INTRODUCTION
“Hee is a better physician that hee keepes diseases off us, than that cures them being on us. Prevention is so much better than healing, because it saves us the labor of being sick.”

- Thomas Adams 17th century

Cancer is one of the most feared diseases that afflict our civilization since ancient times. Paleontologist have shown the presence of tumors in the bones of dinosaurs long before the advent of Homo sapiens. The ancient Egyptian knew of the existence of cancer in man as shown by the existence of bone tumors from autopsies of mummies.

The word Cancer has been actually derived from the Greek word “Karkino” means Crab and the great veins that sometimes surrounded the malady were compared to the claws of the crab.

Cancer is a disease of cells that occurs in all known species of higher animals. Human beings are known to suffer more than 100 forms of cancer in tissues, organs, blood and lymphatic system. It can occur during any time in the life of human being. As the life span is increasing the occurrence of cancer is also increasing.

Today cancer is a major disease accounting more than 7 million deaths per year worldwide (Ames et al., 1995). Currently cancer is the second leading cause of a death in the United States with an observed annual mortality rate increase that parallels the increasing incidence of the disease. (Lenhard. 1996: ACS. 1997).
More than a million cases of cancer are diagnosed annually in the United States and more than half million American die of cancer each year. Though there are many kinds of cancer only a few occur frequently (table 1).

**Table-I : Most Frequent Cancers in the United States**

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Cases per year</th>
<th>Deaths per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>317,000 (23%)</td>
<td>41,000 (7.4%)</td>
</tr>
<tr>
<td>Breast</td>
<td>186,000 (14%)</td>
<td>45,000 (8.1%)</td>
</tr>
<tr>
<td>Lung</td>
<td>177,000 (13%)</td>
<td>159,000 (29%)</td>
</tr>
<tr>
<td>Colon/rectum</td>
<td>134,000 (10%)</td>
<td>55,000 (9.9%)</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>60,000 (4.4%)</td>
<td>25,000 (4.5%)</td>
</tr>
<tr>
<td>Bladder</td>
<td>53,000 (3.9%)</td>
<td>12,000 (2.2%)</td>
</tr>
<tr>
<td>Uterus</td>
<td>50,000 (3.7%)</td>
<td>11,000 (2.0%)</td>
</tr>
<tr>
<td>Skin (melanoma)</td>
<td>38,000 (2.8%)</td>
<td>7,000 (1.3%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>31,000 (2.3%)</td>
<td>12,000 (2.2%)</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>29,000 (2.1%)</td>
<td>8,000 (1.4%)</td>
</tr>
<tr>
<td>Leukemias</td>
<td>28,000 (2.1%)</td>
<td>21,000 (3.8%)</td>
</tr>
<tr>
<td>Ovary</td>
<td>27,000 (2.0%)</td>
<td>15,000 (2.7%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>26,000 (1.9%)</td>
<td>28,000 (5.0%)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>1,156,000 (85%)</td>
<td>439,000 (79%)</td>
</tr>
<tr>
<td>All sites</td>
<td>1,359,000 (100%)</td>
<td>555,000 (100%)</td>
</tr>
</tbody>
</table>


Cancers of 13 different body sites account for approximately 85% of this total cancer incidence. The four most common cancers, accounting for
more than half of all cancer cases are those of prostate, breast, lung and colon rectum. Lung cancer by far the most common lethal is responsible for nearly 30% of all cancer deaths.

In India, 6-7 lakhs new cases of cancer occur every year and the prevalent of this disease has been estimated between 1.5 and 1.8 million. (Murthy, et al., 1990)

While recent advancements on cancer diagnosis and therapy have not significantly improved the quality of life for cancer patients. There is only a slight decrease in the mortality rates for most of the cancer patients (Heyne et al 1991; Ames et al 1995). These limitation highlight an urgent need to develop successful approach that may prevent the occurrence of cancer in the first place.

Cancer control, therefore, is the ultimate objective of cancer research, cancer medicine and cancer health services. Epidemiological and experimental studies in man have provided enough data on several aspects of cancer, which suggest that this disease is a multifactorial, multistaged and multi-mechanistic complex process. Several environmental role and host factors play an important role in it’s inception, progression and manifestation (Pitot and Dragan, 1994)

According to the reports of Doll and Peto (1981). 80% of all cancers are attributed to environmental factors. of which 30% are related to diet and nutrition.

The major environmental factors known to play important roles in the etiology of human cancer include chemical factors (like certain polycyclic aromatic hydrocarbons, aflatoxin B1, N- Nitroso compounds amino azo dyes, arsenic, nickel and cadmium and vinyl chloride etc.) physical factors (like
ionizing radiation and ultra violet radiation, between 280-380 mm wave length) and biological factors (oncogenic DNA and RNA viruses like human papilloma virus (HPV) human hepatitis B and C virus (HBV/HCV), herpes virus, human T cell leukemia virus (HTV) etc.

Thus the environment of man including sunlight, his diet, drinks and drugs are not free from carcinogenic potential. The omni presence of carcinogens give rise to misgiving that human race is immersed in uncontrollable sea of carcinogens.

The chemical carcinogens are extremely diverse in terms of their structure, chemical properties and biological effects. These chemical carcinogens exhibit various species and tissue selectivities. A few of them display profound specificity for certain tissues and others are more dangerous being multi potential carcinogens.

Most of the environmental carcinogens require to undergo metabolic activation that yield reactive electrophilic species which can interact with nucleophilic sites on target molecules of the cell to initiate neoplastic process (Miller and Miller. 1981).

Now a days polycyclic aromatic hydrocarbons (PAH) are the most ubiquitous carcinogen in the human environment. The major source of PAH in the environment arises from various forms of inefficient combustion of carbonaceous materials, cigarette smoking, coke production, fossil fuel combustion, internal combustion engines, like car aeroplanes, and other motor vehicles etc. generate complex polycyclic structures which contaminate drinking water, green vegetables and aquatic life (plankton molluses and fish). Fish with higher fat content have greater capacity to accumulate benzopyrene (Grasso and O'Hare, 1976).
Figure 1: The potential pathway by which environmental carcinogen exposure produces tumor initiation (Brandt-Rauf et al., 1990)
Fig. II: Metabolic pathways of Polycyclic Aromatic Hydrocarbons (PAHs)
The metabolic fate of these hydrocarbons in mammalian system has been studied extensively (Fig. II). Jerina and Daly (1976) and Sims et al. (1974) have shown that PAH become carcinogenic when it is metabolically activated by the complicated cytochrome P-450 containing mono oxygenase system which is also known as Aryl hydrocarbon hydroxylase (AHH) in order to be carcinogenic.

The development of cancer viewed as multistep involves mutations and selection for cells with progressively increasing capacity for proliferation, survival, invasion and metastasis. The complex process of chemical carcinogenesis has been operationally divided into three major phases initiation, promotion and progression (Pitot and Dragan, 1994). These phases or stages of carcinogenesis occur in sequence as a result of genetic and epigenetic events that takes place in the target cells. The main step of chemical carcinogenesis can be summarized as follows (Fig. III).

**Fig. III : Main Steps in Carcinogenesis**

- Neoplastic conversion
  - Chemical carcinogen
    - metabolic activation
    - Ultimate carcinogen
      - + DNA
      - altered receptor expression
  - Neoplastic development
    - Neoplastic cell
      - growth
      - promotion
      - Neoplastic cell
      - progression
      - undifferentiated cancer
1 The biotransformation of carcinogens:

Many chemical carcinogens that enter the body are actually quite innocuous pro-carcinogens. These are metabolically activated by cellular enzymes into ultimate carcinogens.

2 Alteration of Cellular macromolecules:

Alteration of cellular macromolecule due to the attack of highly reactive electrophilic ultimate carcinogens (positively charged) with nucleophile sites of DNA, RNA or protein (negatively charged). The resulting altered or damaged DNA is subject to removal and restoration by repair enzyme systems, while other altered macromolecules are either replaced or disposed off. The cells can be protected from carcinogenic insult at this stage.

3 Fixation of carcinogenic damage:

Fixation of carcinogenic damage happens if the cell replicates while the DNA damage or alteration persists. Permanent alteration in the genome can be produced by mispairing of bases leading to point mutations by erroneous replication yielding frame shift mutations, by transpositions resulting in codon rearrangement or by activating proto-oncogenes into oncogenes. (Cairns, 1981. Land et al 1983. Bishop, 1985).

4 Multiplication of cells with altered genome:

Multiplication of cells with altered genome leads to ‘preneoplastic lesion’ formation during which further alterations of DNA are possible. Thus, the formation of a fully neoplastic cell with an altered program of terminal differentiation is completed.
5. **Growth promotion of initiated cells:**

Growth promotion of initiated cells can be observed at the time when tumor promoters are applied. These produce tissue environment conductive to the selection clonal out growth of initiated cell resulting in a clinically evident premalignant lesion.

6. **Progression of premalignant cells to malignant:**

The final step in the development of cancer is the progression of neoplastic cells to form undifferentiated invasive cells (Williams and Weisburger, 1986). Progression is characterized by demonstrable changes in the neoplastic cells associated with increased growth rate, increase invasiveness, metastasis, biochemical and structural characteristic of the neoplasms. These correlate with changes in the number or arrangement of genes or with visible chromosomal alterations within majority of neoplastic cells that make up the tumour.

Malignancy can result in death because of damage to critical organs, starvation, secondary infections, metabolic problems, secondary malignancies and/or hemorrhage.

Most of the drugs currently used in cancer treatment either damage DNA or inhibit DNA replication. Consequently these damages are toxic not only to cancer cells but also to normal cells particularly those normal cells that are undergoing rapid cell division (e.g. hematopoietic cells, epithelial cells of the gastro intestinal tract, and hair follicle cells). The action of anti cancer drugs against these normal cell populations account for most of the toxicity associated with these drugs and limit their effective use in cancer treatment (Cooper, 1997)
The early detection which is necessary to manage the growth of cancer development is unfortunately delayed by the long period of time between the initial interaction of biological, physical and chemical carcinogens at the cellular level and the appearance of neoplasm. Therefore current trends are shifting more towards the preventive approach of cancer control.

Therefore research is being carried out on prevention of cancer. Cancer chemo prevention means control of the disease by the administration of one or more naturally occurring or synthetic chemical agents (Wattenberg 1985; Morse and Stoner. 1993; Kelloff et al., 1994; Kelloff et al., 1996a; Hong and Sporn 1997).

Among the naturally occurring chemopreventive agents, vegetables, fruits and common beverages as well as several herbs and plants with diversified pharmacological properties have been shown to be a rich source of micro chemicals with the potential to prevent human cancer (Kelloff et al., 1994; Kelloff et al., 1996a; Birt et al., 1996; Lipkin. 1997; Hong and Sporn, 1997). A list of the nutritional influences on the various stages of carcinogenesis induced by chemicals is given in Fig. IV.

Various tumor model systems like skin, lung, breast and cervical have been developed to screen the efficacy of chemo preventive agents in vivo. The mouse skin tumor model systems possibly, in human is a step wise process of at least three distinct stages initiation, promotion, and progression (Agarwal and Mukhtar. 1991; DiGiovanni. 1992; Yuspa. 1994).

For more than 50 years, mouse skin has been used as a conventional model to study the mechanism of carcinogenesis and modulation of sequential steps involved in this process. For the experimental protocol in our study the mouse skin model system was chosen for multifarious reasons.
**Fig. IV : Aspects of the Carcinogenic Process that might be affected by Nutrition**

<table>
<thead>
<tr>
<th>Probable Actions of Carcinogens*</th>
<th>Possible Sites of Nutritional Influence</th>
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<tbody>
<tr>
<td>Carcinogen</td>
<td>Dietary carcinogenesis</td>
</tr>
<tr>
<td>Activation</td>
<td>Induction and inhibition of activating enzymes</td>
</tr>
<tr>
<td>Electrophilic reactant</td>
<td>Detoxification of reactive carcinogenic compounds</td>
</tr>
<tr>
<td>Covalent binding of carcinogen to critical cellular macromolecules</td>
<td>Interference with binding to macromolecules</td>
</tr>
<tr>
<td>Initiation</td>
<td>Modulation of macro molecular damage and repair</td>
</tr>
<tr>
<td>Specific alterations in critical macromolecules</td>
<td>Inhibition of the action of tumor promoters</td>
</tr>
<tr>
<td>Promotion</td>
<td>Modulation of growth control required tumor growth factors</td>
</tr>
<tr>
<td>Expression of altered cellular information</td>
<td></td>
</tr>
<tr>
<td>Growth of altered cells</td>
<td></td>
</tr>
<tr>
<td>Evolution</td>
<td>Influence of diet on tumor behaviour</td>
</tr>
<tr>
<td>Autonomy</td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
</tr>
</tbody>
</table>

* Adapted from (Miller & Miller. 1979)
Introduction

Firstly it has well and clear defined initiation and promotion stages, secondly, the tumor have a relatively short latent period (time lage between the application of the promoting agent and appearance of 50% of tumors). Thirdly, the tumor development can be observed by naked eye and so the animals need not be sacrificed to score tumor. Finally the tumors are non lethal and the number of tumors developed on the skin give an indication of the efficacy of the compound (phytochemical) as potential chemo preventive agent.

In the mouse skin tumourgenesis model, initiation stage is achieved by using a single dose of carcinogen initiating agent, 7,12 dimethyl benz(a) anthracene (DMBA) and promotion stage by repeated applications of tumor promoter (croton oil) following the tumor initiation.

Ingested environmental chemical careingens need to be eliminated from the body. They produce toxic symptom as such or after being transformed in the body. Metabolism of xenobiotics by bio transformation enzymes, is directed at converting lipophilic metabolites/chemical species which can be excreted.

Two classes of enzyme systems have been proposed by William, 1971 for the bio transformation of xenobiotics to hydrophilic metabolites. These are called as the phase I and phase II reactions. Phase I reactions which involves oxidation, reduction and hydrolysis and Phase II reaction, which consist of conjugation or synthetic reactions (Fig. V).

Phase I reaction (functionalization ) introduce or add functional group or polar group (e.g. -OH, -SH, -NH₂, -COOH) into the xenobiotics/ substrate molecules. These reactions are mainly oxidative in nature. Oxidation is the most important reactions of phase I.
Fig. V: Integration of Phase-I and Phase-II Biotransformation Reactions

Phase I

- Xenobiotic
  - Oxidation reductions hydrolysis
  - Exposure or add functional groups
  - Primary product

Phase II

- Secondary product
  - Biosynthetic conjugation
  - Excretion
  - Hydrophilic (Ionizable)

Phase II reactions are biosynthetic in nature where the compound xenobiotic covalently linked with hydrophilic endogenous compound in producing conjugation. Primarily consist of conjugation of polar groups of xenobiotic with hydrophilic endogenous compounds or moieties (water soluble substances) such as glutathione, glucuronic acid, sulfate, glycine, producing water soluble conjugates that can be excreted.

Thus the introduction of a polar group during phase I reactions make it possible for a conjugation reaction to take place in phase II. The products of phase II reactions are highly water soluble and are therefore readily excreted by the organism.

The conjugation moieties or compounds are normally added to endogenous products to promote their secretion or transfer across hepatic, renal and intestinal membranes.

In a number of cases, high reactive metabolites are formed during the biotransformation of chemicals. These reactive metabolites are thought to initiate the events that ultimately result chemically induced cancer and other toxicities.
The biotransformation enzymes are mainly located in cells of tissues through which foreign compounds can be absorbed into the body; namely, skin, gastrointestinal tract, liver, lung and kidney. The liver is the key organ where bulk of the xenobiotic metabolism occur.

Phase I enzymes are located in the endoplasmic reticulum. These reactions may add functional groups by two major oxidative enzyme systems the cytochrome P-450 system (which is also referred to as the mixed function oxidases and the mixed function amine oxidase which is flavin mono-oxygenase).

The phase II reactions are biosynthetic and energetic to drive the reaction. This is accomplished by activating the cofactors like uridine-5'-diphospho-D-glucuronic acid (UDPGA), acetyl coenzyme A,3'-phospho adenosine-5-phosphosulfate (PAPS), S-Adenosyl-methionine (SAM) also, as these cofactors activated by ATP, the energy status of the organ, is important in cofactor availability.

Several plant components which are naturally available, by modifying the cell biotransformation enzyme profile, have demonstrated a decline in the incidence and mortality owing to certain types of cancers in high-risk human population. (Pautuck et al. 1979; Coldin et al. 1985; Boon et al. 1990; Yang et al. 1992). Modulation of phase-I and phase-II enzyme activity significantly affect the end point owing to a carcinogenic insult. Some frequently consumed spices, condiments, vegetables and other plants in India, Japan and other Asian countries have been claimed to exhibit potentials for anti-carcinogenicity and other benefits.  

ethanolic extract of Ocimum sanctum, Liv.-52 (a herbal preparation) and Spirulina have shown chemopreventive properties in skin tumor model system (Prashar et al., 1994; Prashar and Kumar, 1994; Prashar and Kumar, 1995; Mittal et al., 1998).
Mustard Seeds are widely used in the preparation of variety of edible sauces, pickles and pastes. The oil derived from the mustard seed is extensively used as a cooking medium in several countries and is also used for anointing the body.

Many edible species of the Brassicaceae family have been documented to possess cancer chemopreventive potential in rodents using various experimental protocols (Beecher, 1994; Nugon-Baudon and Rabot, 1994). Their modulatory influence on the process of carcinogenesis results from alterations in the level of enzymes that catalyze biotransformation of endogenous as well as exogenous chemical substances including carcinogens. The active phytochemicals, present in several members of the Brassicaceae family that lead credence to chemoprevention include indole glucosinolates aromatic isothiocyanates, dithiolthione and phenols (Nugon-Baudon and Rabot, 1994).

Thus, in the present study mustard seed extract of Brassica compestris (var sarason) has been selected to investigate its chemopreventive action on the DMBA induced skin carcinogenesis in male Swiss albino mice.