INTRODUCTION

Cancer is the price we pay for our multicellularity

Ricki Lewis and Richard Gallagher

1.1 DEFINITION

Tumour (neoplasm) is defined as a lesion resulting from the autonomous or relatively autonomous abnormal growth of cells which persists even after the initiating stimulus has been removed if cell growth has escaped from normal regulatory mechanisms (Currie 1982)

1.2 EPIDEMIOLOGY

Chronic diseases such as cancer and other non communicable diseases are fast replacing communicable diseases in India and developing countries. The burden of cancer is still increasing worldwide despite advances in diagnosis and treatment. It is widely held that 80-90% of human cancers may be attributable to environmental and life style factors. The most frequently affected organs are lung, breast colon rectum stomach and liver (Murthy and Mathew 2004) From the population based registries in India covering 28 30 million population from different parts of the country the age adjusted incidence rates vary from 44 to 122 per 100,000 population in males and 52 to 128 per 100,000 females Cancer incidence is higher in females compared to males (Yeole, 2004)
1.3 CLASSIFICATION OF TUMOURS

1.3.1 Behaviour classification: benign or malignant

i. Benign tumours

- Non-invasive and remain localised
- Slow growth rate
- Close histological resemblance to parent tissue

ii. Malignant tumours

- Invasive and thus capable of spreading directly or by metastasis
- Relatively rapid growth rate
- Variable histological resemblance to the parent tissue

Classification of Malignant tumours

Carcinoma - Tumour of endodermal or ectodermal origin (skin, epithelial lining of internal organs and glands)

Leukemia - Non-solid tumours of cells of the hematopoietic lineage

Lymphoma - Solid tumours of cells of the hematopoietic lineage

Sarcoma - Tumour of mesodermal origin (bone, fat, cartilage)
1.4 INVASION

Invasion is the most important sole criterion for malignancy. The invasiveness of malignant neoplasms is determined by the properties of the neoplastic cells within them. Neoplastic cells show abnormal or increased cellular motility which show loss of the normal mechanism that arrests or reverts normal cellular migration - contact inhibition of migration. Neoplastic cells also secrete enzymes, such as collagenase, which cause the dissolution of the adjacent connective tissue boundaries.

Moreover, factors like immune system response, localization, blood support and the rate of growth play an important role in this process. During invasion, cancer cell presents new or modified antigens on the outer surface of cell membrane. These antigens are cell type, tissue and species-specific molecules, which can identify the cell to other cells and to immune system. The MHC I (Major Histocompatibility Complex), found at the outer membrane of most human cells, can present protein structures built in the cell to the environment. If a cell is producing false proteins due to neoplasia, these proteins will be presented with the MHC I to cytotoxic T-lymphocytes. This T-cells are specialized in killing foreign cells, due to their foreign antigen. Other lymphocytes can activate Macrophages (specialized in killing by digestion) and NK cells (natural killer cells), or produce antibodies (proteins that recognise antigens, produced by B-lymphocytes) which mark a tumour cell like a target for the immune system. Under special circumstances, a cancer cell can stay alive also in a body with normal working immune system (mechanism called immunosurveillance). First, a malignant cell can mask its outer antigens, by a produced or already existing substance like fibrin. Second, malignant cells can produce and excrete molecules, which act as antigens, overload the organism and exhaust the immunity. Third, they can excrete molecular signals, which suppress the activity of the cellular immune system.
The growth rate has the following influence on invasion. First, a fast growing tumour can compress the normal tissue in the neighbourhood, sometimes causing a cell death. The cell death is always followed by an (small) immune reaction with an increase in concentration of immune cells in the tumour area. The immune cells can effect further tumour progression. Second, the fast cell growth can only progress as long as the nutrition support (blood) is guaranteed. A fast growing tumour can compress vessels or grow too fast so that there could be a relative decrease of vascular support in the tumour area. The most fast growing tumours have a centre consisting of dying or dead cells due to this nutrient deficiency but there is also a small amount of (slow growing) tumours which produce angiogenesis factors (factors increasing the vessel growth) which grow with a normal nutrition supply.

1.5 METASTASIS

Metastasis is the process whereby malignant tumours spread from their site of origin (the primary tumour) to form other tumour (secondary tumour) at distant sites.

The routes of metastasis are

- **haematogenous** by the blood stream to form secondary tumours in organs perfused by blood which has drained from a tumour
- **lymphatic** to form secondary tumours in the regional lymph nodes
- **transcoelomic** in pleural, pericardial and peritoneal cavities where this invariably results in a neoplastic effusion
- **implantation** for example by accidental spillage of tumour cells during the course of surgery
1.6 BIOLOGY OF TUMOUR CELL

Neoplastic cells are relatively or absolutely autonomous, unresponsive to extracellular growth control. Tumour cells have abnormal nuclear DNA (nuclear hyperchromaticism). Aneuploidy and polyplodity are associated with increased tumour aggressiveness and are recognisable in histological sections as variations in nuclear size and staining (pleomorphism). Genetic abnormalities are being found with increasing frequency in tumours. Malignant tumours frequently appear to exhibit more mitotic activity than the corresponding normal cell population. In histological sections, mitoses are abundant and mitotic figures are often grossly abnormal showing tripolar and other bizarre arrangements. It is likely that these aberrant mitoses are incapable of proceeding to completion. Cellular proliferation can be estimated by mitosis counting. DNA measurements and other techniques. Tumour cells show a tendency towards anaerobic glycolysis. The known metabolic abnormalities of tumour cells are simply inappropriate to the normal physiological state of the tissue or host.

Tumour cells have a greater negative surface charge than do normal cells, and are also less cohesive. Neoplastic cells show poor cellular cohesion which is due to a reduction in specialised intercellular junctions such as desmosomes. Tumour cells may retain the capacity to synthesise and secrete products characteristic of the normal cell type from which they are derived, often doing so in an excessive and uncontrolled manner (Currie, 1982).
Cancer: General Etiology and Pathogenesis

Acquired (environmental) DNA damaging agents
- Chemicals
- Radiation
- Viruses

NORMAL DAMAGE

DNA Damage

Successful DNA Repair

Failure of DNA Repair

Mutations in the genome of somatic cells

Inherited mutations in
- Genes affecting DNA repair
- Genes affecting cell growth or apoptosis

Activation of growth promoting oncogenes

Alterations of genes that regulate apoptosis

Inactivation of cancer suppressor genes

Expression of altered gene products and loss of regulatory gene products

Clonal expansion
- Additional mutations (progression)
- Heterogeneity

Malignant neoplasm
1.7 TUMOUR DORMANCY

After surgical removal, radiotherapy and/or chemotherapy, there may be no clinically detectable tumour remaining in a patient. This does not mean that the tumour has been completely eradicated, however, as minute deposits can evade detection by even the most sophisticated imaging techniques. These occult tumour foci can remain clinically dormant for perhaps several years before their regrowth causes signs and symptoms. For this reason, it is virtually impossible to speak of a cancer patient as being 'cured', and prognosis can be given only in terms of the probability of survival or the length of the disease-free interval. The prognostic information derived from tumour type, grade and stage is used to predict the patient's chances of surviving, say 5 years (Currie, 1982).

1.8 BREAST CANCER

Breast Cancer is not just one disease, but rather is a general term used to describe a number of different types of cancers that occur in the breast. Breast cancer starts in the epithelium, proliferate at a high rate, causing necrosis of surrounding tissues. As they infiltrate at a high rate, the malignant cells metastasize to regions like lungs, bone, liver, adrenals, brains and even meninges (Velicer et al., 2004).

1.9 EPIDEMIOLOGY OF BREAST CANCER

Breast Cancer is the second leading cause of cancer deaths in women today after cervical cancer and is the common cancer among women. According to the WHO, more than 1.2 million people will be diagnosed with breast cancer this
year worldwide (Jemal et al. 2004) In India the incidence of breast cancer is increasing with an estimated 80,000 new cases being diagnosed annually (Perin et al. 2001).

1.10 Classification of Breast Cancer

Breast Cancer is a heterogeneous disease in terms of clinical course, gross and microscopic pathology and imaging characteristics. According to WHO classification it is classified in the following way.

1.10.1 Noninvasive carcinoma

Breast cancer is usually subdivided into noninvasive (in situ) and invasive cancer. In situ carcinoma is characterized by growth within the ducts without penetration of the basement membrane and thus without the ducts invading the stroma. In situ carcinoma is subdivided into ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS).

a. Ductal Carcinoma in situ

It is often subdivided into comedo and noncomedo types depending on the presence or absence of comedo necrosis growth pattern (e.g., solid, papillary, cribriform) and nuclear grade. The comedo type is characterized by a growth pattern where the cells in the centre of the involved ducts are necrotic and the surrounding viable cells have a high nuclear grade. The central area of necrosis often demonstrates dystrophic calcification. Endocrine ductal carcinoma in situ is a variant growth pattern of ductal carcinoma in situ where the arrangement of cells suggests an endocrine structure. This is a descriptive term which lacks clinical or prognostic significance.
b. **Lobular Carcinoma *in situ***

It is characterized by distension of lobular ducts by a population of uniform discohesive cells which lack nuclear pleomorphism nucleoli or mitotic activity. Involvement of one complete lobule in a biopsy is sufficient for the diagnosis although some consider involvement of most of the lobular ducts sufficient. The lesion is neither palpable nor mammographically evident therefore its diagnosis usually relates to its presence adjacent to a clinically or mammographically detectable lesion. Lobular carcinoma *in situ* is characteristically multicentric and bilateral. It should be considered as an indicator of an increased relative risk for the patient of developing invasive carcinoma.

1.10.2 **Invasive carcinoma**

It denotes neoplastic penetration of the basement membrane of a duct containing ductal carcinoma *in situ* and extension of neoplastic cell aggregates into the mammary stroma.

1.10.3 **Ductal carcinoma**

The ductal carcinoma originates in ducts as opposed clinically or mammographically because of the frequently meager desmoplastic response and the absence of associated microcalcifications.

1.10.4 **Mucinous (colloid) carcinoma**

It is characterized by abundant extracellular mucin surrounding nests of carcinoma cells. Such carcinomas are typically circumscribed and may have adjacent foci of ductal carcinoma *in situ*. This neoplastic growth pattern is associated with a relatively favourable prognosis provided that the carcinoma consists almost entirely of the mucinous growth pattern.
1.10.5 Medullary carcinoma

It is a form of invasive ductal carcinoma characterized by a circumscibed growth pattern, pronounced nuclear pleomorphism and mitotic activity, a syncytial arrangement of the neoplastic cells and a lymphocytic or plasmacelluar infiltrate. Medullary carcinoma, despite its aggressive microscopical appearance, is associated with a relatively favourable prognosis.

1.10.6 Papillary carcinoma

It can refer to a noninvasive or invasive carcinoma. The papillary growth pattern results from the presence of carcinoma cells on delicate fibrovascular stalks. In a noninvasive neoplasm, the papillary lesion is present within a distended duct, and is often attached to more than one site on the duct wall. The neoplastic epithelium on the fibrovascular stalks is multilayered, is often merged with similar epithelium on an adjacent stalk, and may have a cribriform appearance. Such noninvasive carcinomas may be present in a subareolar duct, and be associated with a bloody nipple discharge, or in a cystic duct elsewhere in the breast where a mass lesion results.

1.10.7 Tubular carcinoma

It is a well differentiated form of invasive breast carcinoma. The carcinoma is usually seen to be associated with foci of ductal carcinoma in situ (DCIS), and is characterized microscopically by relatively uniform angulated small ducts which invade mammary stroma. The ducts lack a myoepithelial investment, are lined by cells which lack significant nuclear pleomorphism and are surrounded by cellular connective tissue. Tubular carcinomas are almost always less than 2 cm in greatest dimension. Only nodal metastases are seen in approximately 15% of cases, although fewer than three nodes are usually involved. The neoplasm is associated with an excellent prognosis.
1.10.8 **Adenoid cystic**

A low-grade form of invasive carcinoma characterized by growth in the form of mucincontaining cylinder surrounded by both epithelial and myoepithelial cells. This is the only form of invasive carcinoma which typically demonstrates myoepithelial participation.

1.10.9 **Carcinoma with metaplasia**

While foci of metaplastic change are not uncommon in invasive ductal carcinoma, some tumours consist almost entirely of such a growth pattern. Such neoplasms typically are circumscribed and present in postmenopausal women. The metaplasia may be of varying forms, including squamous cells, spindle cells, as well as chondroid, osteoid or even skeletal muscle growth patterns. In some instances only rare foci of recognizable ductal carcinoma *in situ* or invasive ductal carcinoma are evident, so that diagnostic separation from a primary mammary sarcoma may be difficult. Immunohistochemical studies for high molecular weight cytokeratin may be helpful in resolving the latter situation.

1.10.10 **Paget’s disease of the nipple**

It is an eczematous lesion of the nipple, occasionally involving the areola and adjacent skin of the breast, almost always associated with underlying carcinoma of the breast. The involved area of the nipple is typically scaling and erythematous, and occasionally ulcerated with crusting. The microscopic appearance consists of an epidermal infiltrate of large cells with abundant cytoplasm and prominent nucleoli with large nucleoli. The condition is best considered as involvement of the nipple by ductal carcinoma *in situ* of high nuclear grade.
1.11 GRADING AND STAGING OF BREAST CANCER

a. Tumour Grading

Tumour grade refers to a measure of how malignant tumour cells are differentiated. The grade of differentiation is diagnosed by factors like cell size, cell shape, similarity of cells, number of mitosis (divisions), and similarity to the tissue of origin. Based on these factors, a pathologist commonly describes tumour grade by four degrees (recommended by The American Joint Commission on Cancer) (Eva et al., 2002).

This is the most used method. There have been developed additional grading methods for many tumours.

G1: Well-differentiated malignant tumour (least aggressive behavior in most cases)
G2: Intermediate – differentiated malignant tumour
G3: Poorly – differentiated malignant tumour
G4: Undifferentiated malignant tumour (most aggressive behavior in most cases)
Gx: Grade cannot be assessed.

b. Tumour Staging

The most common method is the TNM-system (tumour, nodes, metastasis), developed by The Union International Cancer Center (UICC) and this is further modified by American Joint Committee on Cancer (AJCC). This classification describes how cancer has spread anatomically, including cancer’s size, lymph nodes involvement and metastasis.
Primary Tumour (T)

TX : Primary tumour cannot be assessed

TO : No evidence of primary tumour

Tis : Carcinoma \textit{in situ} : intraductal carcinoma, lobular carcinoma \textit{in situ}, or Paget's disease of the nipple with no tumour

T1 : Tumour 2 cm or less in greatest dimension

T2 : Tumour more than 2 cm but not more than 5 cm in greatest dimension

T3 : Tumour more than 5 cm in greatest dimension

T4 : Tumour of any size with direct extension (a) to the chest wall (b) skin, only as described below

T4a : extension to chest wall

T4b : edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast

T4c : (a) and (b)

T4d : Inflammatory carcinoma

Regional lymph nodes (N)

NX : Regional lymph nodes cannot be assessed

N0 : No regional lymph node metastasis

N1 : Metastasis in one to three axillary lymph nodes, and / or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
N1mi  Micrometastasis (> 0.2 mm, ≤ 2.0 mm)
N2   Metastasis in four to nine lymph nodes, or in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
N3   Metastasis in 10 or more axillary lymph nodes, or in intracavicular lymph nodes, or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more axillary lymph nodes, or in more than three axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes, or in ipsilateral supravacicular lymph nodes

Stage groupings

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<td>Stage IV</td>
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1.12 ETIOLOGY OF BREAST CANCER

The known risk factors for breast cancer account for only a small proportion of all cases. Such risk factors are classified here into four broad categories: genetic/familial, reproductive/hormonal, lifestyle and environmental factors. Pre-existing breast conditions and height are also known to affect breast cancer risk.
i. **Benign Breast Conditions**

Benign breast disease (also known as fibrocystic breast disease or mammary dysplasia) is a common condition involving benign changes in breast tissue characterized by proliferation of apparently normal cells and cysts. About one-third of benign breast disease can be classified as benign proliferative breast disease, a condition associated with increased breast cancer risk (Byrne 2001). Mammographic breast density also appears to be a risk factor for benign breast disease and is an independent risk factor for breast cancer (Byrne 2000).

ii. **Height**

A pooled analysis of seven cohort studies showed positive associations between height and breast cancer risk among postmenopausal but not premenopausal women (Foulkes 1995).

iii. **Genetic and Familial Factors**

About 5 to 10% of breast cancers in the general population have a hereditary basis (Kelsey, 1991). The major known breast cancer genes, BRCA1 and BRCA2, are involved in DNA repair and transcriptional regulation.

iv. **Hormonal and Reproductive Factors**

Estrogen and progesterone are imperative for normal mammary gland function and growth. Estrogen and progesterone interact with particular receptor proteins in the cell nucleus and can promote both proliferation and malignant transformation of breast cells. Moreover, estrogen can be metabolically activated to cytotoxic and genotoxic products (Hankinson, 1998).
a. **Reproductive Factors**

Human studies support an etiologic role for estrogens in breast cancer. These factors include early age of menarche, late onset of menopause, not having children, late age at first pregnancy, oral contraceptive use, hormone therapy and postmenopausal obesity (which favours conversion of androgen to estrogens in adipose tissue). A pooled analysis six-cohort study showed associations between breast cancer and late menarche, high parity and late age at first birth (Henderson, 2000).

b. **Endogenous hormones**

Among women in prospective cohort studies, high baseline levels of endogenous estrogens (estrone, estradiol, bioavailable estradiol) and their androgenic precursors were associated with increased breast cancer (Toniolo, 1997).

c. **Hormone Therapy**

Breast cancer risk varies with type of HT, and is considerably higher among those using estrogen, progestin combinations compared to estrogen alone (Schairer, 2000). The risk of developing breast cancer by age 70 increased by 23% in women with a history of HT use between the ages of 50 to 60 compared to women with no history of HT use (Kirsh and Kreiger, 2002).

d. **Oral Contraceptive Hormones**

A pooled analysis of 54 studies (over 53,000 cases and over 100,000 controls) concluded that, compared to never-users of oral contraceptives, breast cancer risk is 24% higher among current users and 16% higher among women who ceased use within the past 7 years. Recent use of a synthetic progesterone drug (Depo-Provera) has been associated with a doubling of breast cancer risk, suggesting that the drug may accelerate growth of pre-existing lesions (Muti, 2002).
v. Glucose and related factors

Altered glucose metabolism has been linked to breast cancer incidence and severity. Bio-markers of increased breast cancer risk include plasma glucose, insulin and insulin like growth factor I(IGF-I) levels. Plasma IGF binding protein (IGFBP) levels has been linked to lower risk in some (Kaaks, 2002) but not all studies (Van den Brandt, 2000).

vi. Lifestyle Factors

The fact that most newly diagnosed breast cancer cases are not attributable to established risk factors other than age is consistent with an important role of life style and environmental factors in the etiology of the disease. This observation might also explain the wide variation in breast cancer incidence seen internationally.

a. Diet

The cancer risk can be reduced by changing the individual eating behavior, by eating less salty meat, less fat and more fruits.

1. Broiled Meat

Polycyclic aromatic hydrocarbons are present in high concentrations in charcoal-broiled meats, deposited on the surface during cooking, which is responsible for the initiation of cancer.

2. High fat content

High fat content could induce changes in cell membrane lipid composition and proliferation in the cells and these changes may be related to the
development of tumours. Some fatty acids may have an influence on cell growth and cancer promotion.

3. Obesity and Physical Activity

Studies involving over 10,000 post-menopausal breast cancer cases and controls, showed that weight gain since the lowest adult weight was associated with increased breast cancer risk, while the opposite held for women whose highest adult weight occurred before age 45 (Shoff et al., 2000).

vii. Alcohol Consumption

Breast cancer risk is associated with alcohol consumption, independent of other risk factors and type of beverage, with an average excess relative risk of 9% per daily drink. Alcohol may also be a risk factor for reduced survival after breast cancer (McDonald et al., 2002).

viii. Cigarette Smoking

Premenopausal breast cancer was associated with an onset of smoking among parous women with in 5 years of menarche and among nulliparousr heavy smokers (Marcus et al., 2000).

ix. Environmental Factors

a. Occupational Exposure

There is limite evidence to suggest that breast cancer risk may be increased among women in certain occupations, including dental hygienists, nurses, teachers, beauticians, airline attendants, laboratory technicians, telephone and
telegraph operators, leather and fur processors, glass manufacturing workers, and metal fitters and assemblers. Specific occupational exposures linked to breast cancer include organic solvent and ionizing radiation. Both organic solvent exposure and exposure to ionizing radiation have been shown to be carcinogenic in highly exposed humans and experimental animals (Band et al., 2000).

b. **Ionizing Radiation**

High exposures to ionizing radiation related to medical diagnosis or treatment is a known cause of breast cancer. Women with benign breast disease or a family history of breast cancer may have increased breast cancer risk following relatively low-level exposure to ionizing radiation (Aronson et al., 1999).

c. **Electromagnetic Fields**

A review of epidemiologic studies of breast cancer and power-frequency electromagnetic fields (EMF) concluded that: (1) among 11 studies of occupational EMF exposure, there were significant associations with breast cancer in most of these studies and (2) among 8 studies of residential EMF exposure and studies of electric blanket exposure, results were inconsistent, most showing no significant relationships (Caplan et al., 2000).

d. **Environmental Contaminants**

There is considerable scientific and public interest in the possible role of environmental contaminants in the etiology of breast cancer, particularly those with alleged estrogenic activity such as specific organochlorines. These chemicals include DDT, its metabolites several other pesticides and polychlorinated biphenyls (PCBs). Chemicals known to cause mammary gland tumours in animals include solvents (benzene, methylene chloride, 1,1- and 1,2 – dichloroethane), pesticides
(dischlorvos) and therapeutic drugs (resperprine). Many environmental contaminants vary from ecologic (correlative) studies of breast cancer risk and environmental contaminants vary from ecologic (correlative) studies with no individual exposure information to cohort and case-control studies that measured indices of internal dose such as blood or breast adipose tissue organochlorine levels (Porta et al., 1999).

1.13 IMMUNE SYSTEM IN CANCER

Cancer patients face immune system challenges primarily in two areas. 1. fighting malignancies through immune mechanisms and 2. confronting the immune suppressive effects of the disease and treatment. Originally proposed by Ehrlich in 1909 and elaborated on by Burnet in the 1950s, the "immune surveillance" hypothesis states that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumours and to kill tumours after they are formed. When immune surveillance falters, cancers can more readily develop and progress. The allure of this belief is further heightened by the recognition that tumours often lack co-stimulatory molecules, express decreased level of MHC class I and II antigens, have lesions in the antigen processing pathways, up-regulate expression of proteins inhibiting apoptosis mediated by immune cells, and by the fervent hope that enhancing such immune function should in turn support one's ability to combat cancer (Keith et al., 2002). An important corollary to the immune surveillance theory is that the immune system's capacity for surveillance can be modified through medical interventions.
1.13.1 The Immune System

Living animals are able to fight off infections and tumor growth because of their immune systems. On the other hand, the presence of an immune system can leave an animal suffering from allergies or autoimmune diseases. Immunologists attempt to study how the immune system functions and to characterize and treat malfunctions.

The human immune system can be divided into two main branches, namely the innate system, which is nonspecific or antigen-independent, and the acquired immune system, which elicits specific, antigen-dependent responses (Roitt et al., 1996). The acquired immune system is further subdivided into humoral immunity and cell-mediated immunity. While humoral immunity is dependent on B lymphocytes (B cells) which originate from bone marrow stem cells and are stored in the spleen, cell-mediated immunity is dependent on T lymphocytes (T cells) which also originate from bone marrow stem cells, but mature and are stored in the thymus. Upon exposure to a foreign material, or antigen, activated B cells produce antibodies, or immunoglobulins, that bind specifically to the corresponding antigen. The antibody-antigen complex can then be effectively eliminated via complement-activated phagocytic macrophages. Instead of secreting antibodies, the three major types of T cells can bind directly to antigen presenting cells (APC) (Roitt et al., 1996). The two types of T-helper (TH) cells, called T helper-1 (TH-1) and T helper-2 (TH-2) cells, assist macrophages and B cells, respectively. The third type of T lymphocytes, called T-cytotoxic cells, is capable of destroying cancer cells and cells infected by intracellular pathogens. While TH cells express CD4 molecules, T-cytotoxic cells express CD8 molecules on the cell surface. In addition, all three types of T cells express the CD3 molecule, also called the T cell receptor (TCR) (Roitt et
al., 1996). To become activated, T cells must bind to the APC via the TCR and either the CD4 or the CD8 cell surface receptors. Natural killer (NK) cells are capable of killing tumor cells and virally-infected cells without first having to be activated by a membrane recognition system. NK cells, which make up about 15% of circulating lymphocytes, can also be distinguished by their unique cell surface markers, CD16 and CD56. Resting NK cells also express a portion of certain cytokine receptors such as the interleukin-2 (IL-2) receptor. NK cells, which make up about 15% of circulating lymphocytes, can also be distinguished by their unique cell surface markers, CD16 and CD56. Resting NK cells also express a portion of certain cytokine receptors such as the interleukin-2 (IL-2) receptor. In turn, cytokines represent a group of molecules which are produced by T cells, B cells and / or macrophages to serve as mediators between the different types of immune cells. For example, binding with IL-2 leads to stimulation and subsequent activation of NK cells and T cells. Together, immune cells play an essential role in recognizing and eliminating tumour cells via a highly coordinated immune response. Yet, as part of the natural aging process, proliferation of T lymphocytes, B lymphocytes and NK cells appear to be inhibited. Thus, the fact that older aged women are disproportionately afflicted with breast cancer could be linked to the progressive aging of the immune system (Roitt et al., 1996).

1.14 CHEMICAL CARCINOGENESIS

Yamagiwa and Ichikawa developed the first experimental model for chemical carcinogenesis.

- Coal tar paint on rabbit ears resulted in skin cancer.
Later shown that coal tar contained polycyclic aromatic hydrocarbons

More than 400 chemicals have been demonstrated to cause cancer in animals. Of these, some 30–40 have been directly associated with human cancer. Most chemical carcinogens or their metabolites form covalent bonds with DNA. Some chemicals, however, appear to induce cancer without metabolism.

Observations led to the division of chemical carcinogens into two classes:

a. **Activation Independent**: These compounds bind directly to DNA without being metabolized as alkylating agents.

b. **Activation Dependent (Procarcinogens)**: These compounds require cellular enzymatic metabolism to get converted into an ultimate carcinogen in order to exert their carcinogenic action. The ultimate carcinogenic forms of both of these classes of carcinogens have in common the property of electrophilicity. The electron-deficient electrophilic reactant bind covalently with nucleophilic areas of DNA, RNA, and protein ultimately forming DNA-Carcinogen adduct.

**Examples of Chemical Carcinogens**

**Polycyclic aromatic hydrocarbons (PAHs)**

PAHs were the first group of chemicals shown to be carcinogenic in man. They are produced from the combustion of fossil fuels and tobacco. PAHs are probably the most widespread chemical carcinogens in the environment and some of
the most powerful carcinogens are found in this group. Examples include benzo(a)pyrene and methylcholanthrene.

Aromatic amines

Aromatic amines result from the addition of an amine group to a polycyclic aromatic hydrocarbon. For example, adding an amine group to the non-carcinogen anthracene produces a well-known carcinogen, 2-anthrancine. Other examples include the OSHA regulated carcinogens, benzidine, and 2-naphthylamine.

Aminoazo compounds

Aminoazo compounds were frequently used as dyes in polish, soap, cooking oils, and margarine. An example is 4-Dimethylaminoazofenene.

n-Nitroso compounds

n-Nitroso compounds are widely distributed in the environment and can also form in the body. These compounds may be one of the most important groups of carcinogens in man. Sodium nitrite is a commonly used preservative in meat that is converted by heat to nitrous acid which reacts with amines in the meat to form nitrosamines. A common example is methyl benzyl nitrosamine.

Alkylating agents

Alkylating agents represents one of the largest classes of carcinogens. They are subdivided based on their functional groups and include compounds such as the aziridines, nitrogen, sulfur, and oxygen mustards, epoxides, lactones, and aldehydes. A common aziridine is the OSHA regulated carcinogen ethylenimine.
Alkylating agents commonly used in laboratories include ethylene oxide, propylene oxide, formaldehyde, acetaldehyde, and acrolein.

**Aliphatic halogenated hydrocarbons**

Several of these compounds are commonly used in the laboratory as solvents. Examples include carbon tetrachloride, chloroform, trichloroethylene, methylene chloride, and ethylene dibromide.

**Inorganic metals and minerals**

Several carcinogens are known among metals or their salts. Examples of these include beryllium, cadmium, nickel, cobalt, and chromium. Only two minerals are known to cause cancer; asbestos and arsenic.

**Naturally occurring**

Several natural occurring carcinogens are known. Among these is aflatoxin, probably the most potent of all carcinogens. Aflatoxins are produced by molds that grow on peanuts and corn. Other naturally occurring carcinogens are present in sassafrass and chili peppers.

1.15 **METABOLISM OF CHEMICAL CARCINOGENS**

The transformation of chemicals is important in carcinogenesis, both in bioactivation and detoxification. Many of the general concepts regarding bioactivation, detoxification and genotoxicity are:

1) ‘Reactive intermediates’ do have finite stability and can travel limited distances to alkylate DNA. In the 1970s, a view had developed that
'activated carcinogens' were so reactive that they could not diffuse very far. This view led to the hypothesis that only nuclear enzymes could be involved in the activation of carcinogens (Bresnick, 1979). Subsequent work showed that products reactive with DNA could be generated in hepatocytes and be trapped outside the cells, e.g. with polycyclic hydrocarbons, nitrosamines and vinyl halides as the substrates (Shen et al., 1980; Umbenhauer et al., 1981; Miller et al., 1983). Furthermore, i.p. injection of a benzo[a]pyrene diol epoxide into pre-weanling mice generated lung tumours, arguing that distribution could be widespread even with a compound having a 30s half-life.

2) 'Phase II' enzymes involved in conjugates are not only protective but also activate chemical carcinogens. An example is glutathione (GSH) transferase, which activates 1,2-dihaloethanes (Rannug et al., 1978; Ozawa and Guengerch, 1983). These enzymes can also activate other chemicals (Monks et al., 1990; Anders et al., 1998). Examples of roles of bioactivation are also known for N-acetyltransferase (Grant et al., 1992), UDP-glucuronosyl transferase, sulfotransferase (Boberg et al., 1983) and other Phase II reactions. During the past 20 years evidence has been obtained for roles of kinases in activation of chemical carcinogens (Lin et al., 1995).

3) Humans generally form the same DNA (and RNA and protein) adducts as animal models. Twenty years ago this was still a hypothesis but has now been clearly demonstrated in many cases. Further review is beyond the scope of this review and more relevant to another (Poirier et al., 2000). The point is that the same metabolic pathways are also important in humans and animals; generally the major differences are quantitative.
4) DNA adducts can be generated by the metabolism of 'endogenous' chemicals. Key examples here are the generation of DNA adducts from products of lipid peroxidation (Marnett, *et al.*, 1985; Chung *et al.*, 1996), estrogens (Bolton *et al.*, 1998; Cavalieri *et al.*, 1997) and some other 'endogenous' materials.

5) Metabolism of chemicals to unreactive, non-genotoxic products can be an important issue in tumorigenesis, at least in animal models. A classic example is the oxidation of 2, 2, 4-trimethylpentane to an alcohol, which is stable but is complexed with \(\alpha\)-globulin to produce male rat kidney tumours (Borghoff *et al.*, 1990).

1.15.1 Metabolic Activation of 7,12-dimethylbenz(a)anthracene (DMBA)

The unified theory of Polycyclic aromatic hydrocarbon carcinogenesis, originally formulated by Flesher and Sydnor (1973), views less carcinogenic hydroxymethyl metabolites as important proximate intermediates in the activation of PAH to highly reactive esters and/or halides as ultimate carcinogens. The lessened biological activity of the more polar hydroxymethyl metabolites is explained, according to this theory, by their decreased ability to penetrate cellular membranes (Flesher and Sydnor 1971). This explanation is supported by numerous studies, which indicate that various less polar derivatives, the alcohols, such as acetate derivatives, possess activity rivaling that of the parent hydrocarbons (Fried, 1974).

Alternative theories of activation of DMBA include bay-region dihydrodiol-epoxidation and formation of radical cations via metabolic one-electron oxidation (Jerina *et al.*, 1978; Cavalieri and Rogan, 1976). The bay-region theory of PAH carcinogenesis predicts that PAH possessing an unsubstituted angular benzo
ring will undergo activation to ultimate electrophilic forms through the formation of an M-region dihydrodiol. Activation by biological one-electron oxidation predicts that PAH possessing low ionization potentials will undergo sequential one-electron abstractions and hydrogen losses to form radical cations as the ultimate carcinogenic forms, which react with macromolecules and cause damage or through generation of reactive oxygen species promoting tumour formation. The oxidation reactions are mediated through mixed function oxidases.

1.16 POLYCYCLIC AROMATIC HYDROCARBON - CARCINOGENS WITH IMMUNOSUPPRESSIVE PROPERTY

Our immune systems are continuously assaulted by pollutants in the environment, toxic additives in our food and common chemicals we use and depend on everyday. These assaults can leave us vulnerable to so-called simple diseases, like cold and the flu, as well as serious problems such as cancer and autoimmune diseases.

Polycyclic aromatic hydrocarbons (PAHs) represent an important class of widely distributed environmental contaminants. They are usually formed through the combustion of fossil fuel and the burning of various substances and are found in significant amounts in automobile exhaust, cigarette smoke, various foods, and industrial waste by-products. They can exert major toxic effects, including development of cancers in various tissues, cardiovascular diseases, loss of fertility and immunosuppression. PAHs inhibit murine T and B cell proliferation and alter T cell-related cytokine production and B cell-mediated Ab production (White and Holsapple, 1984). They also suppress mitogenesis of human T lymphocytes and alter B cell lymphopoiesis through triggering pre-B lymphocyte apoptosis. Besides lymphocytes, APCs such as macrophages and dendritic cells can also be affected by
PAHs. Indeed, PAHs impair Ag presentation by mouse macrophages, after T cell-macrophage interaction and suppress phagocytic activity of peritoneal macrophages. PAHs also reduce esterase-positive macrophagic cell population in mouse spleen. In fact, murine splenic macrophages, which have been demonstrated to metabolize PAHs, are considered the cell types targeted by BP among the different splenic cell populations and are responsible for PAH-related suppression of splenic humoral immune response (Ladies et al., 1992). Moreover, it is noteworthy that cigarette smoke condensates, whose major components consist of PAHs, markedly down-regulate functional system.

PAH induced thymic atrophy, decrease resistance to infectious agents and transplantable tumours, reduce bone marrow cellularity, alter lymphocyte homing, impair B and T lymphocyte proliferative responses, inhibit B cell antibody responses, decrease cytotoxic T cell activity, induce cell death in myeloid, B and T cells, inhibit natural killer activity, or decrease cytokine production in animal model systems (Holsapple, 1994).

### 1.16.1 DMBA and Immune Suppression in Cancer

7.12 – Dimethylbenz(a)anthracene(DMBA), a synthetic model of polycyclic aromatic hydrocarbon(PAH), is documented in the literature as immunotoxic, carcinogenic and teratogenic in all mammals examined, including humans (Cheremisinott, 1994). PAHs inhibit both cell-mediated and humoral immune responses, however, their exact immunosuppressive mechanism(s) remain unknown. Since the PAHs are being structurally planar and highly lipophilic, they are capable of intercalating into plasma or organelle membranes and disrupting transduction of transmembrane signals and/or altering the conformation of membrane receptors (Melius et al., 1984).
1.16.2 Proposed Mechanisms of Immune Suppression by DMBA

The p53 tumour-suppressor protein is responsible for regulating cellular responses to DNA damage and progression through the cell cycle. Gupta et al., (1993) had described the importance of p53 in maintaining a normal pattern of cellular proliferation. p53 plays a crucial role as a “gate keeper” or molecular policeman of genomic integrity. p53 acts as a transcriptional activator as a G1 checkpoint control for DNA damage. In tumour cells it is inactivated by mutation. So if excess DNA damage occurs it could not inhibit cell division. Thus, several DNA damaging agents have been demonstrated to induce p53 and activate apoptosis in a p53 - dependant manner.

The B-cell lineage constitutes the main component of the humoral immune system. In the bone marrow, there is a specific differentiation pathway for B cells that begins with the common lymphoid progenitor and ends with immature IgM expressing B cells, which then leave the bone marrow to populate the spleen and the lymph nodes (Hardy and Hayakawa, 2001). B-cell development is driven by the interaction of the progenitor B cells with bone marrow stromal cell that secrete cytokines and express adhesion molecules that drive the differentiation of the progenitor B cells.

Disruption of this interaction via exogenous toxicants could have profound effects on B cell lymphopoiesis and can result in the impairment of antibody production directed against pathogens. According to (Todd J. Page et al., 2003) DMBA disrupts hematopoiesis in the bone marrow and affects normal B-cell development by targeting discreet cell populations along the B-cell lineage continuum and further that this disruption is dependent on the function of p53.
DMBA elicits immunotoxicity in the spleen, thymus and bone marrow. It has been showed to suppress both humoral and cell mediated immune responses in spleen and cultured splenocytes.

DMBA requires metabolic activation for carcinogenicity. The carcinogenic potency of PAH correlates with splenic immunosuppressive potency (White and Holsapple, 1984; White et al., 1985). Thus, PAH mediate two distinct mechanisms, AhR-mediated and metabolic activation.

Microsomal epoxide hydrolase (mEH) is an enzyme catalysing hydrolysis of aliphatic and arene epoxides and these reactions are generally considered the detoxification pathway. The hydrolysis of epoxide derivatives of DMBA is, however, required for a major metabolic pathway for activating carcinogens to the ultimate electrophilic derivatives (Chou and Yang 1978; Huberman et al., 1979; Cooper et al., 1980). In the metabolic activation of DMBA, mEH is the only enzyme that transforms DMBA 3, 4 – epoxide to DMBA-3,4-dihydrodiol (DMBA – 3,4 – diol) and then CYP1A1 or CYP1B1 oxidizes DMBA 3,4-diol-1,2 epoxide (Christou et al., 1989; Pottenger and Jefcoate, 1990; 1990: Savas et al., 1997; Buters et al., 1999).

mEH is expressed not only in liver but also in several extrahepatic tissues including kidney, testis, ovary, lung, thymus and spleen (Oesch et al., 1977; Seidegard and Depirre, 1983). Thus, mEH is believed to play a critical role in the multiorgan carcinogenesis of DMBA. Miyata et al., has provided genetic evidence that the metabolic activation of DMBA in the spleen plays a key role in the splenic immunotoxicity. Thus indicating a critical role for mEH in DMBA induced spleen immunotoxicity. In thymus the reactivity is low but there is also another possibility of receptor mediated mechanism that is independent of metabolic activation.
Another proposed mechanism that continues to receive considerable attention is the possibility that PAHs alter the production of various interleukin proteins, specifically IL-2. These alterations can ultimately result in immunological dysfunction, which is most often manifested as immunosuppression.

Immunosuppression produced by PAHs is mediated in part by the alterations in Ca\(^{2+}\) dependent pathways of B and T-cell activation. Davila have shown that PAHs increase intracellular levels of Ca\(^{2+}\) in various murine and human B and T-cell lines and cells obtained from murine lymphoid tissues (Barbara, 1998). PAHs disrupt Ca\(^{2+}\) homeostasis by two distinct mechanisms. First, it has been demonstrated that 7,12 - dimethyl benz(a) anthracene (DMBA) activates PTKs in T-cells leading to tyrosine phosphorylation of PLC\(_{1}\), the production of IP\(_3\) and the release of Ca\(^{2+}\) from intracellular stores. The second mechanism by which PAHs appear to alter Ca\(^{2+}\) homeostasis in lymphocytes relating to direct or indirect inhibition of Ca\(^{2+}\) - ATPase pumps found in the endoplasmic reticulum (ER) membranes. These Ca\(^{2+}\) pumps, known as SERCAs, play an important role in Ca\(^{2+}\) reuptake following release triggered by intracellular agents such as IP\(_3\).

Second messengers such as tyrosine phosphorylation, cAMP and calcium and their associated signaling pathways and primary targets for a number of diverse classes of chemicals. Because of the central roles that these signal transduction pathways play in the physiology of immunocompetent cells, toxicant induced changes can have consequences that range from subtle changes in immune function to marked immunosuppression and lead to DNA fragmentations of B and T cells.
1.17 CONVENTIONAL CANCER THERAPIES

One of the major drawbacks of the current cancer therapeutic practices, such as chemotherapy and radiation therapy, is suppression of immune system. A wide variety of compounds are capable of potentiating immune responses. Classical adjuvants of bacterial origin such as Bacillus Calmette Guerin (BCG), have been shown to exert therapeutic effects in the treatment of cancer. But the effect is limited due to a number of undesirable side effects in host, like liver dysfunction, induction of hepatic granuloma; enhancement of tumour growth; when large doses of BCG are administered. Chemical agents, such as levamisole and interferons were widely used to treat cancer in the mid 1970's to early 1980s. Despite the immunological effects, adjuvant levamisole treatment or levamisole alone or in combination with interferon showed no significant clinical benefit. Cytokines play a critical role in the induction and effect or functions of both humoral and cell mediated immune responses. The immunomodulating property of IL-2, IL-4, IL-7, etc. promoted their use in the treatment of cancer patients. But their unique and diverse side effects, such as cardiovascular toxicity, pulmonary toxicity, hematological toxicity, etc., made limitation in their use. Immunomodulators, which can be used for long period without or less side effects, are appreciable in the cancer therapy.

1.18 INTEGRATIVE AND ALTERNATIVE MEDICINE

Many carcinogens and mutagens are not active per se and have to be activated into reactive electrophiles before exerting their biological effects (Miller and Miller, 1986). Therefore, vectors which prevent activation of carcinogens and/or increase their detoxification become important as protective agents against cancer.
(Ito et al., 1989). A number of plant and vegetable constituents have been shown to act as desmutagens. In India, a number of spices, leafy vegetables and condiments are used in diet and many of them have diverse medicinal properties (Pruthi, 1979).

Several medicinal herbs have been shown to promote immunity in different ways. They have been shown to augment specific cellular and humoral immune response Kuttan, (2000). have reported the immunomodulatory activity of some plants, such as *Viscum album* *Tinospora cordifolia*, *Withania somnifera*. Cancer therapies have failed to fulfill all the needs of a patient. It is comprised by numerous side effects. Therefore, it is necessary to identify an integrated mode of treatment which could act both as antitumour agent and also as agent that would suppress the side effects.

Our Indian traditional medicine system, Siddha also depicts plant products in the treatment of malignancy. Several Indian Siddha products can reduce chemically induced mammary tumours in rats without any toxicity. Reference show that Siddha drugs could also enhance immune modulation (Tanaka, 1999). One such, which has high value in Siddha system, is *Semecarpus anacardium* nut.

*Semecarpus anacardium* Linn.

*Semecarpus anacardium* Linn. (Family: *Anacardiaceae*) is distributed in Sub-Himalayan region, tropical and central parts of India, Western peninsula and N. Australia (Krithikar, 1975). The fruit is kidneyshaped, drupaceous nut with a fleshy pear-shaped receptacle. The nut is commonly called ‘marking nut’ and in the vernacular as ‘Ballataka’ or ‘Bhilawa’ (Satyavati et al., 1969).
Historical background

In Ayurveda and Siddha (Indian systems of medicine) classics, copious references regarding the properties and uses of *S. anacardium* nuts are found (Sharma *et al.*, 1966). The fruit of *S. anacardium* is acrid, hot, sweetish, edible, aphrodisiac, anthelmintic, causes looseness of bowels, removes ascites, alleviates skin diseases, piles, dysentery, fever; loss of appetite, urinary discharges, heals ulcers, strengthens the teeth and is useful in insanity and asthma (Krithikar, 1975). It is popularly known as "Ardha Vaidya" (multipurpose medicine).

Phytochemistry

A review of literature reveals the presence of biflavonoids, phenolic compounds, bhilawanols, sterols and glycosides in *S. anacardium* nuts. The chemical examination of *S. anacardium* nuts has been carried out in India by Pillai and Siddiqui. The crushed pericarp of the marking nut on extraction with acetone gives 28% dark brown oil which on distillation gives three fractions i.e. (a) light yellow oil known as semecarpol, a monophenol, (b) a golden yellow oil termed bhilawanol with a C_{15}H_{27} side chain at position-3. Bhilawanol is shown to be a mixture of phenolic compounds consisting mainly of 1,2-dihydroxy-3-(pentadecadienyl)-8’, 11’)-benzene. Bhilawanol on dry distillation gives rise to catechol and a hydrocarbon and (c) a tarry non-volatile residue (54% of extract). On the basis of chemical and spectral data several biflavonoids have been characterised, viz. semecarpuflananol, Jeet-furanone (II), galluflananol (III), Nallaflavanone (IV), Semecarpetin, (V) and Anacarduflananol (VI) the first bioflavanone to occur with a methylenedioxy group.
Phytochemical examination revealed 3.68% of total ash, 0.33% of acid insoluble ash, 11.27% alchol soluble extractive, 11.84% water soluble extractive and 12.71% moisture content in S. anacardium nuts. The proximate principles, minerals and vitamin content are given in Table 1.1. Analysis by Bose revealed the presence of iron, copper, sodium, calcium and aluminum in traces. Phenolic substances and resins were also detected. Vijayalakshmi et al., (1996) have reported the presence of carbohydrates, phenols and flavonoids in the Siddha preparation of S. anacardium nut milk extract.

Table 1.1: Proximate Principles, minerals and Vitamins in Semecarpus anacardium nuts (in 100g)²

<table>
<thead>
<tr>
<th></th>
<th>Moisture (g)</th>
<th>3.8</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>Protein (g)</td>
<td>26.4</td>
</tr>
<tr>
<td>3</td>
<td>Fat (g)</td>
<td>36.4</td>
</tr>
<tr>
<td>4</td>
<td>Minerals (g)</td>
<td>3.6</td>
</tr>
<tr>
<td>5</td>
<td>Fibre (g)</td>
<td>10.4</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates (g)</td>
<td>28.4</td>
</tr>
<tr>
<td>7</td>
<td>Energy (Kcal)</td>
<td>587</td>
</tr>
<tr>
<td>8</td>
<td>Calcium (mg)</td>
<td>295</td>
</tr>
<tr>
<td>9</td>
<td>Phosphorus (mg)</td>
<td>836</td>
</tr>
<tr>
<td>10</td>
<td>Iron (mg)</td>
<td>6.1</td>
</tr>
<tr>
<td>11</td>
<td>Thiamine (mg)</td>
<td>0.38</td>
</tr>
<tr>
<td>12</td>
<td>Riboflavin (mg)</td>
<td>0.15</td>
</tr>
<tr>
<td>13</td>
<td>Niacin (mg)</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Pharmacological evaluation

Satyavati et al (1969) and Baipee et al (1970) have reported the antinflammatory activity of S anacardium nuts in acute inflammation of both immunological and nonimmunological origin. A variety of marking nut preparations are used in traditional medicine against numerous tumours. Ghothoskar and Ranadive (1971) have found that even a single injection of SAN-AB another marking nut preparation could bring about complete inhibition of tumour growth in 14/15 rats. The chloroform extract of S anacardium nut possesses antitumour action against L1210 P388 advanced P388 leukemia B16 melanoma and glioma 26 (Cassady et al 1981). Increase in life span was observed in these cases (Chitnis et al 1980). In vitro effect of acetyl derivatives from S anacardium nut indicated that the incorporation of radiolabelled precursors into DNA RNA and protein was considerably inhibited at a concentration ranging from 40-75 μg/ml within 2 hr and thereby biosynthesis of DNA RNA and protein was significantly inhibited (Phatak et al., 1983). Anacardin forte an Ayurvedic marking nut preparation exhibited not only a broad spectrum of anticancer properties in clinical and animal studies but also a wide margin of safety in therapeutic dosage even when used for long periods. It has shown very gratifying results in the cases of the cancer of oesophagus, urinary bladder, liver and chronic leukaemia by giving subjective and objective improvement, alleviation or disappearance of troublesome symptoms and clinical benefits with extension of survival time. This preparation has selective action, attacking only the cancer cells without harming the normal cells (Vad, 1973).

Siddha preparation called 'Serankottai nei' which is a milk extract of S anacardium nuts was subjected to biochemical screening by Premalatha et al., (1997) The antioxidative, membrane stabilizing, tumour marker regulative, glucose
level restoring (via modulation of carbohydrate metabolizing enzymes) and mineral regulation properties in experimental hepatocellular carcinoma (HCC) were observed. Also, *S. anacardium* nut extract is found to detoxify a potent hepatocarcinogen, aflatoxin B₁ and causes its metabolites to be excreted in urine. Considering the pessimistic prospect in case of primary HCC, for which the prognosis is grim with an expected six months span of life, the results achieved in these experiments hold out great promise for future. The nut extract also potentiates the efficacy of widely used anticancer drugs, viz. mitomycin-C, 5-fluorouracil and methotrexate. Immunomodulatory potency of the nut extract in hepatocellular carcinoma was also reported from our laboratory (Premalatha, 2000).

**Toxicity evaluation**

*S. anacardium* nuts can be given orally with milk, ghee, peanut oil, etc. Toxic effects are not observed by such routes of administration. On the contrary, anabolic effects are obtained. Traditional methods recommended in Ayurveda and Siddha should be closely followed so as to get therapeutic effects without toxicity. Various reports have mentioned the range of dosage from 300 to 9000 mg in a graded manner (Sharma et al., 1966). Toxicity studies were carried out by Ghosh et al., (1981) with one Siddha preparation of *S. anacardium* (coded as SKx) and they found that, in rats, there was no adverse effect or mortality up to the oral dose of 2000 mg/kg body weight. Histopathological studies on liver, lung, kidney and heart did not reveal any significant pathological lesions even when the extract was administered at a high dose of 1000 mg/kg body weight (Ghosh et al., 1981). The animals looked healthy and active without any physiological disturbance and loss in body weight. Hematological picture was almost normal. The extract did affect total
WBC count but there was no effect on RBC count and haemoglobin percentage. The LD$_{50}$ dose of 40g/kg in rats and rabbits was determined by Vaishnav et al., 1983).

The toxic-side effects of the very high dose of the drug are diarrhoea and vomiting, swelling all over the body, ulceration and vesication on the skin. The drug should be used cautiously and in lesser doses in hot season (Sharma, 1966). During use, whether external or internal, the least appearance of a rash or redness of the skin or an itchy or uneasy sensation in any part of the body should be considered as a manifestation of undesirable effects and use should be discontinued immediately.

**Pharmacological Activity of Flavonoids**

Polyphenolic compounds exert a variety of physiological effects including antioxidative, immunomodulatory and antigenotoxic effects (Achim Bub et al., 2003). Polyphenols are the major phytochemicals in fruits and vegetables. A variety of studies have shown that polyphenols such as flavonoids have antioxidative (Rice Evans et al., 1996) and immunomodulatory activities (Middleton, 1998). Polyphenols further have a potential to prevent genotoxicity by reducing the exposure to oxidative and carcinogenic factors (Cai et al., Pool-Zobel et al., 1999).

Regulation of leukocytes in inflammatory region has been proved to be an effective strategy for oxidative stress control and cancer chemoprevention (Nakamura et al., 1998). Flavonoids, terpenoids and some phenolics are well known to act not only as oxygen radical scavengers but also to be anti - inflammatory agents and inhibitors of arachidonic acid metabolism (Murakami et al., 2000).

Flavonoids could presumably impair the production of reactive oxygen intermediates by neutrophils and other phagocytic cells. This may be accomplished
by interference with NADPH oxidase, a powerful oxidant — producing enzyme localized on the surface membranes of neutrophils (Tauber et al., 1984). Flavonoids could also inhibit neutrophil myeloperoxidase (MPO), a source of reactive chlorine intermediates (Pincemail et al., 1988).

Thus, the flavonoids inhibit several biochemical events associated with immunosuppression. The flavonoids present in *Semecarpus anacardium* nut extract may act synergistically and may be responsible for the potent immunomodulatory activity in cancer condition. However, not all modes of action are applicable to all flavonoids in the extract or some flavonoids may act in more than one way or may not act at all.

1.19 AIM AND SCOPE

Mobilizing the body's immune system against cancer has long been an elusive goal in cancer medicine. The role of immune function has become increasingly important in our understanding of the mechanisms underlying the body's ability to prevent cancer. There is increasing evidence that alteration of immune function using medicinal plants may be a key component of disease prevention. *Semecarpus anacardium* nut milk extract, a Siddha drug preparation, has been proved to be a potent anti-cancer drug due to its protective actions such as antioxidant, anti-proliferative, anti-inflammatory, enzyme inhibitory and differentiation - inducing properties. But, yet, to date, research examining the effect of nut extract on immune parameters is limited. Since cancer has to be associated with decreased immunocompetence, we studied the effect of *Semecarpus anacardium* nut milk extract on general biochemical parameters and humoral and cell-mediated immunity in control and experimental rats by the following way.
• Evaluation of non-specific immunity and hematological variables in control and experimental animals

• Assessment of the impact of *Semecarpus anacardium* on cell mediated and humoral functions in control and experimental animals

• Investigating the biochemical derangements in immune organs such as spleen and thymus

• The role of *Semecarpus anacardium* on cellular DNA damage and apoptosis