Review of Literature
2. REVIEW LITERATURE

2.1 CERVICAL CARCINOGENESIS

Cervical carcinogenesis is the second most common cancer of the reproductive system in women worldwide, and the most common in women under 50 years of age. It most commonly affects women between 35 and 55 years old (figure 2.1).

2.1.1 Uterine cervix in human beings

Figure 2.2 is a schematic presentation of female reproductive system (human beings). The human uterus is a pear-shaped muscular organ, about 7 cm in length, 4 cm in width, and 2-3 cm in thickness. The upper part receives on each side the openings of the oviducts. The uterine cervix is the lower, tubelike segment of the uterus. Its central cavity, the cervical canal, about 3 cm in length, is continuous, above, with the uterine cavity through a constriction known as the internal os, and opens below into the upper portion of the vagina through the external os. The wall of the cervix consists of three layers: endometrium, myometrium and perimetrium. Endometrium can be differentiated in three parts: endocervix, ectocervix and squamocolumnar junction.

Endocervix: It lined by simple columnar mucus-secreting epithelium that dips down into the underlying stroma to form the cervical glands which secret mucus. The mucosa displays numerous longitudinal and transverse folds called the plicae palmate. It does not slough off during menstruation, although the glands undergo some change in secretory activity.

Ectocervix: This is a vaginal portion covered by stratified non-keratinizing squamous epithelium (figure 2.3).

Squamocolumnar junction: It is also called transformation zone. It is an abrupt border between the stratified squamous and the simple glandular epithelium, usually occurring just inside the cervical canal. Here is where most of the carcinomas in the cervix are developed.
Fig 2.1: Five year age group distribution- cancer cervix
(Source: National Cancer Registry Programme, 1994-1998. ICMR)
Fig 2.2: Female reproductive system (human beings)
Fig 2.3: The different histologic layers of the normal cervical stratified squamous epithelium
2.1.2 Uterine cervix in murine model system

The murine uterus is comparable to the human one (figure 2.4). The uterus of mouse consists of two uterine horns which join at the lower end and form the uterine corpus or body. Then it is narrowed to form the uterine cervix, and it is followed by the vagina. The two lumina of the uterine horns continue inside the corpus where it remains separated by a midline septum. These lumina then join and form the cervical canal.

The histology is essentially the same that the one in human cervix. The lumina of the uterine horns and upper one-half of the corpus is lined with simple columnar epithelium with simple branched and tubular uterine glands projecting down from the lumen; while the rest of the portion of the corpus, the cervical canal and the projected portion of the cervix into the vagina are made up of stratified squamous epithelium.

Experimental carcinogenesis in animal model systems is of great importance in cancer research since it helps to find out the different events which take place in the process of carcinogenesis in both cellular and molecular levels. It also helps to develop new chemopreventive agents in humans.

Due to the similar histology and physiology of the cervix between the human and the murine system, the former one appears as an excellent model system for research in cervical cancer and has been used by many (Rao, 1989; Hussain and Rao, 1992 a and b; Anisimov et al, 2000; Boer, 2001; Lu et al, 2001).

2.1.3 Geographical distribution

International comparisons of age adjusted incidence rates (AAR) for cervical cancer are given in figure 2.5. The incidence of cervical cancer is higher in developing countries like some of Central and South America, Central and South Africa and South-East Asia.
Fig 2.4: Internal structure of adult murine uterine cervix

Solid lines: Stratified Squamous Epithelium
Broken lines: Simple Columnar Epithelium

Endocervical Canal

Upper Vagina

Uterine Corpus

Uterine Cervix
Fig 2.5: International comparisons of age adjusted incidence rates (AAR) for Cervical Cancer
(Source: National Cancer Registry Programme, 1990-1996. ICMR)
Among all these areas, the higher incidence is reported in Colombia and the north-east of Brazil. This may be due to the lower level of social-economic conditions which is one of the risk factors for cervical carcinoma.

The incidence is also quite high in some developed countries like New Zealand (only among the Maori population), East Germany, Romania, Singapore and Hong Kong. In other countries, the cervical cancer incidence is surprisingly low, as in the case of Nigeria, Zimbabwe, Switzerland, non-Maori population of New Zealand, Israel and Japan (National Cancer Registry Programme, ICMR, 2001). Few women in Israel have cervical cancer which could be due to the customs of Jewish population such as monogamy and non-use of contraceptives. Polygamy and use of contraceptives are definitely risk factors for cancer of cervix.

The low incidence found in Japan could be ascribed to their high intake of green tea in diet. The modulatory effect of green tea in cervical carcinoma have been tested in this work of research. In India, cancer of cervix is the most common cancer among women. Madras, Bhopal, Delhi, Bangalore and Barshi have the highest incidence and Jammu and Kashmir has the lowest one (Das, 2000). The trends in cervical cancer from 1994 to 1998 at different centres for National Cancer Registry programme, is shown in figure 2.6.

2.1.4 Process of cervical carcinogenesis

The carcinogenesis consists of three stages. Quite often they overlap. These stages are schematically shown in figure 2.7 and briefly described below.

a) Initiation: It is an interaction of an ultimate carcinogen with the DNA leading to genetic damages which usually are repaired by repair enzyme systems restoring the normal conditions. However, sometimes the replication of the altered DNA leads to the fixation of the carcinogenic damage by causing mutation in specific genes which initiates the process of carcinogenesis. Therefore, cancer is believed to be a mutational event (Weinberg, 1989). The stage of initiation is irreversible and fast.
Fig 2.6: Trends in actual numbers – cancer cervix
(Source: National Cancer Registry Programme, 1994-1998. ICMR)
Fig 2.7: Process of Cervical Carcinogenesis
b) **Promotion**: It has been known that some of the compounds which are non-carcinogenic agents can enhance potential of a carcinogen to induce cancer. These agents could be endogenous or exogenous chemical compounds and enhance the tumor incidences only when administered after a carcinogen. Croton oil is an excellent example of such group of compounds. The promoters selectively increase the proliferation of initiated cells. This process is reversible and epigenetic (Tanaka, 1994).

c) **Progression**: It is the development of the transformed cells to cancer cells. This stage is characterized by changes in the neoplastic cells like a major autonomy from both environment and the host, increase of growth rate, increased invasiveness and metastasis. This is an irreversible process and it is associated with an increased frequency of genetic alteration.

The process of carcinogenesis can be very fast or slow, and even it may take the major part of the life span of the individual. The transitions between successive stages can be enhanced or inhibited by various agents.

**2.1.5 Precancerous lesions, stages of cervical carcinogenesis and histopathological criteria for classification**

The cancers that develop from uterine cervix are of two types namely squamous cell carcinoma (SCC) and adenocarcinoma. Squamous cell carcinomas (SCC) develops from squamous epithelium and represents about 85-90 % of all the cervical cancers. Adenocarcinoma arise from glandular lining of endocervical canal and represent the 10-15 % of the cancers developed in the cervix.

Squamous cell carcinoma is preceded by well-recognized epithelial changes, the precancerous lesions, which develop through several grades (figure 2.8) mentioned below:
Cervical Intraepithelial Neoplasia (CIN)

- Normal
- CIN I
- CIN II

Diploid or polyploid cell population.

Appearance of atypical cells in the lower layers of the squamous epithelium, with persistent and abnormal differentiation towards the higher cell layers. N/C increases, loss polarity, more mitosis, more abnormal mitosis, hyperchromasia.

Stage 0 = CIN III (carcinoma in situ, non-invasive carcinoma)

More loss of differentiation involving more layers, till no surface of differentiation.

Invasive cervical carcinoma

- Stage I: early stromal invasion with the cancer confined to the cervix
- Stage II: cancer involving the cervix and the upper part of the vagina
- Stage III: cancer involving the cervix, entire vagina and the pelvic wall
- Stage IV: cancer involving surrounding organs or with distant metastasis

Fig 2.8: Process and Stages of Cervical Cancer
a) **Mild dysplasia or Cervical Intraepithelial Neoplasia I (CIN I):** In the lower layers of the epithelium a tetraploid (4c) cell population appears and the proliferation rate increases.

b) **Moderate dysplasia or CIN II:** The proliferation rate of cells is increased as well as the number of tetraploid cells and the nuclei/cytoplasm ratio (N/C). There is hyperchromasia. Atypical cells appear in the 2/3 lower layers of the squamous epithelium, with persistent and abnormal differentiation toward the higher cell layers, there is loss of polarity in the epithelium.

c) **Severe dysplasia or CIN III:** High proliferative activity; the tetraploid population in the mild and moderate dysplasia is shifted increasingly to an aneuploid cell population with until 8 dotations of DNA (8c) indicating pronounced genetic instability.

The process of cervical carcinogenesis can be differentiated into several stages (figures 2.8 and 2.9):

a) **Carcinoma in situ (CIS):** The loss of cell differentiation involves more layers until it affects the whole thickness of the epithelium. The basal layer of the epithelium is intact and the cancer cells are confined within the epithelium (Heselmeyer et al, 1996). CIS is usually known as Stage 0 of cervical carcinogenesis.

b) **Invasive carcinoma:** Increasing number of chromosomal aberrations can be seen, the basal layer is broken and the cancer cells invade the surrounding and distant organs (metastasis). This metastasis can be differentiated in four stages.

Stage I: early stromal invasion with the cancer confined to the cervix

Stage II: it involves the cervix and the upper part of the vagina

Stage III: involving the cervix, the entire vagina, and the pelvic wall.

Stage IV: there is metastasis, it involves surrounding organs and distant ones.

These above mentioned histological process of cancer development are shown in figure 2.10 for clarity.

Hyperplasia is another indicator of abnormal but non-pathological growth of the epithelium. It consists of an increased mitosis rate and thicker epithelium. Although in
Fig 2.9: Comparison of Normal, Pre-cancer and Cancer histology
All normal cells (nc) 

Basement membrane (bm) 

INITIATION

PROMOTION

PROGRESSION

INVASION

PRENEOPLASTIC CELLS

MALIGNANT CELLS

MALIGNANT cells

METASTASIS

INITIATED CELLS

Radiations

Chemicals

Viruses

Basement membrane (bm)
hyperplasia the number of cells are increased they are normal. Under some specific conditions the epithelium may become hyperplastic but later on regresses to the normal thickness. Hyperplasia has also been examined in the present work.

2.1.6 Different nomenclatures

The three different nomenclatures for cervical abnormalities are given in Table 2.1. An appropriate way of classifying the precancerous intraepithelial lesions was the concern of many until the Bethesda System was introduced in the year 1988 (Wright and Kurman, 1996). The Bethesda System of classification correlates with the previous nomenclatures (which are still in use) as given below:

Low-grade squamous intraepithelial lesion (LSIL): It covers mild dysplasia, or cervical intraepithelial neoplasia 1 (CIN 1), as well as koilocytosis, flat condyloma and koilocytic atypia.

High-grade squamous intraepithelial lesion (HSIL): It covers moderate dysplasia, severe dysplasia and carcinoma in situ, or CIN 2 and CIN 3.

For lesions of undetermined significance, the term ASCUS (atypical squamous cells of undetermined significance) is used (Kruger-Kjaer et al, 1998).

2.2 INDUCTION OF CERVICAL CARCINOGENESIS IN MODEL SYSTEMS

Cervical carcinogenesis has been induced in a number of animal species such as mice, rats and rabbits. However mice is the most commonly used. Cervical cancer is induced using various carcinogens and different methods. Hormones, different chemicals and virus are used to induce cervical cancer.

2.2.1 Hormones: Loeb et al (1936) demonstrated the possibility of inducing cervical carcinoma in mice by the prolonged systemic treatment of estrogenic hormones. Subsequently, many others have induced cervical tumours in mice using hormones as
Table 2.1: Three different nomenclatures for cervical histological abnormalities

<table>
<thead>
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<th>Hyperplasia</th>
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<th>Dysplasia III</th>
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<td>Hyperplasia</td>
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<td>CIS</td>
<td>Invasive Carcinoma</td>
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<tr>
<td>3</td>
<td>Hyperplasia</td>
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CIS: Carcinoma in situ
CIN: Cervical intraepithelium Neoplasia
LSIL: Low-grade Squamous Intraepithelial Lesion
HSIL: High-grade Squamous Intraepithelial Lesion
carcinogens. Gardner (1959) induced cervical and vaginal tumors using stilbestrol-cholesterol pellets attached to nylon thread and dipped in collodion, and then placed in the vagina. The tumors were also induced after intravaginal installation of stilbestrol three times weekly. Van Nie (1961) also reported the induction of tumors in 26 out of 42 mice in about fifteen months time by subcutaneous implants of testosterone pellets twice a week in C57B1 x DBA female mice. Some of the other hormones used in laboratory animals to induce cervical carcinogenesis are 17 β-estradiol, diethylstilbestrol and chlormadinone (Rose, 1976).

2.2.2 Chemicals: Several chemical compounds have been used to induce cervical tumors in animals. Benzo[a]pyrene (BP), methylcholanthrene (MCA) and dimethylbenzanthracene (DMBA) are most commonly used carcinogens (figure 2.11). There are two methods i.e. string and painting to induce cervical cancer.

The string method has been developed by Murphy (1953) and Vellios and Griffin (1955). Placement of MCA plus beeswax (in the ratio 1:3)-impregnated threads in the vagina of C3H mice led to the occurrence of malignant neoplasm of the cervix. In almost all the animals the carcinogenesis involved the cervix and there was metastasis to the uterus and upper vagina. A few showed metastasis to paraaortic lymph nodes, adrenal and lung (Reagen et al, 1955).

The painting of BP solution in acetone on the cervix with cotton tipped wire loops was shown to induce cervical tumors (Von Haan and Scarpelli, 1955). Glucksman (1971) induced cervical tumors using the painting method with DMBA.

When compared the string and painting methods with MCA and with BP in CaH female mice, it was observed that MCA-string method gave the highest incidence of cervical tumors followed by BP string (Scarpelli and Von Haan, 1957). The main advantage of the string method has been the localization of tumors in the cervix only, while painting could give many vaginal lesions in addition to cervical tumors. Many tumors were invasive and extended to pelvic nodes, with metastasis seen in lung and liver. The lesions developed,
(BP) Benzo[a]pyrene

Active metabolite of B(a)P

(MCA) 3-Methylcholanthrene

Active metabolite of 3-Methylcholanthrene (9, 10-diol-7,8 epoxide form)

(DMBA) 7, 12-Dimethylbenz(a)anthracene

Active metabolite of 7, 12-Dimethylbenz(a)anthracene (3,4-dihydrodiol 1,2 epoxide form)

Fig 2.11: Chemical structure of some chemical carcinogens
showed a marked resemblance to human cervical cancer. DMBA has also been used to induce cervical tumors using knotted string method (Vellios and Griffin, 1955) or painting (Glucksman, 1971).

Since the last two decades, the method most commonly used for induction of cervical cancer in mice has been the string impregnated in a mixture of beeswax and methylcholanthracene (MCA). The investigations carried by Hussain and Rao (1992 a and b) using 300 µg and 600 µg of MCA per animal and getting 60.5% and 72% of tumors respectively are excellent examples; Hussain et al (1990), Sengupta et al (1991) and Das et al (1988) also reported similar results.

2.2.3 Virus: Herpes Simplex Virus type 2 (HSV-2) has been used in an attempt to induce cervical carcinomas. HVS-2 were inoculated in the reproductive tract of the female mouse with the help of formalin. Mucosa alterations and tumors were observed in the animals. Non invasive lesions of the cervix were identified in 76.8% and invasive adenocarcinoma detected in 30.2% of the mice (Wentz et al, 1975). However in other studies this attempt was not successful (Sever, 1973). Rabbits infected by applying the virus to the cervix with the help of cotton wool swabs died with paralysis and encephalitis. Munoz (1973) also used HSV-2 in mice without success.

2.2.4 Radiation: Several generalizations about radiation carcinogenesis can be made: 1) a single exposure is sufficient to elevate cancer incidence many years later; 2) radiation-induced cancer cannot be distinguished from naturally occurring cancer, which means that there is not unique radiogenic cancer; 3) leukemia is the most prominent radiogenic tumor followed by breast and thyroid; 4) age at radiation exposure is probably the most important host factor influencing subsequent cancer risk; 5) the percentage increase in cancer incidence per rad is not the same for all cancers; 6) dose-effect curves are often linear, but curvilinearity is also observed; 7) interestingly, although most cancers appear to be increased after irradiation, cervical cancer represents an exception together with chronic lymphocytic leukemia, possibly Hodgkin’s disease and few others (Boice, 1981).
2.3 CAUSES OF CERVICAL CARCINOGENESIS IN HUMAN BEINGS

2.3.1 Human Papillomaviruses (HPV)

For over a century, it has been believed that cervical cancer is associated with sexual behavior, indicating the involvement of a sexually transmissible infectious agent. At the beginning, for many years, herpex simplex virus type 2 (HSV-2) was considered as the possible candidate.

This believe hinged on the presence of serum antibodies against HSV-2 in women, and the knowledge that these viruses produced lymphoproliferative disease in chickens and an adenocarcinoma in frogs. Due to the widespread nature of the virus the appearance of serum antibodies in women is common. However, extensive controlled studies, involving 10,000 women with cervical cancer, have revealed no correlation between the neoplasm and herpex simplex antibodies. More studies showing the absence of HVS-2 DNA in most cervical tumors ratify that HSV-2 was not directly involved in cervical cancer development.

Although papillomaviruses particles were first observed by electron microscopy in human warts in 1949, the possible involvement of human papillomavirus (HPV) in human cervical cancer was investigated only in the early 1980s. HPV genomes were cloned from cervical carcinomas and genital warts (Gissman and Hausen, 1980; Gissman et al, 1982; Durst et al, 1983; Boshart et al, 1984), and the causal role of specific types of HPV in cancer of cervix and their precursor lesions was finally established (Doeberitz et al, 1992; Munoz et al, 1992). Walboomers et al (1999) demonstrated the presence of HPV DNA in almost all invasive cervical carcinomas.

The current epidemiological data strongly supports that HPV infection is the primary risk factor playing a central role for development of invasive human cervical cancer as well as a variety of benign lesions, warts and intraepithelial neoplasia (Das et al, 2000; Kirwan and Herrington, 2001). Recently several case-control studies have shown a greater odds ratio for the association between HPV and cervical carcinoma than for smoking and lung
cancer (Kirwan and Herrington, 2001). HPV DNA is present in at least 90% of the cervical cancers (Yoshinouchi et al, 1999; Franco et al, 2001). However, HPV while being necessary it is not a sufficient cause of most cases of cervical cancer and the association of other risk factors are needed (zur Hausen, 1991; Green et al, 2003).

Current research has focused on the determinants of infection with oncogenic HPV types, the assessment of prophylactic and therapeutic vaccines and the development of screening strategies incorporating HPV testing and other methods as adjunct to cytology.

### 3.1.1 Different HPV types involved in cervical carcinogenesis

Classification is usually based on partial sequencing of the E6 and/or L1 region of the virus. Different HPV types have less than 90% homology, subtypes 90-98% and variants less than 2% variance (Van Ranst et al, 1993).

There are over 200 types of HPV, of which approximately 50 infect the genital area (Heley, 2003). There are some HPV associated with cervical cancer. HPV types 16 and 18 are considered to be the high risk types and are associated with anogenital invasive tumors and their precursor lesions (Durst et al, 1983; IARC monographs, 1995). HPV types 6 and 11 are the low-risk types which rarely progress to malignancy and are mainly associated with benign growths such as genital warts and condylomas (Gissman et al, 1982). Globally, the geographical distribution pattern of HPV appears to be similar in different countries: 60 to 65% positivity for HPV 16; 4 to 20% for HPV 18; and a low prevalence of other HPV types (Das, 2000).

#### 3.1.2 HPV genome

HPV belong to a large family of DNA tumor viruses: papovaviridae. Papillomaviruses are small DNA viruses, approximately 55 nm in diameter consisting of a non-enveloped cosahedral outer protein coat of 72 capsomeres, which surrounds a circular genome of double stranded DNA. The genome consists of approximately 8,000 base pairs replicating as an episome in the nucleus of host cells.
HPV genome is divided into early (E1, E2, E4, E5, E6, E7) and late (L1 and L2) genes. The early genes are responsible for DNA replication, transcriptional regulation and transformation. Early genes products E6 and E7 encode the major transforming proteins. The late genes control the formation of the capsid coat. The early and late genes are separated by a transcriptional long control region (LCR). It contains viral promoters as well as several enhancer elements which control viral replication and transcription of E6 and E7 genes, leading to malignant transformation and maintenance of tumorigenic phenotype (Das, 2000).

2.3.1.3 Infection, replication and transmission of HPV

HPV are epitheliotrophic and are responsible for several mucous and skin lesions (Villers, 1998). HPV, particularly the high-risk types, have been detected in more anogenital cancers besides the cancer of the cervix. Several additional human cancers too have been linked to human papillomavirus infection. The presence of HPVs in tumors of oral cavity, larynx, tonsil, nasal sinus and anogenital carcinomas including penile and anal carcinomas have been recorded in 20-60% cases (Das, 2000).

The vast majority (over 80%) of HPV infections are transient, being cleared by the immune system within a few months without any residual detectable lesion. The remaining 20% persist and go on to promote the development of cervical intraneoplasia (CIN). Again most of these cells are able to clear the virus and therefore sanction regression of these low grade lesions, often with seropositivity. This regression is associated with low risk HPV and is age-related being more common in the under 30’s. In older women, persistence and subsequent progression to a high grade lesion are more frequent. Approximately one-third of such high grade lesions go on to invasive disease (Kirwan and Herrington, 2001). The relative risk for women with HPV 16/18 developing CIN is 11 compared to women without HPV infection (Koutsky et al, 1992).

Infection is initiated when a virus gains entry to the basal cells of the epithelium. Minor trauma, as would occur in sexual intercourse, allows access to target cells at or near the
cervical transformation zone, possibly through small abrasion in the tissue. The ensuing virus life cycle is then linked to keratinocyte differentiation. HPV infection involves coordinated expression of early viral proteins in lower epithelial layers with a switch to late gene expression as viral replication takes place leading to koilocytosis, nuclear enlargement, multinucleation, dyskeratosis and in some cases squamous intraepithelial neoplasia. The viruses multiply exclusively in the nuclei of infected cells and are then released with exfoliation of the surface epithelium (Southern and Herrington, 1998; Stoler, 2000).

A clonal growth of HPV infected cells showed a requirement for a highly specific intracellular environment for the development of lesions, possibly provided by cells that had undergone specific genetic alterations, since presence of HPV DNA has been demonstrated in clinically symptom-free epidermal and mucosal sites of cervix, larynx and skin (Das, 2000).

Viral gene expression is generally regulated by several viral and host-cell transcription factors, which bind to the LCR. These factors include nuclear factor-1 (NF-1), activator protein-1 (AP-1), octamer-binding factor-1 (Oct-1), progesterone receptor, Yin and Yang factor-1 (YY-1), SP-1, KRF-1 and glucocorticoid receptor, etc. (Bauknecht et al, 1992).

The clinical manifestations of HPV infection depend on the viral subtype and molecular variant, the HPV load, persistence of HPV infection, the immune status of the patient, and environmental co-carcinogens (like coinfection with other sexually transmitted disease agents) (Mayrand et al, 2000).

Anogenital infections are mainly transmitted by sexual contact since HPV DNA is rarely detected in sexually inexperienced young women. Anogenital HPV are also transmitted digitally from one epithelial site to others. Skin infections by HPV appear also from contacts with contaminated materials, walking barefoot on an abrasive surface, or by acquiring accidental epithelial wounding with contaminated equipment (Das, 2000). There is discrepancy in the opinions regarding the possibility of maternal-fetal virus transmission.
2.3.1.4 Physical state in the host cell

When the HPV enters an epithelial cell of the basal layers it is in episomal form. Integration of HPV DNA into host genome occurs early in cancer development and is probably an important event in malignant transformation of cervical cancer. The frequency of integration of the virus genome into host chromosome, regardless of type, correlates with lesion grade, being rare in CIN I, common in CIN III (Southern and Herrington, 1998), and an event in almost all cervical carcinomas (Yoshinouchi et al, 1999). During a common infection and in most premalignant lesions HPV is in an episomal state. However, most cervical carcinomas maintain the HPV genome in an integrated form, or both integrated and episomal forms (Cullen et al, 1991; Lukaszuk et al, 2003). Thus, integration has been proposed as an activation mechanism for progression from preinvasive to cervical cancer (Cullen et al, 1991; Bosch et al, 1995; Vernon et al, 1997). May be due to that, integration provides a selective advantage to cervical epithelial precursors of cervical carcinoma (Jeon et al, 1995). Few viral integration sites have been mapped (Choo et al, 1987; Tomlinson and Bodmep, 1995).

Integration of HPVs usually disrupts or deletes the E2 open reading frame, which results in the loss of expression of the corresponding gene products. Disruption of E2 gene also leads to overexpression of the E6 and E7 oncoproteins (Durst, et al, 1985; Jeon and Lambert, 1995), since the E2 gene product can repress activities from the HPV promoters that direct the expression of the E6 and E7 genes (Romanczuk et al, 1990; Thierry and Howley, 1991).

Figure 2.12 describes the types, HPV genome, infection and physical state in host cell of the human papillomavirus.

2.3.1.5 Genetic changes associated with HPV infection

HPV can transform the epithelial cells to a malignant phenotype through activation of cellular oncogenes and/or loss of tumor suppressor genes (Southern and Herrington, 1998). Changes in almost all chromosomes have been shown in cervical intraepithelial
1. **TYPES:**
   - HPV
     - Low-risk types: 16, 18
     - High-risk types: 6, 11

2. **HPV GENOME:**

   ![HPV Genome Diagram]

   Exemplified by HPV 18
   (Source: Das et al, 2000)

3. **INFECTION:**

   ![HPV Infection Diagram]

   Basal layer
   
   Growth direction of the epithelium

4. **PHYSICAL STATE IN HOST CELL**

   ![HPV Physical State Diagram]

   Basal cell (HPV in episomal form)
   
   HPV DNA integration in host genome
   
   Cancer Cell

**Fig 2.12: Human Papillomavirus (HPV)**
neoplasias, with the number of chromosomal aberrations increasing with stage and grade of disease (Lazo, 1999). Several studies have identified loss of heterozygosity on chromosome 3p (Rader et al, 1990; Mullokandov et al, 1996) and gain of chromosome 3q in progression from CIN to invasive disease (Heselemeyer et al, 1996). There is also evidence that activation of oncogenes, such as c-Has-ras, is important in progression of malignancy (Riou et al, 1988). Abnormal DNA methylation patterns have been observed in tumors of cervix (Li et al, 2003).

The E6 oncoprotein of high-risk type HPV 16 has been shown to bind to the p53 tumor suppressor gene and stimulate its degradation by a ubiquitin-dependent protease system leading to apoptosis failure and carcinogenesis (Moodley et al, 2003). The low-risk E6 proteins lack this activity although they bind p53 (Tommasino et al, 2003). The E7 protein of high-risk type HPV 16/18 forms a stable inactivating complex with retinoblastoma-susceptibility gene (Rb) product, p105 (Das, 2000).

E6 and E7 oncoproteins activate telomerase activity leading to maintenance of telomere length and thus cell immortalization. Snijders (1998) was able to identify telomerase activity in 96% of invasive of squamous carcinomas and in 40% of CIN III, but not in lower grades of CIN, suggesting activation may be linked to progression.

2.3.2 Risk factors related to HPVs infection

Epidemiological studies did demonstrate the association of several risk factors with the development of cervical cancer. Some of them are clearly related to the sexual transmission and infection of HPV:

a) Early age at first intercourse: In India, the association of the infection of high risk HPVs with the age of marriage below 18 years has been found to increase the risk of cervical carcinoma by 22 fold (Das, 2000). Kruger-Kjaer et al, (1998) published an interesting study where they showed that not only young age at first intercourse of the woman is important, but also young age of the first male partner.
b) **Multiple sexual partners:** Since there will be more chances to acquire the sexual transmitted infection, it is considered to be high risk factor.

c) **Sexual behavior of the woman’s male partner:** If the male partner have in his turn multiple partners, there will be more chances for him to acquire the viral infection and transmit it. It was pointed out by Franco *et al* (2001).

d) **Multiparity:** Pregnancy can alter the immune response to the HPV infection. There is no consensus regarding parity as risk factor for cervical carcinoma. In some studies like the one of Schiffman *et al* (1993) and Kjaer *et al* (1996 a), parity appears to be significantly associated to cervical intraepithelial neoplasia. However, there are some publications affirming the opposite (Brinton *et al*, 1989; Kjaer *et al*, 1992).

e) **HPV persistence:** Persistence of HPV infection is an important risk factor for progression to clinically relevant preinvasive and invasive carcinoma (Mayrand *et al*, 2000). It is often defined as the detection of the same HPV type in consecutive samples obtained at 3 to 6 month intervals. It is related to older age, high risk HPV types and infection with multiple HPV types (Van Ranst *et al*, 1992). Only women with persistent high risk HPV positive CIN will develop carcinoma of the cervix (Ho *et al*, 1995; Remmink *et al*, 1995; Nobbenhuis *et al*, 1999).

### 2.3.3 Cofactors with HPV

As stated above, although HPV is essential to the transformation of cervical epithelial cells, it is not sufficient, and a variety of cofactors influence the process of development of cervical cancer. Oral contraceptives and tobacco smoking are thought to be important cofactors with HPV infection.

#### 2.3.3.1 Oral contraceptives

More than 100,000,000 women currently use oral contraceptives or have used them in the past. Most oral contraceptives contain a combination of synthetic estrogen and synthetic
progesterone. The combination, like the hormone balance of normal pregnancy, suppresses the hormonal signal from the pituitary gland for the ovaries to release an egg. A minority of pills contain only a synthetic progesterone and act mainly by causing changes in the mucus that prevent the ascent of sperm.

In different doses, combination pills and certain other hormonal preparations can be used after coitus. They prevent pregnancy up to two or three days after the fertilizing intercourse, primarily by rendering the lining of the womb unsuitable for the attachment (implantation) of a fertilized egg (Safra, 1998).

Hormones, particularly oestrogens, have been known to be carcinogenic for experimental animals for over 50 years. More recently an association has been found between human cancer and both hormone replacement therapy and the use of oral contraceptives. From animal studies it has been possible to conclude that tumor of cervix can be induced by hormones (Drill, 1979; Moodley et al, 2003).

The study of the relation between oral contraceptives (OC) use and cervical carcinoma risk is not easy because OC use is highly correlated with sexual and reproductive factors and with screening behavior. In spite of it many studies support the use of OC as a cofactor with HPV for cervical cancer. The increased cervical carcinogenesis risk for OC users has been supported (Beral et al, 1999; Villiers, 2003).

Arbeit et al (1996) suggest a model where chronic estrogen exposure and HPV 16 cooperate to elicit cervical carcinogenesis. Estrogen treatment induces benign proliferation of squamous epithelial cells in the cervix and vagina, presumably by signaling through the estrogen receptor. While estrogen is thought to directly up regulate viral transcription in humans infected by HPV 16, they suggest that proliferating squamous cells in the reproduction tract may modestly increase (or maintain) the levels of HPV oncogenes expression in other indirect ways. They suggest that HPV oncoproteins transform proliferating cells in estrogen-induced hyperplastic lesions in the genital epithelium.
Current users of combined estrogen-progestin OC have an almost 4-fold increased risk compared with non-users; and there is an increasing risk for cervical carcinoma *in situ* with increasing duration of use (Ylitalo *et al.*, 1999). Most of the effects of OC on mortality occur in current or recent users and few effects persist ten years after stopping use. These results relate predominately to use of combined OC containing 50 μg oestrogen (Beral *et al.*, 1999).

### 2.3.3.2 Smoking

Epidemiological studies have yielded conflicting results regarding the role of smoking as a risk factor for cervical carcinoma *in situ*. Some studies have shown an association of smoking with an increased risk for cervical cancer (La Vecchia *et al.*, 1986; Brock *et al.*, 1989; Becker *et al.*, 1994 a; Brisson *et al.*, 1994; Kjaer *et al.*, 1996 b). Whereas others failed to support such association (Morrison *et al.*, 1991; Schiffman *et al.*, 1993; Liaw *et al.*, 1995).

In more recent studies the influence of smoking in the development of cervical cancer appears more clear. Ylitalo *et al* (1999) demonstrated that there was a 2 fold higher risk for cervical carcinoma *in situ* among ever smokers compared with never smokers. No significant trend was found with increasing tobacco consumption (duration, intensity, pack-years); but they found a strong age-dependent association between smoking and cervical carcinoma *in situ*.

In a case control study to compare risk factors for ASCUS (atypical squamous cells of undetermined significance), LSIL (low-grade squamous intraepithelial lesions) and HSIL (high-grade squamous intraepithelial lesions), Kjaer *et al* (1998) showed that smoking remained a significant determinant of risk for all disease categories, although most strongly for HSIL; among HPV-positive women HSIL was associated also with smoking. In this study, the risk among ever smokers tended to increase with the number of cigarettes smoked per day, whereas the duration of smoking (number of years) appeared less important.

Smoking could increase risk for cervical neoplasia through a number of biological mechanisms. One of the mechanisms, which is highly supported, is an
immunosuppressive effect of smoking, which increases persistence of HPV infection, which in turn has been shown to be another risk factor. Several investigators have reported a lowered number of Langerhans' cells in the cervical epithelium of smoking women with in situ cervical carcinoma (Moreli, et al, 1993; Morris et al, 1993), a finding that might explain an impaired cellular immunity (Barton et al, 1988). More convincingly, high contents of smoke-derived nicotine and cotinine have been found in cervical mucus of smokers (Sasson et al, 1985; Schiffman et al, 1987). In vitro studies have shown that polycyclic aromatic hydrocarbons present in cigarette smoke inhibit normal cell proliferation and that other smoking derivatives adduct with DNA and are therefore genotoxic (Simons et al, 1993).

2.3.4 Other risk factors

Besides HPV infection, smoking and use of oral contraceptives there have been proposed other risk factors like the following ones:

a) Genetic predisposition: It was observed by Schiffman et al (1987) and Apple et al (1994).

b) Environmental mutagens and carcinogens

c) Low socio-economic status: There is a correlation between various indicators of decreasing social class and increasing incidence of cervical cancer. These indicators include level of education, income and occupation (Faggiano et al, 1997). There are several factors that could be the reason why low socio-economic condition is a risk factor for cervical cancer, among them: poor hygiene, diet poor in vitamins and less access to early diagnosis or cytological screening for cervical cancer.

d) Poor hygiene: Since it increases the possibility of infections

e) Diet poor in vitamins: This risk factor has been well recognised world wide (Franco, 2001).

f) Coinfection with other sexually transmitted infectious agents: Herpes simplex virus2 (HSV-2) (Becker et al, 1994 b); Chlamydia (Kjaer et al, 1998). And also with human immunodeficiency virus (HIV): cervical cancer is the most common
AIDS defining neoplasm in women; HIV alters the natural history of HPV infection, with decreased regression rates and more rapid progression to high grade and invasive lesions, which are refractory to treatment (Clarke and Chetty, 2002)

g) Diethylstilbestrol (DES): Perinatal DES exposure is associated with several reproductive tract abnormalities and increased of vaginal and cervical cancer risk in women. (Li et al, 2003).

2.4 EARLY DETECTION

In both developed and developing countries, more than 90% of the new cases are due to sexually transmitted HPV infection of the cervix. If cervical cancer is detected in an initial asymptomatic stage it is nearly always curable by surgery or radiotherapy. More importantly, early detection of precancerous lesions through cytological screening is the main strategy for global control of the disease.

According to the experts, the success of screening programmes, practiced in a number of developed countries, varies from country to country. Nevertheless, mass screening programs, in which women have cervical smear tests at least every three to five years, have proved effective in reducing cervical cancer mortality and morbidity rates. In British Columbia (Canada) and Finland, for example, organized screening made it possible to reduce mortality rates by up to 70% and over (Press release WHO/47).

The cervical smear, also called Papanicolau or Pap smear, consists of the examination of exfoliated cervical and vaginal cells for the detection of premalignant and malignant disease. The sample should contain adequate number of representative well-preserved epithelial cells, including cells from the transformation zone. The cells are smeared on one or more slides and stained with the Papanicolau stain which stains differently the nucleus and the cytoplasm. Abnormal cells are then identified and classified by cytotechnologists and pathologists. The results are communicated utilizing a format and terminology formulated by the National Cancer Institute workshop (developed in 1988
and revised in 1991) and referred to as the Bethesda System (National Cancer Institute Workshop, 1993).

In the majority of developing countries screening programmes can not be organized for lack of funds and qualified personnel.

The HPV DNA testing is another method being actively investigated, which can complement and improve the cytological detection of cervical disease, especially in patients with equivocal Pap smear results. This test has been shown to be an effective tool in determining the need for referrals of such patients to colposcopy.

Colposcopy is a technique already widely used in cervical cancer diagnosis, to determine the nature and extent of the disease. For a number of experts it is not considered cost-effective for routine screening.

In the pretreatment assessment due consideration should be given to the stage of the disease, the patient’s age, her desire to preserve fertility and the presence of other medical conditions. The options are colposcopy-directed biopsy, complete hysterectomy, combination of surgery and radiotherapy, combination of radiotherapy with chemotherapy. Some of the agents used in chemotherapy are cisplatin and pemetrexed.

2.5 CERVICAL CANCER PREVENTION

Organized cytology screening programs have been successful in developed countries. However, in developing countries where 80% of the global burden of cervical cancer occurs, these programs lack coverage, accessibility, effectiveness and acceptability. These conditions are not likely to change soon because of competing public health priorities (Franco et al, 2001).

Although testing for HPV in detection, monitoring and preventing invasive disease is important, the ultimate aim is suggested to be eradication of high risk HPV by vaccination (Kirwan and Herrington, 2001).
2.5.1 Vaccines

The immune system of the body controls viral infections by neutralizing the virus with antibodies or by killing the virus-infected cells. These processes utilizes either antigen-dependent cellular immune response or antigen-independent phagocytosis by macrophages. Clinical and histopathological observations however, indicate an important role of immune system in controlling HPV infections. It is now clear that papillomaviruses can elicit both humoral as well as cell-mediated immune responses. At the same time, persistence occurrence of HPV infection in immunocompetent hosts indicates that perhaps no efficient antiviral immune response is induced, or else the infected cells escape immune surveillance (Das, 2000). The attempts to control the HPV-induced diseases are aimed to find preventive immunotherapies, and also to develop HPV vaccines to prevent infection with high-risk HPV.

There are two main strategies being developed to control the preneoplastic and neoplastic cervical lesions caused by HPV infection:

Prophylactic vaccines: This is one of the strategies to prevent HPV infection and consequently the various HPV-associated diseases. DNA-free virus-like particles synthesized by self assembly of fusion proteins of the major capsid antigen L1 has been found to induce a strong humoral response with neutralizing antibodies. These virus-like particles are thus the best candidate immunogen for HPV vaccine trials. Protection seems to be type specific, which will require the production of virus-like particles for a variety of oncogenic types. Such vaccines are already being evaluated in phase I and II trials in different populations (Franco, 2001).

Therapeutic vaccines: These vaccines are developed with the aims of acquiring the regression of precancerous lesions or remission of advanced cervical cancer. They aim the transforming proteins E6 and E7. The candidates include purified early viral proteins, various specific peptides, etc. (Kirwan and Herrington, 2001).
The most attractive vaccines would be the ones that were multivalent, cheap, easily administered and had both prophylactic and therapeutic potential. It is appropriate to point out here that, as HPV cannot be grown in conventional cell cultures, genetic engineering methods are required.

2.5.2 Angioprevention

Angiogenesis is the formation of new blood vessels to irrigate the cancer cells. It is a process parallel to the one of carcinogenesis since the cancer cells need more oxygen and nutrients for their high mitotic activity.

The potential to block tumor growth by inhibition of the neoangiogenic process represents an intriguing approach to the treatment of solid tumors. The high proliferation rate in the tumor deprived of proper vascularization would be balanced by cell death due to lack of diffusion of nutrients and oxygen. Matrix metalloproteinases (MMPs), angiogenesis growth factors, and their receptors are the main targets of an increasing number of clinical trials approved to test the tolerance and therapeutic efficacy of antiangiogenesis agents (Tosetti et al, 2002).

It has been shown that some of the well known chemopreventive agents have antiangiogenic properties (Tosetti et al, 2002).

2.5.3 Chemoprevention

Cancer prevention is based on the evidence that our environment could contain not only carcinogenic compounds but also natural and synthetic substances able to prevent, inhibit or reverse the process of carcinogenesis. Chemoprevention of cancer is a way of cancer control in which the occurrence of this disease is prevented by the administration of one or several chemical compounds (Wattenberg, 1985) either as individual drugs, or as intakes of medicinal herbs (Wargovich and Uda, 2001) or as naturally occurring constituents in diet (Tanaka, 1994).
2.5.3.1 With synthetic products
Among the synthetic drugs that show chemopreventive properties are tamoxifen, raloxifene, fenretinide (Sporn and Suh, 2000), finasteride (Hakama, 1998), and the hormone melatonin (Anisimov et al, 2000). However, there is a tendency to prefer natural to synthetic products for the prevention and treatment of any illness.

2.5.3.2 With natural products
Among the natural products that may prevent cancer can be found the nutraceuticals and the medicinal plants. Nutraceuticals have a nutritional role in the diet and the benefits to health may arise from long-term use as food. In contrast, many medicinal plants exert specific medicinal actions with short- or long-term use, without having a nutritional role in the human diet. There is abundant scientific evidence (both epidemiological and experimental) that change of lifestyle, including diet, affects the risk for many cancers. In general, a lower risk for cancers is observed specially when there are big intakes of fruits and vegetables.

The chemopreventive potentials of numerous diet derived agents have been studied. These agents are briefly described below:

a) Macronutrients:
Carbohydrates: Dietary fiber is considered to have a preventive effect against colorectal cancer, since by increasing stool bulk, it dilutes any toxic material (World Cancer Research Fund/ American Institute for Cancer Research, Washington DC, 1997). There may be a preventative action for pancreatic and breast cancer. Conversely, high starch diets or those high in refined starch may increase risk for stomach cancer (Bemanier, 2002).

Fats: populations consuming diets richer in olive oil have a lower breast cancer incidence (Trichopoulos et al, 1995; La Vecchia et al, 1995). In some cases higher fish consumption was associated with possibly decreased risk for cancer of the larynx, pharynx, liver, colon, endometrium, and kidney (Caygill and Hill, 1995; Schloss et al,
1997). There are many confounding factors in such studies, but the indications are that olive and fish oils are beneficial, while the saturated fats from most meats and some of the Ω-6 unsaturated fats from many oil seeds may enhance the action of exogenous or endogenous carcinogens.

A fatty acid with antimitogenic and anticarcinogenic effects in animal systems is conjugated linoleic acid (CLA) which is found in grilled ground beef and other ruminants, and in dairy products such as cheese. In animal experiments, CLA has been effective as an inhibitor of tumors from application of polycyclic aromatic hydrocarbons, in decreasing the action of tumor promoters, decreasing cell proliferation, reducing the activation of heterocyclic amine carcinogens for some organ sites but not others, and modulating the action of protein kinase C proteins involved in signal transduction (Ip et al, 1991; Cesano et al, 1998). CLA was effective at a dietary level of 0.1%, one-hundredfold less than the level of dietary fish oil needed to inhibit animal tumors.

Proteins: Dietary use of soy products has been suggestive for a lower risk of breast and endometrial cancer in Asian women, although it seems to be due to its content in flavonoids more than the soy protein in itself. In animal studies, low protein diets tend to inhibit cancer, probably due to slower growth, while a high intake tends to enhance cancer development at various sites (Bemanier, 2002). Of all the aminoacids, only methionine appears to be involved in physiological processes which have a chemopreventive action against cancer.

b) Vitamins and Minerals:

Vitamin A: It is also called retinol, usually obtained from carotenoids which occur in vegetables, mostly green, yellow and dark green leafy vegetables. The carotenoids are converted to Vitamin A in the intestinal tract. Investigations in different animal species have shown that retinol esters and beta-carotene can inhibit the effects of various carcinogens through modulating DNA stability and decreasing lipid peroxidation (Duthie et al, 1998).

As for humans there have been many epidemiological studies of retinol and carotens, with conflicting results. There was no protective effect against cancer of lung, stomach,
breast, and cervix. An IARC (International Agency for Research of Cancer) group concluded that there is little evidence that Vitamin A intake has a substantial cancer-preventive effect (Vainio and Rautalahti, 1999).

The situation differs for dietary carotenoids, where there is evidence for a modest to weak protective action against lung cancer (Albanes, 1999), and a possible decreased risk for esophageal (Zhang et al, 1997), stomach, colorectal, breast and cervical cancers (Longnecker et al, 1997; Verhoeven et al, 1997; Zhang et al, 1999) as well as an effect against cancers of thyroid and salivary gland. Dietary intake of beta carotene rather than intake of vitamin A itself is better correlated with decreased cancer incidence.

Another carotenoid which is not a vitamin A precursor, namely lycopene, responsible for the color of tomatoes, has shown a protective action against chemical carcinogens in animals (Krinsky, 1998). The protective effects of carotenoids on carcinogenesis might be related to biological functions including antioxidant property, free radical scavenging property and anti proliferative property.

**Vitamin B:** Both hypo- and hypermethylation of DNA are markers of the early stages of carcinogenesis. By maintaining a normal methylation pattern, folic acid (vitamin Bc) may aid in decreasing cancer risk. For example, high dietary folate intake was weakly associated with decreased risk of colon cancer (Ma et al, 1997).

**Vitamin C:** It is an ascorbic acid and has been widely studied both in animal systems and in epidemiological trials. In animal experiments, vitamin C had a beneficial action against skin or mammary cancer with dimethylbenz(a)anthracene, benzo(a)pyrene, or against estrogen-induced kidney cancer in hamsters. Epidemiological trials have been less definitive. Most studies have shown some possible decrease in cancers of many organs systems, but the effects were not dramatic (Bernanier, 2002)

**Vitamin D:** The effect of vitamin D is linked to the level of dietary calcium. Both animal and human studies lend support to the concept that increased intake of calcium and vitamin D can reduce the risk of colon cancer associated with high dietary fat (Newmark and Lipkin, 1992).
**Vitamin E:** In humans, vitamin E possibly protects against cancer of the mouth and pharynx, esophagus, pancreas, stomach, colon and rectum, cervix and prostate (Moyad et al, 1999). For breast cancer, the epidemiologic results have been inconclusive. The mechanism of its action, apart from the antioxidant properties, has not been elucidated.

The cancer preventive potential of vitamins is summarise in Table 2.2.

**Calcium:** It is one of the more abundant essential minerals of the body. The main dietary sources are milk and dairy products. As for any chemopreventive action against cancer, a number of studies show a beneficial effect, but conflicts remain. A protective association for pancreatic cancer was noted in one study (Farrow and Davis, 1990), and for colorectal cancer, the weight of the evidence points toward an inhibitory action, as do some animal tests (Lipkin and Newmark, 1995; Holt et al, 1998). However conflicting results were also noted (Pritchard et al, 1996). Higher intake of calcium may reduce breast cancer risks (Franceshi, 1997), but the data on prostate cancer and calcium intake levels are conflicting (Newmark and Lipkin, 1992; Chan et al, 1998).

**Iron:** The essential micronutrient iron is mostly present in the body in the hemoglobin of the blood and the myoglobin of muscle tissue. Good sources of iron are egg yolks, liver, wheat germ, lentils and spinach. Deficiency of iron has been associated with cancer of esophagus. Conversely, the risk of various other types of cancer increases in association with higher body iron stores. High serum ferritin levels were associated with an increased risk of colorectal adenomas or cancer (Nelson et al, 1994), and a high concentration of iron in the liver was linked to a greater risk of liver cancer (World Cancer Research Fund/American Institute for Cancer Research, 1997). The suggestion has been made that high dietary intakes of iron enhance the generation of free radicals, which are implicated in the initiation or promotion phases of carcinogenesis. As with other essential micronutrients, a balance between a deficiency and an excess of iron is needed to maintain good health.
Table 2.2: Cancer preventive action of Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Species</th>
<th>Organ/Tissue</th>
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<tbody>
<tr>
<td>A</td>
<td>Mouse</td>
<td>Skin</td>
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<td></td>
<td>Rat</td>
<td>Mammary gland</td>
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<td></td>
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<td>Urinary bladder</td>
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<td>B</td>
<td>Mouse</td>
<td>Lung</td>
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<td></td>
<td>Human</td>
<td>Colon (weakly)</td>
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<tr>
<td>C</td>
<td>Mouse</td>
<td>Colon</td>
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<td></td>
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<td>Lung</td>
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<td></td>
<td>Rat</td>
<td>Mammary gland</td>
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<td></td>
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<td>Colon</td>
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<td></td>
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<td>Liver</td>
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<td></td>
<td>Hamster</td>
<td>Kidney</td>
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<td>D</td>
<td>Mouse</td>
<td>Skin</td>
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<td></td>
<td>Rat</td>
<td>Colon</td>
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<td></td>
<td>Human</td>
<td>Breast</td>
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<td>Colon/rectum</td>
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<td></td>
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<td>Prostate</td>
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<td>E</td>
<td>Mouse</td>
<td>Skin</td>
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<td>Colon ?</td>
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<td></td>
<td>Rat</td>
<td>Mammary gland</td>
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<td>Stomach</td>
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<td></td>
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<td>Colon ?</td>
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<td></td>
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<td>Ear duct</td>
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<td>Liver</td>
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<td></td>
<td>Hamster</td>
<td>Buccal pouch</td>
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<td></td>
<td>Human</td>
<td>Mouth (possibly)</td>
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<td></td>
<td></td>
<td>Pharynx (possibly)</td>
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<td></td>
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<td>Esophagus (possibly)</td>
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<td>Pancreas (possibly)</td>
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<td>Stomach (possibly)</td>
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<tr>
<td></td>
<td></td>
<td>Colon/rectum (possibly)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cervix and prostate (possibly)</td>
</tr>
</tbody>
</table>

(Bernanier, 2002)
Selenium: It was recognized only about 30 years ago as an essential micronutrient. It is a component of the glutathione peroxidase system and occurs in organ meats, seafood, and in cereals and seeds. An excess of selenium is toxic. Animal studies with selenium, mostly as the selenite salt, have shown an inhibitory action against tumor development in various organs (World Cancer Research Fund/American Institute for Cancer Research, 1997). In humans, the data are often conflicting. High blood levels of selenium were correlated with esophageal cancer in a Chinese population (World Cancer Research Fund/American Institute for Cancer Research, 1997), while in other studies, high body selenium was protective against stomach cancer. Data for liver and pancreatic cancer are somewhat conflicting, but overall there was some inhibitory effect. Evidence for a protective action against colorectal and breast cancer in humans is limited. Higher body stores of selenium were associated with reduced risk of advanced prostate cancer (Yoshizawa et al, 1998). Accordingly, although selenium suppresses most types of cancer and has a beneficial action, the toxicity of selenium limits the amount that can be administered.

Zinc: It is an essential trace element, as it is a component of several enzymes, and at the same time it activates other enzymes. Zinc deficiency causes serious disorders, but high zinc intake is toxic. Many animal experiments have shown that dietary zinc deficiency as well as zinc supplementation can increase the incidence of some carcinogen-induced tumors and decrease the incidence of others (Bemanier, 2002). However there are no definitive epidemiologic studies that associate human cancers with either a deficiency or an excess of zinc.

c) Nonnutritive Components:

Fiber: of all the nonnutritive food constituents, dietary fiber has been the one most extensively investigated in humans. The evidence is suggestive that high dietary fiber decreases the risk of stomach, pancreatic and breast cancer, and is protective against colorectal (and possibly endometrial) cancer (Bagga et al, 1995; Goodman et al, 1997; Slattery et al, 1997 a). Several possible reasons exist for the beneficial action of dietary
fiber. The fiber may physically trap or attach to various carcinogens, and sweep them out of the intestinal tract. Fiber may also trap hormonal constituents and thus help decrease breast cancer. Further, some fiber is eventually fermented by intestinal bacteria to butyric acid, which regulates cell cycles (Goldin and Gorbach, 1994; Stoll, 1996).

Flavonoids, Isoflavones and Polyphenols: Flavonoids and isoflavones are present in plants and their various parts in combination with sugars (glycosides). The common property of many chemopreventive plant products is the presence of several hydroxyl groups in the molecule; thus the designation as polyphenols is appropriate for a wide range of these substances. Many polyphenols from foods have demonstrated preventive effects against chemical carcinogens in animal experiments. Examples include ellagic acid (Lesca, 1983), quercitin, which is found in most plant materials (Hertog and Hollman, 1996), the flavones or epicatechins common in tea (Conney et al., 1997) and curcumin in turmeric and mustard. Further, several plant phenolics can inhibit nitrosamine formation in vivo and thus has a chemopreventive action. The soybean compounds genistein and daidzein are examples of isoflavones. Various studies have indicated a lower risk of breast and possibly endometrial cancer in women who consume soybean products (Stoll, 1997; Wu et al., 1998). The mechanism probably resides in the weak estrogenic effect of these compounds which then bind to estrogen receptors, thus blocking the action of the more potent natural estrogens (Nagata et al., 1997). Plant lignans, which also are beneficial, appear to bind weakly to estrogen receptors.

Indole-3-carbinol (I3C): It was one of the first specific cancer chemopreventive compounds to be isolated from a cruciferous vegetables, namely Brussels sprouts (Wattenberg and Loub, 1978). Many animal studies have shown its ability to suppress the effects of chemical carcinogens, presumably through its induction of detoxifying enzymes (Organesian et al., 1997).

Isothiocyanates: They occur naturally in the form of their glucosinolate conjugates in a variety of cruciferous vegetables, and are generally responsible for the sharp taste associated with cruciferous vegetables such as mustard, horseradish and watercress.
Animal studies with isothiocyanates have shown definitive and often quite specific inhibition of the action of some carcinogens, largely through suppressing metabolism to an activated intermediate (Hecht, 1995).

**Sulfides:** The numerous sulfides and their selenium analogs occur in “Allium” vegetables. Animal tests have shown an inhibitory action of garlic and onion constituents, specially diallyl sulfide, on experimental carcinogenesis of the skin, esophagus and colon. Epidemiologic studies have noted the same trend (that Allium vegetables protect against stomach and colon cancer) but no such action was noted for breast and lung cancer (Wargovich and Uda, 1996).

**Terpenoids:** Examples of terpenoids are: carvone, p-cymene, geraniol, limonene, linalool, nerol, perillyl alcohol, pinene and thymol, among others. Their inhibitory action was noted several decades ago. More recent efforts have confirmed that d-limonene, the putative active component of sweet orange oil, has several inhibitory mechanisms (Bernanier, 2002). It suppresses the activation of nitrosamines (Wattenberg and Coccia, 1991) and azoxy methane (Kawamori et al, 1996), induces glutathione-S-transferase, and inhibits oncogene activation by depressing the isoprenylation of oncogene products.

The cancer preventive action of non-nutritive principles of food is also summarised in Table 2.3.

### 2.5.3.3 Mechanisms of cancer chemoprevention

A major recent advance in cancer research has been the acquisition of a new body of knowledge that elucidates fundamental molecular and cellular mechanisms involved in the development of malignancy. Rational approach to prevention, based on the use of agents whose mechanism of action is understood, can be used to develop drugs for chemoprevention. (Kelloff et al, 2000). Possible chemopreventive mechanisms and associated agents is given in Table 2.4.
Table 2.3: Cancer preventive action of non-nutritive principles of food

<table>
<thead>
<tr>
<th>Substance</th>
<th>Species</th>
<th>Organ/Tissue</th>
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<tr>
<td>Fiber</td>
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<td>Stomach</td>
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<td>Pancreas</td>
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<td>Breast</td>
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<td>Colon/rectum</td>
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<td>Endometrium</td>
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<td>Rat</td>
<td>Colon</td>
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<tr>
<td>Flavonoids, isoflavones and</td>
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<td>Breast</td>
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<tr>
<td>polyphenols</td>
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<td>Endometrium</td>
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<td>Rodents</td>
<td>Mammary gland</td>
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<tr>
<td></td>
<td></td>
<td>Skin</td>
</tr>
<tr>
<td>Indole-3-carbinol</td>
<td>Mouse</td>
<td>Forestomach</td>
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<td></td>
<td>Rat</td>
<td>Mammary gland</td>
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<tr>
<td>Isothiocyanates</td>
<td>Mouse</td>
<td>Lung</td>
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<td></td>
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<td>Forestomach</td>
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<td>Rat</td>
<td>Mammary gland</td>
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<td>Sulfides</td>
<td>Human</td>
<td>Stomach</td>
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<td>Colon</td>
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<td>Esophagus</td>
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<td>Thyroid</td>
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<td>Terpenoids</td>
<td>Mouse</td>
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<td></td>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Mammary gland</td>
</tr>
</tbody>
</table>

(Bernanier, 2002)
Table 2.4: Mechanisms for chemoprevention by diet-derived agents with possible molecular targets

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Possible molecular targets</th>
<th>Representative agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti mutagenesis</td>
<td>Bile acids (bind)</td>
<td>Calcium</td>
</tr>
<tr>
<td>Inhibit carcinogen uptake</td>
<td>Cytochromes P450 (inhibit)</td>
<td>PEITC, tea, indole-3-carbinol, soy isoflavones</td>
</tr>
<tr>
<td>Inhibit formation/activation of carcinogen</td>
<td>PG synthase hydroperoxidase, 5-lipoxygenase (inhibit)</td>
<td>Curcumin</td>
</tr>
<tr>
<td></td>
<td>Bile acids (inhibit)</td>
<td>Ursodiol</td>
</tr>
<tr>
<td>Deactivate/detoxify carcinogen</td>
<td>GSH/GST (enhance)</td>
<td>NAC, garlic/onion disulfides</td>
</tr>
<tr>
<td>Prevent carcinogen-DNA binding</td>
<td>Cytochromes P450 (inhibit)</td>
<td>Tea</td>
</tr>
<tr>
<td>Increase level or fidelity of DNA repair</td>
<td>Poly(ADP-ribosyl)transferase (enhance)</td>
<td>NAC, protease inhibitors (Bowman-Birk)</td>
</tr>
<tr>
<td>Modulate hormone/growth factor activity</td>
<td>Steroid 5a-reductase (inhibit) IGF-1 (inhibit)</td>
<td>Soy isoflavones, tea</td>
</tr>
<tr>
<td>Inhibit oncogene activity</td>
<td>Farnesyl protein transferase (inhibit)</td>
<td>Perillyl alcohol, limonene, DHEA</td>
</tr>
<tr>
<td>Inhibit polyamine metabolism</td>
<td>ODC induction (inhibit)</td>
<td>Retinoids, curcumin, tea</td>
</tr>
<tr>
<td>Induce terminal differentiation</td>
<td>TGFb (induce)</td>
<td>Retinoids, vitamin D, soy isoflavones</td>
</tr>
<tr>
<td></td>
<td>Cyclooxygenases (inhibit), T,NK lymphocytes (enhance)</td>
<td></td>
</tr>
<tr>
<td>Restore immune response</td>
<td>Langherans cells (enhance)</td>
<td>Tea, curcumin, selenium, tea, vitamin E</td>
</tr>
<tr>
<td>Increase intercellular communication</td>
<td>Connexin 43 (enhance)</td>
<td>Carotenoids (lycopene), retinoids</td>
</tr>
<tr>
<td>Induce apoptosis</td>
<td>TGFb (induce), RAS farnesylation (inhibit), telomerase (inhibit), arachidonic acid (enhance), caspase (activate)</td>
<td>Retinoids, soy isoflavones, vitamin D, perillyl alcohol, limonene, DHEA, retinoic acid, curcumin, tea</td>
</tr>
<tr>
<td>Inhibit angiogenesis</td>
<td>FGF receptor (inhibit tyrosin kinase), thrombomodulin (inhibit)</td>
<td>Soy isoflavones, retinoids</td>
</tr>
<tr>
<td>Correct DNA methylation imbalances</td>
<td>CpG island methylation</td>
<td>Folic acid</td>
</tr>
<tr>
<td>Inhibit basement membrane degradation</td>
<td>Type IV collagenase (inhibit)</td>
<td>Protease inhibitors</td>
</tr>
<tr>
<td>Inhibit DNA synthesis</td>
<td>Glucose 6-phosphate dehydrogenase (inhibit)</td>
<td>DHEA</td>
</tr>
</tbody>
</table>

Abbreviations: PEITC, phenethyl isothiocyanate; PG, prostaglandin; GSH, glutathione; GST, glutathione-S-transferase; NAC, N-acetyl-L-cysteine; IGF, insulin-like growth factor; DHEA, dehydroepiandrosterone; ODC, ornithine decarboxylase; TGFb, transforming growth factor b; NK, natural killer; RAS, ras oncogene protein product; FGF, fibroblast growth factor.
2.5.3.4 A classification of chemopreventive agents

Absolute classification of all chemopreventive agents is difficult due to the fact that the precise mechanism(s) of action of the agents are not known for many compounds. One means of providing an organizational framework is to classify inhibitors according to the time in the carcinogenic process at which they are effective (Wattenberg, 1985).

To fully understand this classification there is a need to get details of the process of xenobiotic metabolism.

In the metabolism process, there are a number of complex enzyme systems to protect against toxic effects. Three major demands should ideally be met by a xenobiotic metabolizing enzyme system:

a) The metabolites should have sufficient water solubility to allow sufficient excretion via urine.

b) The metabolites should not have adverse biological activity, to avoid toxicological effects.

c) The enzyme system should have a broad substrate specificity, to be able to deal with any newly encountered compound.

The liver is considered to be the major organ of detoxification, though other organs are also known to have detoxification machinery. The common strategy of detoxification is to improve water solubility of foreign compounds by conjugating lipophilic substances with hydrophilic building blocks. This conjugation step requires the presence of suitable functional group in the molecule that often has to be introduced or uncovered by one or more steps. In the classical model of xenobiotic metabolism, this functionalization is called phase I, while conjugation is called phase II. Phase I reactions introduce polar groups into xenobiotic compounds; most of them are oxidative reactions. The main enzyme involved in phase I reactions is cytochrome P450. In the phase II metabolism, the metabolic products from phase I undergo conjugation reactions with glucuronide, sulfate and glutathione. The products of these reactions, being hydrophilic, are readily excreted from the body. Glutathione-S-transferase is the main enzyme in phase II reactions.

The chemopreventive agents can be classified (figure 2.13) on the basis of the time within the carcinogenic process at which they are effective:
**Category of inhibitors**

**Inhibitors of carcinogen formation**
- ex: ascorbic acid (vitamin C), phenols like caffeic acid and ferulic acid, alpha-tocopherol (vitamin E)

**Blocking agents**
- ex: flavones, coumarins, ellagic acid, butylated hydroxyanisole (BHA), benzyl isothiocyanate, indol-3-carbinol, 5,6-benzoflavone

**Suppressing agents**
- ex: retinoids, plant sterols, protease inhibitors, beta-carotene, alpha-tocopherol, ascorbic acid, glutathione, selenium, vitamin A

**Sequence leading to neoplasia**

- Precursor compounds
- Ultimate carcinogen
- Interactions with critical molecules
- Promotion
- Progression
- Neoplastic manifestations

**Fig 2.13: Three categories of chemopreventive agents and their time of action**
a) Inhibitors of carcinogen formation
These are the compounds that prevent the carcinogen formation from their precursors. For example, the majority of compounds that inhibit the formation of carcinogens are known to prevent the formation of nitrosamines. Ascorbic acid, when present in appreciable amounts, can decrease nitrosamine formation (Mirvish, 1981). Other compounds that inhibit nitrosamine formation include phenols such as caffeic acid and ferulic acid (Kuenzig et al., 1984) as well as different sulphydryl compounds (Shenoy and Choughuley, 1992). Inhibitors of carcinogen formation may have limited utility since there is a lot of human exposure to carcinogens already formed. However a potential use of such compounds would be their incorporation into the diet for population with suspected high rates of endogenous formation of nitrosamines.

b) Blocking agents
Blocking agents prevent carcinogen from reacting with critical targets sites. They act on direct-acting carcinogens which do not require prior activation. Genotoxic carcinogens must be metabolically activated to electrophilic forms that can damage DNA. In general overall metabolism of foreign compounds is directed towards producing chemical species that can be excreted. According to the mechanism of their action the vast majority of blocking agents can be assigned to one or more categories namely (Morse and Stoner, 1993):

*Inhibitors of cytochrome P450*: This group of compounds act simply by inhibiting the activation of carcinogen to its ultimate carcinogenic form. One of the first cytochrome P450 inhibitors shown to have chemopreventive activity was disulfiram, which inhibits the activation of dimethylhydrazine (Fiala et al., 1977).

*Inducers of cytochrome P450*: Inducers of cytochrome P450 can act as blocking agents as well, by increasing the production of activated metabolites at resistant non-targets tissues or by enhancing oxidative detoxification at any tissue site. Indol-3-carbinol is a potent inducer of cytochrome P450 enzyme and has chemopreventive activity in a number of animal models (Morse et al., 1990; Tanaka et al., 1990).
**Phase II enzyme inducers:** Specific inducers of phase II enzymes are preferred to cytochrome P450 inducers as chemopreventive agents, since they inhibit a greater range of target carcinogens and are less likely to produce cancers themselves (Morse and Stoner, 1993).

**Nucleophilic compounds acting as scavengers of electrophiles:** Trapping agents or scavenging agents are compounds that physically react with the activated (electrophilic) forms of carcinogens. Physiological nucleophiles, like GSH fall into this group. Ellagic acid has been shown to be a potent scavenger of the active form of benzo(a)pyrene.

c) **Suppressing agents**

Suppressing agents are compounds that inhibit carcinogens when they are administered subsequently to the course of carcinogen administration, which would result in the occurrence of cancer. Current suppressing agents can be categorized as inhibitors of polyamine metabolism, inhibitors of oncogen expression, inhibitors of protein kinase C and inhibitors of oxidative DNA damage. The most extensively studied suppressing agents are the retinoids (Moon et al, 1994). However most of their mechanisms of action are still poorly understood.

**2.5.3.5 Qualities of a chemopreventive agent**

The ideal chemopreventive agent should have the following qualities:

i) **Little or no untoward toxic effects:**

In any chemopreventive dosing regimen, healthy individuals are administered a drug chronically, possibly for life. Only certain high risk populations can be reasonably expected to endure mild toxicity or discomfort in the use of chemopreventive agent.

ii) **High efficacy:**

The efficacy should be rigorously demonstrated, first by success in *in vivo* animal models, and then in clinical trials.

iii) **Capability of oral administration:**

It allows self-medication.
iv) A known mechanism of action:
Knowledge of the precise mechanism(s) of action of the prospective chemopreventive agent will decrease the possibility of untoward interactions with other administered drugs or dietary constituents.

v) Low cost:
Cost of chemopreventive regimen must be low, since the agent(s) will be chronically administered.

2.5.3.6 Target population
Presently the projected target populations for cancer chemoprevention are high-risk groups, such as:
i) Individuals who are engaged in risk-taking behaviors of lifestyles (e.g. smokers and snuff users)
ii) Individuals who have received occupational exposure to known carcinogens (e.g. asbestos workers)
iii) Those who are known to be genetically predisposed to the development of cancer (e.g. individuals with familial colonic polyposis)
iv) Individuals who possess premalignant lesions (e.g. oral leukoplakia)
v) Survivors of primary cancers with a high degree of recurrence or a high tendency towards formation of second primary tumors.
vi) Cancers survivors who received chemotherapy and/or radiation therapy (Morse and Stoner, 1996).

2.6 CANCER CHEMOMODULATORS

2.6.1 *Trigonella foenum-graecum* (fenugreek, methi)

**Family name:** Leguminosae.

**Description:** Fenugreek is an erect, legume, strongly scented, robust, annual plant, about 30 to 60 cm high. It has compound, smooth and thin leaves of light green color, axillary yellow flowers and thin pointed pods 10 to 15 cm long. Each pod contains 10 to 20 seeds,
which are smooth and oblong, about 3 mm long. They emit a peculiar odor and have flavor of their own. They are used as a spice.

**Origin and distribution:** Fenugreek is considered to be a native of south-Eastern Europe and West Asia. It is also found growing widely in North-Western India. It has been used since ancient times both as a food and medicine. It is extensively cultivated throughout Africa, India, and Central and South America.

**Nutritive value/composition:** An analysis of fenugreek seeds shows them to contain moisture 6.3%, protein 9.5%, fat 10.0%, minerals 3.0%, crude fibre 18.5% carbohydrates 42.3%, ash 13.4%, calcium 1.3%, phosphorus 0.48%, iron 0.011%, sodium 0.09%, potassium 1.7%, vitamin A 1.401 IU/100gm, vitamin B1 0.41 mg/100g, vitamin B2 0.36, vitamin C 12.0 and niacin 6.0 mg/100 g, and calorific value 370 calories/100 g. Fenugreek seeds also contain gums 23.6%, mucilage 28.0%, trigonelline (0.13-0.28%) and total saponin (1.7%). The seeds are rich in essential amino acids. It has high level of steroids. Fenugreek seeds have a high phenolic content (518±36 mg/100g) and high levels of flavonoids (103±16 mg/100g) (Nair *et al*, 1998).

**Medicinal and other use:** Fenugreek was used by the Ancient Egyptians in embalming and for incense. The Romans grew it as a fodder for their animals, which is still the practice in India today, with the benefit that it restores nitrogen to the soil and acts as a natural fertilizer. Used in Middle Ages as a cure for baldness, fenugreek is still used in Indonesia as a hair tonic. Fenugreek is traditionally used to stimulate the metabolism and to help control blood-sugar levels in cases of diabetes (Sharma *et al*, 1991; Raju *et al*, 2001). It is also given to assist with stomach and digestive orders, and lowering blood pressure. Having a valuable iron content, fenugreek was also given in cases of anaemia. Fenugreek seeds contain substantial amount of steroidal substance diosgenin, which is used as starting material in the synthesis of sex hormones and oral contraceptives (Pareek and Gupta, 1981). The powder is sometimes used as a dye. In India fenugreek is used as a spice and vegetarian cooking, adds nutritive value and flavor to foods.
2.6.2 Brassica sp. (mustard seeds)

Family Name: Cruciferae.

Description: There are over 150 species of mustard of annual or biennial herbs. Several of these species are cultivated as oilseed crops or as vegetable or fodder crops. The seeds of only three of these species have condiment value: Brassica alba (white), Brassica juncea (brown), Brassica nigra (black). The plant of brown mustard produces flowers, which are a pale yellow, and seed pods, which are larger than those on the other mustard plants. Dry mustard seeds are small, measuring about 1 mm in diameter. They are round and darkish-brown or grayish-brown in color; the internal part is yellowish and fatty. They have no smell but when pounded or moistened with water, they emit a peculiar pungent odor. The taste of mustard seed is bitter and pungent.

Origin and distribution: It has been used by Greeks, Romans and Indians from ancient times. During the Middle Ages, mustard was introduced into Spain by Arab traders, and it was soon carried throughout Europe. Now mustard is native of Europe, Asia and North America. The plant is cultivated as a field crop in most temperate countries (Morris, 2000; Bakhru, 2001)

Nutritive value / composition: An analysis of mustard seed shows it to consist of moisture 8.5%, protein 27.0%, fat 39.7%, minerals 4.2%, fibre 1.8% and carbohydrates 23.8%. Its vitamin and mineral contents are calcium 490 mg, phosphorous 700 mg, iron 17.9 mg, carotene 162 mg, thiamine 0.65 mg, riboflavin 0.26 mg and niacin 4.0 mg per 100 grams. Black mustard seeds contains about 1% sinigrin (allylglucosinolate), a thioglycoside-like compound (a so-called glucosinolate) of ally isothiocyanate with glucose; in presence of water, by action of the enzyme myrosinase (also present in the seed), allyl isothiocyanate, a pungent, lachrymatory and volatile compound is liberated. This oil is responsible for the pungent, bitter smell and taste. Besides allyl isothiocyanate, in Brown mustard seed another related compound is found, namely crotylisothiocyanat (2-butenylisothiocyanata)
(Bakhru, 2001). Brassica sp. has a high phenolic (674±60 mg/100g) and flavonoid (145±12 mg/100g) content (Nair et al, 1998).

Medicinal and other uses: Mustard seeds are antibacterial and decongestant. Its paste made with water, is applied as an analgesic in muscular pains. It is used for hair growth (Bakhru, 2001).

2.6.3 Cuminum cyminum (cumin, geera)

Family name: Umbelliferae.

Description: Cumin is a small, annual herbaceous plant with a smooth surface and perpendicular root. It grows up to a height of 35-45 cm. Cumin flourishes best in sunny climes with some rainfall. The small white or pink flowers grow on small compound umbels. Harvesting takes place about 4 months after planting. The plant has aromatic seed-like fruit, commonly known as cumin seed. It is approximately 6 mm long, oval in shape and brown-yellow in color. It has strong aromatic smell and warm, bitterish taste, which is due to the presence of a volatile oil.

Origin and distribution: Cumin is indigenous to upper Egypt, but it was cultivated from early times in Arabia, India, China and countries bordering the Mediterranean. Evidence shows that it was known to the Egyptians 5,000 years ago and it was found in the pyramids; they used it both to season meats, fish, stews and to mummify their dead. The Romans used it as a substitute of pepper. In the Middle Ages, when cumin was very popular, Europeans believed it would keep poultry from wandering away, and even ensure fidelity between couples. Cumin is now extensively cultivated in India, Iran, Morocco, China, Southern Russia, Indonesia, Japan and Turkey. It is cultivated in all states of India except Assam and West Bengal. The main producing centers are Punjab and Uttar Pradesh.
**Nutritive value / composition:** An analysis of cumin seeds shows that it contains moisture 11.9%, protein 18.7%, fat 15.0%, crude fiber 12.0%, carbohydrates 36.6%, and mineral matter 5.85. Their mineral and vitamin contents are calcium 1080 mg, phosphorous 511 mg, iron 11.7 mg, sodium 0.16 mg, potassium 2.1 mg, thiamine 0.55 mg, riboflavin 0.36 mg, and niacin 2.6 mg, vitamin C 3 mg, and vitamin A 175 I.U. per 100 grams. On steam distillation, the crushed cumin seeds yield a valuable volatile oil whose constituents are: cumin aldehyde, thyme, cuminol, carvone, cynol and terpene (Bakhru, 2001). Cumin seeds have a high content of phenolic compounds (1536±74 mg/100 gr), and also a moderate level of flavonoid content (76±4 mg/100 gr).

**Medicinal and other uses:** As flavourant due to its aromatic odour and its spicy and somewhat bitter taste. Cumin seeds are used as a condiment and form an essential ingredient in all mixed spices and curry powders for flavouring soups, pickles and for seasoning breads and cakes. In medicine they are considered stimulant, carminative (helpful in flatulence), stomachic (opens the appetite), astringent and useful in diarrhea and dyspepsia (difficulty in digestion). They are now very much used in veterinary medicine. The oil is used in perfumery and for flavoring liqueurs. The residue left after the extraction of volatile oil can be used as a cattle feed.

### 2.6.4 **Piper longum (pipli)**

**Family name:** Piperaceae.

**Description:** Long pepper (*Piper longum*) is a small, slender, trailing or climbing aromatic plant with perennial woody roots and thin and erect branches. The leaves are seven-ribbed, smooth, 5 to 9 cm long and 3 to 5 cm wide. The plant has minute flowers, small berries, 2.5 to 4 cm egg-shaped, oblong, shiny and red in color, when ripe. Long peper consists of the dried fruits of the plant. Its spikes are long and can be distinguished from the other important species of the genus, *P. nigrum* L., whose fruits are round.
**Origin and distribution:** Long pepper is indigenous to India, specifically to the region of Magadh i.e. North Bihar. It occurs in the hotter parts of India from central Himalayas to Assam, Khasi and Mikir Hills, lower hills of Bengal and evergreen forests of the Western Ghats from Konkan to Travancore (Kerala). It also occurs in Nicobar islands. It is cultivated extensively in many places of Tamil Nadu, West Bengal and Assam, especially Cherrapunji regions which receives very heavy rains from the end of March to the middle of September and where humidity is relatively high. It was familiar to the Greeks and Romans but now it is difficult to find, sometimes even in India.

**Composition:** Long pepper contains alkaloids, piperine 4-5 % and piplartine m.p. 124-250. It also contains resin and a volatile oil. The dried fruit of long pepper on steam distillation gave 0.7 % of an essential oil of spicy odor resembling that of pepper and ginger oils.

**Medicinal and other uses:** The fruits are used as a spice and also in pickles and preserves. They have a pungent pepper-like taste and produces salivation and numbness of the mouth when eaten or munched. In Chhota Nagpur, the root is used to ferment rice-beer. In Andaman Islands, the leaves are chewed like betel leaves. The fruits as well as the roots are credited with numerous medicinal uses and are used as an important drug (piplamul) in Ayurvedic and Unani systems. They may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma, etc; as counterirritants and as analgesic when applied locally for muscular pains and inflammation. It is used also as a carminative and as a sedative in insomnia.

One of the active components present in *Piper longum* is piperine, an alkaloid which is claimed to cure jaundice. Piperine present both in *Piper longum* and *Piper nigrum*, is found to posses antihepatotoxic activity. This plant principle exerted a significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both *in vitro* and *in vivo* lipid peroxidation and by preventing the depletion of GSH and total thiols in the intoxicated mice.
2.6.5 *Camellia sinensis* (green tea)

Family name: Theaceae.

Description, origin and distribution: Tea is an evergreen shrub. Tea is made from the young leaves and leaf buds of the tea plant *Camellia sinensis*. Two principle varieties are used, the small leaved China plant (*Camellia sinensis sinensis*) and the large-leaved Assam plant (*Camellia sinensis assamica*). Native to Southeast Asia, but currently cultivated in over 30 countries and consumed worldwide. Of the total amount of tea produced and consumed in the world, 78% is black tea, 20% is green tea, and less than 2% is oolong tea. Green tea is consumed primarily in China, Japan, India and a few countries in Africa and the Middle East.

Manufacturing process: Green, black and oolong teas undergo different manufacturing processes. To produce green tea, freshly harvested leaves are rapidly steamed or pan-fried to inactivate oxidating enzymes, thereby preventing fermentation. The leaves are then subjected to further heating and are dried. For the production of black and oolong teas, the fresh leaves are allowed to wither until their moisture content is reduced to approximately 55% of the original leaf weight, which results in the concentration of polyphenols in the leaves. The withered leaves are then rolled and crushed, initiating fermentation of the polyphenols. During this processes, the catechins are converted to theaflavins and thearubigins. Oolong tea is prepared by firing the leaves shortly after rolling to terminate the oxidation and dry the leaves. Normal oolong tea is considered to be about half as fermented as black tea. The fermentation process results in oxidation of simple polyphenols to more complex condensed polyphenols to give black and oolong tea their characteristic colors and flavors.

Composition: The composition of the tea leaves depends of a variety of factors, including climate, season, horticultural practices and the age of the plant. The chemical composition of Green tea is similar to that of the leaf. Green tea contains polyphenolic compounds, which includes flavanols, flavandiols, flavonoids, and phenolic acids and
account for 30% of the dried weight of green tea leaves. Most of the polyphenols in green tea are flavanols, commonly known as catechins that account for its characteristic color and flavor; the major catechins in green tea are (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin and (-)-epigallocatechin-3-gallate (EGCG). In black teas, the major polyphenols are theaflavin and thearubigin (Katiyar et al, 2000; Mukhtar and Ahmad, 2000).

**Medicinal and other uses:** *Camellia sinensis* has anti-inflammatory effects, anticarcinogenic effects and antioxidant properties (Katiyar et al, 2000). Green tea has been reported to reduce lung, forestomach, esophagus, pancreas, breast, duodenum, skin, colon and liver risk cancer (Fujiki et al, 1999; Mukhtar and Ahmad, 2000); it has been found to be useful for encouraging the efficacy of chemotherapy (Sadzuka et al, 1998); green tea consumption has shown a preventive effect against coronary heart diseases high blood cholesterol concentration, and high blood pressure (Mukhtar and Ahmad, 2000). Much of the cancer preventive effects of green tea are mediated by EGCG, the major polyphenolic constituent of green tea.

*Camellia sinensis* is also a good cutaneous photo chemoprotector (Ahmad and Mukhtar, 2001) and this is the reason why many pharmaceutical and cosmetic companies are supplementing skin care products with green tea extracts.

**Mechanisms of biological effects of tea:** Some of the mechanisms through which green tea accomplishes its beneficiary properties are: green tea induces apoptosis and cell cycle arrest; EGCG suppresses extracellular signals and cell-proliferation through epidermal growth factor receptor binding; EGCG inhibits the enzyme urokinase (u-plasminogen activator) one of the most frequently expressed enzymes in human cancers (Mukhtar and Ahmad, 2000).

**2.6.6 Glycine max (soybean seeds)**

**Family name:** Leguminosea.
Description: Annual legume; erect, branching plant ranging in height from several centimeters to more than two meters. The self-fertilizing flowers are white or a shade or purple. Seeds can be yellow, green, brown, black or bicoloured. The soyabean may be cultivated in most types of soil, but it thrives in warm, fertile, well-drained, sandy loam.

Origin and distribution: Originated in China. The beans were introduced in the American colonies in 1765. Large scale of soybeans in the United States appears to have started during the 1850s.

Composition: Good source of protein; the seeds contain up to 48% proteins and up to 22% oil. Storage proteins are the predominant ones. 7S and 11S globulins are two storage proteins that constitute 80% of the total protein content. Other less abundant storage proteins in soybean are 2S, 9S and 15S globulines. The seeds also contain bioactive proteins including cytochrome c, lectin, lipoxygenasa, protease inhibitors of trypsin (Kunitz inhibitor) and of chymotrypsin and trypsin (Bowman-Birk inhibitor), as well as secondary metabolites including isoflavones (the main one in soybean is genistein), saponins, phytic acid, flatus-producing oligosaccharides, goitrogens, and also other components as minerals, ascorbid acid and fiber (Garcia et al, 1997; Friedman and Brandon, 2001).

Usage in culinary art: In East Asia the bean is extensively consumed in the forms of soybean milk, a whitish liquid suspension, and tofu, a curd somewhat resembling cottage cheese. Soybeans are also sprouted for use as a salad ingredient or as a vegetable and may be eaten roasted as a snack food. Soy sauce, a salty brown liquid, is produced from crushed soybeans and wheat that undergo yeast fermentation in salt water for six months to a year or more.

Soybean proteins are used in human foods in a variety of forms, including infant formulas, flours, protein isolates and concentrates, and textured fibers. Another soy food is vegetarian meat substitutes.
**Medicinal use:** Consumption of soy foods is increasing because of reported beneficial effects on nutrition and health. These effects include: lowering of plasma cholesterol, prevention of cancer, diabetes, obesity, protection against bowel and kidney disease.

**Other uses:** Industrially the oil is used as an ingredient in paints, adhesives, fertilizers, sizing for cloth, linoleum backing, insect sprays, and fire extinguisher fluids.

Survey of literature suggested that not much information is available particularly on the chemopreventive potential of the plants used in the present study against cervical carcinogenesis. Table 2.5 shows their chemopreventive properties against different types of cancers. It may be mentioned that very few plants have been tested for their modulatory effects against cervical carcinogenesis (Table 2.6).
Table 2.5: Studies on the cancer chemopreventive effect of plant modulators against carcinogenesis

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Species</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>Trigonella foenum-graecum</strong> (fenugreek)</td>
<td></td>
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</tr>
<tr>
<td>Colon</td>
<td>Rat</td>
<td>Devasena and Menon, 2002 and 2003</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Human</td>
<td>Hibasami et al., 2003</td>
</tr>
<tr>
<td>(protodioscin isolated from fenugreek)</td>
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<td></td>
</tr>
<tr>
<td>Ehrlich ascites carcinoma</td>
<td>Mouse</td>
<td>Sur et al., 2001</td>
</tr>
</tbody>
</table>

| Brassica sp. (mustard seeds) |         |                                                 |
| Skin                         | Mouse   | Quiblawi and Kumar, 1999                       |
| (ethanolic extract)          |         |                                                 |
| In F1                         | Mouse   | Hashim et al., 1998                            |
| (oil from mustard seeds, prevention against transplacental and translactational carcinogenesis) | | |

| Cuminum cyminum (cumin)      |         |                                                 |
| Forestomach                  | Mouse   | Gagandeep et al., 2003                         |
| Cervix                       |         |                                                 |
| Liver                        | Rat     | Sinha et al., 2003                             |
| SCC in stomach               | Rat     | Salim and Fukushima, 2003                      |
| (volatil oil)                |         |                                                 |
| SCC in stomach               | Mouse   | Nalini et al., 1998                            |
| Liver                        |         | Aruna and Sivaramakrishna, 1992                |

| Piper longum (pipli)         |         |                                                 |
| Dalton's lymphoma ascites   | Human   | Sunila and Kuttan, 2004                         |
| Ehrlich ascites carcinoma   |         |                                                 |
| (alcoholic extract)         |         |                                                 |
| Lung                         | Mouse   | Pradeep and Kuttan, 2002                        |
| (piperine isolated from Piper longum) | | |

SCC: Squamous Cell Carcinoma

Continued...
<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Species</th>
<th>References</th>
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Table 2.6: Studies on cervical carcinogenesis chemopreventive effect of different plants

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<th>Plant used</th>
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<td><em>Curcuma longa</em> (curcumin)</td>
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