INTRODUCTION

Cancer is a multi-factorial diseases, which is characterized by varying degree of morphological disorientation, uncontrolled over growth, invasive and metastasis (Wynder et al., 2006). This uncontrolled division can compromise the function of the host and ultimately may cause death. Cancer can appear as a result of different causes which can be both external factors such as tobacco, infectious organisms, chemicals, radiation and also internal factors like hormones, immune conditions and mutations which occur from metabolism in a variety of sites within the body and that each type of cancer displays its own growth rate, prognosis and treatability (Iacobuzio et al., 2009).

CARCINOGENESIS

Carcinogenesis is the process of genetic mutations induced by physical or chemical agents. Conceptually, this process can be divided into three distinct stages such as initiation, promotion and progression (Vincent et al., 2008). Tumour Initiation results from irreversible genetic damage. The initial genetic change occurs as the result of carcinogen DNA interaction which as termed tumor initiation. A carcinogen causes a mutation by modification of the molecular structure of DNA. It is brought about by formation of an adduct between the carcinogen or one of its functional groups and nucleotide in DNA. A positive correlation is found between the amount of carcinogen-DNA adducts that can be detected in system (Luo et al., 2010).

Three important steps involved in the initiation stage are carcinogen metabolism, DNA repair and cell proliferation. Many chemical agents must be metabolically activated before they become carcinogenic. Most carcinogens or their active metabolites are strong electrophiles and bind to DNA to form adducts that must be removed by DNA repair mechanisms. Failure to repair chemical adducts, followed by cell proliferation, results in permanent alterations or mutations in the genome that can be lead to
oncogene activation (Kotsopoulos et al., 2005). Promotion is a reversible process in which chemical agents stimulate proliferation of initiated cells.

![Figure 1. Stages of carcinogenesis](image)

Tumor promotion comprises the selective clonal expansion of initiated cells since the accumulation rate of mutations is proportional to rate of cell division. It follows the clonal expansion of mutated cells which produces a larger population of cells that are at risk of further genetic changes and malignant conversion. Thus initiated cells are irreversibly altered and are at greater risk of malignant conversion than normal cells (Lambert et al., 2010). The epigenetic effect of tumor promoters facilitate the clonal expansion of initiated cells. This selective clonal growth advantages results in the formation of pre-neoplastic cells. It is possible that may tumor promoters by their ability to reduce the latency period for tumor formation after exposure of tissue to a tumor initiator or to increase the number of tumors formed in that tissue (Laurie et al., 2004).

Malignant conversion is the transformation of a pre-neoplastic cell into the malignant phenotype. This process requires further genetic changes. These changes result from infidelity of DNA synthesis (Daniel et al., 2010). The relatively low probability of malignant conversion can be increased by exposure of pre-neoplastic cells to DNA damaging agents and this process is
mediated through the activation of proto-oncogenes and inactivation of tumor suppressor genes (Sharma et al., 2011). Progression refers to the process of acquiring additional mutations which lead to malignancy and metastasis. Many initiating agents can also lead to tumor progression, which lends strong support for the notion that further mutations are needed for cells to acquire the phenotypic characteristics of malignant tumor cells. Chemicals are converted into positively charged metabolites which binds to negatively charged groups on molecules like proteins and nucleic acids. This result in the formation of DNA adducts which if not repaired, lead to mutations (Klezovitch et al., 2004). The result of these mutations coupled with an increased growth rate, invasiveness, metastasis and an alteration in biochemistry and morphology (Rose et al., 2012).

The continuing magnitude of the cancer and the failure of conventional chemotherapy of advanced invasive disease are belived to effect major reduction in the mortality rates for the common forms of cancer such as carcinoma of lung, colon, breast, prostate and pancreas which indicate that the requirements of new approaches to control and treat the cancer.

BREAST CANCER

Breast cancer is a malignant growth which begins in the tissues of the breast primarily from the epithelial component of the gland where it proliferates at high rate, causing necrosis of surrounding tissues (Jemal et al., 2010). As they infiltrate at high rate, the malignant cells metastasize to regions like lungs, bone, liver and brain. An accumulation of genetic and epigenetic alternations convert normal breast cells to cancer cells. The structure of the female breast is important in understanding this cancer (fig.2).

The interior segment of the female breast consists mostly of fatty and fibrous connective tissues. Breast tissue is a complex network of lobules (small round sacs that produce milk) and ducts (canals that carry milk from the lobules to the nipple openings during breastfeeding) in a pattern that looks like bunches of grapes. These “bunches” are called lobes. Adult
women have 15 to 20 lobes in each breast and each lobe has 20 to 40 lobules (Fang et al., 2009). Small ducts are attached to the lobules. These ducts join together like branches of grape stems into increasingly larger ducts. There are about ten duct systems in each breast, each with its own opening at the nipple (Osborne et al., 2011). The ducts carry the milk through the breast and converge in a collecting chamber located just below the nipple.

**Figure 2. Structure of the Breast**

Blood and lymph vessels run throughout the breast the blood nourishes the cells and the lymph drains the waste. Breast cancer usually start in cell that line the milk ducts(ductal cancer) or milk producing lobes(lobular cancer) a few other are forms of breast cancer are also exit. It is reported that 15% to 20% of breast cancers fall into the category of non-invasive cancer or carcinoma *in situ*- tiny growth that have not spread across the wall of the milk ducts or lobules. More advanced cancers are called “invasive” which have spread beyond the ducts and lobules.
Breast cancer grows at different rates but according to an estimation of oncologists that the average tumour doubles in size every 100 days. Since cancer start with one irregular cell, even with this doubling time, they may not be palpable for years. Mammography can find tumours that are too small to be felt, but even the tumours have probably been growing for years they are large enough to be visible on a Mammogram. Breast cancer cells migrate to the lymph nodes under the arm (axillary), in the neck (cervical), or those just below the collarbone (supra-clavicular). The most common sites of breast cancer metastasis are skin, distant lymph nodes, bone, lung and liver (Britta Weigelt et al., 2005).

**DUCTAL CARCINOMA IN SITU**

Ductal carcinoma *in situ* (DCIS) is an early stage of cancer. It is non-invasive, which means it has not spread beyond the milk ducts to other parts of the breast and to the lymph nodes in the under arm, or to other parts of the body. In addition, several types of DCIS are also exist. If not removed, it develops into an invasive cancer. Others may never progress to this stage and DCIS is highly curable.

**LOBULAR CARCINOMA IN SITU**

Lobular carcinoma *in situ* (LCIS) is a non-invasive growth limited to the milk lobules of the breast. According to the National Cancer Institute, women with LCIS have about a 1% risk of developing invasive breast cancer per year. At 20 years, this risk is about 18%.

**INVASIVE DUCTAL CARCINOMA**

In more advanced stages, breast cancer cells cross the lining of the milk duct or lobule and begin to invade or infiltrate adjacent tissues. In this stage, the cancer is called “infiltrating cancer”. Invasive ductal carcinoma (also known as infiltrating ductal carcinoma) is most common kind of invasive breast cancer. More than half of all cases are of this type.
OTHER TYPES OF BREAST CANCER

Medullary Carcinoma

This is the type of invasive ductal carcinoma which appears well confined, but often has infiltrated the lymph nodes. Medullary carcinoma may grow large but has a better than average prognosis.

Mucinous Carcinoma

This is a type of invasive ductal carcinoma which produces a gelatinous-like tumour. These cancers have a very good prognosis.

Tubular Carcinoma

This is a type of cancer which produces many small glands and tubules that closely resemble normal ductules.

Invasive Lobular Carcinoma

This cancer arises at the ends of the ducts or in the lobules and may cause widespread breast thickening rather than a specific lump. The prognosis is better than average.

Paget’s disease

This is very rare type of cancer. It appears as an itchy rash around the nipple and areola. It should not be mistaken for a benign skin conditions such as eczema or contact dermatitis.

Inflammatory Carcinoma

This is the most serious breast cancer. The skin over the breast becomes very inflamed and swollen because the skin lymph vessels are blocked by cancer. While the prognosis has improved considerably with new treatments, this cancer still has the least favourable prognosis.

EPIDEMIOLOGY

Breast cancer is the second leading cause of deaths in women and is the most common cancer among women, excluding non melanoma skin cancers. Breast cancer incidence varies widely within regions and countries which has been increasing in the general population all over the world due to differences in racial and ethnic make-up, health resources and lifestyle
patterns. Worldwide, the incidence of breast cancer varies from 3.9/100,000 in Mozambique to as high as 101.1/100,000 in the U.S. Geographic variation in breast cancer incidence can be attributed to racial and genetic differences, cultural differences as well as environmental exposures that vary throughout the world (Marugame et al., 2006).

Breast cancer is the most common cancer of women in the United States and European countries. According to the American Cancer Society, breast cancer rates have risen about 30% in the past 25 years in western countries, due in part to increased screening which detects the cancer in earlier stages. Approximately 2.4 million women living in the U.S. have been diagnosed with and treated for breast cancer. Breast cancer continues to be the most commonly diagnosed cancer in women in the United States, accounting for 26% of all female cancers. In 2013, an estimated 232,340 new cases of invasive breast cancer are expected to be diagnosed among women, as well as an estimated 64,640 additional cases of in situ breast cancer. Of these, approximately 85% will be ductal carcinoma in situ (DCIS). The incidence rate of the in situ breast cancer increased 2.8% per year from 2005 to 2009. An estimated 40,030 breast cancer deaths (39,620 women, 410 men) are expected in 2013 (American cancer society, 2013).

![Figure 3: Current Worldwide Breast cancer incidence rates](image-url)
The lifetime probability of developing breast cancer is about 4.8% in developed countries whereas 1.8% in developing countries. The lowest breast cancer incidence is reported from far Eastern and South-East Asian countries (Agarwal et al., 2007). In the developing countries of Asia, the health care burden on account of breast cancer has been steadily mounting. Breast cancer is the most common neoplastic disease amongst women in the UK and Wales, accounting for more than 12,000 deaths each year (Woods et al., 2007).

According to McKay et al., (2004) about 36 women were diagnosed with breast cancer every day in Australia. In Nigeria, about two-thirds of women with breast cancer are diagnosed at advanced stage of cancer (Adebamowo et al., 2003). In Greece, the annual incidence is more than 3000 per year and the annual incidence is increased over the last 20 years with 1.3%. An increase in incidence rate was reported every year in Japan, Singapore and Korea (Knaul et al., 2009). Reports from Pakistan showed an increasing incidence of Estrogen, Progesterone negative breast cancer among women populations (Kakarala et al., 2010).

Breast cancer is the commonest cancer of urban Indian women and the second commonest in the rural women (Murthy et al., 2009). In India, 75,000 new cases of breast cancer are reported every year and gradually increase in the incidence has been reported in metropolitan cities (Agarwal et al., 2008). Breast cancer is the most common cancer among Indian women in urban registries of Delhi, Mumbai, Ahmadabad, Calcutta and Trivandrum where it constitutes 30% of all cancers in females. It has been reported that the age standardized incidence rates for breast cancer range varies from 6.2 to 39.5 per 100,000 in Indian women. However, the highest incidence was reported in the Parsi community of Mumbai women (Yeole et al., 2003). Owing to the lack of awareness of this cancer and in absence of a breast cancer screening program, the majority of breast cancers are diagnosed at a relatively advanced stage.
Figure 4: Age standardised incidence rates (ASR) for breast cancer in India

AGE AND SEX DISTRIBUTION

Breast cancer is about 100 times more common in women than in men, but survival rates are equal in both sexes. The older woman is the greater chances of developing breast cancer. Approximately 77% of breast cancer cases occur in women over 50 years of age. Annual breast cancer rates are eight fold higher in women who are at 50 years old, in comparison with women who are at 30 (Kroman et al., 2003). If a woman has already had breast cancer, she has a greater chance of developing a new cancer in an other breast.
RISK FACTORS

The established risk factors for breast cancer may enhance the chances of getting the cancer. Breast cancer as it is a complex disease with often many different contributing causes. The greatest risk factor for developing breast cancer is gender (female) and the age factor (Hsu et al., 2010).

![Diagram of breast cancer risk factors]

SIGN AND SYMPTOMS

The most common symptom of breast cancer is a mass or lump in the breast. In early stages, breast cancer usually has no symptoms. The earliest sign of breast cancer is often an abnormality detected on a Mammogram, before it can be felt by the woman or a health care professional. Larger tumors may become evident as a painless mass (Rim et
al., 2008). The symptoms of advanced stage of breast cancer are bone pain, weight loss, swelling of an arm, and skin ulceration.

Other signs of breast cancer are swelling of part of the breast, skin irritation or dimpling, nipple pain or the nipple turning inward, redness or scaliness of the nipple or breast skin, a nipple discharge other than breast milk, a lump in the underarm area, pain or tenderness in the breast, change in the contour and texture or temperature of the breast, abnormal modifications in the appearance or sensation of the nipple area including the retraction/enlargement of the nipple, a purulent appearance, a bloody clear to yellow or green fluid secretion that weeps from the nipple and itching sensations.

SCREENING AND DIAGNOSIS

The screening of breast cancer usually refers to screen healthy women for breast cancer, in an attempt to achieve an earlier diagnosis. The assumption is that early detection will improve outcomes. A number of screening tests have been employed, which include clinical and self-examinations of breast, Mammography, Genetic screening, Ultrasound, and Magnetic Resonance Imaging (MRI) (Perry et al., 2008).

STAGING

‘Staging’ is a method which has been developed to describe the extent growth of cancer. Breast cancer is ‘staged’ usually based on the surgical and other findings. In addition, staging is based upon findings from imaging studies such as chest X-ray, abdominal Ultrasound (images produced by high-frequency sound waves), Computed Tomography (CT or CAT scan; computer-assisted technique that produces cross-sectional images of the body) and bone scans.

Pathologists use a specific system to the breast cancer staging. This method is known as the TNM system which was devised by the American Joint Committee on Cancer (AJCC) in collaboration with the National Cancer Institute (NCI) (Greene et al., 2002). Within the TNM system, “T” refers to tumor size, “N” refers to lymph node involvement and
“M” refers to the extent of metastasis. To simplify this information, the TNM classifications are grouped within four basic stages, labeled stage 0 through stage IV (0-4).

Stage 0: It is an early stage of breast cancer refers to lobular carcinoma in situ (LCIS) or ductal carcinoma in situ (DCIS)

Stage I: It is an early stage of invasive breast cancer. The tumour is no more than 2cm across. Cancer cells have not spread beyond the breast.

Stage II: The cancer has spread to the lymph nodes under the arm. The tumour is between 2 and 5 centimetres. The cancer may have spread to the lymph nodes under the arm.

Stage III: The tumour in the breast is larger than 5 centimetres. The cancer has spread to under arm lymph nodes or the cancer may have spread to lymph nodes behind the breast bone.

Stage IV: It is a distant metastatic cancer. The cancer has spread to other parts of the body.

TREATMENT

The treatment of breast cancer is determined by many factors, and depends on stages of tumor, type and characteristics. Further the person’s general health and medical conditions that may influence treatment (Herbst et al., 2006).

Surgery

The primary goal of the surgery of breast cancer is to remove the cancer from the breast and lymph nodes. Once the tumor is identified as malignant, surgery becomes the safe option to remove the malignant lump. Surgery could involve removing whole of the breast or just the area where lump has formed. In a lumpectomy, only cancerous tissue plus a rim of normal tissue is removed. Lumpectomy is almost always followed by six to seven weeks of radiation therapy. Simple or total mastectomy includes removal of the entire breast. Both lumpectomy and mastectomy are often
accompanied by removal of regional (axillary) lymph nodes to determine if the disease has spread beyond the breast. Surgery is often combined with other treatments such as radiation therapy, chemotherapy, hormone therapy and/or monoclonal antibody therapy.

**Radiation Therapy**

Radiation may be used to destroy cancer cells remaining in the breast, chest wall, or underarm area after surgery or to reduce the size of a tumor before surgery.

**Hormone Blocking Therapy**

Some types of breast cancers require estrogen to continue growing. They can be identified by the presence of estrogen receptors (ER+) and progesterone receptors (PR+) on their surface. These ER+ cancers can be treated with drugs which block the production of estrogen or block the receptors, such as Tamoxifen or an aromatase inhibitor. Women whose breast cancers with positive for estrogen or progesterone receptors can be given hormone therapy to block the effects of estrogens on the growth of breast cancer cells. Tamoxifen, the most commonly used antiestrogen drug, has been shown to provide a 26% annual reduction in recurrence and a 14% annual reduction in deaths. Hormone therapy is effective in both post-menopausal and pre-menopausal patients whose cancers are positive for steroid hormone receptors. Estrogen replacement therapy (ERT), also known as hormone replacement therapy (HRT) is used by many older women to relieve the symptoms of menopause. However some studies indicates that ERT may increase the risk of breast cancer after long-term use (10+ years).

**Systemic Therapy**

Systemic therapy includes both the chemotherapy and hormone therapy. Adjuvant systemic therapy is used after all visible cancer has been surgically removed in order to destroy any undetected tumor cells that may have migrated to other parts of the body.
Chemotherapy

Chemotherapeutic drugs are given with the hope that micrometastases will be eliminated before they spread to other tissues. Many chemotherapeutic drugs interfere with cell division or other metabolic processes. Therefore, they are most harmful to rapidly dividing cancer cells, although normal cells may also be damaged. Chemotherapy typically is delivered in the form of shots or pills. It may be the only treatment used if breast cancer has spread to other parts of the body. Commonly, chemotherapy is given as an adjuvant (assisting therapy) to reduce the chance of cancer recurring after surgery, radiation therapy or both. Research has established that combinations of several drugs are more effective than just one drug alone.

Chemotherapy medications for breast cancer include Paclitaxel, Doxorubicin, Paraplatin, Cyclophosphamide, Epirubicin, Gemcitabine, and Vincristine. In early stage breast cancer, standard chemotherapy regimens lower the risk of the cancer recurrence. In advanced breast cancer, chemotherapy regimens make the cancer shrink or disappear in about 30-60% of people treated. Targeted therapy, also called as biologic therapy, is a newer type of cancer treatment. This therapy uses special anticancer drugs that target certain changes in a cell that can lead to cancer. One such drug is Trastuzumab (Herceptin). It may be used for women with HER-2-positive breast cancer.

Prevention of Breast Cancer

Modifiable factors that are associated with a lower risk of breast cancer include breast feeding, moderate or vigorous physical activity and maintaining a healthy body weight. Two medications such as Tamoxifen and Raloxifene have been approved to reduce breast cancer risk in women at high risk (Lin et al., 2008). Raloxifene appears to have a lower risk of side effects such as uterine cancer and blood clots. In women with estrogen-receptor
positive breast cancer additional treatment with Tamoxifen reduces the risk of recurrence of secondary breast cancers by about half.

**Chemoprevention**

Cancer chemoprevention is defined as the use of natural or synthetic agents to reverse, suppress or prevent carcinogenic progression. Chemopreventive agents that are derived from natural resource are considered pharmacologically safe (Tsao *et al.*, 2004). Primary chemoprevention mainly focuses on the prevention of cancer in populations who are at higher risk of cancer development. Secondary chemoprevention mainly involves the prevention of cancer in populations with pre-malignant or pre-cancerous condition. Tertiary chemoprevention deals with the prevention of secondary cancers in patients treated for primary cancer or individuals who have been treated for pre-malignant lesions (Takemura *et al.*, 2013). Chemopreventive agents can be placed into three broad categories. Blocking agents which prevent carcinogenic agents from reaching or reacting with critical target site, thus act by exerting a barrier function. Resisting agents which decrease the vulnerability of target tissue to carcinogenic stimuli. Suppressing agents prevents the evolution of neoplastic process in tissues or otherwise it would become malignant (Hail *et al.*, 2005).

**POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)**

Polycyclic Aromatic Hydrocarbons (PAHs) are an important class of chemical carcinogens that are widespread in the ambient environment due to combustion of fossil fuel for energy production, transportation and industry (Haritash *et al.*, 2009). PAHs also found in tobacco smoke and foods such as charred and broiled meat. The human mammary gland is exposed to the agents capable of inflicting DNA damage and thereby initiating tumor. The high lipid content of the mammary gland allows an accumulation of lipid soluble compounds including possible carcinogens for the epithelial cell ducts. It is reported that PAHs are potent mammary carcinogen in experimental bioassays (Mafuvadze *et al.*, 2011). Carcinogen-DNA adduct
are considered as a necessary, but not sufficient event in the development of malignancy (Poirier et al., 2012). It is possible to monitor exposure to PAHs by measuring PAH-DNA adducts. With the availability of newer genetic techniques to quantify chemical carcinogens which bind to human DNA. Moreover the PAH-DNA adducts have been detected in breast tissue suggesting that mammary gland epithelial cells may be exposed to cancer initiating events from PAH exposure in the environment (Kathleen et al., 2009). Hence, the genetic damage from environmental source of PAH may be a risk factor for breast cancer and progression.

It is reported that several tons of polycyclic aromatic hydrocarbons are spilled into the environment every year and these are drastically affect the normal life of the living organisms. Sometimes these effects are stable because of the accumulation of the metabolites from one tropic level to another. It is well known fact that xenobiotics enter into the environment and cause various disorders (Vijayavel et al., 2005). In this regard, the world health organization (WHO) has sent a warning signal that “an increased xenobiotic or environmental pollutant is responsible for the toxicities in human population world wide” (Murray et al., 2001).

**7,12 DIMETHYLBENZ(a)ANTHRACENE**

7,12 Dimethylbenz(a)anthracene (DMBA) is a polycyclic aromatic hydrocarbon (PAH) which is commonly found in our environment and they can be isolated from diesel exhaust, barbequed meat, tobacco smoke and overheated cooking oil etc., (International Agency for Research on Cancer, 1983). Reactive oxygen species (ROS) are potentially dangerous by-products of cellular metabolism and also predominant reason for many oxidative stress mediated diseases which is generated by various environmental contaminants among which DMBA is an important one. DMBA is a potent organ specific carcinogen which is widely used in the study of breast cancer etiology and are not chemically reactive but are altered under metabolism and which is employed to induce mammary carcinoma in female Sprague Dawley rats
DMBA induced mammary carcinogenesis is therefore, an ideal model to study the chemopreventive and chemotherapeutic effects of natural and synthetic agents (Manoharan et al., 2009a). Since it closely resembles human breast cancer both in histology and morphology. DMBA is a fat soluble compound and because of this property it accumulates and persists in the adipose tissue of the mammary gland and increases the exposure of mammary epithelium to the chemical carcinogen (Carroll et al., 2010).

DMBA is an indirectly acting carcinogen which requires metabolic activation to yield an ultimate carcinogenic form (Sathish et al., 2011). DMBA is oxidized to DMBA-3,4-epoxide by phase I enzymes especially the CYP (Nadine et al., 2010).

Figure 5. Metabolic activation of 7, 12 Dimethylbenz(a)anthracene

Another phase I enzyme, epoxide hydrolase then converts the epoxide to DMBA-3,4 diol, the proximate carcinogen. Subsequent oxidation by CYP leads to the formation of DMBA-3,4-diol-1,2-epoxide, the ultimate carcinogen (Kirubha et al., 2012) which then reacts with DNA to form adducts which is responsible for mutagenicity and carcinogenicity.
SELECTION OF RAT STRAIN

The ultimate use of the experimental animal models are to understand the behavioural, body weight changes and pathology of the disease in humans and to provide necessary information about the prevention and treatment. Animal model is a living organism with an inherited naturally acquired or induced pathological process which are closely resembles the same phenomenon occurring in human beings in many aspects (Mahmood et al., 2005). The laboratory rat has been one of the most widely used species in breast cancer research because of the exquisite sensitivity of its mammary gland to the carcinogenic effects of chemical compound. Sprague Dawley rat has been a favoured laboratory animal to study chemically induced mammary tumorigenesis because of their extreme sensitiveness to the effects of chemical carcinogenesis in terms of high incidence in short period. There is a positive association for a long period between the Sprague-Dawley rats and chemically induced mammary tumorigenesis.

The chemical carcinogens such as 7,12-dimethylbenz(a)anthracene (DMBA) and N-methylnitrosourea (NMU) are widely used to induce breast cancer in experimental rats. DMBA or NMU-induced mammary tumorigenesis is age-dependent approximately between 45–60 days and has maximum susceptibility of rat mammary gland towards the administered chemical carcinogen which is correlated with the sexual maturity period of Sprague-Dawley (SD) rats where high rate of epithelial cell proliferation and differentiation of alveolar duct occurs with high rate of DMBA activation to initiate carcinogenesis (Yang et al., 2013). Initiation of neoplasm requires stable binding of carcinogen to the DNA and permanent alteration of DNA which occurs at S-phase where maximal DNA synthesis occurs. If the altered DNA is not repaired during G1 phase, the genomic insult is passed on to the daughter cells. DMBA is metabolized by mammary epithelium to both polar and phenolic metabolites in the terminal end buds (TEBs) of virgin rats (Russo et al., 1983) and the rate of formation of polar metabolites is higher in
the TEB epithelial cells and hence the binding of carcinogen to DNA is also higher. However, the removal of DNA adduct with carcinogen in TEB is very low, indicating the poor repair capacity of DNA damaged by DMBA.

**APOPTOSIS AND CANCER**

Apoptosis or programmed cell death represents a universal and exquisitely efficient cellular suicide pathway that removes aging or injured cells from the body (Ghavami et al., 2009). It describes the orchestrated collapse of a cell characterized by membrane blebbing, cell shrinkage, condensation of chromatin and fragmentation of DNA followed by rapid enlargement of the collapse by neighboring cells (Irene et al., 2005). In cancer, the balance between proliferation and programmed cell death is disturbed and defects in apoptotic pathways allow cells with genetic abnormalities to survive (Judith Henry et al., 2004). Hence, in cancer the therapeutic goal is to trigger tumor selective cell death. Studies have demonstrated that a wide range of anticancer agents including chemotherapeutic agents induce apoptosis in malignant cells (Tianfeng et al., 2010). The efficacy of cancer treatment depends not only on the cellular damage they cause but also on the cells ability to respond to the damage by inducing apoptotic machinery (Tomris Ozben, 2007).

The mitochondrial pathway is thought to be important in response to cancer treatment and is mediated by Bcl-2 family proteins. The final execution of cell death is performed by the caspase cascade, which is triggered by release of cytochrome C from mitochondria. The most studied genes related to apoptosis are the tumor suppressor gene p53, the anti-apoptotic gene Bc1-2 and pro-apoptotic gene Bax (Vogler et al., 2011). p53 has a dual role, it can either increase apoptosis or arrest growth and thereby increases drug resistance. Over expression of Bc1-2 could provide a survival advantage for cancer cells but in vivo, Bc1-2 expression has been associated with a more favorable prognosis in many malignant diseases. In pre-clinical
studies Bax, the proapoptotic gene has been reported to restore sensitivity in drug and radiation induced apoptosis (Chandra-Kuntal et al., 2013).

NATURAL COMPOUNDS AS ANTICARCINOGENS

Chemotherapeutic drugs, which cause DNA damage and generally used to destroy the tumor cells by apoptosis and provide some of the most effective anticancer agents used in the clinic today. There are however, significant limitations in their use. Many tumors are intrinsically resistant to particular drugs and over a periods of time others develop an acquired resistance to previously effective agents when presented again as a second round of therapy. Most of the drugs for breast cancer that are currently available in the market show limited efficacy against advanced disease and are also associated with severe side effects which are more severe with higher doses and increases over the course of treatment. Common side effects include nausea, vomiting, diarrhea, hair loss and fatigue. Serious short and long-term complaints can also occur and may vary depending on the specific agents used which includes anemia, neutropenia, liver and kidney damage and also drop in WBC counts (Morrow et al., 2006).

Considering these facts, it is of interest that innovative new strategies will be required to treat breast cancer. Therefore, searching for effective chemotherapeutic agents is important to improve the survival rate of patients with advanced or recurrent breast cancer. There is an urgent need for the evaluation of new active drugs against breast cancer. In recent years, attention has been focused on the identification of naturally occurring plant derived compounds as possible chemopreventive and chemotherapeutic potential in a variety of bioassay systems and animal models. An effective and acceptable chemopreventive or anticancer agent should have certain properties such as (i) no toxic effects in normal and healthy cells, (ii) high efficacy against cancers, (iii) capability of oral consumption, (iv) known mechanism of action, (v) low cost, (vi) acceptance by the human population, (vii) ability to inhibit initiation, (viii) capability to inhibit promotion or progression of tumor, (ix) capacity to block DNA adduct formation, (x) lack
of genotoxicity, (xi) not to be a carcinogen precursor and (xii) lack of enhancing activity at any stage of carcinogenesis (Aziz et al., 2003).

In this connection, several studies have also shown that certain naturally occurring plants contain active compounds that possess substantial antimutagenic and anticarcinogenic effects against variety of chemicals (Huang et al., 1983). Therefore, development of novel drugs of natural origin or from plant derived compounds is desired. In support of this, number of research work has been carried out to evaluate the therapeutic effect of medicinal plants from natural sources for the cure of various human diseases, including cancer (Prakash and Gupta, 2000).

D-PINITOL

The National Cancer Institute has highlighted a number of foods for which there is evidence from epidemiology or experimental studies of an association with a reduced risk of cancer, amongst which soybeans are most soybeans (Zuo et al., 2008). A number of studies have reported that consumption of soy and soy products is associated with some degree of protection against either induced or spontaneous cancers in animals as well as reduced cancer risk in several human epidemiological studies (Andres et al., 2011).

Soy diet has been associated with various beneficial effects in human beings. It contains various biologically active components and has received much attention for their potential beneficial effects in cancer prevention, osteoporosis, diabetes and obesity, menopausal symptom relief and reducing risks in cardiovascular disease, as well as concerns of toxicity due to their potential to act as endocrine disruptors (Adlercreutz, 2002).

D-Pinitol is a compound of the soybean and the methyl ether of D-chiro-inositol found as large quantities in soy foods and it occurs in about 1% of dry weight of soybean meal (Phillips et al., 1982). It is an active low-molecular cyclitol isolated from the seed coat, cotyledon and embryo axis of
soybean seeds (Kuo et al., 1997). D-Pinitol is also known as 3-O-methyl-chiro-inositol, D-(+)-chiro-Inositol, Inzitol, D-(+)-Pinitol, (+)-Pinitol, Sennitol and Pinnitol. The role of D-Pinitol in plants is often associated with salt and drought stress (Keller et al., 1993), osmoprotectant (Paul et al., 1989), embryo development (Gomesa et al., 2005) and nodulation (Streeter et al., 1980).

D-Pinitol has been reported to have an insulin-like effects, driving creatine in addition to other nutrients into the muscle cells (Davis et al., 2000). D-Pinitol may also possess multifunctional properties. Shin et al.,(2004) have shown that pinitol has a protective effect on fatty changes, infiltration of inflammation cells and tissue necrosis of the liver induced by carbon tetrachloride in rats. Kim et al.,(2005) have reported the anti-inflammatory activity of pinitol against carrageenan and cotton pellet-induced acute and sub-acute inflammation in rats and in addition, it could prevent cardiovascular diseases. Furthermore, Lee et al., (2007) have demonstrated that pinitol could inhibit ovalbumin-induced airway inflammation in rats. D-pinitol has also Antiviral(Zhan et al.,2006) and Larvicidal activity (Chaubal et al.,2005).

Additionally, Sethi et al., (2008) have suggest that pinitol is not only effective as an anti-inflammatory agent but also as a therapeutic agent through regulation cell proliferation, apoptosis, invasion and angiogenesis in various human cell lines. Further, Sivakumar et al., (2010) are of the opinion that D-Pinitol has better anti-oxidant activity and also able to suppress the inflammation in diabetic treated rats. All of these functions are connected with the ability of pinitol to attenuate or suppress oxidative stress and the inflammatory process both in vitro and in vivo studies however suggest that pinitol is quite safe.

D-Pinitol is one of the natural therapeutic agents and has gained much attention due to their diverse biological activity. Hence, in the present
investigation D-Pinitol was selected as a compound from natural origin to evaluate anticancer potency.

![Figure:6 Structure of D-Pinitol (C$_7$H$_{14}$O$_6$)](image)

IUPAC name: (1S, 2S, 4S, 5R)-6-Methoxycyclohexane-1, 2, 3, 4, 5-pentol

Even though, D-Pinitol showed its various excellent therapeutic uses against various diseases and there is a paucity of information on the usage of D-Pinitol especially on DMBA induced mammary carcinoma in experimental rats. Therefore, it is of interest to investigate the anticancer property of D-Pinitol on DMBA induced mammary carcinoma and to provide the scientific rationale for use the drug D-Pinitol as an effective chemotherapeutic agent against breast cancer.

**CELL LINE STUDIES**

In general the *in vitro* cell culture is the most suitable for evaluating the plant-derived anticancer agents. In this connection, a cytotoxicity assay is a rapid tool to screen the plant-derived compounds before *in vivo* studies. Many researchers are concentrating in the area of programmed cell death in cancer treatment. Thus, chemicals that can modify apoptosis are likely to be potentially useful drugs.

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation - 7, referring to the institute in Detroit where the cell line was established by Herbert Soule *et al.*, (1973). MCF-7 cells are useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. Although easy to propagate the cells are generally slow-growing in nature. These include the
ability for MCF-7 cells to process estrogen in the form of estradiol via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line.

The MDA-MB-231 breast cancer cell line was obtained from a patient in 1973 at M. D. Anderson Cancer Center. With epithelial-like morphology, the MDA-MB-231 breast cancer cells appear phenotypically as spindle shaped cells. *In vitro*, the MDA-MB-231 cell line has estrogen receptor (ER) negative control cell line and an invasive phenotype. The MDA-MB-231 cell line is also able to grow on agarose, an indicator of transformation and tumorigenicity, and displays a relatively high colony forming efficiency. The MDA-MB-231 cells form mammary fat pad tumors in nude mice *in vivo*.

The two different cell lines (MCF-7 and MDA-MB-231) are suitable model to study and understanding mammary carcinogenesis and for drug targeting studies. Hence, in the present investigation both the human breast cancer cell lines such as MCF-7 and MDA-MB-231 were such in order to evaluate the anticancer effect of D-Pinitol.

**AIM AND OBJECTIVES OF THE STUDY**

Researchers are exploring ways to reduce the side effects of treatment and improve the quality of patient's lives and reduce the pain. The successful therapy of breast cancer will have to identify natural products that have significant chemotherapeutic and preventive potential without toxic effects. The aim of the present study has been to overcome these problems and to find out an alternative drug. The D-Pinitol may be used clinically in the process of cancer treatment, which also enable to develop as a promising chemotherapeutic adjuvant for the treatment of mammary cancer and could be subjected to human trials. Hence the following biochemical and some molecular changes were studied in both *in-vivo* and *in-vitro*. 

**IN VIVO STUDIES:**

**EFFECT OF D-PINITOL ON DMBA INDUCED MAMMARY CARCINOMA IN RATS**

The following parameters were analyzed in control and experimental groups.

- Body weight and tumor weight were estimated in the experimental groups.
- Cellular constituents like DNA and RNA were estimated.
- Lipid peroxidation, the unique primary property of the cancer cells was estimated by its product MDA.
- Enzymic and non-enzymic antioxidants which play a characteristic role in malignancy were assayed in control and experimental animals.
- As malignancies have hyperlipidemia as a secondary complication, hypolipidemia effect of the drug was assessed by evaluating the levels of lipids and activities of lipid metabolising enzyme in the treated and untreated conditions.
- Since malignant cells alter the membrane integrity and its functions, the membrane-stabilizing effect of D-Pinitol can be assessed by analyzing the activities of lysosomal enzymes, membrane bound ATPases and levels of glycoproteins in the diseased and treated animals.
- Microsomal enzymes, involved in carcinogen metabolism, were assessed to evaluate the detoxification efficacy of D-Pinitol.
- Carcinoembryonic antigen and CA 15-3 which are specific marker for breast cancer and was estimated in drug treated and untreated animals to assess the antineoplastic property of D-Pinitol.
- pro-inflammatory cytokines levels were estimated in control and experimental animals.
Estradiol and progesterone levels were determined in control and experimental animals.

Protein expressions of p53, Bcl-2, Bax, Caspase-3 and NF-κB were studied by Western blotting.

p53, Caspase-3 and NF-κB genes expressions were studied by RT-Polymerase chain reaction.

Immunofluorescence analysis of p53, Bcl-2, Caspase-3 and NF-κB were observed in breast tissue of control and experimental animals.

Histopathological changes of breast and liver tissues were carried out to assess the curative potential of D-Pinitol.

Transmission Electron Microscopic examination were studied in breast of control and experimental animals.

**IN VITRO STUDIES:**

**EFFECT OF D-PINITOL IN HUMAN MAMMARY CARCINOMA CELL LINES (MCF-7 AND MDA-MB-231)**

The following parameters were analyzed in control and D-Pinitol treated MCF-7 and MDA-MB-231 cells.

- Effect of D-Pinitol on cell viability (MTT assay) and LDH leakage assay to assess the cytotoxicity nature of D-Pinitol.
- Reduced glutathione (GSH) level was estimated.
- Light and fluorescence microscopic observations with reference to the morphological changes of MCF-7 and MDA-MB-231 cells.
- Mitochondrial membrane potential was carried out.
- DNA fragmentation was performed to study the apoptosis.
- Protein expressions of p53, Bcl-2, Bax and NF-κB were studied by Western blotting.