CHAPTER 2

Review of Literature

2.1 Background

Cancer is not a disease of modern age and its existence can be dated back to the early human civilization. The pre-historic evidence of cancer is found among fossilized bone tumors, human mummies etc. around the world. [31] Oldest description of cancer was first discovered and deciphered in the late 19th century. The "Edwin Smith" and "Goerge Ebers" Papyri, is perhaps the earliest reference to tumor-like swelling, written around 1600 BC. The Papyrus is actually a copy of the part of an ancient Egyptian textbook on trauma surgery (3000 BC), which describes 8 cases of breast cancers that were removed by cauterization with fire drill. [32]

The origin of the term 'cancer' is credited to the "Father of Medicine" Hippocrates (460-370 BC). He coined the term *carcinos* and *carcinoma* to describe non-ulcer forming and ulcer-forming tumors. The name comes from the appearance of finger like projection from cancer. Later on, the Greek term *carcinos* was translated into Latin form *cancer* by Celsus (25 BC-50AD). The term *Oncos* was given by Galen (130-200 AD), which is now a days used as *Oncology* to describe the study of cancer. [33]

Scientists in the beginning of the 15th century, developed greater understanding of the human body employing scientific methods for various diseases and dissection of human body was accepted to discover the cause of illness. The German professor Wilhelm Fabry (1560-1634) believed milk clot in mammary duct as causation of breast cancer. The chemical foundation of cancer was laid by Dutch professor Francois de la Boe Sylvius (1614-1672), who believed that diseases are the outcome of chemical processes, and considered the acidic lymph fluid as the cause of cancer. Another Dutch surgeon Nicolaes Tulp (1593-1674), believed that cancer is a poison that slowly spreads, and concluded that it was contagious. [34] The scientific foundation of oncology was laid by Giovanni Morgagni (1682-1771) of Padua in 1761 by doing autopsies to relate the patient's illness to pathologic findings. [33] The causation of cancer was first identified by British surgeon Percivall Pott (1714-1788) in 1775 by studying the cancer of scrotum among chimney sweepers. John Hunter (1728-1793) a surgeon from Scotland, suggested surgical method to remove the cancer, but the rate of survival after surgery was very less which might be due to problems with hygiene and

post-operative pain and infections. The development of anesthesia in 18th century, allowed surgery to flourish and classic cancer operations such as the radical mastectomy were developed. [33-35]

The 19th century witnessed the birth of scientific oncology with use of the modern microscope for studying diseased tissues. The first evidence of metastasis was formulated by English surgeon Campbell De Morgan (1811-76) between 1871 and1874 who stated that the 'cancer poison' eventually spreads from the primary tumor through the lymph nodes to other sites. Rudolf Virchow (1821–1902), often called the founder of cellular pathology, provided with the the histological basis of cancer. [33-35] Thereafter, tremendous development in modern science provide insight into the biochemical and molecular aspect of cancer development and therapeutic modalities.

2.2 Present scenario of cancer

2.2.1 World cancer statistics

In the present scenario of disease burden, cancer stands as one of the most aggressive and lethal disease throughout the world. It is the second most common cause of disease related death in the world after cardiovascular (CV) disease and is expected to surpass the CV disease in next few decades.

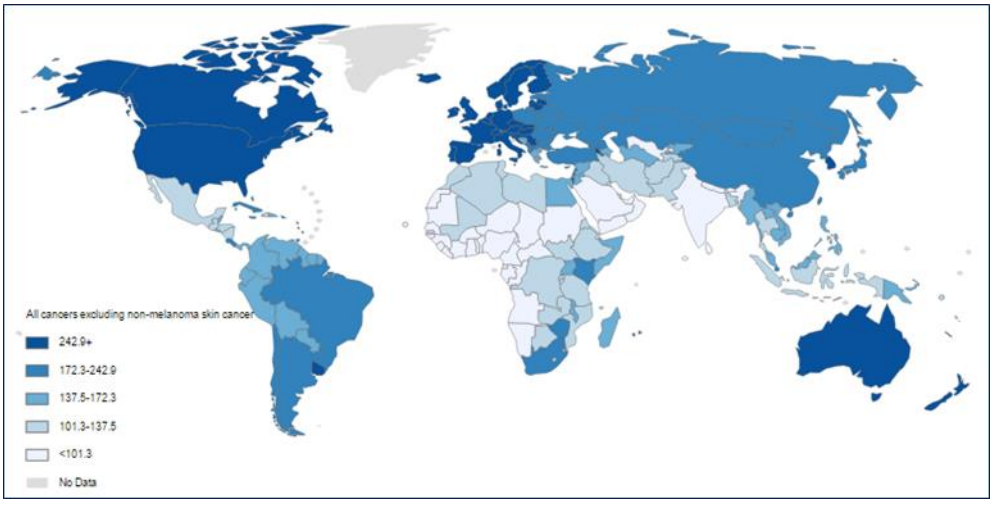


Figure 1: World map showing the distribution of age standardized rate of cancer incidence (Source: Globocan 2012, http://globocan.iarc.fr/Default.aspx) [2]

Recent data estimates that there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.5 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. The prevalence of this disease in developing countries is estimated to be around 15.6 million (48%), with 8 million (57%) new cases and 5.3 million deaths (65%) (Table 1). The overall age standardized cancer incidence rate is almost 25% higher in men than in women, with rates of 204.9 and 165.2 per 100,000, respectively. In men, the overall age standardized morality rate is also 52% higher than

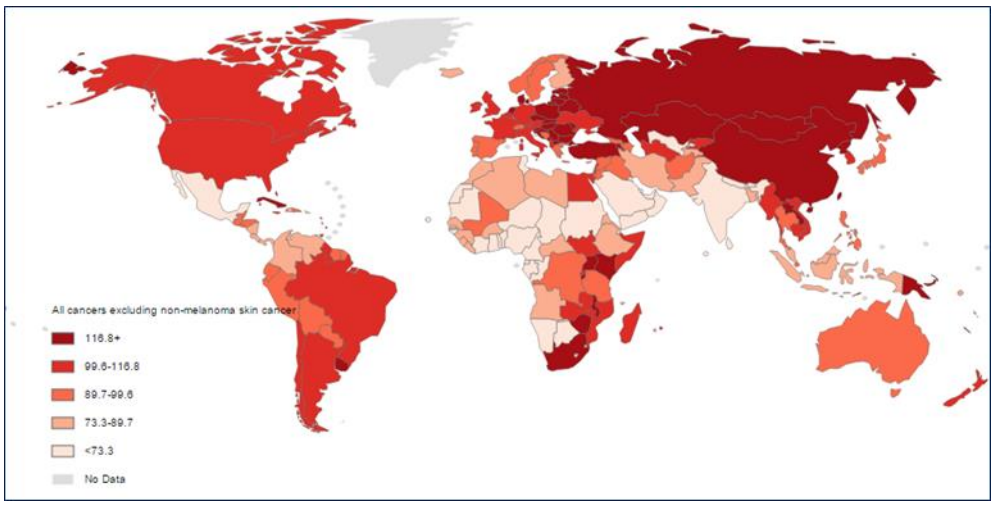


Figure 2 World map showing the distribution of age standardized rate of cancer mortality (Source: Globocan 2012, http://globocan.iarc.fr/Default.aspx) [2]

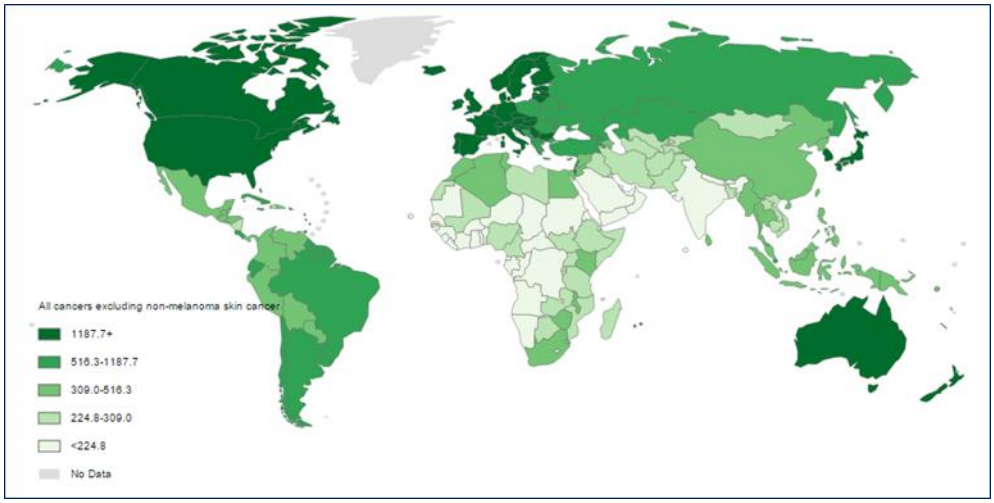


Figure 3: World map showing the distribution of prevalence of cancer (5-year, proportions per 100,000) (Source: Globocan 2012, http://globocan.iarc.fr/Default.aspx) [2]

in women, with rates of 126.3 and 82.9 per 100,000 respectively. However, proportion of population living with cancer (5-year prevalence) is 12% higher among women (660.5 per 100,000) than among men (589.4 per 100,000). [2]

Table 1: Estimated incidence, mortality and 5-year prevalence of top five ranked cancer: both sexes [2]

Cancer	Incidence			Mortality			5-year prevalence		
	Numbers	%	ASR(W)	Numbers	%	ASR(W)	Numbers	%	Prop.
Lung	1824701	13	23.1	1589925	19.4	19.7	1893078	5.8	36.5
Breast	1671149	11.9	43.1	521907	6.4	12.9	6232108	19.2	240
Colorectum	1360602	9.7	17.2	693933	8.5	8.4	3543582	10.9	68.2
Prostate	1094916	7.8	30.7	307481	3.7	7.8	3857500	11.9	149
Stomach	951594	6.8	12.1	723073	8.8	8.9	1538127	4.7	29.6

Abbreviation: Prop. : Proportion; ASR: Age standardized rate. Incidence and mortality data for all ages; 5-year prevalence for adult population only; ASR (W) and proportions per 100,000

The most common type of cancer in the world irrespective of sexes is lung cancer with 1.8 million (13%) new cases, 1.6 million (19.4%) cancer deaths and 1.9 million (5.8%) people living with cancer (within 5 years of diagnosis) in 2012 [Table 1]. The lung cancer is also the most commonest cancer in men too, with around 1.2 million (16.8%) new cases, 1.1 million (23.6%) cancer deaths and 1.3 million (8.3%) people living with cancer (within 5 years of diagnosis) in 2012 [Table 1]. However, in women, the most common cancer is breast cancer accounting 1.7 million (25.1%) new cases, 0.5 million (14.7%) deaths and 6.2 million (36.3%) people living with cancer (within 5 years of diagnosis) in 2012 [Table 3]. The overall age standardized mortality rate is higher in lung cancer irrespective of sex and in men too [Table 1,2]. However, among women the highest mortality is due to breast cancer [Table 3]. The 5-year prevalence is higher in case of breast cancer irrespective of sex (6.2 million, 19.2%) and in women too [Table 1,3]. The 5-year prevalence of prostate cancer is higher among men (3.9 million, 25.2%) [Table 2]. Its ever increasing incidence and mortality rates estimates that more than 17 to 19 million will people be diagnosed with cancer and 10 to 11 million people will die of cancer in between 2020 and 2025. [2]

2.2.2 Indian cancer statistics

India is burdened with 1.0 million (94.0 per 100,000) new cancer cases, 0.7 million (64.5 per 100,000) cancer deaths and 1.8 million (202.9 per 100,000) people living with cancer (within 5 years of diagnosis) in 2012. The overall age standardized cancer prevalence rate is 55.8% higher in women than men, with rates 262.5 and 146.4 per 100,000 respectively. The overall age standardized incidence rate is also 5.4% higher in women than in men, with rates of 97.4 and 92.4 per 100,000, respectively. In men, the overall age standardized mortality rate is also 15.8% higher than in women, with rates of 69.7 and 60.2 per 100,000 respectively. [2]

Table 2: Estimated incidence, mortality and 5-year prevalence of top five ranked cancer: men [2]

Cancer	Incidence			Mortality			5-year prevalence		
	Numbers	%	ASR(W)	Numbers	%	ASR(W)	Numbers	%	Prop.
Lung	1241601	16.8	34.2	1098702	23.6	30	1266696	8.3	48.8
Breast	1094916	14.8	30.7	307481	6.6	7.8	3857500	25.2	148.6
Colorectum	746298	10.1	20.6	373639	8	10	1953431	12.8	75.3
Prostate	631293	8.5	17.4	468970	10.1	12.8	1030787	6.7	39.7
Stomach	554369	7.5	15.3	521041	11.2	14.3	453345	3	17.5

Abbreviation: Prop. : Proportion; ASR: Age standardized rate. Incidence and mortality data for all ages; 5-year prevalence for adult population only; ASR (W) and proportions per 100,000

The most common type of cancer incident in India irrespective of sexes is cancer of breast (14.3%), cervix uteri (12.1%) and Lip, oral cavity (7.6%) with age standardized rate 25.8, 22.0 and 7.2 per 100,000 respectively. Cancer related mortality is highest in case of breast (10.3%), cervix uteri (9.9%) and lung (9.3%) cancer, with age standardized rate 12.7, 12.4 and 6.3 per 100,000 respectively. The 5-years prevalence is higher in case of breast (22.2%), cervix uteri (17.3%) and Lip, oral cavity (6.6%) with age standardized rate 92.6, 72.0 and 13.5 per 100,000 respectively. [2]

The lung (11.3%) and lip, oral cavity (11.3%) cancer is most common cancer incident in men. In men, cancer related mortality is highest in case of lung (13.7%) and stomach (11.4%) cancer. However, in terms of 5-year prevalence, the top ranked cancer is lip,

oral common form cancer incident (27.0% and 22.9%), mortality (21.5% and 20.7%) and prevalent (35.3% and 27.4%) form of cancer in women. It has been predicted that around 1.2 to 1.4 million people will be diagnosed with cancer and 0.84 to 0.95 million die of cancer in between 2020 and 2025. The predicted new cancer cases in between 2020 and 2025 will be around 0.65 to 0.73 million women and 0.59 to 0.67 million men. In between 2020 and 2025 around 0.44 to 0.50 million men and 0.40 to 0.65 million women will be die of cancer. [2]

Table 3: Estimated incidence, mortality and 5-year prevalence of top five ranked cancer: women [2]

Cancer	Incidence			Mortality			5-year prevalence		
	Numbers	%	ASR(W)	Numbers	%	ASR(W)	Numbers	%	Prop.
Lung	1671149	25.1	43.1	521907	14.7	12.9	6232108	36.3	239.9
Breast	614304	9.2	14.3	320294	9	6.9	1590151	9.3	61.2
Colorectum	583100	8.8	13.6	491223	13.8	11.1	626382	3.7	24.1
Prostate	527624	7.9	14	265672	7.5	6.8	1547161	9	59.6
Stomach	319605	4.8	8.3	76160	2.1	1.8	1216504	7.1	46.8

Abbreviation: Prop. : Proportion; ASR: Age standardized rate. Incidence and mortality data for all ages; 5-year prevalence for adult population only; ASR (W) and proportions per 100,000

2.3 Currently available therapies and their limitations

The development in the basic and medical sciences revolutionized therapeutic approaches to cancer. Ancient world was largely dependent on removal of cancer affected parts of the body, but in the late 19th century, radiation and chemotherapy emerged as selective therapeutic modalities to treat cancer. However, development of resistance to chemotherapeutic drugs by cancer cells and non-specific behavior of radiation therapy limits their use. Thereafter several other therapeutic regimes such as targeted therapy, biological therapy, immunotherapy, hormonal therapy etc. and more recently, bone marrow transplantation, stem cell therapy, hyperthermia, photodynamic therapy, laser therapy, gene therapy etc. were developed for effective management of cancer. Unfortunately, many of these treatment modalities were restricted by their own set of limitations. [36,37,38] Some of the very common forms of limitations are as follows:

- Types, size, stages and locations of malignancy.
- Efficacy: complete removal of cancer is not possible.
- Specificity: lack of specificity damages the surrounding normal tissues, proximate and distant organs.
- Toxicity: less toxicity to cancer cells and higher toxicity to normal cells.
- Resistance: resistance to existing treatment regimes.
- Recurrence: recurrence of primary secondary tumors.
- Cost, availability, accessibility etc.

Despite having huge strides in cancer diagnostic and therapeutic modalities, cancer still continues to be a formidable disease, which led the scientists to reshape the basic assumption about nature of cancer and to develop more intensive therapeutic modalities to combat this disease.

2.4 Chemoprevention: promise for cancer

Since the currently available preventive and treatment regime cannot combat cancer, the research on cancer therapeutics has been shifted towards a more realistic and holistic strategy called chemoprevention. The term chemoprevention was coined by Sporn and co-workers in 1976. [18] The term chemoprevention describes pharmacological intervention of earliest stages of carcinogenesis, before the invasive cancer appears.

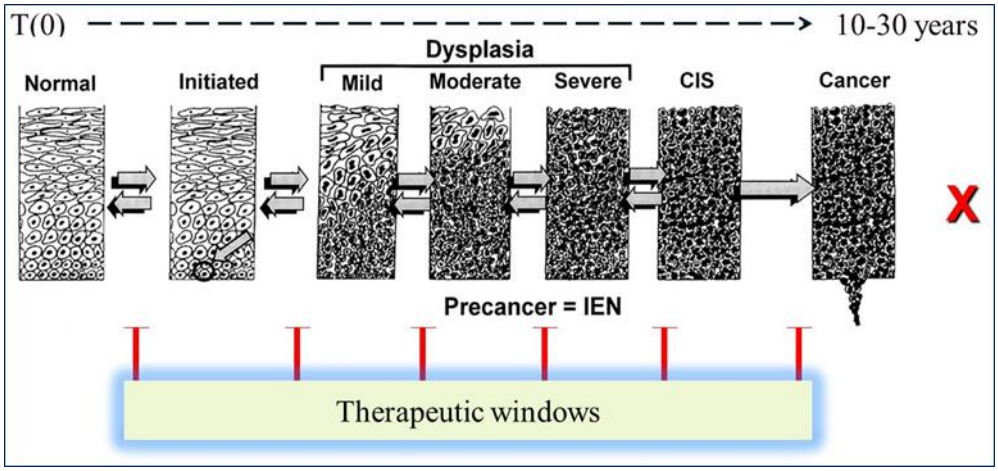


Figure 4: Basic Progression Model of cancer and its windows for chemoprevention Abbreviation: CIS: Carcinoma *in situ*; IEN: Intraepithelial neoplasia. (O'Shaughnessy *et al*., 2002) [39]

Cancer chemoprevention basically involves use of non-toxic naturally occurring or synthetic or biological entities in order to inhibit or reverse and/or delay the carcinogenesis process. [18] The concept of chemoprevention was originally developed in 1970s, but have gained impetus in recent times, when Noble Laureate Michael Bishop in 1999 predicted that chemoprevention will take rightful place as premier means to control cancer. [40]

In the present context of cancer therapeutics, chemoprevention has established itself as one of the most promising area of cancer research due to failure of the "Conventional War on Cancer" and cost effective safety and potency. [41] The chemoprevention received greater attention due to our precise understanding of cancer pathophysiology. The study of carcinogenesis has led to the current dogma that human carcinogenesis is a multi-year process. As shown in Figure 4, multi-stage carcinogenesis provides therapeutic window to intervene multiple biochemical and molecular signaling pathways, prior to accumulated mutations or phenotypic changes. Also, the time required to become malignant provide long therapeutic windows. Beside this, several studies indicated that cancer develops as a result of complex interplay between genetic make-up of an individuals and environmental factor. The environmental factors that drive carcinogenesis can at least be manipulated by appropriate life style modification in order to eliminate and /or minimize the cancer burden. [17]

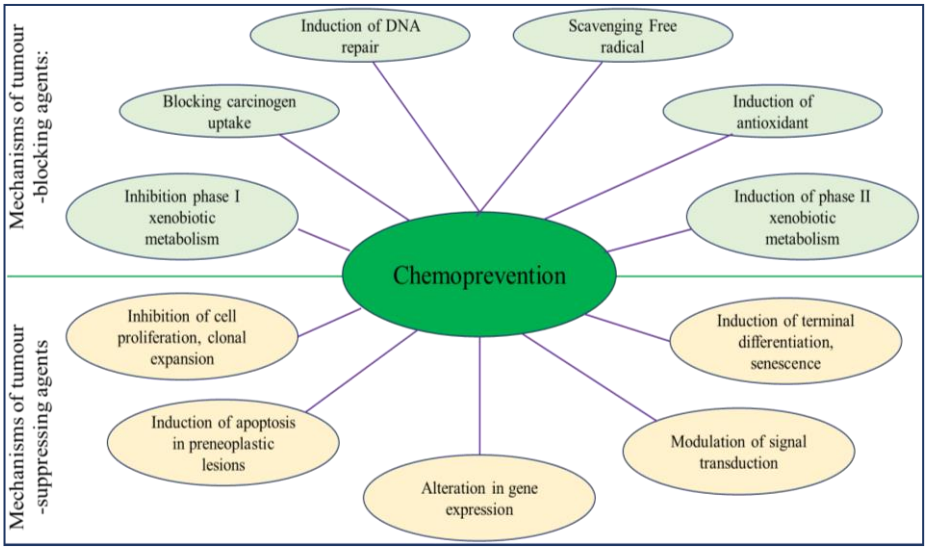


Figure 5: Mode of action of chemopreventive agents

2.5 Mechanism of chemoprevention

As the carcinogenesis process itself provides several therapeutic window to intervene, chemoprevention by virtue of action perturbate various stages such as initiation, promotion and progression. Chemopreventive agents are known to modulated the cellular biochemical and /or molecular pathways that are directly or indirectly involved in carcinogenesis. The mechanism of chemopreventive action greatly varies from cancer to cancer and agents to agents. Each of the agents owe to distinctive mechanism of action. Broadly, the mechanism of action can be summarized as shown in Figure 5. [42]

Chemopreventive agents can block or reverse or retard the pre-malignant stages of multistep carcinogenesis by blocking the uptake of pre-carcinogens/carcinogens and metabolic conversion of pre-carcinogens into carcinogens by inhibiting phase I xenobiotic metabolism enzymes or eliminating xenobiotics from the body by inducing phase II xenobiotic metabolism enzymes. From the detrimental effects of carcinogens, chemopreventive agents can protect the body by scavenging free radicals and inducing antioxidant systems or cellular repair systems. Beside this, chemopreventive agents may also inhibit cell proliferation, clonal expansion or induce apoptosis, terminal differentiation or modulate signal transduction and gene expression involved in carcinogenesis. [43,44]

2.6 Biochemical and molecular targets in chemoprevention

The substantial progress in cancer research have identified several intracellular biochemical and molecular events associated with carcinogenesis that can be targeted in order to prevent the carcinogenesis. Table 4 summarizes some of the chemopreventive agents and their molecular targets.

2.6.1 Role of xenobiotic metabolizing enzymes

Animal systems are continuously exposed to various forms of xenobiotics and some of them are potent active carcinogens and others are pre-carcinogens. However, the risk for development of cancers primarily depends on the ability of the animal system to neutralize and/or eliminate the xenobiotics. The load of xenobiotics/carcinogens is

usually tackle by the enzymes of phase I and II and thereby protect from the detrimental effects. The enzymes of phase I xenobiotic metabolizing enzymes mainly includes Cytochromes P450 (CYPs) are the key enzymes that either activate precarcinogens into active form or participate in inactivation of anticancer drugs. [45] In contrast to phase I, the enzymes of phase II such as Glutathione-*S*-transferase (GST) play role in the detoxification of xenobiotics by forming complex between xenobiotic and cellular reduced glutathione (GSH). [46] The DT-diaphorase (DTD), another enzyme of phase II metabolism, detoxify quinnones and their precursor polycyclic aromatic hydrocarbons. [47,48] Several studies have showed that increased activity of GST and DTD provide protection from the detrimental effects of xenobitics by decreasing their bioavailibity. [47,50-52]

2.6.2 Role of antioxidants

Oxidative stress, generated as result of imbalance between oxidant and antioxidant is known to plays vital role in multistage carcinogenic process through the modification of various biological micro and macromolecules such as DNA, RNA, proteins, lipids etc. To counterbalance the oxidative stress, biological system possess antioxidant system consisting of enzymatic and non-enzymatic component. [53,54] Reduced glutathione (GSH), the non-enzymatic antioxidants in conjugation with GST detoxify the electrophilic compounds. [55] The level of cellular GSH is maintained by an enzyme glutathione reductase (GR) by recycling the cellular oxidized glutathione (GSSG) to reduced glutathione (GSH). [56,57] The superoxide radicals generated in the cellular system is reported to being detoxified by the action of superoxide dismutase (SOD) which converted into into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2^{*-}). [58] The glutathione peroxidase (GPx) and catalase (CAT) convert the excess level of cellular hydrogen peroxide into water. [59] In response to oxidative stress or other xenobiotic insult, certain members of helix-loop-helix bZIP family of nuclear transcription factor such as nuclear receptor factor (NRF1, NRF2) binds to the antioxidant response elements (ARE) located to 5′-flanking of the promoter regions for genes encoding detoxifying and antioxidant enzymes such as glutathione S-transferases (GST) and NADPH:quinone oxidoreductase (NQO1). These enzymes exert their protective effects either by directly removing the xenobiotics from the body or by

masking their deleterious effects. Therefore, inducers of NRF as well as ARE are considered as potential chemopreventive agents. [17,43] However, in many cases of cancer cellular levels of antioxidants were found to be depleted causing severe threat for the free radical mediated cellular damages. The realization of harmful effects of free radical and inadequate antioxidant system, has triggered attentions to explore antioxidant in order to maintain healthy cellular environment and subsequently to protect from several life threatening diseases.

2.6.3 Role of mitogen activated protein kinases (MAPKs)

The mitogen-activated protein kinases (MAPKs) pathways link extracellular signals to control downstream machinery that control several fundamental cellular process such as growth, proliferation, differentiation, migration and apoptosis. Till date 6 different extracellular signal-regulated kinase *viz.* extracellular signal regulated kinases ERK1/2, ERK3/4, ERK5, ERK7/8, p38 isoforms alpha/beta/italic gamma (ERK6)/delta and Jun N-terminal kinase (JNK)1/2/3 have been characterized in mammals. [60] The ERK1/2 is known to plays prominent role in cancer and their upregulation and mutations in receptor tyrosine kinases (RAS, BRAF, CRAF, MEK1 or MEK2) drive inappropriate proliferation and survival of cells independent of growth factor in many types of cancer. Therefore, MEK1/2 has been recognized as a potent target in cancer therapeutics. Several studies have indicated that ATP-competitive inhibitor of ERK1 and ERK2 such as VTX11e, SCH772984 etc. prevent the phosphorylation of ERK1 and ERK2 by MEK1 and MEK2 and thus inhibit uncontrolled proliferation of cancer cells. BVD-523, RG7842 and CC-90003 etc. are the analogue of selective inhibitors of SCH772984 that have entered the clinical trials recently. [61,62] Acquired and *de novo* resistance to chemotherapeutic drugs is challenge in clinical outcome of many cancers. Chemotherapeutic drug Cisplatin activates p-ERK1/2 and p-Akt in lung cancer A549 cells and their inhibition by chemical inhibitors sensitized A549 cells to cisplatin-induced apoptosis and reduced cell viability through down regulation Bcl-2. [63] Buonato *et al.* (2014) demonstrated that prolonged exposure to MEK or ERK inhibitors helps in restraining epithelial-mesenchymal transition (EMT) to overcome chemoresistance of EGFR-targeted therapy in the non-small cell lung carcinoma

(NSCL). [64] Hypoxia inducible factor (Hif-1 α) activate ERR1/2 that confer chemoresistance to gemcitabine in pancreatic cancer cells through over expression of ABCG2 and inhibition of ERK1/2 and Hif-1 α sensitize cancer cells to gemcitabine resistance. [65] Thus the inhibition of ERK1/2 alone or in combination of other cellular signaling molecule could be an effective target for management of cancer.

Table 4: Selected chemopreventive agents with their molecular targets

Chemopreventive agents	Sources	Mechanism of action	Ref.
Apigenin	Numerous plant sources	Inhibit protein kinase C (PKC) activity, c-jun and c-fos expression	66
Benzyl isothiocyanate	cruciferous vegetables	Inhibit STAT3	67
Brevilin A	*Litsea glutinosa*, *Centipeda minima*	Inhibits JK activity and blocks STAT3 signaling	68
Celecoxib	Synthetic	Inhibit COX2	69
Curcumin	*Curcuma longa*	Up regulates Nrf2 signaling; Induces apoptosis; Inhibits NF-Kb; signaling; decreases cell invasion and motility; Inhibit STAT3	43, 70
Epigallocatechin gallate	Numerous plant sources	Inhibit AP1	71
Isothiocyanate/ Sulphoraphane	cruciferous vegetables	Increases Nrf2 expression	43
Luteolin	*Terminalia chebula*	Inhibits MMP-9 expression	43
Nastrozole	Synthetic	Inhibit CYP19 activity	43
Resveratrol	*Vitis vinifera*	Increases expression of E-cadherin; Inhibits signal transduction through PI3K/Akt; Inhibits NF-κB signalling; Inhibit β-catenin	17, 43, 72

2.6.4 Role of mammalian target of rapamycin (mTOR)

Mammalian target of rapamycin (mTOR), a conserved serine/threonine kinas, is associated with the phosphatidylinositol 3-kinase (PI3K) cell survival pathway which plays an important role in the regulation of cell growth and proliferation by monitoring mitogenic signals, nutrient availability, oxygen and cellular energy levels. [73,74] Anomalous activation of the PI3K pathway has been widely implicated in many cancers and its increased activity is often associated with resistance to cancer therapies. [75] There are two different multi-protein complexes for mTOR, TORC1 and TORC2, sensitive to rapamycin, activates S6K1 and 4EBP1, which are involved in mRNA

translation hence regulates the synthesis necessary proteins for cell growth, proliferation, angiogenesis, and other cellular endpoints. Deregulation of different elements of the mTOR pathway (PI3K amplification/mutation, AKT overexpression, PTEN loss of function and S6K1, 4EBP1 and eIF4E over expression) has been reported in several types of cancers including melanoma, where alterations in the key components of the mTOR pathway were reported to have considerable effects on tumour progression. [76] The evidence of dysregulation of mTOR signaling pathways in premalignant and early malignant cancers in human suggests mTOR as a promising target for chemoprevention of cancer. [77] Several studies have indicated that PIK3/Akt/mTOR is the most frequently altered pathway in many cancers. [77] Several anticancer drugs such as everolimus and temsirolimus are known to inhibit PI3K/AKT/mTOR and have become an effective strategy in cancer treatment. Recent preclinical studies have demonstrated that inhibitor of mTOR in combination with estrogen receptor (ER) inhibitor helps in overcoming resistance in Breast cancer. [78]

2.6.5 Role of nuclear factor kappa B(NF-κB)

The NF-κB family of transcription factors play pivotal link between inflammation and cancer. The prototypical pro-inflammatory signaling pathway is largely based on the activation of NF-kB by pro-inflammatory cytokines, such as interleukin 1 (IL-1) and tumor necrosis factor a (TNFa). [79] Furthermore, it regulates a colossal variety of target genes by generating sophisticated feedback circuits, comprising of all elements of cellular regulators such as intracellular signaling molecules, growth factors, cytokines, adhesion molecules, transcription factors as well as miRNAs. Thus NF-κB and members of its signaling network have essential roles in the complete flux of biological information from transcription to regulation of RNA function and turnover, the synthesis of proteins, their functions and their degradation. [80,81] Additionally, NF-κB is increasingly recognized as a critical player in different steps of cancer initiation and progression. During these latter processes NF-κB cooperates with manifold signaling molecules and pathways. [81] Studies have demonstrated that NF-kB regulates energy homeostasis via direct engagement of the cellular networks governing glycolysis and respiration, with profound implications beyond metabolic diseases, including cancer, ageing and anticancer therapy. [82]

2.6.6 Role of Kelch-like ECH-associated protein 1 (KEAP1)

The KEAP1 act as a negative regulator of NRF. The KEAP1 binds to the NRF in cytoplasm and prevent NRF to translocate into the nucleus. The KEAP1-NRF2 complex is an intracellular sensor that recognizes redox signalling by detecting electrophiles or ROS and this ROS cause KEAP1 to release NRF2 which later on translocate to the nucleus and transcribe genes of stress response. So, the inhibitor of KEAP1 helps in translocation of NRF2 and thereby protect from oxidative stress or deleterious effects of xenobiotic. [17,83]

2.6.7 Role of signal transducers and activators of transcription (STATs)

The signal transducers and activators of transcription (STAT), a latent cytoplasmic transcription factor are known to be activated by cytokines with their cognate receptors, whose biological activities ultimately regulates many critical aspects of cell growth, differentiation and survival. [84-86] Among the seven mammalian STAT proteins, relentless activation of STAT3 followed by STAT5 is often detected in majority of human cancer cell lines and tumor tissues. Although in normal cells STAT3 expression is very transient and strongly regulated, persuasive evidences suggest that, its persistent activation has been confirmed clinically in majority of tumor samples. This persistent activation of STAT3 plays a vital role in tumorigenesis and provides complimentary conditions to the tumor cells to undergo metastasis while being involved in cellular proliferation, invasion, migration, and angiogenesis. These include breast cancer, lung cancer, pancreatic cancer, head and neck cancer, prostate cancer, ovarian cancer, melanoma, leukaemias, and lymphomas. [86] Dysregulation of the JAK-STAT pathway is frequently observed in many primary human tumors, reflecting the importance of this pathway in the maintenance of cellular integrity. [87] In light of the contrast to this, blocking STAT3 signaling in tumor cells inhibits tumor growth, angiogenesis, and metastasis without affecting the normal cells, thus confirming STAT3 as a prospective target for cancer therapy.

The chemopreventive agents are also known to targets Cytochrome P450s, matrix metalloproteinase (MMPs) etc. in chemoprevention of several types of cancers. [17,43]

Most of the agents exerts their effect by inducing or inhibiting a single molecular target or multiple target. Since, cancer is a multi-factorial disease, agents that affects multiple targets may have advantages.

Table 5: List of chemopreventive and anticancer drugs that are in advance stages of clinical trial (Retrieved from CenterWatch. http://www.centrewatch.com/)

Drug Name (Trade Name)	Generic Name	Company	Cancer (Current status)	Mechanism of action
Abemaciclib, LY2835219	Abemaciclib, LY2835219	Eli Lilly, USA	Breast cancer(III); Lung Cancer (III)	CDK inhibitor
ADI-PEG 20, arginine deiminase	ADI-PEG 20, arginine deiminase	Polaris Pharm., USA	Hepatocellular Carcinoma (III)	Arginine deiminase (ADI)
AGS-003, RNA-loaded DC vaccine	AGS-003, RNA-loaded DC vaccine	Argos Therapeutics, USA	Renal Cell Carcinoma (III)	RNA-loaded dendritic cell (DC) vaccine
Atezolizumab, MPDL3280A	Atezolizumab, MPDL3280A	Genentech, USA	Bladder Cancer (III); Breast Cancer (III); Lung Cancer (III); Renal Cell Carcinoma (III)	Targets programmed death-ligand 1 (PD-L1) and kills cancer cells
Avelumab, MSB0010718C	Avelumab, MSB0010718C	Merck KGaA, Germany	Lung (III)	Targets programmed death-ligand 1 (PD-L1) and kills cancer cells
Bavituximab	Bavituximab	Peregrine Pharma., USA	Lung Cancer (III)	Anti-phosphatidylserine antibodies

2.7 Clinical basis of chemoprevention

From clinical point of view chemoprevention is of three type *viz.* primary, secondary, or tertiary. The primary chemoprevention is targeted to the general population and population at high risk of cancer. The secondary chemoprevention is meant for patients with premalignant lesion that may progress to an invasive disease. In contrast to primary and secondary, the tertiary chemoprevention prevents the cancer recurrence and primary secondary cancer. However, the primary and secondary chemoprevention is clubbed under "primary chemoprevention" as it actually prevents carcinogenesis.

The creditability of chemopreventive approach to control cancer gained impetus in recent past and several clinical trials has been conducted till date to show the efficacy of several naturally occurring and synthetic agents. Among them Tamoxifen, Raloxifen, 4-hydroxyphenylretinamide (fenretinide) have shown to be effective against

prevention of breast cancer. [18] Table 5 shows some of the chemopreventive and anticancer agents that are under clinical trials in 2015-16 and Table 6 that have recently approved by Food and Drug Administration (FDA), USA.

Table 6: Chemotherapeutic drugs approved by FDA, USA in 2015

Trade name	chemical name	Company	Treatment	Mechanism of action
Farydak	Panobinostat	Novartis	Multiple myeloma	Inhibition of histone deacetylase (HDAC)
Ibrance	Palbociclib	Pfizer	ER-positive, HER2-negative breast cancer	Inhibitor of cyclin-dependent kinase (CDK) 4 and 6
Imlygic	Talimogene laherparepvec	Amgen	Unresectable recurrent melanoma	Production of the immune stimulatory protein GM-CSF
Lenvima	lenvatinib	Eisai	Thyroid cancer	Inhibition of receptor tyrosine kinase (RTK)
Lonsurf	Trifluridine and tipiracil	Taiho Oncology	Metastatic colorectal cancer	Inhibition of nucleoside metabolism pluc thymidine phosphorylase
Odomzo	Sonidegib	Novartis	Locally advanced basal cell carcinoma	Inhibition of hedgehog pathway
Onivyde	Irinotecan liposome Inj.	Merrimack	Metastatic pancreatic cancer	Inhibition of topoisomerase

2.8 Model system for chemoprevention study

The process of carcinogenesis and their prevention can be studied in several preclinical models starting from cell culture to animal models. However, animal model of carcinogenesis is advantageous over cell culture as it mimics the complete and complex *in vivo* environment. Numerous animal models such as transplanted tumor model, genetically engineered tumor model and chemically induced carcinogenesis models are developed till date. But, none of the individual animal models can completely mimic the complex human cancer. [88-90] Table 7 shows some of the chemically induced carcinogenesis model that are extensively used to evaluate chemopreventive potentials.

2.8.1 DMBA induced and croton oil promoted skin papillomagenesis

The 7,12-Dimethylbenz(a)anthracene (DMBA)/Croton oil model of mice skin carcinogenesis is an example of excellent model of chemically induced carcinogenesis. It is best suited for evaluating chemopreventive potentials of dietary factors/dietary manipulations and other chemicals (both natural and synthetic) at all the three phases of carcinogenesis *viz.* initiation, promotion and progression. In this model, DMBA is tropically applied that targets the *Hras1* gene in epidermal keratinocytes, cause A:T to T:A transversions binding to the N^6 position of deoxyadenosine in 61 codon of *Hras*. Repetitive tropical application of Croton oil, helps in clonal expansion of initiated cells resulting into clonal outgrowths of the skin carcinomas. The active components of croton oil is 12-O-Tetradecanoylphorbol 13-acetate (TPA) that bind to protein kinase C (PKC) and the activated PKC induces proliferation of cells through activation of the downstream mitogen-activated protein Kinase/ Extracellular signal-regulated kinases (MAPK/ERK) pathway.

Activated PKC also targets c-Jun N-terminal kinase (JNK), epidermal growth factor receptor (EGFR), vimentin and RASSF1A etc. Phosphorylation of vimentin, promotes integrin recycling, cell motility, whereas phosphorylated RASSF1A (Ras association domain family 1A) allow cell cycle progression. The TPA also promote the carcinoma by inducing inflammation as the activated inflammatory cells produce reactive oxygen species (ROS) and nitric oxide resulting in oxidative stress. This DMBA/Croton oil skin carcinogenesis model is extensively utilized to address various questions of epithelial cancers as it truly recapitulates multi-stage carcinogenesis of human at genetic and molecular level such as activation of ras protein, EGFR, STAT3, Akt-mediated signaling pathways, elevation in the expression of transforming growth factor β1 (TGF β1) etc. The rational of utilization of this chemically induced mice model is also supported by multiple low dose exposure of carcinogens, long latency period in human. [88-93]

2.8.2 Benzo(a)pyrene induced forestomach papillomagenesis

The Benzo(a)pyrene induced forestomach carcinogenesis in mice is one of the routinely used experimental model in chemoprevention of cancer. This model employ oral gavage administration of B(a)P. The B(a)P is a potent pro-carcinogen of

polyaromatic hydrocarbon (PAHs) type, present in the environmental pollutant, generated from the combustion sources such as diesel exhaust, cigarette smoke, industrial coke production and known to initiate and promote papilloma. Following inhalation or oral administration B(a)P readily gets absorbed, binds to aryl hydrocarbon receptor (AhR) and translocate to the nucleus. In the nucleus, it forms s heterodimer with Ah Receptor Nuclear Translocator (ARNT), binds to the promoter region of xenobiotic response element (XRE) and induces expression of CYP1A and CYP1B genes. The cytochrome P450-dependent monooxygenase and epoxide hydrolase metabolically convert B(a)P into its active form (+)-anti-7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene [(+)-anti-BaPDE]. The active form of B(a)P covalently interact with nucleophilic sites in DNA, block DNA polymerase activity and expression various types of genes such as DNA repair gens, cell cycle regulatory genes resulting into papilloma formation in forestomach. Since, PAHs are environmental pollutant, evaluation of chemopreventive potential using this model helps in judging real scenario of human and their environmental encounter. [94-96]

2.9 Phytochemicals as chemopreventive and anticancer agents

Plant derived substances have traditionally played important roles in the treatment of human diseases. Today about 80% of the world population residing in third world countries still rely almost on the plant products for their primary health care. The remaining 20% of individuals living in the first world use pharmaceuticals which have been directly derived from the plant products. [119, 120] Also the evidences from several epidemiological studies have established that regular consumption of diet rich in phytochemicals reduces cancer risk. Block *et al.,* (1992) reviewed ~200 epidemiological studies that described the relationship of fruits and vegetable consumption with cancer risk and found that 128 studies out of 156 have clear demonstration of protective effects of fruits and vegetable consumption. The study also revealed 2-fold higher risk of cancer in person with low intake of fruits and vegetables as compared to high intake. [121,122] Thereafter plant derived natural compounds have gained impetus as novel chemopreventive and anticancer agents.

Table 7: Chemically induced carcinogenesis models that are currently being used in chemoprevention study

Organ	Carcinogen	Species	Route	Endpoint measured	Mechanism	Ref.
Breast	DMBA	Mice, Rat	Oral gavage	Adenocarcinomas and adenomas	Complete carcinogenesis	97, 98
	MNU	Rat	Oral gavage	Adenocarcinomas	Complete carcinogenesis	99
	PhIP	Rat	Intragestric *Inj*.	Adenocarcinomas	Complete carcinogenesis	100
Skin	DMBA /Croton oil	Mice	Tropical application	Squamous cell carcinomas	Initiation/Promotion	101
Lung	NNK	Mice, Rat	Oral gavage, subcutaneous	Adenomas and squamous cell carcinomas	Complete carcinogenesis, Initiation	102, 103
	MCA	Mice	Intraperitonea l (IP) *Inj*.	Adenomas	Initiation	104
	B(a)P	Mice	Oral gavage	Adenomas and adenocarcinomas	Complete carcinogenesis	105
	DENA	Rat	Instillation	Adenomas and adenocarcinomas	Initiation	106
Liver	DENA/PB	Mice	IP/*Ad libitum*	Carcinoma	Initiation/Promotion	107
	DMN	Rat	Oral gavage	Carcinoma	Initiation	108
	MeIQx	Rat	Oral gavage	Carcinoma and adenomas	Initiation	109
Stomach	B(a)P	Mice	Oral gavage	Carcinoma	Complete carcinogenesis	101
	MNNG	Rat	Oral gavage	Adenocarcinoma	Complete carcinogenesis	110
Renal	DENA /Fe-NTA	Rat	IP *Inj*.	Carcinoma	Initiation/Promotion	111
Bladder	BBN	Mice, Rat	*Ad libitum*, oral gavage	Transitional cell carcinomas	Complete carcinogenesis	112, 113
Prostate	MNU	Rat	IP *Inj*.	Adenocarcinomas	Initiation	114
Cervix	MCA	Mice	Uterine cervical *Inj*.	Carcinoma	Complete carcinogenesis	115
Oral cavity, esophagus	4NQO	Mice, Rat	Topical application	Squamous cell carcinomas	Complete carcinogenesis	116, 117
	DMBA	Hamster	Topical application	Squamous cell carcinomas	Complete carcinogenesis	118

Abbreviations: DMBA: 7,12-Dimethylbenzanthracene, MNU: N-Methyl-N-nitrosurea, PhIP: 2-Amino-1-methyl-6- phenylimidazo[4,5-b]pyridine, NNK: 4-(Methylnitrosamino)-1- (3-pyridyl)-1-butanone, MCA: 3-Methylcholanthrene, B(a)P: Benzo[a]pyrene, DENA: N-Nitrosodiethylamine, PB: Phenobarbital, DMN: Dimethylnitrosamine, MeLQx: 2-Amino-3,8-dimethylimidazo- [4,5-f]quinoxaline, MNNG: N-Methyl-No-nitro-Nnitrosoguanidine, Fe-NTA: Ferric nitrilotriacetate, BBN: N-Butyl-N-(4-hydroxybutyl) nitrosamine, 4NQO: 4-Nitroquinoline 1-oxide.

The secondary metabolites are the organic compounds produced by plants that are not generally required for normal growth and development of plants. These metabolites basically serves as plant defensive agents and humans use them as medicine, flavouring agents, and recreational drugs etc. The plant secondary metabolites are known to possess various biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer and chemopreventive etc. which make them attractive resources for development of therapeutic and preventive medicine for human welfare. [123]

2.9.1 Phenolic compounds

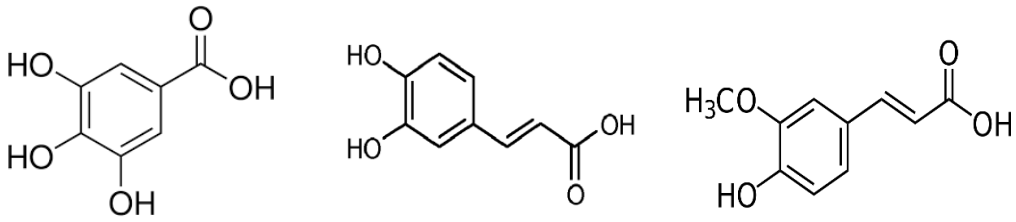
The phenolics are the largest structural classes of plant secondary metabolites characterized by the presence of one (monophenolic) or more (polyphenolic) phenol structural unit with one or more hydroxyl groups attached. Most of the phenolics are synthesized within the plant cells from amino acids such as phenylalanine or tyrosine. The chemical structure of phenolics varies from simple, low molecular weight, single unit phenolic to large, complex and multiunit tannins. More than 8000 different types of phenolic have been identified and many of them possess significant anticancer and chemopreventive potentials. [124,125]

2.9.1.1 Gallic acid

Gallic acid is one of the most widely used hydroxybenzoic acid type of phenolic compound founds in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants. It is known to possess anticarcinogenic, antimicrobial, antimutagenic, antiangiogenic and anti-inflammatory activities besides their use in treating critical diseases like depression, cancer, microbial infections, lipid-related diseases, etc. [126] Raina *et al.*, (2008), demonstrated that oral feeding of gallic acid retarded the growth and progression prostate cancer to advanced-stage adenocarcinoma in TRAMP mice by inhibiting progression of cell cycle, proliferation and increasing apoptosis. [127] Gallic acid also exert anticarcinogenic activity against 1,2-dimethyl hydrazine induced colon carcinogenesis in rat by modulating enzymes of xenobiotic metabolism and antioxidants. [128]

2.9.1.2 Caffeic acid

This is also one of the commonly found hydroxycinnamic acid type of phenolic compound, present in many food sources such as coffee, blueberries, apples etc. Caffeic acid is known to possess antioxidative, free radical scavenging, antibacterial and chemopreventive activities. It is also know to prevent atherosclerosis and other cardiovascular diseases. [129] Caffeic acid and its derivatives caffeic acid phenethyl ester (CAPE) cause complete regression of hepatocellular carcinoma and metastasis by selective suppression of MMP-9 enzyme activity and transcriptional down-regulation by the dual inhibition of NF-κB as well as MMP-9 catalytic activity. [130] Caffeic acid induces apoptosis by inhibiting Bcl-2 activity, leading to strong anticancer activity in human cervical cancer cells (HeLa cells). [131] Caffeic acid also inhibit growth of ER(+) MCF-7 and ER(-) MDA-MB-231 breast cancer cells and act as a chemosensitizer to tamoxifen and reduces breast cancer. [132]



Gallic acid Caffeic acid Ferulic acid

Figure 6 : Chemical structure of few chemopreventive and anticancer phenolic compounds

2.9.1.3 Ferulic acid

Ferulic acid, a hydroxycinnamic acid type of phenolic compound is present in large quantities in various fruits and vegetables and known to possess a wide range of therapeutic potential such as antioxidant, anti-inflammatory, antiaging, neuroprotective, hepatoprotective and anticarcinogenic effects. [133] Chemopreventive potentials of Ferulic acid against DMBA induced skin papillomagenesis, mammary papillomagenesis, buccal pouch papillomagenesis has also been reported. [134-136] Manoharan et al., (2014) also showed that ferulic acid suppresses oral tumor formation by down regulating the expression of COX-2, NF-κB and VEGF during DMBA induced oral carcinogenesis. [133]

2.9.2 Flavonoids

The flavonoids are a class of plant and fungus secondary metabolites containing a general structure of 15-carbon skeleton (C6-C3-C6): two phenyl rings and a heterocyclic ring. It is most abundant plant phenolic, distributed ubiquitously and found in high concentrations in the leaf epidermis and fruits skins. Flavonoids play role in pigmentation, protection against UV-radiation and resistance to the disease. Flavones, flavonols, isoflavonols, flavonones etc are major subtype of flavonoids. Several structural sub-constituents such as hydroxyl groups, sugar moiety, methyl, isopentyl etc. remain attached to the flavonoids. Most of the flavonoids generally exist as glycosides. The methyl and isopentyl groups increases lipid solubility, whereas sugars and hydroxyl groups increase the water solubility of the flavonoids. [124, 137]

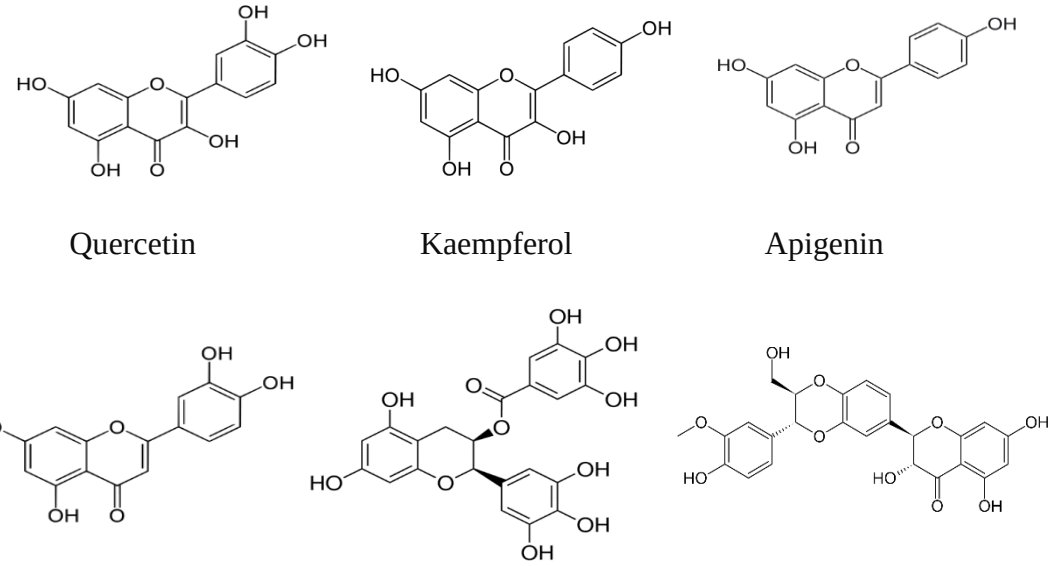
2.9.2.1 Quercetin

Quercetin is a flavonols type of flavonoid present in several vegetables, fruits, seeds, nuts, tea and red wine etc. It is an excellent free-radical scavenger and antioxidant and its activity in cellular system depends on the availability of GSH. Beside this quercetin also have strong anticancer and chemopreventive activities against several cancer. Quercetin exerts direct pro-apoptotic effects on the cancer cells and inhibit the growth and proliferation of several human cancer cell line by targeting cell cycle. [57] It is also reported to reduces the B(a)P induced lung cancer and N-nitrosodiethylamine induced hepatocarcinoma in *Swiss albino* mice and rat respectively, by increasing the activity/levels of GST, GSH, SOD, CAT and decreasing the level of LP. [138,139] Quercetin also reduces the distal metastatic invasions of liver and bone by upregulating forehead box O1 (FOXO1) and NFKBIA (IkappaBalpha) genes in nude mice. [140]

2.9.2.2 Kaempferol

Kaempferol is also a flavonols type of flavonoids extensively found in fruits and vegetables. Recent studies indicated that diet rich in kaempferol, reduces cancer risk in smokers by interrupting the aryl hydrocarbon receptor (AHR) pathway. Kaempferol has been also reported to augment the body's antioxidant defence against free radicals, inhibit cancer cell growth and angiogenesis, induce apoptosis in various types of

cancers without affecting normal cell. [141] Kaempferol exerts antiproliferative and cytotoxic effects on breast cancer MCF-7 cells by inhibiting glucose uptake by decreasing GLUT1-mediated glucose uptake. [142]



Quercetin Kaempferol Apigenin

Luteolin Epigallocatechin gallate Silibinin

Figure 7: Chemical structure of few chemopreventive and anticancer flavonoids

2.9.2.3 Apigenin

Apigenin is a flavones type of flavonoid abundantly present in common fruits and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts etc. and known to have remarkable anti-inflammatory, antioxidant and anti-carcinogenic properties. [143] Apigenin effectively suppresse progression of prostate cancer in TRAMP mice by targeting PI3K/Akt/FoxO signaling pathway and induces apoptosis in 22Rv1 cells by initiating reactive oxygen species and p53 activation. [144,145]

2.9.2.4 Luteolin

Luteolin is a flavones types of flavonoid found in yellow-green plant tissues and its best sources are celery, green peppers, carrots, olive oil etc. and is known to exhibit anticancer activity by inducing apoptosis, inhibiting cell proliferation, angiogenesis and metastasis. Luteolin act as sensitizer to tamoxifen in drug resistance breast cancer MCF-7 cells via the inhibition of cyclin E2 expression. [146] Besides, anticancer,

Luteolin have anti-inflammation, anti-allergy, antioxidant activities. [147] Luteonin also inhibits the incidence of tumor and decreases tumor volume in DMBA induced mammary carcinogenesis model in Wistar rat. [148]

2.9.2.5 Epigallocatechin gallate (EGCG)

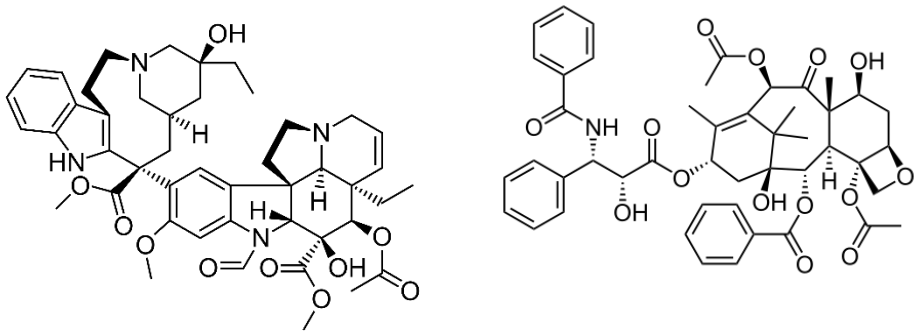
The EGCG is a flavonol types of flavonoids commonly found in tea, coffee, apple skin, plums, onions etc. It is one of the mostly investigated flavonoids known to possess potent antioxidant, chemopreventive and anticancer, anti-inflammatory, activity. [149] EGCG modulated differential ROS production is reported as one of the mechanism of its action. EGCG is found to exert differential prooxidant effects in cancer and normal cells through SIRT3, a novel potential target. [150] Epigenetic modification by inhibition of methyltransferase activity and histon acetylation leading to induction of apoptosis is reported. [151-153]

2.9.2.6 Silibinin

Silibinin is the major active constituent of silymarin, present in extract of the milk thistle *Silybum marianum* seeds. It exhibit strong antioxidant, free radical scavenging, hepatoprotective activity. Silibinin is demonstrated to possess chemopreventive, anticancer, antiangiogenesis, antimetastic etc. efficacy in various pre-clinical cancer models including skin, bladder, colon, prostate, lung etc. Due to its potent results, it has been tested in human cancer patients in phase I-II pilot clinical trials which indicated non-toxic behavior and promising plasma and target-tissue bioavailability. [154-156]

2.9.3 Alkaloids

The alkaloids are diverse group of phytochemicals containing ring structure and nitrogen atom in common and have a wide distribution starting from bacteria, fungi, to higher plants. The alkaloids are basically synthesized by plants for their protective function and some are poisonous for human health. However, several alkaloids are reported to have significant biological activities such as antiarrhythmic, anticholinergic, analgesic, muscle relaxant including anticancer and antitumor activity. [157]



Vinca alkaloid (Vincristine) Paclitaxel)

Figure 8: Chemical structure of few chemopreventive and anticancer alkaloids

2.9.3.1 Vinca alakloid

The vinca alkaloids are a class of organic compounds present in periwinkle plant *Catharanthus roseus* G. Vinblastine (VBL), vinorelbine (VRL), vincristine (VCR) and vindesine (VDS) are the four major vinca alkaloids. Vinca alkaloids are known for their strong antitumor activity and in fact, the discovery of vinca alkaloid have made breakthrough in the field of anticancer drug discovery. They act as an inhibitor of cell cycle progression by binding to the microtubule and subsequently inhibits spindle formation. There were also reports available that demonstrated that vinca alkaloid inhibit DNA repair and RNA synthesis by blocking DNA dependent RNA polymerase. [158,159]

2.9.3.2 Taxen

The alkaloid Taxanes are mainly found in the genus Taxus such as *T. baccata*, *T. brevifolia*. They are most effective and widely used chemotherapy agents. Taxen exerts anticancer activity mainly through depolymerization of microtubule and thereby inhibiting cell proliferation. Beside this, taxen were also found to act as readiosensitiser. However, poor water solubility, permeability and low bioavailability limits their use. In a recent study, Mognetti *et al.*, (2012) encapsulated paclitaxel in Nanosponges which enhanced the cell permeability. [160,161]

2.9.4 Saponins

The saponins are amphipathic glycoside type toxic compound present in soapwort which makes foam upon shaken with water. Recently several studies have demonstrated their anticancer activities in various cancer model. Saponin extracted

from *Allium chinense* exhibited anticancer activity against B16 Melanoma and 4T1 Breast carcinoma cell through inhibition of proliferation, migration and invasion of cancer cells. [162] Another study demonstrated that sapnonin extracted from the *Anemone flaccida* induces apoptosis via COX-2/PGE2 pathways in human BEL-7402 and HepG2 hepatoma cell lines. [163] Saponin was also found to exert anticancer activity against EAC cells and antiangiogenic activity in rat air sac, peritoneal and CAM model. [164]

2.10 Traditional knowledge and drug discovery

Traditional and indigenous knowledge includes subject areas from art to agriculture, as well as use of medicinal plants and traditional medication. It may exist in indigenous or local communities as secret oral traditions, passed down over generations, but it may also be documented in publicly available written or even electronic media. [165] The anticancer and chemopreventive potentials of plants and/or plant derived compounds have been recognized for centuries. Thereafter several plants were being studied for their chemopreventive and anticancer potentials. It was addition of the Vinca alkaloid vincristine or oncovin (isolated from *Catharanthus roseus*, Apocynaceae) to mechlorethamine, prednisone and procarbazine the first cures in a human cancer were achieved. [21,166] The combination of epipodophyllotoxin etoposide (derived from the mandrake plant *Podophyllum peltatum* and wild chervil *P. emodi*, Berberidaceace), bleomycin and cisplatin is currently a highly active and curative in testicular cancer. [167,168] Etoposide is also most active against small cell lung carcinoma. [168,169]

Several reports are available on the chemopreventive and anticancer properties of medicinal plants found around the world. Kumar *et al.*, (2004) reported the anticancer and immuno-stimulatory compounds from *Andrographis paniculata*. [170] Extract from the Neem leaves (*Azadirachta indica*) showed the anti-inflammatory, antioxidant and anticancer properties. [171] Choudhary *et al.*, (2008) showed chemopreventive potential of *Tinospora cordiolia* on skin carcinogenesis. [172] Mahanine isolated from the *Muraya Koenigii* has been reported to be effective against the human prostate cancer cells. [173,174] Choudhary *et al.*, (1998) have reported the antioxidant properties of curcumin isolated from the *Curcuma longa*. [175]

2.11 Biodiversity hotspot of North East India

The biodiversity of India is one of the 12 mega biodiversity countries in the world. Though, this country constitutes only 2.4% of the world's land area, but it contributes 11% of flora and 6.5% of fauna to the world biodiversity. The north eastern region of India is a part of both Himalayan as well as Indo-Burma biodiversity hotspots. The

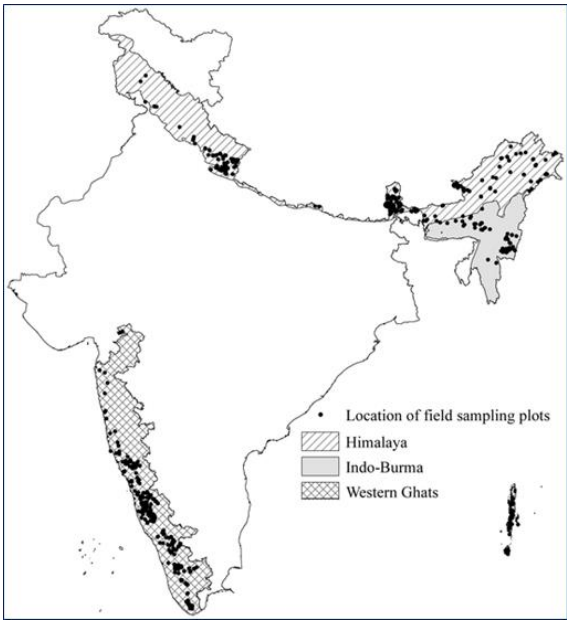


Figure 9: Map of India showing biodiversity hotspots (Chitale *et al*., 2014) [177]

Himalayan biodiversity is located between 25°39′28″ and 35°49′48″ N Latitude; 73°08′04″ and 97°24′44″ E Longitude along Northwest boundary, while the Indo Indo-Burma biodiversity is located Eastern and Southern side in between 10°30′34″-26°55′50″ N Latitude; 89°51′16″-95°22′48″ E Longitude. [176,177] This region possesses a large potential for rich biodiversity due to different climatic conditions and physiographic and altitudinal variations. It has the plethora of floral and faunal diversity encompasses about 50% of India's biodiversity. [178,179] This region harbour 8,000 out of 15,000 species of flowering plants and highest floral diversity is reported from the states of Arunachal Pradesh (5000 species) and Sikkim (4500 species) amongst the North Eastern States. The unique physio-geographical condition also harbours many endemic and rare plants. Botanical Survey of India (BSI) in its Indian Red Data Book mentioned that 800 Nos. of flowering plants from this region is endangered. [180]

2.12 Conclusion

The region of North East India is inhabited by more than 200 tribes of different ethnic groups and carries rich and unique traditional and indigenous knowledge on use of edible plants as source of household remedies against various forms of ailments.[180] The unique and diversified knowledge on medicinal plant can be a better source for discovery of lead molecule/drug with chemopreventive and anticancer potentials. Therefore rich medicinal plant knowledge needs to be explored in a scientific way and systematic pharmacological studies done in suitable experimental models. With this background in the present study, we have made an attempt to identify medicinal plant based on the traditional and indigenous knowledge from the north eastern region of India and tried to evaluate their chemopreventive and anticancer potentials in pre-clinical cancer models. Further, identification of active principle responsible for the activity were also pursued.