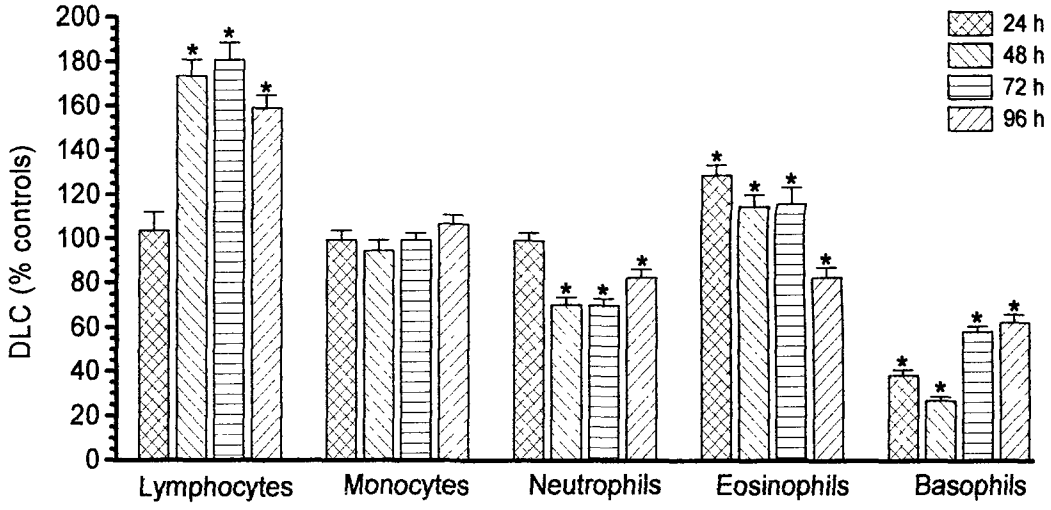


Figure 12.
*Histogram showing the percent changes in DLC of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

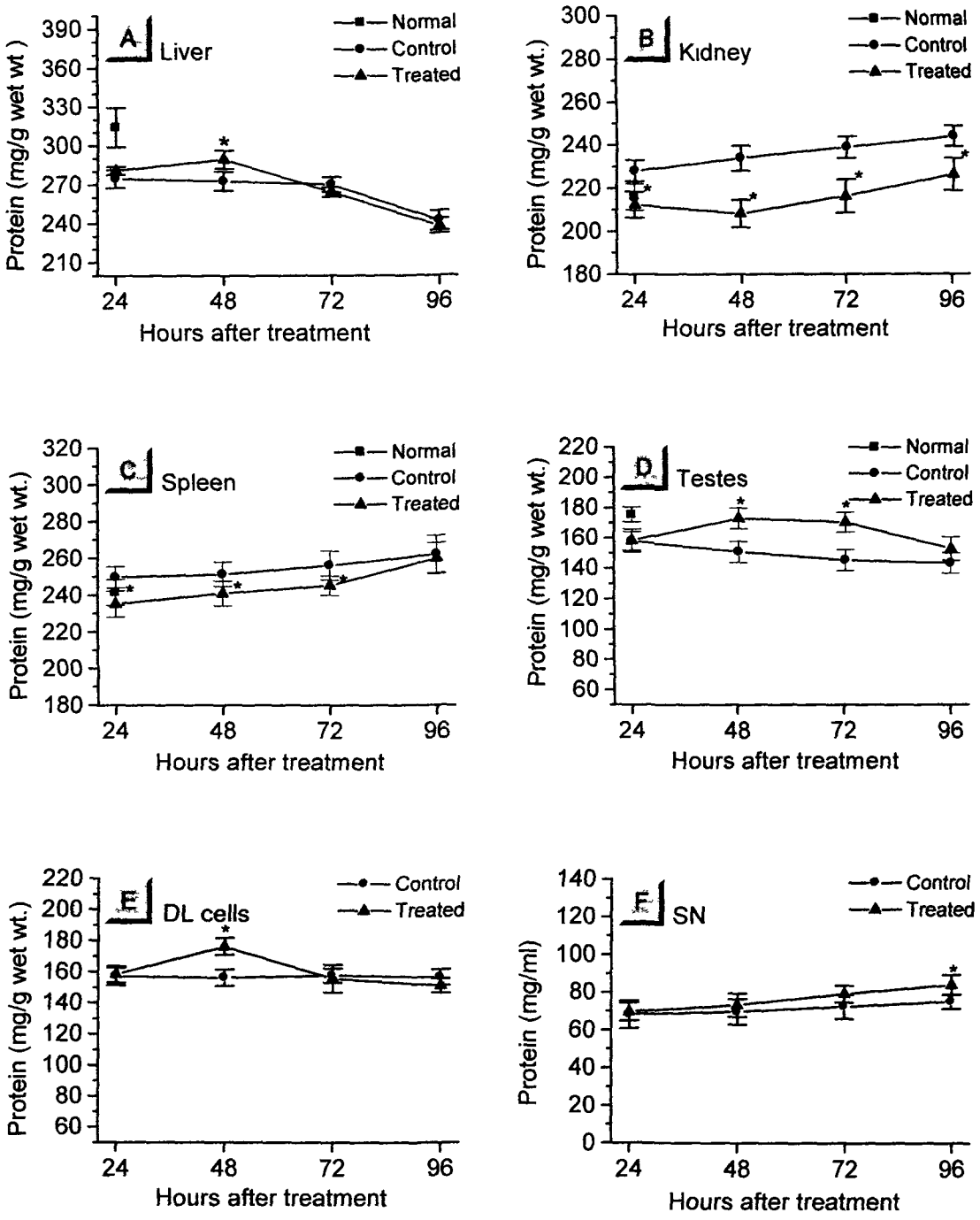


Figure 13.

*Graph showing the changes in protein concentration (mg/g wet wt.) in the tissues, liver (A), kidney (B), spleen (C), testes (D), DL cells (E) and ascites supernatant (F, (mg/ml)) of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

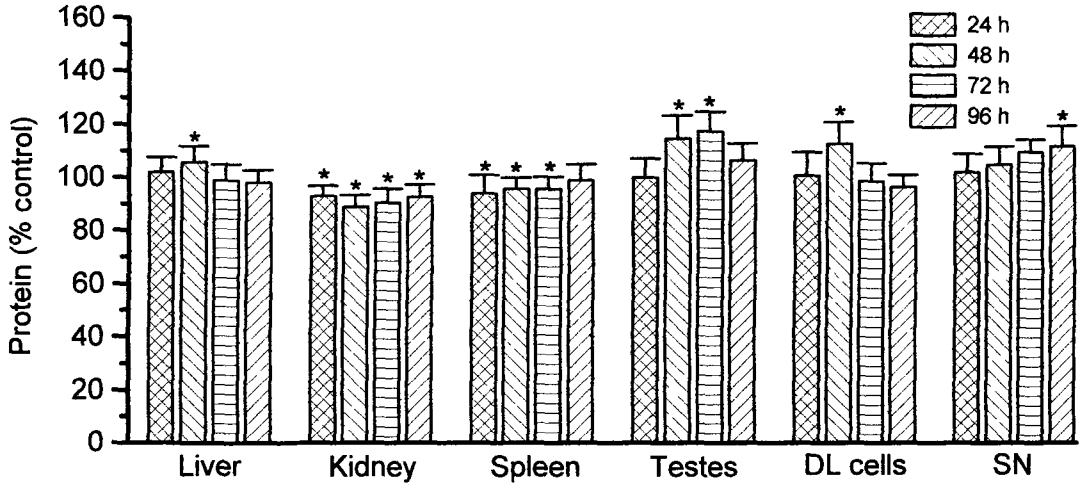


Figure 14.
*Histogram showing the percent changes in the protein content in the tissues, DL cells (mg/g wet wt.) and ascites supernatant (mg/ml) of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *$p \leq 0.05$.*

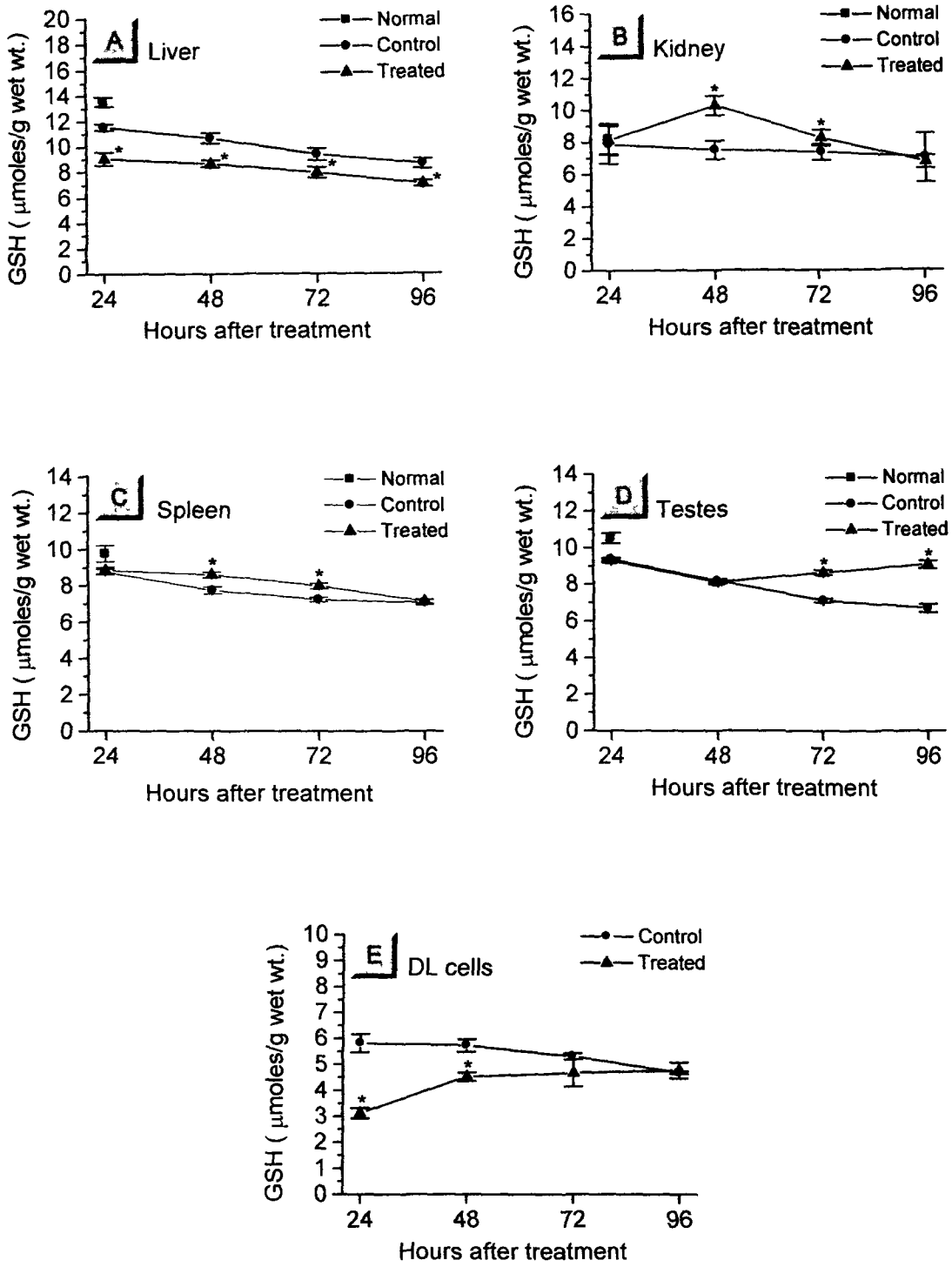


Figure 15.

*Graph showing the changes in the total GSH concentration (µmoles/g wet wt.) in the liver (A), kidney (B), spleen (C), testes (D) and DL cells (E) of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

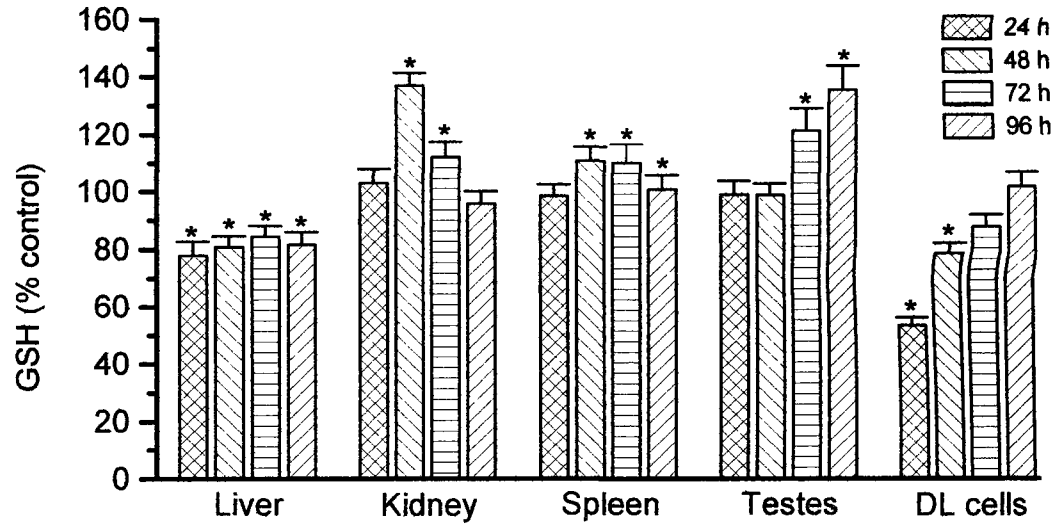


Figure 16.
*Histogram showing the percent changes in the total GSH content (μmoles/g wet wt.) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

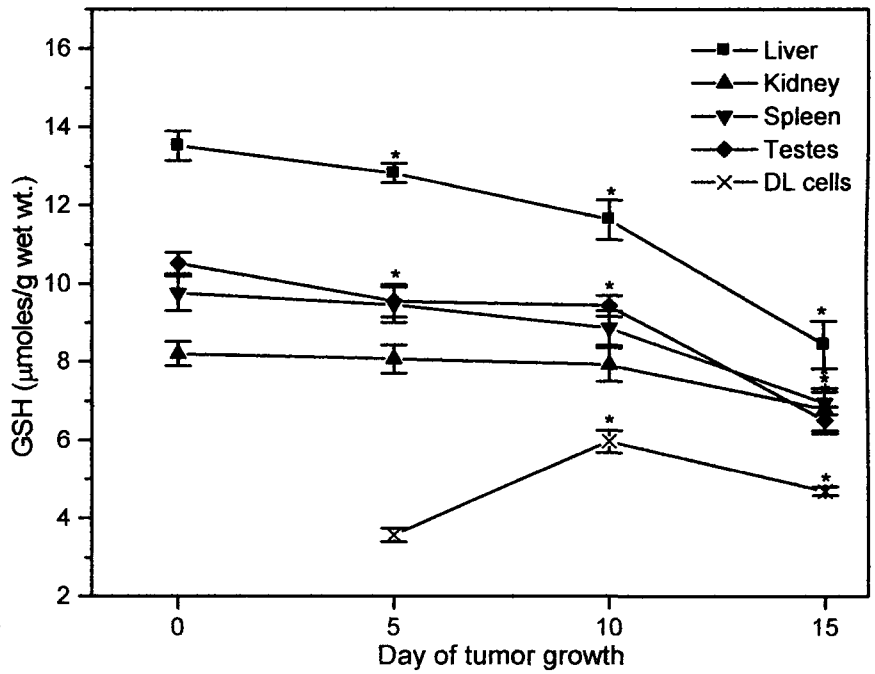


Figure 17.
Figure showing changes in glutathione levels in different tissues of tumor-bearing mice at different stages of tumor growth. Results were expressed as mean ± S.D. Student's *t*-test; as compared to the normal tissue counterpart except DL cells where comparison was done with the fifth day of tumor growth, n = 5, *P<0.05.

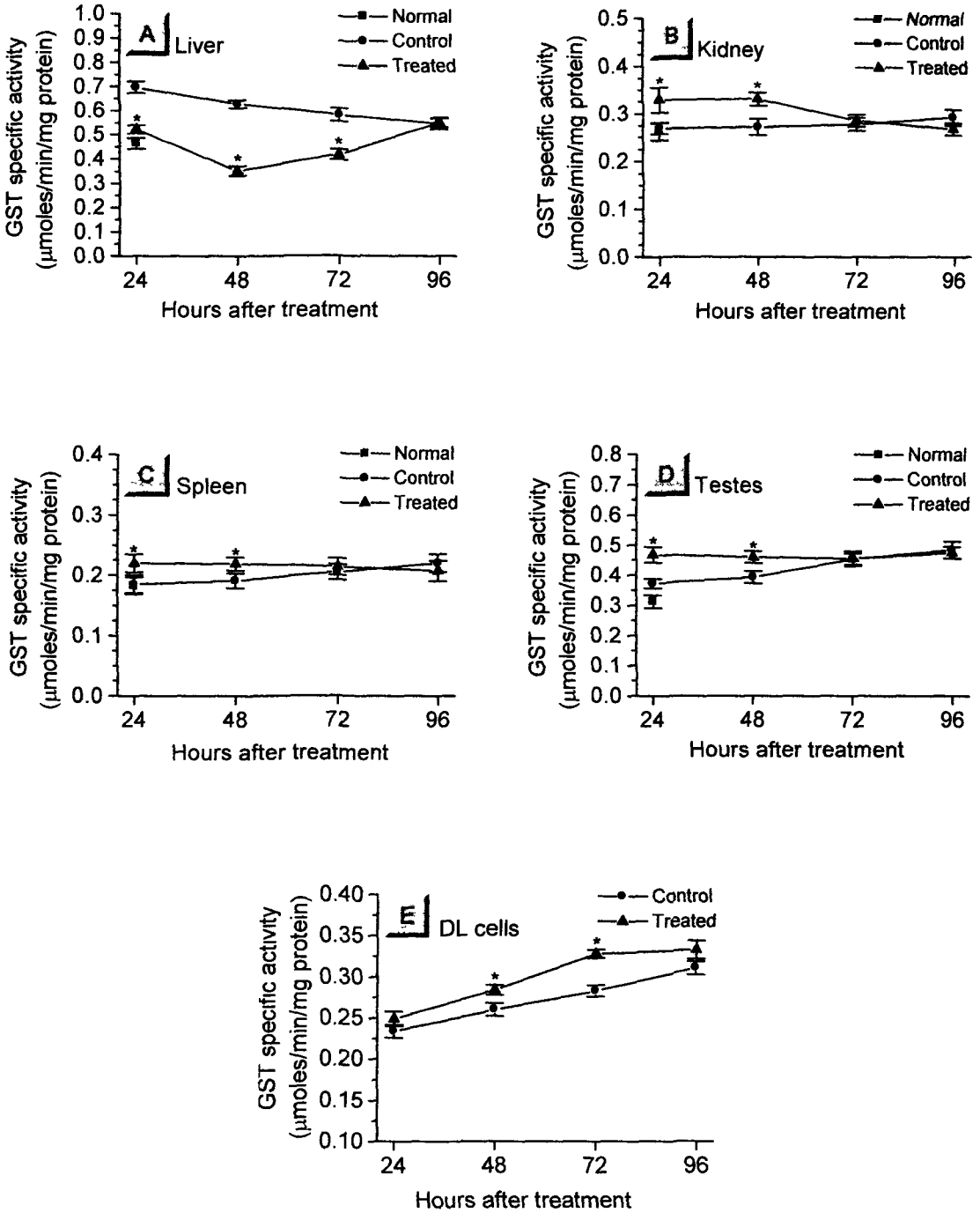


Figure 18.

*Graph showing the comparative changes in the specific activity of GST in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

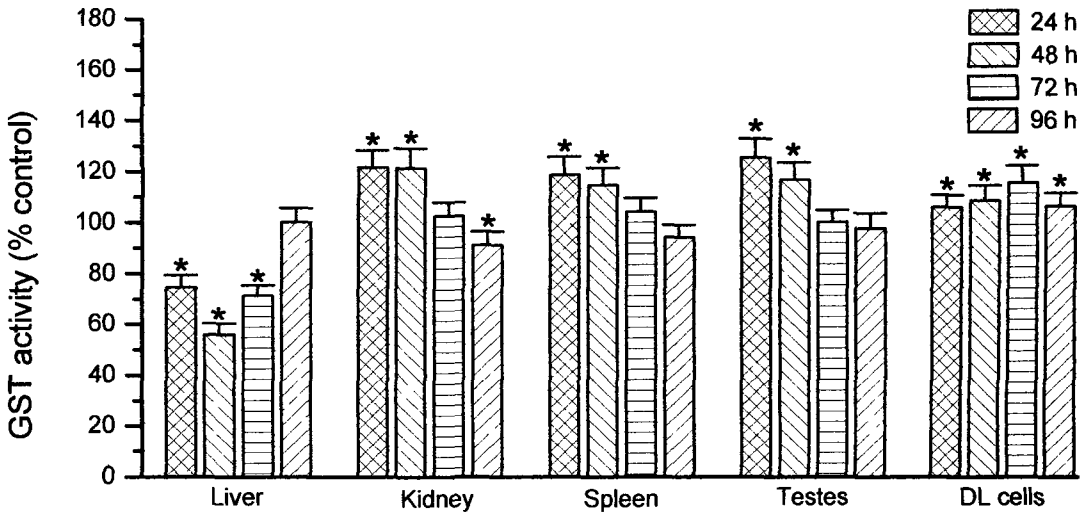


Figure 19.
*Histogram showing the percent changes in the specific activity of GST (μmoles/min/mg protein) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

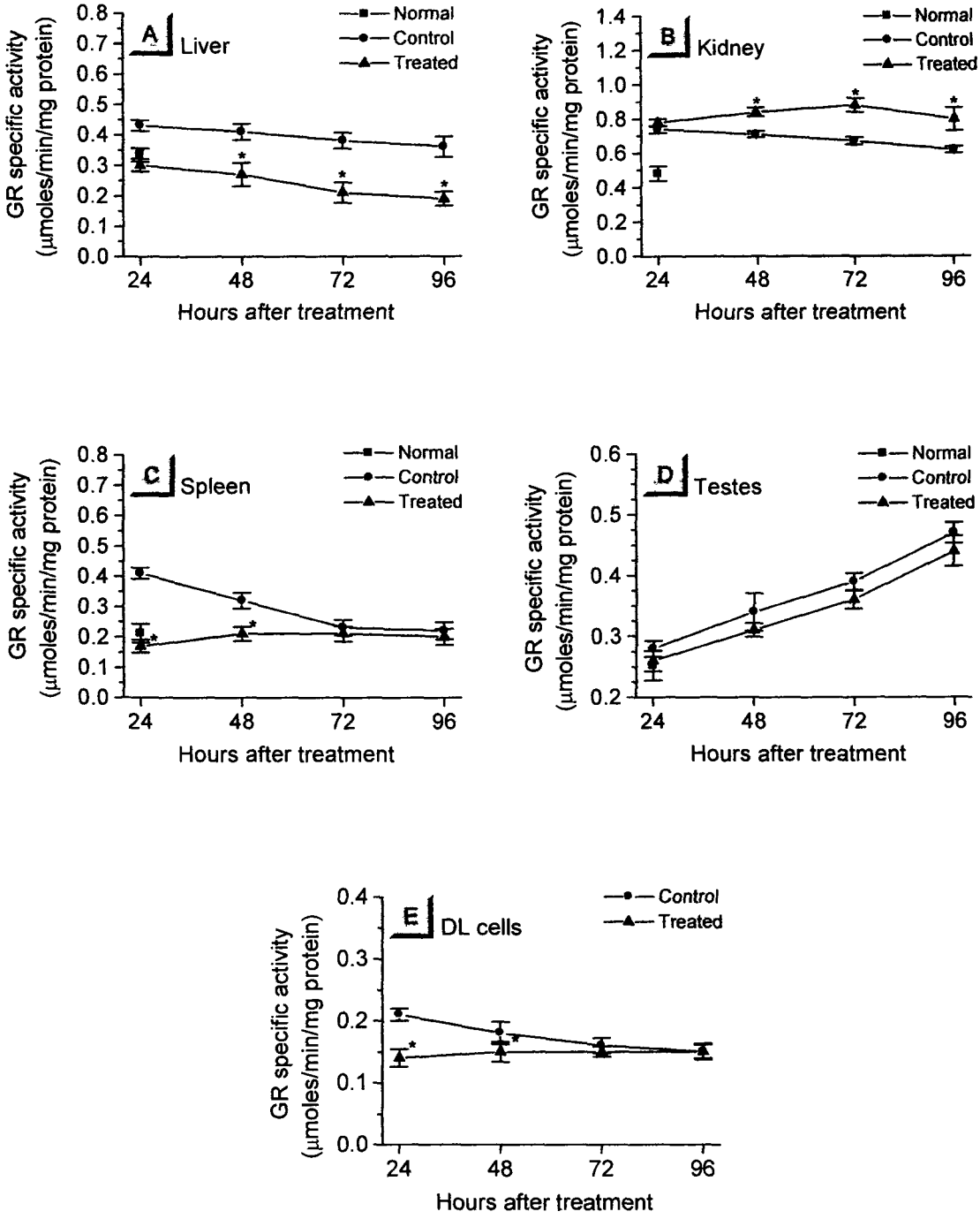


Figure 20.

*Graph showing the comparative changes in the specific activity of GR in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

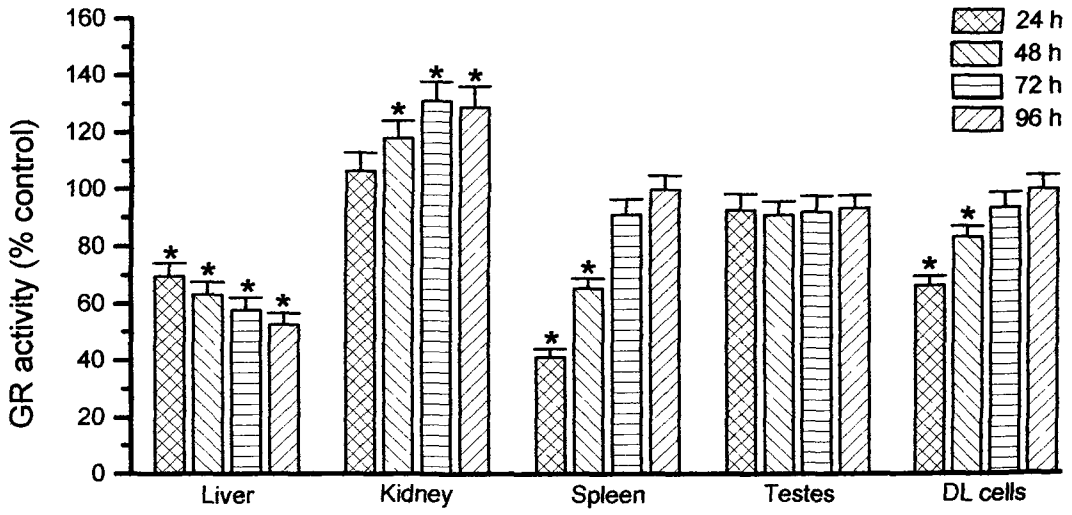


Figure 21.
*Histogram showing the percent changes in the specific activity of GR (μmoles/min/mg protein) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

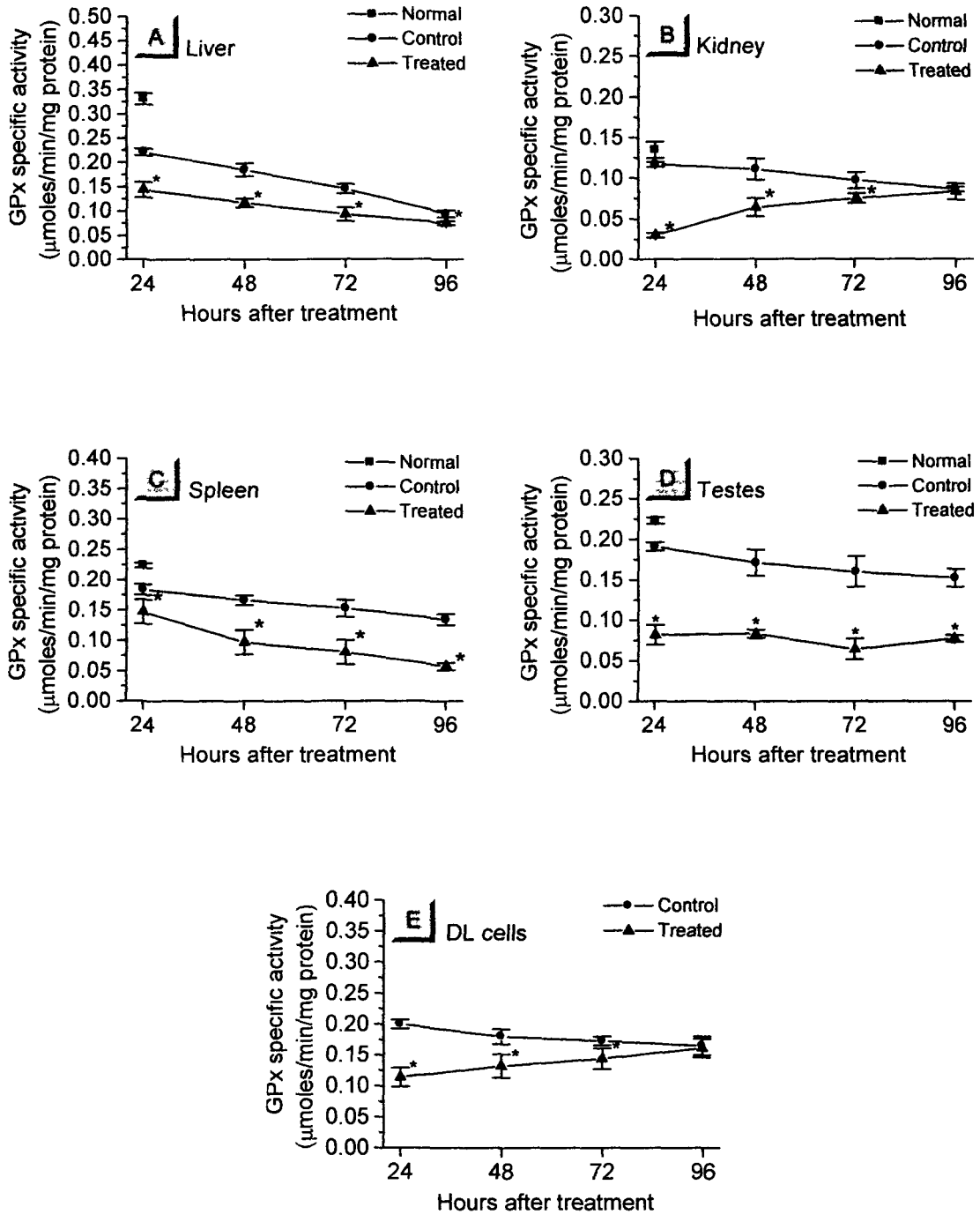


Figure 22.

*Graph showing the comparative changes in the specific activity of GPx in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

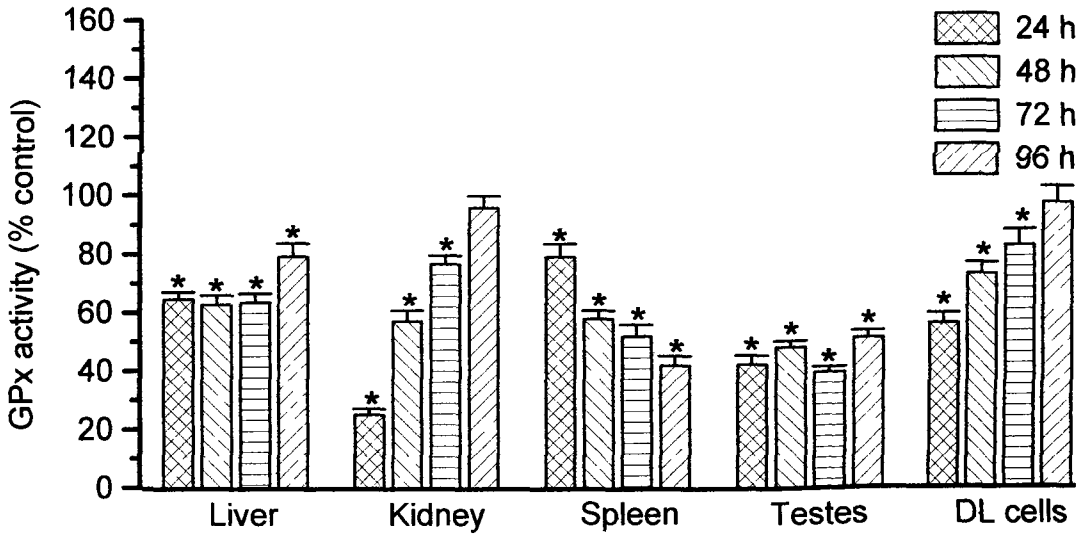


Figure 23.
*Histogram showing the percent changes in the specific activity of GPx (μmoles/min/mg protein) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

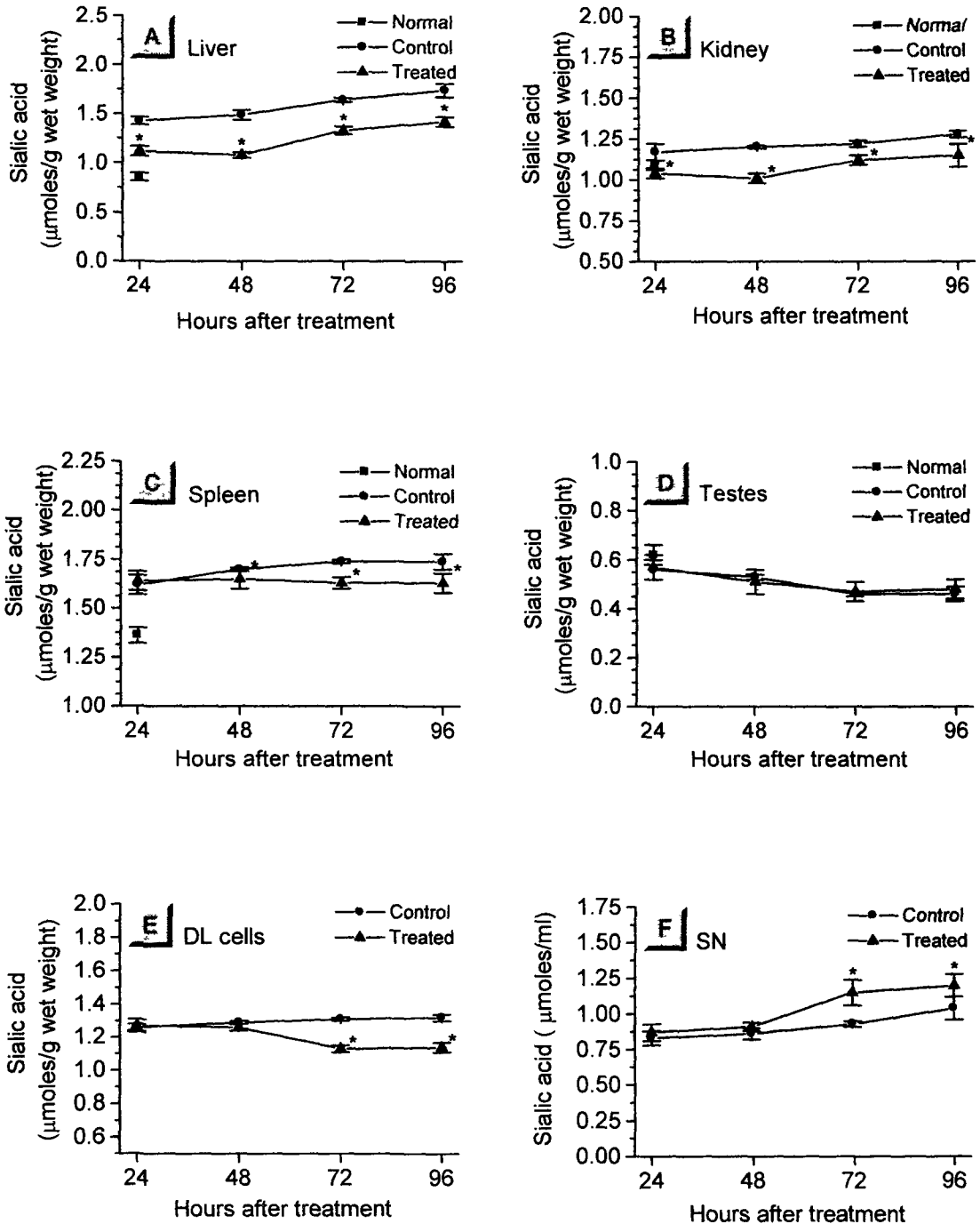


Figure 24.

*Graph showing the changes in the total sialic acid concentration in the liver (A), kidney (B), spleen (C), testes (D), DL cells (E) and ascites supernatant (F) of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

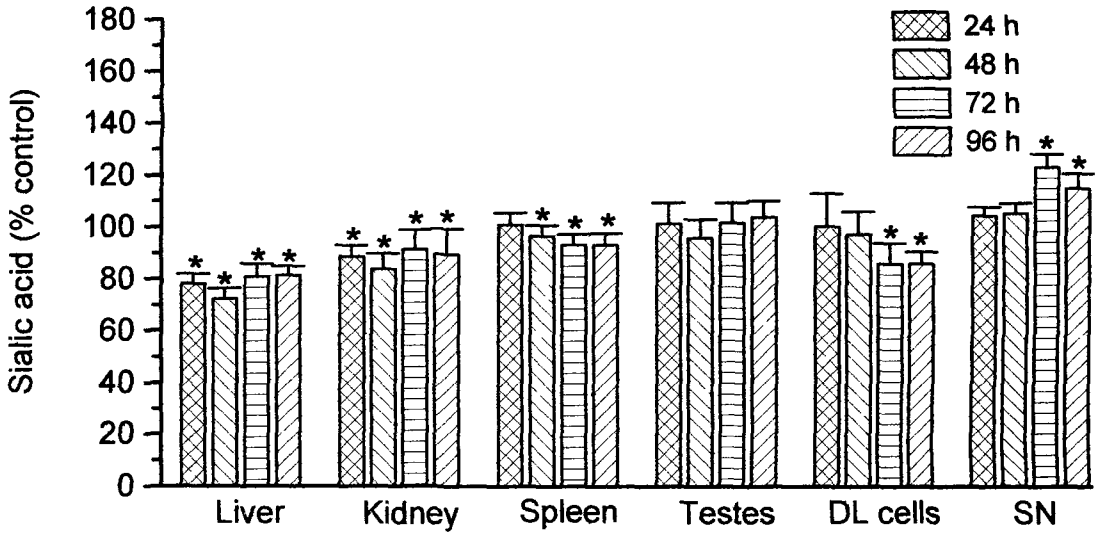


Figure 25.

*Histogram showing the percent changes in the total sialic acid contents in the tissues, DL cells (μmoles/g wet wt.) and ascites supernatant (μmoles/ml) of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

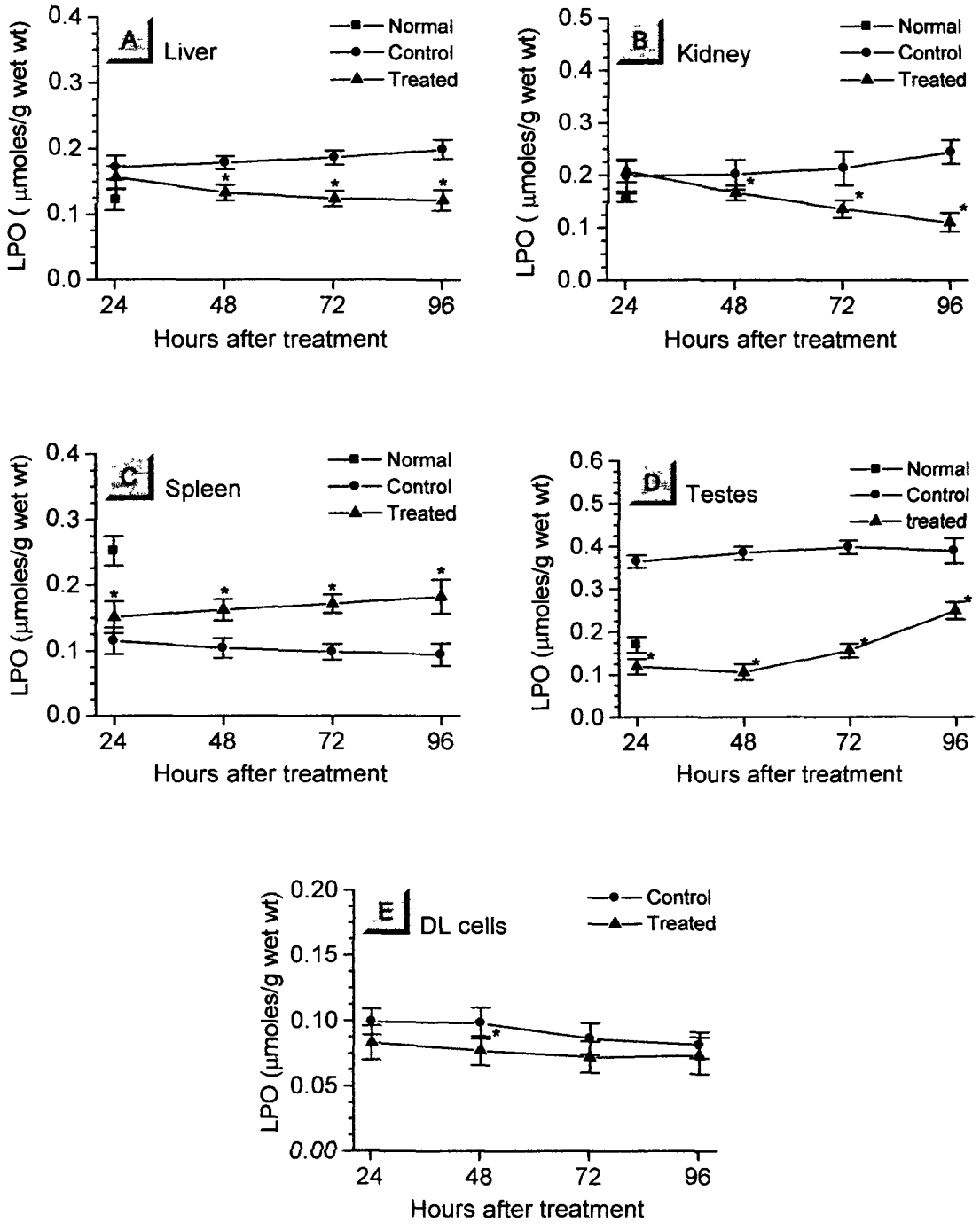


Figure 26.

*Graph showing the comparative changes in the level of LPO (µmoles/g wet wt.) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

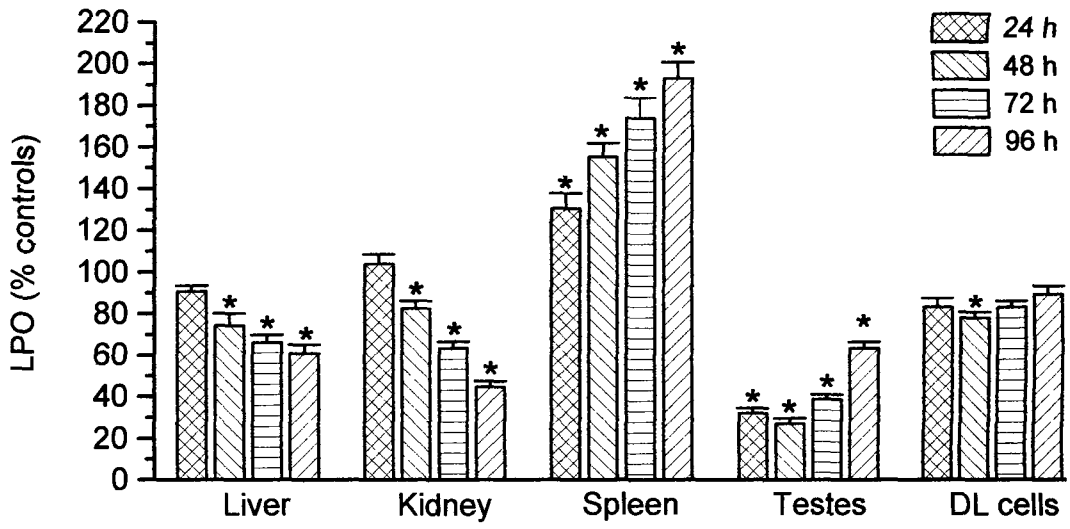


Figure 27.
*Histogram showing the percent changes in the level of LPO in the tissues and DL cells (μmoles/g wet wt.) of tumor-bearing mice after DPE treatment (20 mg/kg b. wt.). Results are expressed as mean ± S.D. Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

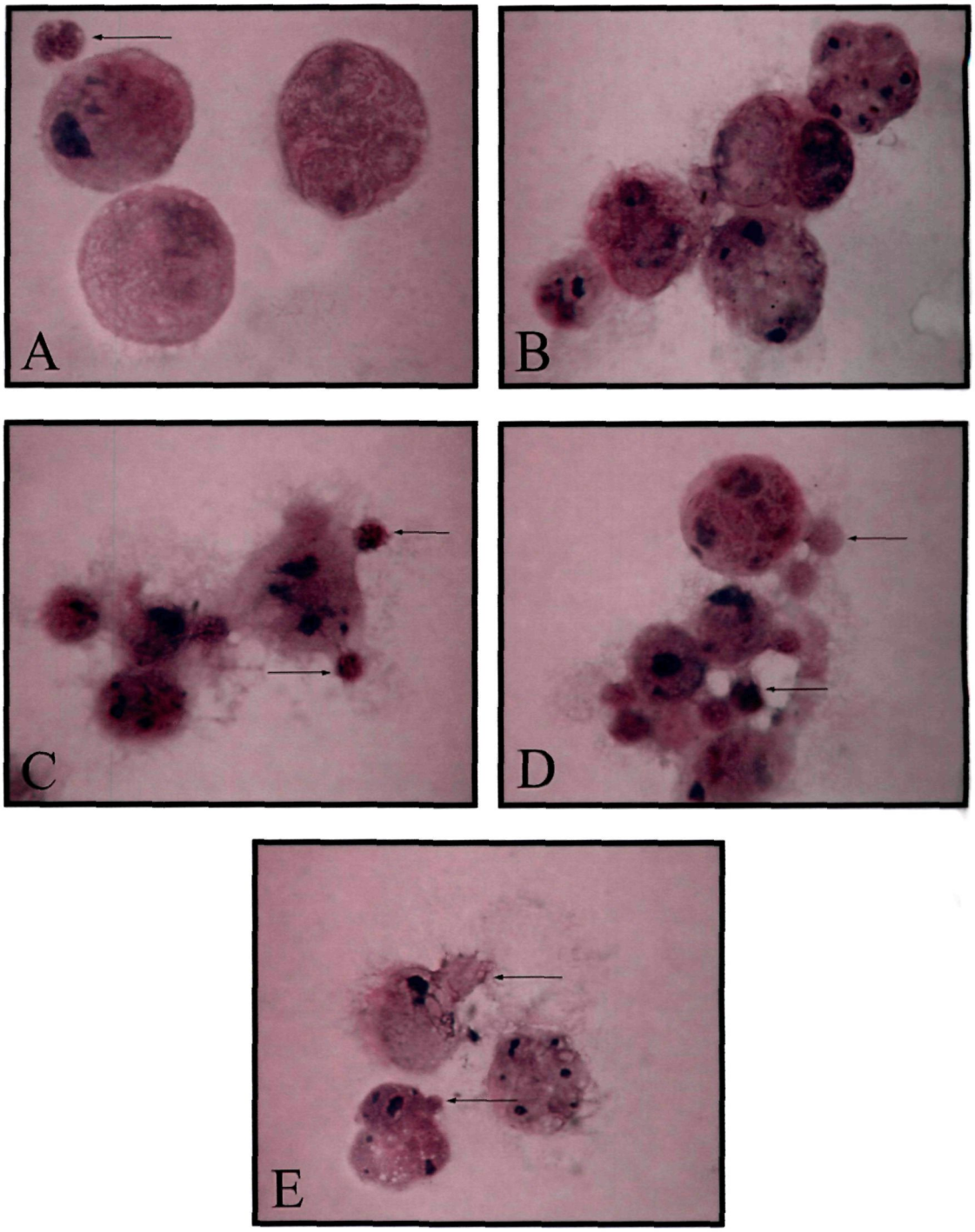


Figure 28.
Light micrographs of tumor cells under different treatment conditions with DPE. Control tumor cells (A) are rounded in shape with very few surrounding leukocytes. 24 h of DPE treatment (B) showing the infiltration of leukocyte towards the tumor cells. DPE treatment for 72 and 96 h (C,D) showed more infiltration of leukocytes towards the tumor cells and gradual disintegration of plasma membrane with membrane vacuoles (E).

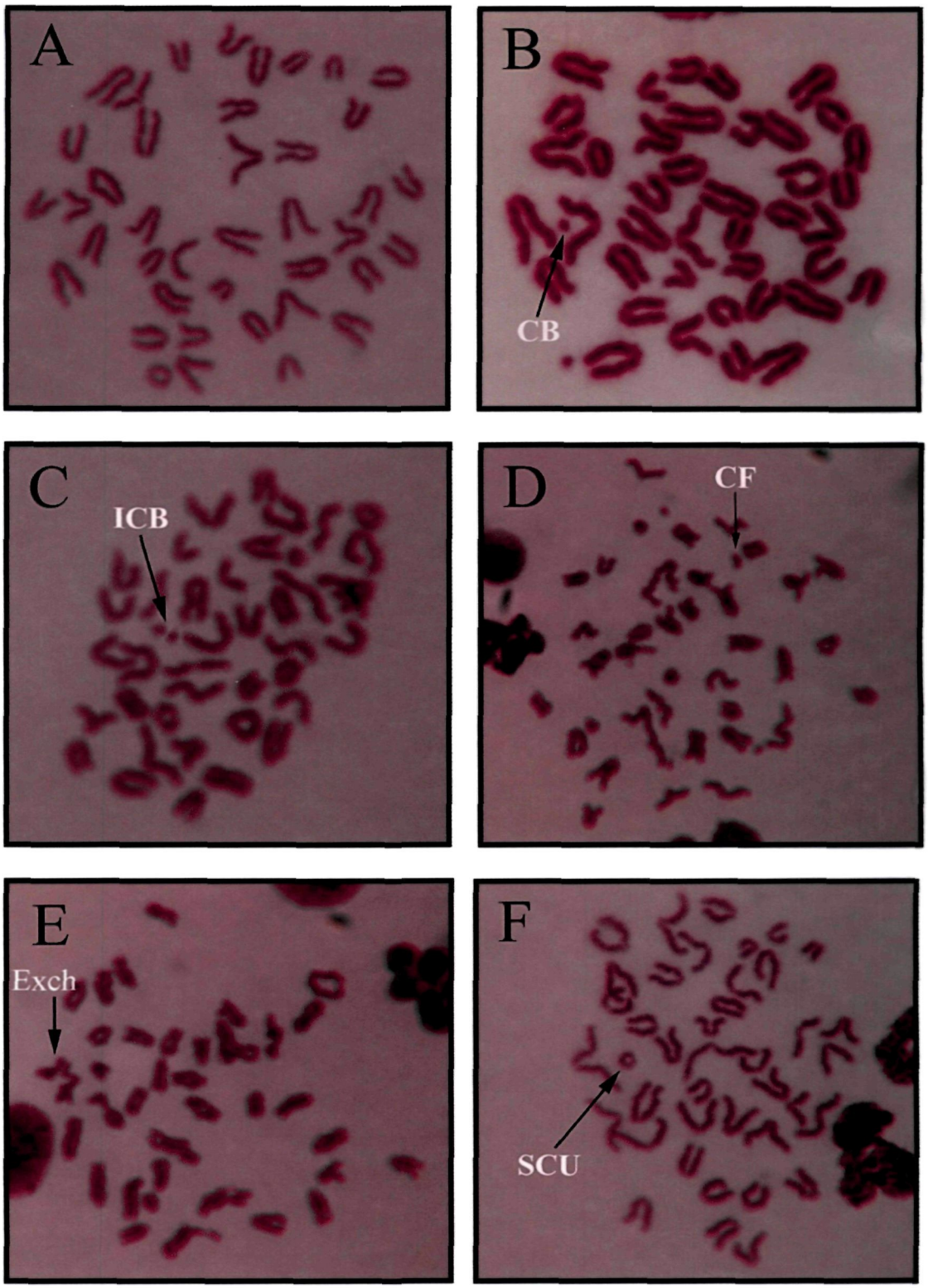


Figure 29.
Photomicrographs of bone marrow metaphase chromosome spreads of mice showing normal set of chromosomes (A) and different types of chromosomal aberrations (B – F), chromatid break (CB), isochromatid break (ICB), chromosomal fragment (CF), exchange (Exch), sister chromatid union (SCU), induced by B[a]P/CIS.

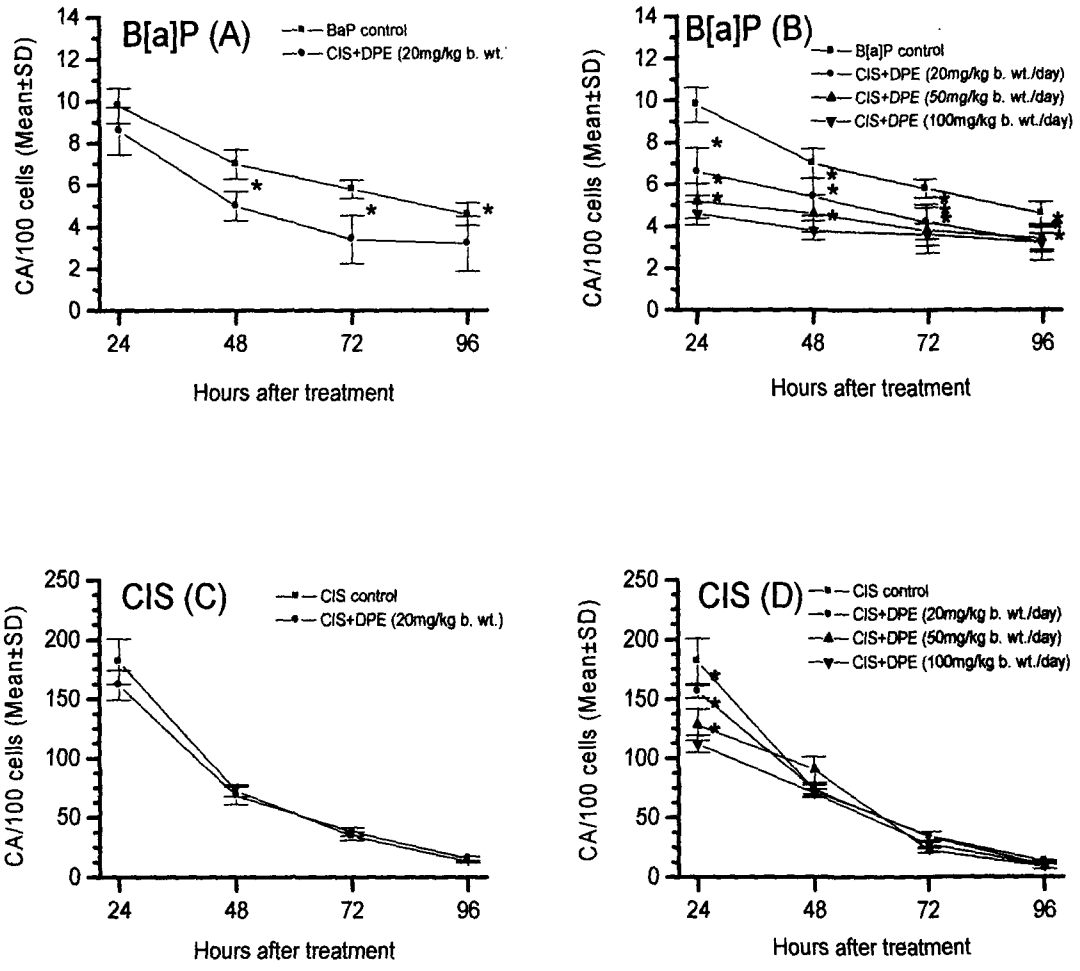


Figure 30.

*Graphs showing the effect of single (A,C) and multiple pre-treatment (B,D) with DPE (7 consecutive days prior to mutagen) on the frequency of bone marrow chromosomal aberrations induced by B[a]P and CIS. Results are expressed as mean ± S.D., Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

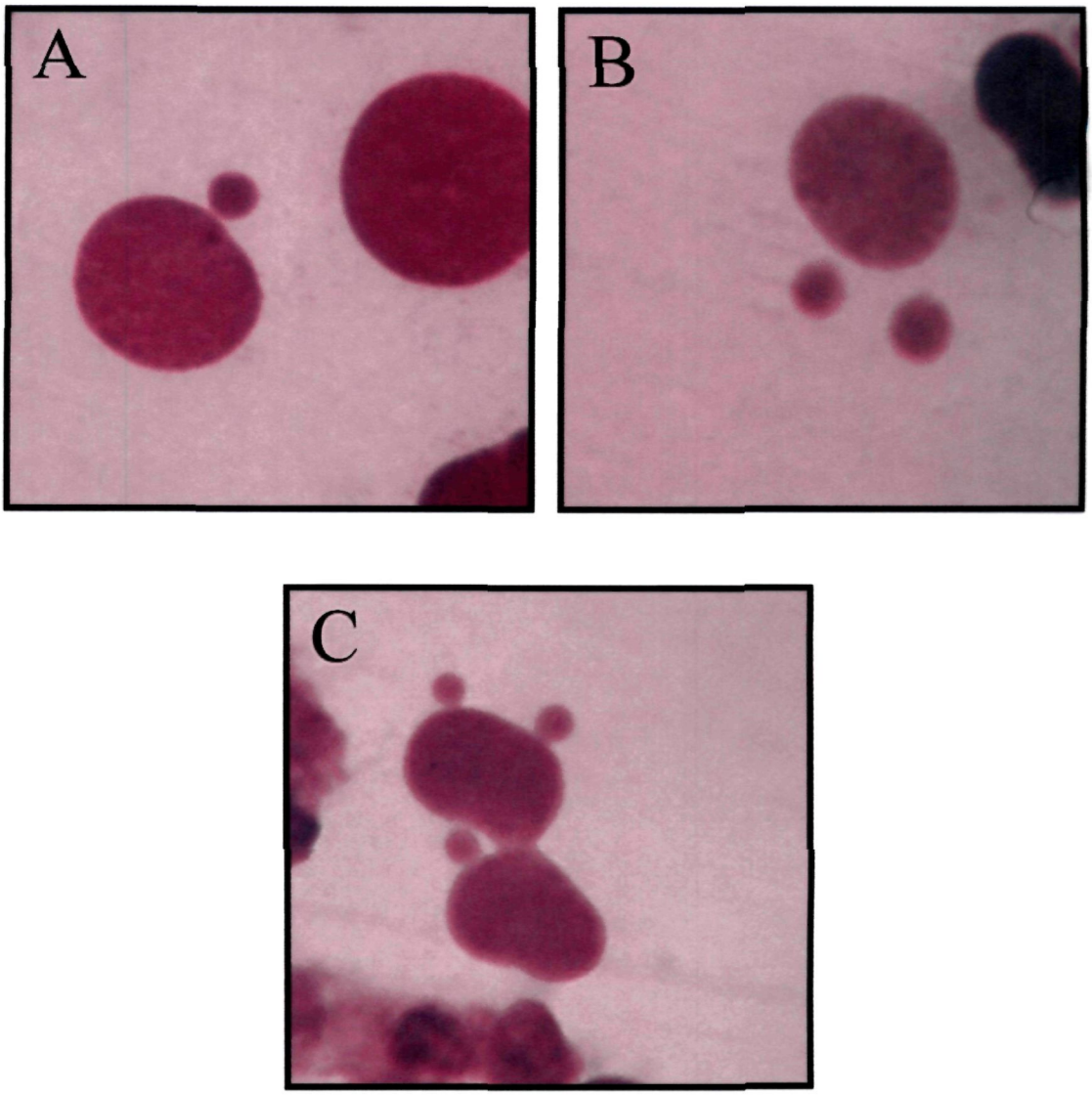


Figure 31.
Photomicrographs of bone marrow cells of mice showing micronuclei induced by B[a]P/CIS.

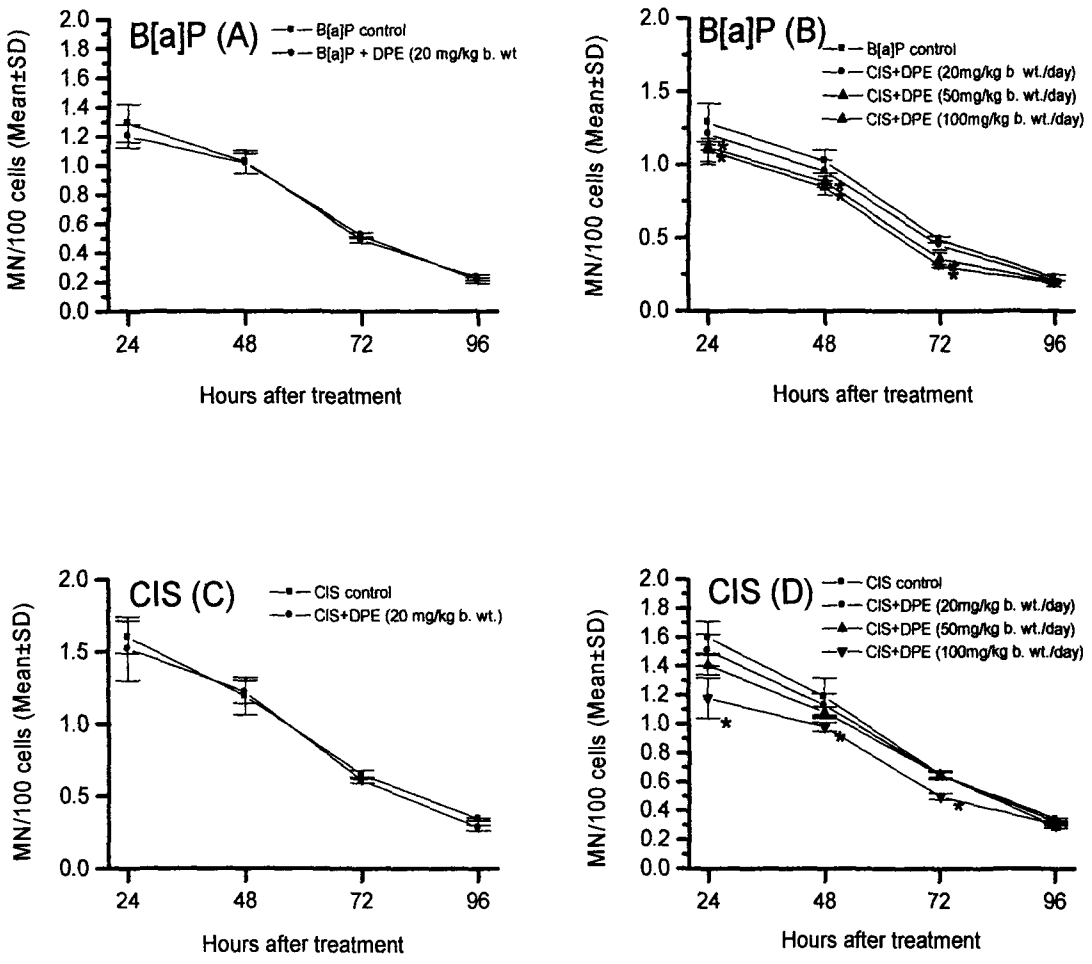


Figure 32.

*Graphs showing the effect of single (A,C) and multiple pre-treatment (B,D) with DPE (7 consecutive days prior to mutagen) on the frequency of bone marrow micronuclei induced by B[a]P and CIS. Results are expressed as mean±S.D., Student's t-test, n = 6, as compared to the corresponding control, *$p \leq 0.05$.*

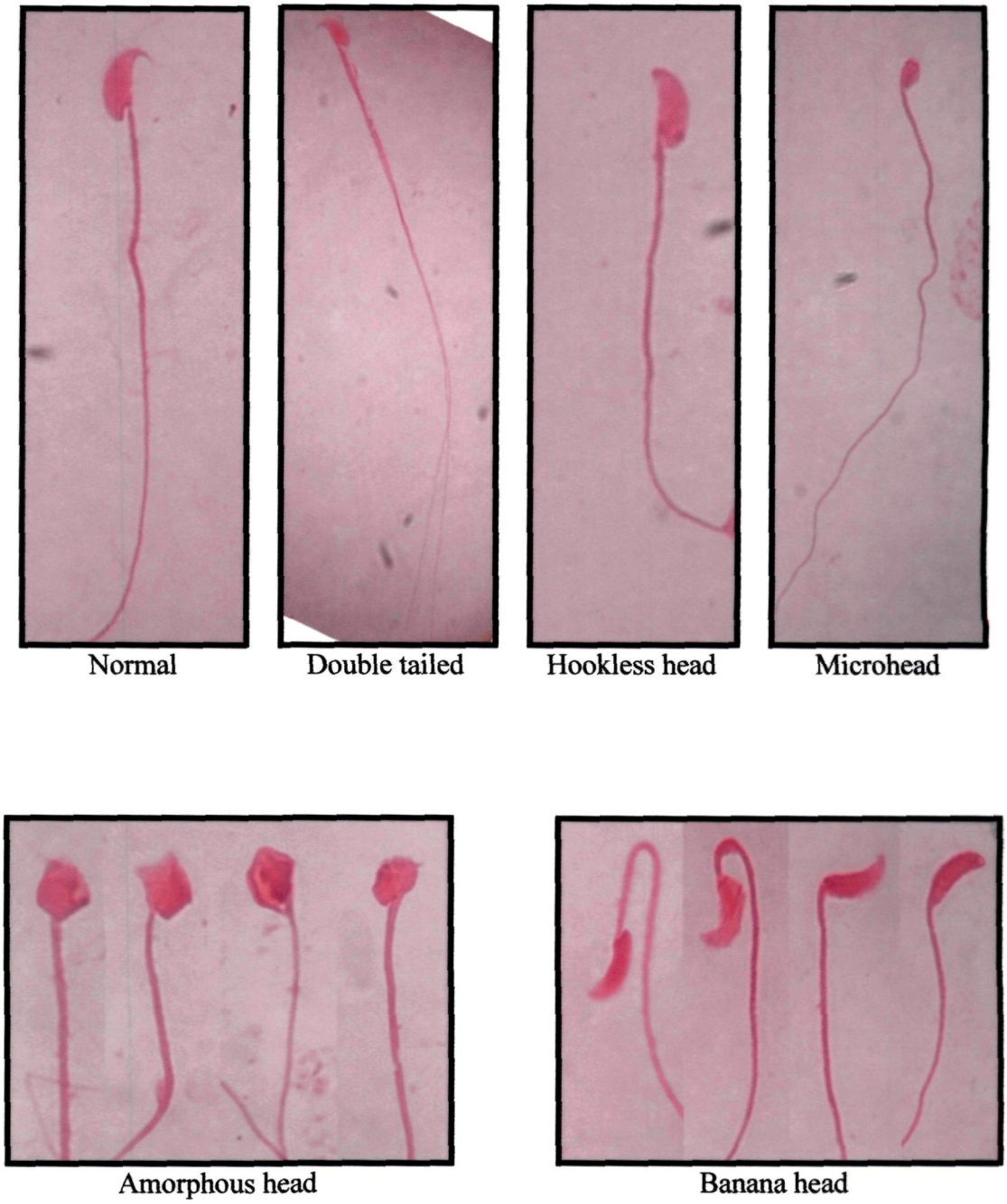


Figure 33.
Photomicrographs of mice spermatozoa showing different types of abnormalities induced by B[a]P/CIS.

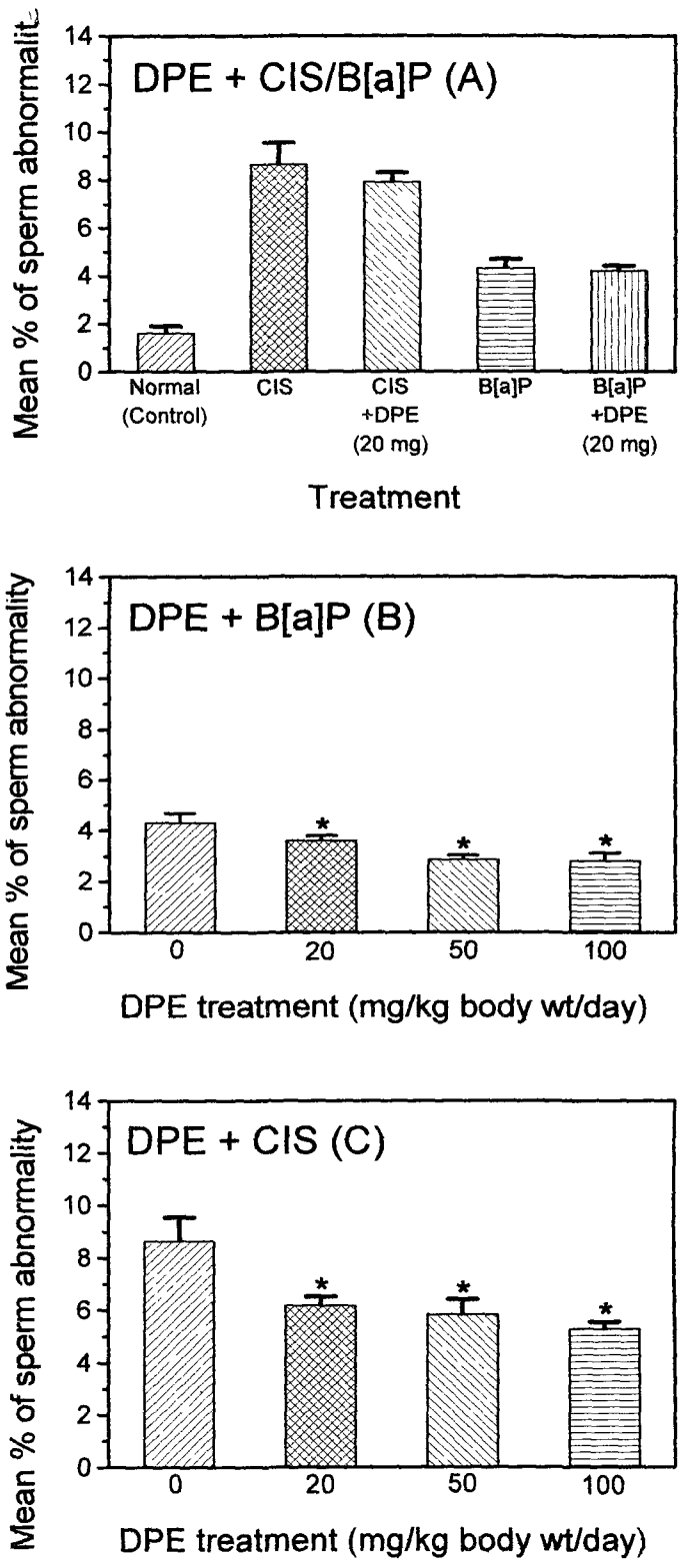


Figure 34.

*Histogram showing the effect of single (A) and multiple pre-treatment (B,C) with DPE (7 consecutive days prior to mutagen) on the frequency of sperm abnormalities induced by B[a]P and CIS. Results are expressed as mean±S.D., Student's t-test, n = 6, as compared to the corresponding control, *p ≤ 0.05.*

DISCUSSION

After survey and screening of possible anticancer medicinal plants from Meghalaya and Mizoram through literature search and consultation with elders and some local herbal practitioners, five plants were selected for the evaluation of possible antitumor activity against Dalton's lymphoma in mice (Table 1; Figure 1). In Meghalaya, *Potentilla fulgens* (shrubby cinquefoil, locally called 'langniang') has been traditionally used for the treatment of colic pain, spasmodic trouble, pyorrhea and tumor, and *Taxus baccata* (common yew, locally called 'soh blei') for the treatment of aphrodisiac, epilepsies, irregular menstruation and tumor. In Mizoram, *Ageratum conizoides* (goat weed, locally called 'vailenhlo') was known to be used for the treatment of tumor and in cuts and wounds as haemostatic, *Blumea lanceolaria* (locally called 'buarze') for the treatment of tumor, asthma, stomachache, wounds and scabies and *Dillenia pentagyna* (simpoh, locally called 'kaihzawl') for the treatment of tumor, stomachache and diarrhea.

In various antitumor studies, murine ascites Dalton's lymphoma has been commonly used as an important experimental tumor model (Prasad and Giri, 1994; Nicol and Prasad, 2002). Dalton's lymphoma was originated in the thymus gland of a DBA/2 mouse at the National Cancer Institute, Bethesda, M D, in 1947. Then, an ascites form was developed by repeated intraperitoneal transplantation of the tumor (Goldie and Felix, 1951). The results of our preliminary screening for antitumor activity (Table 3 and 4; Fig. 5 and 6) indicate that out of five plants used in the present study, only three plants' test parts such as aqueous extract of the root of *A. conyzoides* and *P. fulgens,* and methanol extract of stem bark of *D. pentagyna* showed

comparatively better antitumor activity against ascites Dalton's lymphoma in mice. Among the aqueous extracts of five plants studied, *A. conyzoides* and *P. fulgens* showed antitumor activity (Table 3), while among the methanol extracts of these plants only *D. pentagyna* showed potent antitumor activity (Table 4). Comparison of the antitumor potentials of these plants depicted in the form of survivability showed that antitumor activity against murine ascites Dalton's lymphoma was highest with methanol extract of stem bark of *D. pentagyna* (Table 4). Moreover, out of different doses (50, 100 and 200 mg/kg body wt/day) used, maximum antitumor activity of *D. pentagyna* was observed with 50 mg/kg body wt/day. Therefore, further dose determination was carried out using doses lower than 50 mg/kg body wt/day, and maximum survivability of tumor-bearing mice (ILS ~71%) was noted at a dose of 20 mg/kg body wt/day (Table 5; Fig. 7). With the aqueous extract of the root of *A. conyzoides* and *P. fulgen*, maximum survivability were observed at a dose of 100 and 50 mg/kg body wt/day respectively indicating that different plant extracts have to be used in different doses. From the collected information it was known that the traditional healers used primarily water as the solvent, but we found in this study that the plant extracted by methanol provided better antitumor activity compared to that extracted by water. While the most potent antitumor activity was observed with the methanol extract of *D. pentagyna*, its aqueous extract did not show significant antitumor activity. It appears that the active component of *D. pentagyna* was not fully extracted in hot water or it may have been destroyed by heat during extraction with boiling water. On the other hand, methanol extraction may be more favorable for the stability of the active compound(s) present in this particular plant, or this difference in the antitumor activity might have resulted from the lack of solubility of the active

constituents in water than in methanol. Tumor growth may also be expressed in terms of changes in body weight on different days of treatment. As compared to normal mice, a steady increase in the body weight of tumor-bearing mice was observed. As compared to the control tumor-bearing mice, the methanol extract of *D. pentagyna* caused comparatively less increase in body weight suggesting retardation of tumor growth rate leading to increased survivability of tumor-bearing hosts (data not included). Thus, considering the observed better antitumor efficacy, methanol extract of stem bark of *Dillenia pentagyna* at a dose of 20 mg/kg body wt. was selected for further hematological, biochemical, enzymatic, microscopical and antimutagenic studies. Various reports on the mechanism behind the antitumor activity of various plant extracts indicate that different plant extracts exhibited their antitumor activities through different mechanism of action in the host (Sakagami et al., 1987; Chaudhuri et al., 1998; Sllchenmyer and Von Hoff, 1991; Tanaka et al., 1996; Das, 2004). Since isolation of the active principle has not been done and the extract as such showed antitumor activity, this antitumor activity may involve the combined effect of active compound(s) in the extract.

The association between altered immunity and the occurrence of cancer has been reported in a variety of animals (Burns and Leventhal, 2000). Depression in erythrocytes (anemia) is a frequent complication of cancer diseases. For example, the growth of Ehrlich ascites carcinoma has been reported to be accompanied with a decrease in some haematological values like haemoglobin and RBC counts (Pal et al., 1993). In the present study also a significant decrease in the haematological parameters i.e. RBC, PCV and Hb, was observed in tumor-bearing animals and the DPE treatment of tumor-bearing animals caused significant increase in these

haematological values (Table 6). It has been suggested that this decreased erythrocyte life span and anemia may be correlated with decreased blood antioxidant capacity (Durak et al., 1994). In addition to oxygen transport, RBCs also function as conveyors of nutrients, and serve as targets for drugs, pathological factors and environmental xenobiotics (Pikula et al., 1996). Solid tumors, both primary lesions and metastases, are infiltrated by tumor-associated leukocytes which are a heterogeneous population of cells consisting of various (and variable) subsets of T cells (helper, suppressor and cytotoxic), B cells, natural killer (NK) cells, and macrophages (van-Ravenswaay-Claasen et al., 1992). Although the exact mechanism underlying DPE-mediated increase in haematological values has not been explored, it may be suggested that the observed improvement in this haematological values should be involved in strengthening hosts' immunity, thereby increasing host survivability.

As compared to the normal mice there was a significant decrease in the protein concentration in the tissues of tumor-bearing mice except in spleen (Table 8; Figure 13). DPE treatment of tumor-bearing hosts caused significant decrease in the protein contents in kidney and spleen, and increase in ascites supernatant. These changes may involve some alterations in the rate of protein synthesis or decreased uptake of protein in these tissues. On the other hand, initial (48 h) increase in protein level in DL cells and liver, and a decrease particularly during later period (72-96 h) of DPE treatment may be due to cumulative cytotoxic effects in the tumor cells and possibly some toxic effect in the liver of the host.

Reduced glutathione, an endogenous intracellular thiol-containing tripeptide (L-γ-glutamyl-L-cysteinyl-glycine, GSH), is an important cellular antioxidant. It is

ubiquitous in animals, plants and microorganisms (Kosower and Kosower, 1978; Lomaestro and Malone, 1995). Under normal physiological conditions, mammalian cells maintain more than 98% of intracellular GSH in the reduced form (GSH) with the help of GSSG reductase and maintain at intracellular concentration of 0.5 to 10 mM. In healthy cells, oxidized glutathione (GSSG) rarely exceeds 10 percent of total cell glutathione. The key functional element of GSH is the cysteinyl moiety, which provides the reactive thiol group and is responsible for the many functions of GSH including maintenance of protein structure and function by reducing the disulphide linkages of proteins, regulation of protein synthesis and degradation, maintenance of enzyme activity and immune function, protection against oxidative damage, detoxification of xenobiotics and in drug metabolism (Wang and Ballatori, 1998). Liver is the major site of its biosynthesis (Meister and Tate, 1976; Deleve and Kaplowitz, 1991). The enzymes responsible for its breakdown are dipeptidases and γ-glutamyl transpeptidase (γGT), which are membrane-bound proteins located predominantly on the apical surface of epithelial tissues. γGT is the only enzyme that removes the γ-glutamyl moiety from GSH under physiological conditions. Dipeptidase removes the glycyl moiety. The breakdown products can be reabsorbed into the cell for GSH synthesis in the cytosol (Wang and Ballatori, 1998). GSH has been the focus of interest in cancer chemotherapy (Arrick and Nathan, 1984; Khynriam and Prasad, 2003).

Present studies showed variations in GSH concentrations in different tissues of normal and tumor-bearing mice and also at different stages of tumor growth. As compared to the tissues of normal mice, GSH concentrations decreased in all the tissues of tumor-bearing mice (Table 9; Figure 15). It was observed that GSH level in

DL cells increased gradually with the tumor growth, reaching maximum level on the 10^{th} day followed by a slight decrease over the next 4-5 days while it decreased in liver, kidney, spleen and testes (Figure 17). In Ehrlich ascites tumor also an increase in GSH level has been reported during tumor progression reaching maximum level on day 7 followed by a decrease on the 14^{th} day, which was correlated with a decrease in cell proliferation (Estrela et al., 1992). The increase of GSH in DL cells of tumor-bearing control could be involved in many ways, thereby facilitating the proliferation and metabolism of tumor cells in the host as it has been reported that GSH controls the onset of tumor cell proliferation by regulating protein kinase C activity and intracellular pH (Terradez et al., 1993).

GSH and its dependent enzymes work with other antioxidants and antioxidant enzymes to protect cells against reactive oxygen intermediates (ROIs) (Sies, 1986). Therefore, changes in the rate of cancer cell proliferation are accompanied by changes in their intracellular GSH levels and, consequently, these could be reflected by changes in the antioxidant machinery. Elevation of intracellular GSH levels has also been suggested to be involved in the resistance of cancer cells to oxidative stress, radiotherapy and chemotherapy (Navarro et al., 1999; Mitchell and Russo, 1987), while a depletion of GSH levels could increase the cytotoxicity of a variety of antitumor agents (Arrick and Nathan, 1984; Khynriam and Prasad, 2003) which in turn could induce the apoptotic cell death also (Kane et al., 1993). As the GSH level in DL cells was observed to increase with tumor growth (Figure 17), it became an investigating interest to find the changes in the GSH level of various tissues, if any, after the extract treatment. DPE treatment of the hosts showed differential pattern of effects in different tissues (Table 9). The observed DPE-mediated increase in GSH

level in kidney, spleen and testes of tumor-bearing host (Table 9; Figure 15 and 16) may play a role in protection mechanism in these tissues. However, the decrease in GSH level, particularly in DL cells by the DPE treatment, may be a noteworthy step in the antitumor activity of the extract of *D. pentagyna* against Dalton's lymphoma. Therefore, it can be suggested that, DPE-mediated decrease in GSH in DL cells may have a role in the antitumor activity of *Dillenia pentagyna* by increasing DL cell's susceptibility to oxidative stress, thereby, increasing host survivability. Studies in human and rodent tumor cell lines have shown that resistance to alkylating agents may, in certain instances, be due to elevated tumor GSH concentrations. Depletion of cellular GSH by buthionine sulfoximine (BSO) has been shown to sensitize tumor cells to irradiation and to certain chemotherapeutic agents *in vitro* (Mitchell et al., 1983; Shrieve et al., 1985; Clark et al., 1984; Arrick et al., 1983; Meister, 1983; Russo and Mitchell, 1985). Therefore, treatment strategies involving GSH depletion should also be taken into consideration in order to maximize the therapeutic efficacy of anticancer agents, and the proposal of combination therapy with *Dillenia pentagyna* and other anticancer agents may also be useful in providing better antitumor activity.

Glutathione-related antioxidant enzymes have been reported to be involved in the detoxification of peroxides, xenobiotics, hydroperoxides and drugs (Chasseaud, 1997; Meyer et al., 1998), and some of the excess oxygen radicals such as hydrogen peroxide, superoxides, hydroperoxy and hydroxyl radicals are shown to be implicated in a variety of disorders including cardiovascular disease, reperfusion injury, rheumatoid arthritis, immune injury and cancer (Ross, 1988). In the detoxification of these reactive free radicals, some of the GSH related enzymes such as glutathione s-transferase (GST), glutathione reductase (GR) and glutathione peroxidise (GPx) are

involved in the intracellular defence mechanisms (Tew, 1994; Noctor and Foyer, 1998; Teramato et al., 1999; Ohkuwa et al., 1997). In the present study, activity of these three glutathione-related enzymes (GST, GR and GPx) was investigated in different tissues of tumor-bearing mice under different treatment conditions.

The super family of glutathione *S*-transferases (GSTs, EC 2.5.1.18) represents an integral part of the phase II detoxification system which catalyze the reaction of conjugation of electrophilic compounds with GSH, and are also a family of multifunctional enzymes present in the cytosol of most cells as homodimeric or heterodimeric proteins, with subunit molecular weights ranging from 24,000 to 27,500 daltons (Armstrong, 1987; Mannervik, 1985; Hayes and Mantle, 1986). These intracellular proteins are found in most eukaryotes and prokaryotes, and protect cells against oxidative- and chemical-induced toxicity and stress by catalyzing the S-conjugation between the thiol group of GSH and an electrophilic moiety in hydrophobic and often toxic substrates. The resultant GSH conjugates can be exported from animal cells by putative membrane ATP-dependent pump system (Ishikawa, 1992), after which they are usually metabolized and eliminated by multiple pathways (Commandeur et al., 1995). GST-mediated conjugation of bifunctional alkylating agents to GSH has been reported to contribute to resistance of certain tumors to such agents. *In vitro* studies have shown that human and rodent GSTs catalyze the conjugation of GSH with chlorambucil and melphalan, representatives of bifunctional alkylating agents (Dulik, et al., 1986; Ciaccio et al., 1991). Furthermore, certain tumor cells have been found to be sensitized to the action of cytosolic drugs by co-administration of GST inhibitors (Tew, et al., 1988; Smith, et al., 1989; Hansson, et al., 1991), and overexpression of certain GSTs has been found to accompany

increased tumor resistance to such chemotherapeutic drugs (Smith, et al., 1989; Wang and Tew, 1985; McGown and Fox, 1986; Dahllof, et al., 1987; Lewis, et al., 1988). Increased activity of GST has been reported in several human tumors (Saydam et al., 1997).

In the present studies, GST activity in liver decreases during 24 – 72 h of DPE treatment and a slight increase thereafter (Table 10; Figure 18). DPE treatment resulted in increase in GST activity in kidney, spleen, testes (24 – 48 h) and DL cells (24 – 96 h) (Table 10; Figure 18 and 19). An increase in GST activity in the tissues after DPE treatment may reflect the utilization of glutathione in detoxification reactions. Since GST activity in DL cells increases, the observed decrease in glutathione levels in DL cells after DPE treatment (Table 9) may suggest the involvement of GST in glutathione-mediated detoxification process.

Glutathione reductase (GR, EC 1.6.4.2) is a ubiquitous enzyme, which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH) in the presence of NADPH (Sweet and Blanchard, 1991). It is essential for the glutathione redox cycle that maintains adequate levels of reduced GSH in cells (Dolphin et al., 1989). This reduction reaction is essentially irreversible, and accounts for the very high GSH/GSSG ratios found in cells. By generating GSH, GR indirectly participates in the protection of cells against oxidative stress and cytotoxic compounds and is deeply involved in the maintenance of the redox status of cells. Although early studies suggested that this enzyme can also catalyze reduction of several mixed disulfides between GSH and other compounds including proteins, it now appears that only GSSG, mixed disulfides between GSH and γ-glutamylcysteine, and between GSH and

coenzyme A are significant substrates (Meister and Anderson, 1983). Flavonoids have been described to inhibit glutathione reductase isolated from different sources (Elliott et al. 1992; Zhang et al. 1997; Lio et al. 1993; Kurata et al. 1992). Elliott et al. (1992) showed that the most potent inhibitors of yeast glutathione reductase are luteolin and quercetin and they estimated the IC_{50} values (170 μM and 280 μM respectively). Therefore, modulation of glutathione reductase activity Thus, GR seems to play an important role in cellular defence against oxidative stress. Therefore, any modulation in GR activity affected by DPE may help to understand the involvement of GSH in cellular defence. DPE treatment caused a significant decrease in GR activity in liver, spleen and DL cells, and an increase in kidney (Table 11; Figure 20 and 21). The observed increase in GR activity in kidney could be in response to maintain the intracellular ratio of reduced and oxidized glutathione. GR plays an essential role in the cellular defense against oxidative stress and a controlled decrease of the level of GR in human fibroblasts results in lowering the cell viability (Chavkova et al., 2001). When GR activity is impaired; the ability of the cell to reduce GSSG to GSH may be devastated, leading to GSSG accumulation within the cytosol. GR activity in DL cells was noted to be decreased at 24-48 h (Figure 19E) which may affect the conversion of GSSG to GSH, thus, causing to decrease cellular GSH levels. Therefore, DPE-mediated decrease in GR activity in DL cells could be one of the other possible steps involved to decrease the GSH level, thus, affecting cellular antioxidant machinery and reduced cell viability facilitating antitumor activity.

Glutathione peroxidase (GPx, EC 1.11.1.9) catalyzes the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG), and in the presence of peroxides it protects various organisms/cells from oxidative stresses. Intermediates such as O_2^-

and H_2O_2 are formed extensively in biological systems, and these produce reactive oxygen species that can lead to organic peroxide formation. The GPx/GR system is critical as a cellular defence mechanism against oxidizing species such as hydrogenperoxide (H_2O_2) and hydroperoxides of fatty acids and phospholipids formed as a result of reactive oxygen species. In studies using hepatocytes (Jones et al, 1981), lung epithelial cells (Suttorp and Simon, 1986) and tumor cells (Nathan et al, 1981), the GPx/GR system has been shown to be important in protection against H_2O_2-induced cytotoxicity. Thus, GPx is remarkably effective in preventing cell death from peroxide-mediated oxidation. Sarmishtha and Sukta (2001) have shown a significant decrease in the activity of GPx during carcinogenesis. The present study also showed a significant decrease in GPx activity in liver, kidney, spleen, testes and DL cells after DPE treatment (Table 12; Figure 22 and 23) reflecting that the system of defence mechanisms against free radicals may be affected in the tissues. Therefore, it can be suggested that decreased GPx activity in DL cells may allow accumulation of free radicals inside the cell leading to tumor cell death, thereby increasing host survivability.

Sialic acids consist of a family of acidic nine-carbon sugars that are typically located at the terminal positions on a variety of glycoconjugates. The largest structural variations of naturally occurring sialic acids are at carbon 5, which can be substituted with either an acetamido, hydroxyacetamido or hydroxyl moiety to form 5-*N*-acetylneuraminic acid (Neu5Ac), 5-*N*-glycolylneuraminic acid (Neu5Gc) or deaminoneuraminic acid (Kdn), respectively (Tiralongo and Schauer, 2004). Prasad (1986) reported that the cell surface sialic acid moieties have been implicated in the lectin-mediated agglutination behavior of normal and malignant cells. It has been

reported that sialic acids could be used as a sensitive biomarker for lung cancer although their specificity is low (Kakari et. al., 1991). Sialic acid has been reported for the specificity in diagnosis and response to treatment of cancer (Chen et al. 1979; Shamberger, 1984; Stringou et al. 1992). However, a definite correlation of the change in sialic acid concentration with malignancy has not been reported because of the various reports indicating an increase (Ingraham and Alhadeff, 1978) or a decrease (Onodera et. al., 1976) of sialic acid different malignancies.

The present findings showed an increase in sialic acid concentrations in all the tissues except testes with tumor growth in mice (Table 13). The increase of sialic acid in DL cells with tumor growth may be due to enhanced activity of enzymes involved in sialic acid synthesis. Some reports have indicated a 3-5 times increased sialyl transferase activity in various virally transformed cells as compared to the corresponding normal cells, an event that may be associated with the increase in the amount of sialic acid in the transformed cells (Onodera et al., 1976). The elevated sialic acid levels in malignant cells have also been observed for murine Yoshida ascites sarcoma (Rao and Sirsi, 1973). The influence of sialic acid on the oncogenecity of tumor cells may be based on a negative charge determining constituent on the cell surface, resulting in the loss of contact inhibition, an antigen-masking agent and a component of the cell surface involved in the adherence of tumor cells to the mesothelial membrane prior to their dissemination to form metastasis (Jeanloz and Codington, 1976). Furthermore, the observation of increased sialic acid content in the tissues of tumor bearing mice could be helpful for DL cells in the host since sialic acid has also been known to be important in the transport of proteins, amino acids and ions to cancer cells. As far as the effect of DPE on the quantitative

changes in the sialic acid of DL cells and tissues is concerned, it was noted that DPE treatment of tumor-bearing mice for 24 h to 96 h caused a decrease of sialic acid in liver, kidney, spleen and DL cells while an increased level was observed in ascites supernatant (SN) (Table 14; Figure 24 and 25). The decrease in sialic acid content in DL cells after DPE treatment may be associated with an increase in tumor cell immunogenicity thereby enhancing host's immune response. The increase in the sialic acid content in the SN should be associated with the release of sialic acid moieties from DL cells after the treatment. Along with the sialic acid decrease in DL cells, sialic acid decrease in other tissues in tumor-bearing mice should also help to bring out restoration of the functional activity of the tissues closer to normalcy in the host, thereby facilitating survivability of the host.

Lipid peroxidation, the oxidative breakdown of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death, and is a major contributor to the loss of cell function under oxidative stress situations. Oxidative stress leads to various types of damage at the molecular and cell level (Loft and Poulsen, 1996). Numerous studies have shown that toxicity of superoxide and hydrogen peroxide is highly dependent on the presence of iron or copper and that the nature and extent of damage initiated by these species is related to the subcellular location of these metals (Halliwell, 1992). All cellular components are susceptible to attack by reactive oxygen species (ROS), particularly by OH. Attack on proteins can lead to the modification of amino acids, oxidation of sulfhydryl groups leading to conformational changes, altered enzymatic activity, crosslinking, peptide bond cleavage as well as carbohydrate modification in glycoproteins, loss of metal in metalloproteins, altered antigenicity, and increased proteolytic susceptibility

(Stadtman, 1992; Sies, 1993). ROS attack also causes DNA strand breaks and base modifications (Sies, 1993). Lipid peroxidation can alter vital membrane protein structure and function. The presence of lipid hydro peroxides in a membrane disrupts its function by altering fluidity and allowing ions such as Ca^{++} to leak across the membrane, the consequences of which include activation of phospholysis, membrane blebbing and eventual membrane rupture. The antioxidants in such cases can act as stabilizers of homeostasis. Cancer cells can generate large amounts of hydrogen peroxides which may contribute to their ability to damage normal tissues (Szatrowski and Nathan, 1991). Some chemical drug treatment is associated with induction of oxidative stress by generation of free radicals and reactive oxygen species (McCall and Balz, 1999; Manda and Bhatia, 2003), and the potential role of dietary antioxidants, such as ascorbic acid, tocopherol, β-carotene etc. to reduce the activity of free radical-induced reactions has drawn increasing attention (McDermott and Powell, 1996). In the present studies, as compared to the corresponding normal tissues, an increased level of lipid peroxidation was observed in liver, kidney and testes of mice during tumor progression (Table 15), while treatment of tumor-bearing mice with DPE resulted in a significant decrease of lipid peroxidation in the tissues except spleen (Table 16; Figure 26 and 27). Therefore, these results may suggest that the increased level of lipid peroxidation noted in the tissues of tumor-bearing mice was decreased by DPE thereby minimizing tissue damage which might be occurring by oxidative processes.

Cell membrane/surface changes may influence the structural and functional properties of malignant cells (Hynes, 1979; Gallagher, 1985). In solid tumors and few ascitic tumors, cell–cell contacts have also been observed (Hoshino, 1963; Hayashi

and Ishimaru, 1981; Gupta et al, 1985; Prasad and Arjun, 1991). Cell associations in malignant cells regulate the pattern of growth and malignancy in tumors (Curtis, 1973). In control ascites tumor very few leukocytes were seen among tumor cells which were round in shape (Figure 28 A). The percentage ratio of leukocytes to tumor cells increased after treatment with DPE (Figure 28B and C). This increase in the number of leukocytes in tumor cell population after DPE treatment suggests the infiltration of many leukocytes towards tumor cells. The infiltration of lymphocytes and macrophages after potent antitumor drug, cisplatin treatment also has been noted in murine fibrosarcoma and Dalton's lymphoma (Prasad and Giri, 1994). The plasma membrane disintegration observed after 96 h of DPE treatment could lead to the lysis of tumor cells. Singh and Sodhi (1988) reported that murine peritoneal macrophages treated with cisplatin *in vitro* showed increased binding to DL cells through distinct cytoplasmic extensions, which transfer the lysosomes from the cytoplasm of macrophages to the tumor cell cytoplasm. It has been suggested that lymphocytes kill tumor cells by the release of toxic factors that disrupt the cell membrane (Old, 1977; Young and Cohn, 1991). The present study also revealed that the disintegration in the plasma membrane of tumor cells surrounded/connected by leukocytes (Figure 28E) could also be due to the release of some toxic factors by the leukocytes. *In vitro* cisplatin treatment of murine peritoneal exudates cells (macrophages) has shown the increased secretion of lysozyme, hydrogen peroxide, superoxide anions and interleukin-1 (Gupta and Sodhi, 1988). The formation of membrane vacuoles on the tumor cells (Figure 28E) following DPE treatment could be an indication of tumor cell lysis, eventually leading to cell death. Significant modifications of cell surface and disintegration of the plasma membrane of rat hepatoma cells have been reported

after bacterially fermented mistletoe preparation (BFMP) treatment (Ribereau-Gayon et al., 1986). Prasad and Sodhi (1982) reported that cisplatin treatment removes the cell surface sialic acid moieties and acid mucopolysaccharides which may in turn enhance the antigenecity of tumor cells. In the present study also the observed decrease in sialic acid content of DL cells may have a role in enhancing DL cells antigenecity, thus facilitating immunological recognition.

The development of chromosomal aberration, micronucleus and sperm abnormality has been used as reliable biological indicators in the mutagenic bioassay of drugs (Tandon and Sodhi, 1985; Giri et al., 1998). Since blood cells originate in the bone marrow cells, the incidence of chromosomal aberration and micronucleus were analyzed in the bone marrow cells. In the present study, development of these mutagenic parameters were observed after cisplatin (CIS) and benzo[a]pyrene (B[a]P) treatment of mice *in vivo* supporting the earlier findings of their mutagenic properties (Giri et al., 1998; Overbeck et al., 1996; Pillaire et al., 1994; Sharma et al., 2000). The chromosomal pattern in bone marrow cells revealed that chromatid breaks occurred more frequently after CIS and B[a]P treatment, and the total number of aberrant metaphases as well as chromosomal aberrations were noticed to be maximum during 24 h of both CIS and B[a]P treatment (Table 15 and 17). It has been reported that chemicals generally produce maximum frequency of chromosomal aberrations in rodents during 24 h of single exposure, which roughly coincides with the normal length (22-24 h) of the cell cycle (Schmid, 1973). Decreased aberrations during later periods could be due to death of damaged cells, clearance of mutagen from the body, post-replication repair process and other unknown processes (Sorenson and Eastman, 1988).

The therapeutic efficacy of CIS has been limited by its dose dependent side effects (Krakoff, 1979) and its mutagenic potentials have also been reported in bacteria as well as in mammalian cells (Overbeck et al., 1996; Cross et al., 1996; Giri et al., 1998; Zwelling et al., 1979; Tandon and Sodhi, 1985; Pillaire et al., 1994). 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). B[a]P also induced mutagenicity in the strain YG1024 (Watanabe et al., 1990). Carcinogens bind to the cell macromolecules resulting in mutagenic events leading to cell transformation and neoplastic changes. A single treatment with DPE did not show significant changes in the frequency of bone marrow chromosomal aberrations induced by CIS or B[a]P, while pre-treatment for 7 consecutive days decreased the frequency of chromosomal aberrations in a dose dependent manner (Table 15, 16, 17, 18). Thus, these results showing significant reduction in CIS and B[a]P-induced chromosomal aberrations in presence of *D. pentagyna* may suggest its protective role against CIS and B[a]P-mediated mutagenic potentials.

Micronuclei are chromatin masses that arise from chromosome fragments of intact whole chromosomes lagging behind at the anaphase stage of the cell division (Czyzewska and Mazur, 1995). Micronucleus can be easily accredited in the cytoplasm of immature polychromatic erythrocytes (Schmid, 1976). A dose-dependent increase of micronuclei have been reported in the blastocytes and bone

marrow cells after treatment with anticancer drugs and B[a]P (Giavini et al., 1990; Sharma et al., 2000). In the present study, the incidence of micronuclei was found to be increased after CIS and B[a]P treatment (Table 19, 21). As observed for CA, single treatment with DPE did not show significant changes in the frequency of micronuclei induced by CIS and B[a]P. However, pre-treatment for 7 consecutive days dose dependently decreases the frequency of micronuclei (Table 19, 20, 21, 22). Therefore, *D. pentagyna*-mediated reduction in CIS and B[a]P-induced micronuclei also suggest the protective role of *D. pentagyna* on CIS and B[a]P mutagenic potentials. Since the micronuclei in young erythrocytes arise mainly from chromosomal fragments, the observed significant decrease in the incidence of micronuclei after DPE treatment may be considered to be related with the observed inhibitory effect of DPE on chromosomal aberrations induced by CIS and B[a]P.

Cisplatin has been reported to cause sertoli cells dysfunction and embryotoxicity in rats (Pogach et al., 1989; Aydiner et al., 1997; Giavini et al., 1990). In the present study, sperm abnormality analysis in mice showed that treatment with CIS and B[a]P caused a significant increase in sperm abnormality. Among different types of sperm abnormalities observed, amorphous heads occurred more frequently after CIS and B[a]P treatment (Table 23, 24). As observed for CA and MN, in the sperm abnormality analysis also a single dose of DPE did not cause significant changes, while pre-treatment for 7 days with DPE at all the three doses significantly decreased the frequency of sperm abnormality induced by both CIS and B[a]P. This DPE-mediated reduction in the incidence of sperm abnormality also supports the protective role of *D. pentagyna* on CIS and B[a]P mutagenic potentials, It is also suggested that *D. pentagyna* may be useful as cancer chemo-preventive agent

particularly against B[a]P. *D. pentagyna* may exert its antimutagenic potentials by inducing some of the antioxidant enzymes that detoxify mutagens, or by acting as a free radical scavenger. However, other contributory steps may also be involved in its antimutagenic/antigenotoxic potentials. Thus, the findings from the present study suggest that pre-treatment with *D. pentagyna* extract can lead to moderate protective effects against cisplatin and benzo[a]pyrene-induced *in vivo* mutagenicity.

Conclusion: Thus, based on the various aspects of studies undertaken and results, following important conclusions may be derived:

- Out of five plants and their test parts selected and used in the study, the most potent antitumor activity against murine ascites Dalton's lymphoma was observed with the methanol extract of *D. pentagyna* (DPE). Moreover, out of different doses of DPE, maximum antitumor activity (%ILS ~ 71%) was observed at a dose of 20 mg/kg body wt/day.

  Considering the observed better antitumor effect of *D. pentagyna*, further study was carried out using this plant extract treatment in the host (20 mg/kg body weight).

- DPE treatment (20 mg/kg body wt) of tumor-bearing mice causes significant increase in the haematological values (RBC, WBC, Hb and PCV). It may be suggested that the observed improvement in these haematological values may be involved in enhancing host immunity, thereby increasing host survivability.

- DPE treatment increased the protein content in the tissues except kidney and spleen. These changes may involve variations in the rate of protein synthesis/ degradation.
- DPE-mediated decrease in GSH level in DL cells may cause a diminished protective role in the cells, thereby leading toward cell death to increase hosts survivability. A decrease in GR activity in DL cells could be one of the possible steps involved to decrease the GSH level, thus, affecting cellular antioxidant machinery. Similarly, decreased GPx activity in DL cells, which converts H_2O_2 into water, may also aid in increasing cell susceptibility to oxidative damage. DPE-mediated increase in GSH level in kidney, spleen and testes of tumor-bearing host may play a role in protecting tissues from oxidative damage caused by reactive chemicals.
- DPE treatment showed a decrease in sialic acid, the main component of the cell surface, in DL cells. This may be involved in changing/increasing their antigenicity which should also help to facilitate host survivability. The increase of sialic acid in the ascites supernatant may be due to the release of sialic acid from DL cells, therefore, its decrease in DL cells. DPE-mediated decrease in the lipid peroxidation in the tissues of tumor-bearing mice (except in the spleen) may indicate its possible protective function against tissue damage caused by oxidative stress in tumorous condition, thereby increasing host survivability.
- DPE treatment causes more infiltration of leukocytes towards DL cells, appearance of membrane disintegration and formation of membrane vacuoles on the tumor cells which may result in the tumor cell lysis.

- The findings support the mutagenic potentials of CIS and B[a]P as established previously. However, the findings of the present study demonstrate the antimutagenic potential of *D. pentagyna* pre-treatment against CIS and B[a]P-induced mutagenicity. *Dillenia pentagyna* may also be useful as cancer chemo-preventive agent against B[a]P in particular and others in general.
- Lastly, based on all the present findings, it may be proposed that methanol extract of stem bark of *Dillenia pentagyna* could be a very useful agent for cancer chemotherapy and chemo-prevention. However, the isolation and characterization of the active principle needs to be examined.

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