# Chapter – 1

# INTRODUCTION

## 1.1 History of Herbal Medicine:

All ancient civilizations have their own historical references to medicinal plants. In the earliest records, herbal medicine is linked with magic and is repeatedly viewed as being a gift from the Gods allowing the people to overcome evil power on earth. Chinese Pen T'cao (Great Herb) of Shen Nung, dating back to 2800 BC, in which over 360 species are listed including *Ephedra sinica* (MaHuang) the source of Ephedrine now being widely employed in allopathic practice of medicine. Ancient Papyri show that in Egypt from about 2000BC onwards there was large number of physicians who treated diseases with plants. [1] Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of products derived from plant parts that elicit a pharmacologic effect. [2] The development of drugs from plants continues with drug companies engaged in large-scale pharmacological screening of herbs. Modern western herbalist emphasis the effects of herbs on individual body systems.

Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare. [3] In the last twenty years in the United States, increasing public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of herbal medicines. In Germany, roughly 600 to 700 plant-based medicines are available and are prescribed by approximately 70% of German physicians. The pharmaceutical research of developing countries like India is looking towards plant based medicine. [4]

In laboratory settings plant extracts have been shown to have a variety of pharmacological effects including anti-inflammatory, vasodilatory, anti-microbial, anti-convulsant, sedative and anti-pyretic effects. [5] Human studies also confirm the specific therapeutic effects of particular herbs: randomized controlled trials supported the use of ginger for treating nausea, vomiting and ginkgos for cerebral insufficiency and dementia. The best-known evidence about an herbal product concerns St John's wort (*Hypericum perforatum*) for treating mild to moderate depression. A systemic review of 23 randomized controlled trials found to be significantly superior to placebo and

therapeutically equivalent to but with fewer side effects than anti depressants such as amitriptyline. [6]

### 1.2 Herbal Heritage of India:

Charka (1000BC) and Sushruta (800BC) the two eminent physicians in India mentioned in their somhitas about 700 plant species as therapeutic agents of which about 500 are being mentioned in Indian flora. Rigveda (4500-1600BC) and the Atharvaveda (2000-1500BC) have also mentioned some medicinal plants. Kritikar and Basu (1935) estimated about 604 plants species for the treatment of various ailments. Chopra and his co-workers (1956 & 1969) included about 1800 plant species in the Glossary of Indian Medicinal Plants. [7] India is a leading exporter of medicinal plants in the world trade, eg, poppy husk and seeds, opium crude, psyllium (*Plantago ovate*) husk and seeds, senna leaves and pods, *Glycerrhiza glabra* dried rhizomes, Ipecacuanha dried rhizomes and roots, nuxvomica seeds etc. Phytochemicals like betaionones, papain, quinine sulphate, atropine sulphate, quinine hydrochloride, berberine hydrochloride, emetine, strychnine, and ephedrine hydrochloride are the major phytoconstituents exported to USA, Japan, Germany, France, and Switzerland. [8]

### 1.3 Medicinal Plants in Sikkim:

Sikkim with its geographical area of 7098 sq m (latitude 27°N-28°N, Longitude 88°N-89°N) has a general relief range of 350-8579m altitude. The northern boundary and a part of eastern boundary are bounded by Tibetan plateau and to the south is the Darjeeling Gorkha Hill Council of West Bengal. River Teesta originates from the Cholhamu Lake in North at 5259m altitude. It traverses the state from northeast to south to meet river Rangeet at Triveni near Melli. The Rathong glacier in the West is one of the main sources of Rangeet. [9]

Sikkim is rich in valuable medicinal plants, nurtured by the Buddhist Gompas for the traditional Tibetan pharmacopoeia. According to the believe of the people of Sikkim, the causes of illness can be classified in to two categories among the Buddhist and Hindus.

(1) Diseases caused by supernatural beings like deities, spirits, ghosts and other non-material entities

(2) Diseases caused by magical means, witchcraft and sorcery.

### 1.3.1 Medicinal Plant Groups:

In Sikkim, most of the rural folk and even a good percentage of educated people living in urban areas have faith on herbal charms. Local plant-based drugs can easily be seen in most of the villages, rural and urban markets. Out of the primitive people's diligent trial and error the plants having potent remedial action have come up as identified and is still faithfully prescribed after several centuries. [10]

The medicinal plants found in the Sikkim Himalaya can be classified in terms of their value in usage and marketing potentials. A general screening with regard to potency and consequent value of plant has given us five major groups of medicinal plants in the region. [11]

**Group A:**

The species that constitute this group are time-tested in its efficacy and marketed in large scale. A majority of them grow in the altitude of 1000-2000m, which is also the most populated zone in the hills. A few of them are found in the higher altitudes of this group. The commercially exploited ones are *Swerta chirata, Nardostachys jatamansi, Picrorhiza kurrooa, Aconitum ferox,* etc.

**Group B:**

In this group plants are not marketed in large quantity. Many of these plants are not available in plenty. The *Terminalia* species being trees by habit has been facing greater pressure in this group.

**Group C:**

In this group herbal products (direct extract, decoction, etc) are difficult to preserve and because of this reason the plants hardly has any scope to enter the markets. eg, *Artemisia vulgaris, Eupatorium cannabinum, Clematis buchananiana, Urtica dioica*

**Group D:**

Recently exploited plants are included in this group. eg, *Taxus baccata* (Taxaceae), *Panax pseudo-ginseng* (Araliaceae). Taxus from the Himalaya has been found more important in recent tests even its needles are also used for taxol extraction. There is evidence of more effectiveness of Sikkimese ginseng than that of Korean ginseng.

**Group E:**

Plants in this group assumed to possess therapeutic properties only such as *Oroxylum indicum* (hypertension), *Fraxinus floribunda* (gout) etc which need further testing. Much possibility of further explorations in phytopharmacognostical values may be expected from this group.

### 1.4 Inflammation Pathophysiology - An Overview:

Inflammation is defined as the local response of living mammalian tissue to injury due to any agents like physical, chemical and biological. The cardinal signs of inflammation are Rubor (redness), Tumor(swelling), calor (heat), dolor (pain). [12] Inflammatory responses occur in three distinct phases.

- An acute transient phase characterized by local vasodilation and increasing capillary permeability
- A delayed sub-acute phase characterized by infiltration of leukocytes and phagocytic cells.
- A chronic proliferative phase, in which tissue degeneration and fibrosis occur.

### 1.4.1 Types of Inflammation:

**1. Acute inflammation:**

Acute inflammation is of short duration and represents the early body reaction and is usually followed by repair. The main features of acute inflammation are (1) Alterations in vascular caliber that lead to an increase in blood flow (2) Structural changes in the microvasculature that permit the plasma proteins and leukocytes to leave the circulation and (3) Emigration of the leukocytes from the microcirculation and their accumulation in the focus of injury

(A) Vascular events:

Changes in vascular flow and caliber begin early after injury and develop at varying rates, depending on the severity of the injury. The changes occur in the following order. [13-14]

1. After an inconstant and transient vasoconstriction of arterioles, lasting a few seconds, vasodilation occurs. Vasodilation first involves in the arterioles and then results in opening of new capillary beds in the area. Thus leads to increased in blood flow, which is the cause of the heat and the redness.

2. Slowing of the circulation is brought about by increased permeability of the microvasculature, with the outpouring of protein rich fluid in to the extra vascular tissues.

3. A stasis develops, appearance of peripheral orientation of leukocytes, principally neutrophils, along with the vascular endothelium a process called Leukocytic margination.

(B) Cellular events:

A critical function of inflammation is the delivery of leukocytes to the site of injury. Leukocytes ingest offending agents, kill bacteria and other microbes and degrade necrotic tissue and foreign antigens. Leukocytes may also prolong inflammation and induce tissue damage by releasing enzymes, chemical mediators and toxic oxygen

radicals. [15] The sequence of events in the journey of leukocytes from the lumen to the interstitial tissue, called extravasation, which can be divided into the following steps: (1) In the lumen: margination, rolling and adhesion (2) Transmigration across the endothelium and (3) Migration in interstitial tissues towards a chemotactic stimulus. After extravasation, leucocytes emigrate in tissues towards the site of injury by a process called chemotaxis.[16-17] Exogenous and endogenous substances can act as chemo-attractants. The exogenous agents are bacterial products and the endogenous chemical mediators are (1) Components of complement system C5a, (2) Products of lipoxygenase pathway (LTB4) (3) Cytokines-chemokines family. In addition to stimulating locomotion many chemotactic factors induce Leukocyte activation, it includes production of Arachidonic acid metabolites, degranulation and secretion of lysosomal enzymes and activation of the oxidative burst, finally modulation of leukocytes adhesion molecules. Phagocytosis and the release of enzymes by neutrophils and macrophages constitute two of the major benefits derived from accumulation of leukocyte at the inflammatory focus. [18] It involves three distinct but inter-related steps - recognition and attachment of particle to be digested by the leukocytes, engulfment and killing (or) degradation. The effects of different inflammatory mediators are given in **Table 1.1.**

**Table 1.1 Effect of different inflammatory mediators**

| **Mediators** | **Events/Effects** |
|---|---|
| PGs, NO | Vasodilation |
| Vasoactive amines, C3a, C5a, $LTC_4$, $D_4$, $E_4$, PAF, Substance P | Increased vascular permeability |
| C5a, Chemokines, $LTB_4$, Bacterial products | Chemotaxis, leukocyte activation |
| $IL_1$, IL6, TNF, PGs | Fever |
| PGs, Bradykinin | Pain |
| Neutrophils, macrophages, lysosomal enzymes, oxygen metabolites, NO | Tissue damage |

**2. Chronic inflammation:**

It is considered to be inflammation for prolonged duration whereas in active inflammation, tissue destruction and attempts to repair proceeded simultaneously.

Chronic inflammation arises under the following settings

- Persistent infection by *Tubercle bacilli*, *Treponema pallidum* and certain fungi evoke immune reaction
- Prolonged exposure to potentially toxic agents either exogenous or endogenous.
- Autoimmune diseases such as rheumatoid arthritis, lupus erythematosus

Histological features of chronic inflammation

- Infiltration of mononuclear cells which includes macrophages, lymphocytes and plasma cells.
- Tissue destruction induced by inflammatory cells.
- Attempts of healing by connective tissue replacement of damaged tissue are accomplished by proliferation of small blood vessels.

### 1.4.2 Arachidonic acid Pathway:

The arachidonic acid pathway for synthesis of mediators and the drug targets for anti-inflammatory activity is represented in the **Figure 1.1.** COX, the enzyme that catalyses the synthesis of cyclic endoperoxides from arachidonic acid was isolated in 1976 and cloned in 1988. [19]

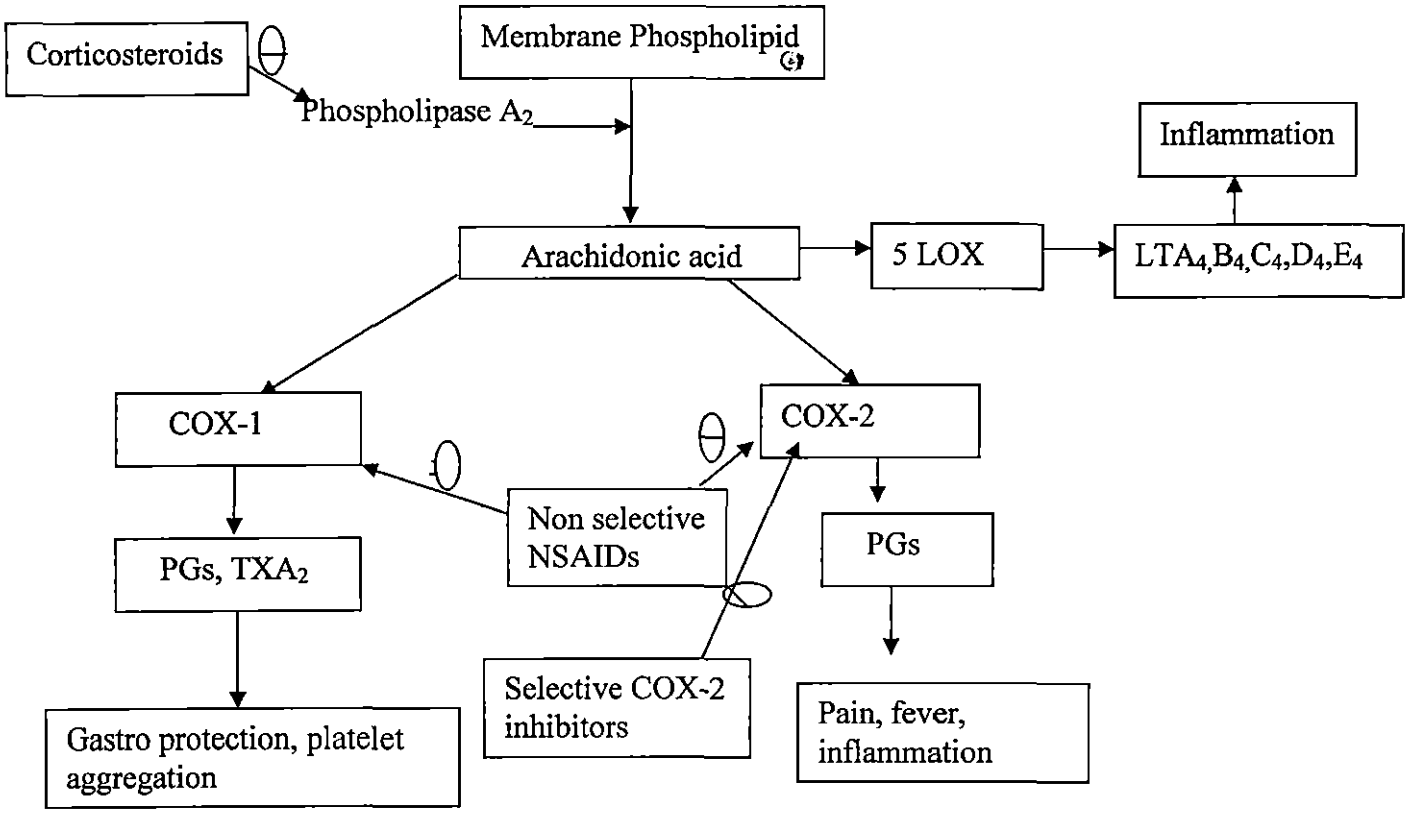


**Figure 1.1 Arachidonic acid pathway and drug targets**

### 1.4.3 Role of COX and 5-LOX in Inflammation:

Cyclooxygenase (COX) converts arachidonic acid to prostaglandin $H_2$ ($PGH_2$) which, in turn is transformed into a series of final active products in different cell types and with different expressions. Two COX isoforms, COX-1 and COX-2, have been identified. They are encoded by two different genes [20] and it has been postulated that while COX-1 is expressed in mammalian cells, particularly in endothelium, platelets, and kidneys in physiological conditions, COX-2 is inducible in pathological conditions by inflammatory stimulation. [21] It has, therefore, been suggested that constitutive COX-1 is involved in homoeostatic processes, whereas COX-2 plays a major role in the inflammatory process and the pain associated with it. The differences between COX-1 and COX-2 are represented in **Table1.2.**

**Table 1.2 Differences between COX-1 and COX-2 enzymes**

| Characteristics | COX-1 Enzyme | COX-2 Enzyme |
|---|---|---|
| 1. Location in genome | Chromosome 9 | Chromosome 1 |
| 2. Molecular weight (kDa) | 69,054 | 69,093 |
| 3. Tissue location | Gastric mucosa, intestine, kidney, platelets, endothelia cells | Macrophages,fibroblasts,chondrocytes,epithelial cells, endothelial cells, CNS |
| 4. Control of Expression | Constitutive | Rapidly inducible: Inflammation |
| 5. Biological function | Gastrointestinal mucosal integrity, platelet aggregation | Inflammation development, renal function, reproduction |
| 6. Kinetics of Inhibition by NSAIDs | Immediate, competitive inhibition | Non-competitive, time dependent |

The final and biologically active metabolites of the 5-Lipooxygenase (5-LOX) cascade are $LTB_4$ and the so-called cysteinyl LTs ($LTC_4$, $LTD_4$, and $LTE_4$), formerly known as a slow reacting substance related to anaphylaxis, which are derived from the unstable intermediate $LTA_4$. Leucotrienes are potent mediators of inflammation. $LTB_4$ is a potent stimulator of leukocyte activation, and adhesion of these cells to vascular endothelium, elicits chemo kinetic and chemo tactic responses. [22] Both the conventional NSAIDs and the selective COX-2 inhibitors primarily exert their activity by reducing the production of PGs induced in the inflammatory process. In recent years, it has been clarified that PG synthesis is only one part of the arachidonic

acid pathway, this precursor being a substrate that gives rise to many other lipid mediators, such as the LTs and the LXs. Leucotrienes themselves have a major role in the development and persistence of the inflammatory process, and it is now clear that PGs and LTs have complementary effects, whereas the production of LXs can counteract the inflammatory actions of LTs. The biological properties of LTs, together with their formation in a variety of diseases, suggest that 5-LOX inhibitors should have a therapeutic potential in a range of allergic and inflammatory conditions, such as asthma, rheumatoid arthritis, ulcerative colitis, etc. [23-24]

In view of these concepts, it has been suggested that blocking of both LT and PG production might have synergistic effects and achieve optimal anti-inflammatory activity. In addition, taking into account of the roles of $LTB_4$ and cysteinyl LTs (against which neither selective nor non-selective NSAIDs are effective) in the inflammatory process, dual inhibition of the COX and 5-LOX pathways could produce a wider spectrum of anti-inflammatory effects.

The dual 5-LOX/COX inhibitors act by blocking the formation of both PGs and LTs without affecting LX formation. The sparing effects on the gastric mucosa are probably due to the inhibition of the synthesis of 5-LOX products. It can, therefore, be expected that dual blockers induce an enhanced anti-inflammatory effect without damaging the GI mucosa. [25]

### 1.4.4 Anti-inflammatory Agents:

NSAIDs are the main category of anti-inflammatory drugs. On the basis of their mechanism of action they can be classified as Non selective COX inhibitors, preferential COX-2 selective NSAIDs and COX-2 selective inhibitors

(A) Non selective COX Inhibitors are

1. Salicylates: Aspirin, Salicylamide, Benorylate, Diflunisal
2. Pyrazolone derivatives: Phenyl butazone, Oxyphen butazone
3. Indole derivatives: Indomethacin, Sulindac

4. Propionic acid derivatives: Ibuprofen, Naproxen, Ketoprofen, Fenoprofen
5. Anthranilic acid derivative: Mephenamic acid
6. Aryl acetic acid derivative: Diclofenac, Tolmetin
7. Oxicam derivative: Piroxicam, Tenoxicam, Meloxicam
8. Pyrrolo-pyrrole derivative: Ketorolac
9. Para aminophenol derivative: Paracetamol
10. Pyrazolone derivatives: Metamizol, Propiphenazone
11. Benzoxazocine derivative: Nefopam
12. Alkanone: Nabumetone

(B) Preferential COX-2 selective NSAIDs are

1. Nimesulide
2. Etodolac
3. Meloxicam

(C) Selective COX-2 Inhibitors are

1. First generation Coxibs: Celecoxib, Rofecoxib
2. Second generation Coxibs: Valdecoxib, Parecoxib, Etoricoxib, Lumiracoxib

### 1.4.5 Adverse Drug Reactions of NSAIDs:

The most common adverse effects of NSAIDs involve in the GI tract. All NSAIDs are the potential cause of GI bleeding. [26]The most common sites of GI injury are the gastric and duodenal mucosa. COX-2 selective inhibitors pose a decreased risk of GI toxicity compared to nonselective NSAIDs. Other side effect includes renal insufficiency, hyperkalemia, and hypersensitivity reactions. [27]

In September 2004, Merck and Co., the innovators of rofecoxib announced the voluntary withdrawal of the drugs from the market, worldwide, because of the fact that it produced an increased risk of cardiac toxicities. [28-29]

### 1.4.6 Anti-inflammatory Agents Derived From Plant Source:

The Plants like *Aconitum napellus, Fraxinus excelsior, Betula verrucosa, Ananas comosus, Capsicum frutescens, Symphytum officionale, Zingiber officinale, Curcuma longa* etc have been well utilized for their anti-inflammatory property. The examples for some established anti-inflammatory phytoconstituents and their biochemical target [30] are given below in **Table 1.3.**

**Table 1.3 Examples of some phytoconstituents having anti-inflammatory activity with biochemical target**

| Phytoconstituent | Chemical nature | Biochemical Target |
|---|---|---|
| 1. Rutaecarpine | Indole-Alkaloid | COX-2 |
| 2. Ardisiaquinone | Quinone | 5-LOX |
| 3. Astringenin | Stilbene | COX |
| 4. Eugenol | Phenol | COX |
| 5. N-Cis feruloytyramine | Phenolic acid | COX |
| 6. Gossypin | Flavanol-O-glycoside | 5-LOX |
| 7. Myricetin | Flavanol | 5-LOX |
| 8. Resveratrol | Stilbene | COX |
| 9. Achillin | Triterpine | LOX |
| 10. Atractylon | Sesquiterpine | LOX |
| 11. Oleanolicacid | Triterpine | COX-1, COX-2 |
| 12. Ursolicacid | Triterpine | COX-2, 5-LOX |

The importance of herbal medicine in inflammatory disorders is due to their low toxicity and devoid of GI related adverse actions.

## 1.5 Cancer Pathophysiology – An Overview:

### 1.5.1 Epidemiology of Cancer:

Cancer is the second common cause of death in the developed countries next to cardiovascular diseases. According to WHO, out of an estimated total of 50 million deaths annually in the world, more than 5 million are attributed to cancer and the number

of deaths due to cancer throughout the world is increasing. [31] The causes of cancer vary world wide. In developed countries, tobacco is considered as a major culprit, causing 1 in 3 cancer deaths. In the developing world, infection plays major role, which is responsible for almost 1 in 4 cancer deaths. The deadly impact of tobacco use in richer countries is now being propagated globally. Another is the high prevalence of chronic infection in the developing world, in particular, with human papillomavirus, cause cervical cancer. Helicobacter pylori, implicated in stomach cancer, and hepatitis B and C viruses, cause the liver cancer. These agents account for over 90% of cancer related to infection. [32] While in the developed countries lungs, colorectal, breast, stomach, and prostate are the major parts of cancer, in developing countries the most common cancer attacked parts are those of the stomach, lungs, liver, breast, and cervix. [33]

In India the record of cancer case as per the National Cancer Registry Program (NCRP), in 1997 varies between 52.9 and 81.5 per 1, 00,000 men and between 56.8 and 95.6 per 1,00,000 women.[34] Cancer case in Indian men is about half to one third of the incidence recorded in USA & Europe. A global comparison shows that India has high rate of cancer case in oral cavity, pharynx, and cervix.

### 1.5.2 The Etiology of Cancer:

Cancer is a disease characterized by uncontrolled cellular growth, local tissue invasion and distant metastases. The processes that normally regulate cell division and tissue growth are not controlled during the proliferation of cancer cells. In discussing the difference between the proliferation of normal cells and that of cancer cells, one needs to consider the cell cycle of dividing cells

**Carcinogenesis:**

Cancer or neoplasm is thought to be developed from a cell in which the normal mechanisms for control of growth and proliferation are altered. Current evidences support the concept of carcinogenesis as a multi step process that is genetically regulated. [35] The steps are as under

1. Initiation
2. Promotion

3. Conversion or transformation

4. Progression

**Initiation:**

Initiation requires exposure of normal cells to carcinogenic substances. These carcinogens produces genetic damage that if not repaired results irreversible cellular mutation. This mutated cell has an altered response to its environment and a selective growth advantage, giving it the potential to develop into a clonal population of neoplastic cells.

**Promotion:**

Carcinogens or other factors alter the environment to favour the growth of mutated cell population over normal cells. The primary difference between initiation and promotion is that promotion is a reversible process; the phase may be the target of future chemoprevention strategies including changes in lifestyle and diet.

**Conversion or transformation:**

In this phase the mutated cells become cancerous. It may take 5-20 years from carcinogenic phase to clinically detectable cancer depending on the type of cancer.

**Progression:**

It involves further genetic changes leading to increased cell proliferation. The critical element of this phase includes tumor invasion into local tissues and the development of metastases. Some of the causative agents of cancer are listed in **Table1.4.**

**Table 1.4 Carcinogens and cancer**

| Carcinogens | Type of cancer |
|---|---|
| **Chemicals** | |
| Aniline dye | Bladder cancer |
| Benzene | Leukemia |
| Asbestos | Lung, gastrointestinal tract |
| Vinyl chloride | Angiosarcoma, liver |
| **Viruses** | |
| Epstein-Barr virus | African Burkett's lymphoma |
| Hepatitis B | Hepatocellular cancer |
| **Drugs** | |
| Alkylating agents (chlorambucil, mechlorethamine, melphalan) | Leukemia |
| Anabolic steroids | Liver |
| Ant estrogens (tamoxifen) | Endometrium |
| Epipodophyllotoxins | Leukemia |
| Immunosuppressant | Lymphoma, skin |

**Genetic basis for cancer:**

There are two major classes of genes involved in carcinogenesis: oncogenes, tumor suppressor genes. [36-37] The flow diagram of genetic basis of cancer is presented in **Figure 1.2.**

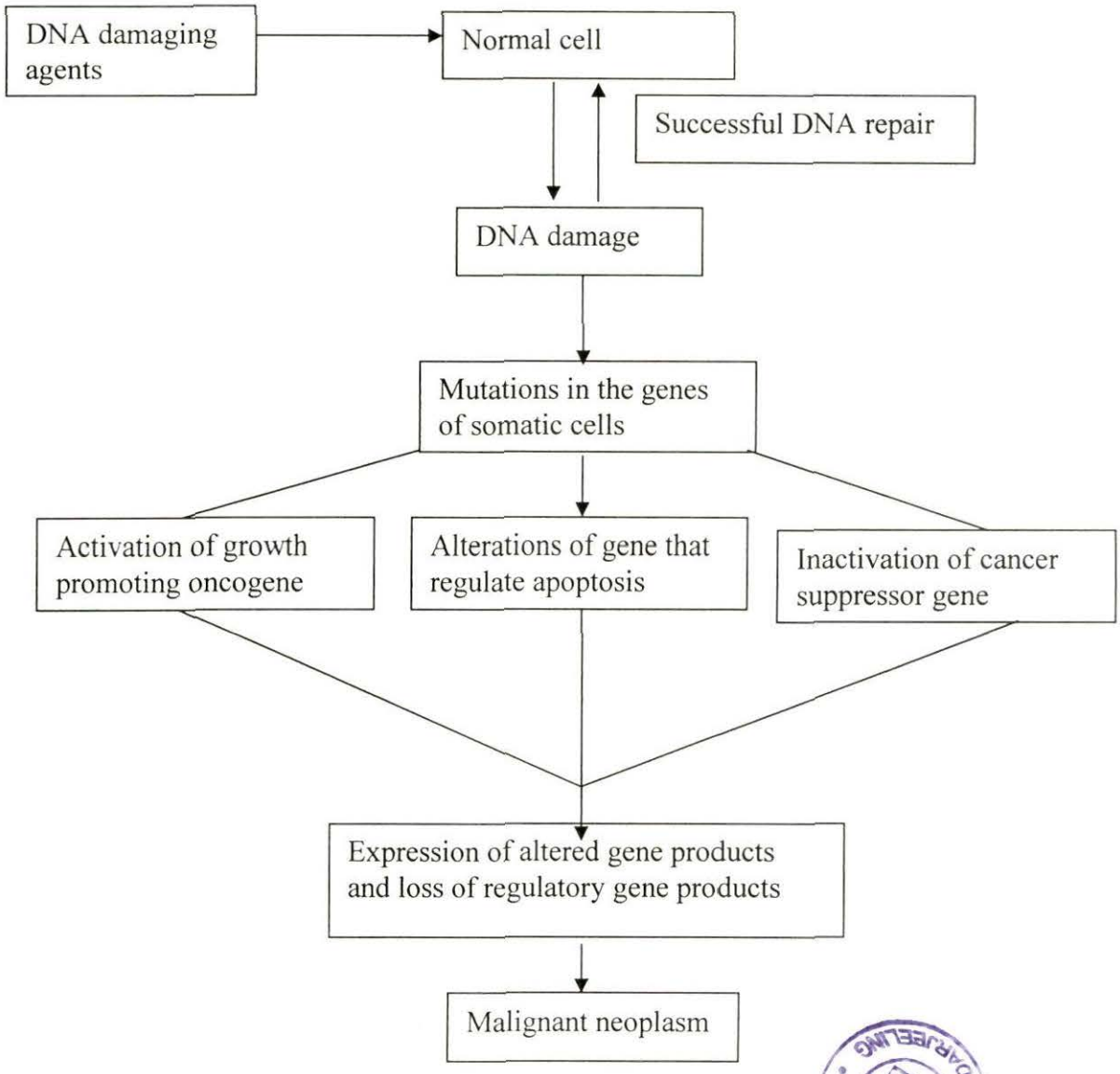


**Figure 1.2 Genesis of cancer**

Oncogenes develop from the normal genes called proto-oncogene. Genetic alteration of the proto-oncogene through point mutation, chromosomal rearrangement or gene amplification activates the oncogene. Carcinogenic agents such as radiation, chemicals or viruses may cause these genetic alterations or they may be inherited. Examples of some oncogenes are represented below

*EGFR*- Codes for epidermal growth factor-Associated with glioblastoma, breast cancer, and squamous carcinoma

*K-RAS & N-RAS*- Code for guanine nucleotide-proteins with GTPase activity-Associated with lung, ovarian, colon cancers

*BCL-2* – Codes for protein that block apoptosis - Associated with indolent B-cell lymphomas

Tumor suppressor genes regulate and inhibit inappropriate cellular growth and proliferation. Two common examples are retinoblastoma gene and $p^{53}$ gene. The normal gene product of $p^{53}$ is responsible for negative regulation of the cell cycle, allowing the cell cycle to halt for repairs, corrections, and response to other external signals. Examples for some tumor suppressor genes are given below

*APC*-Genes for protein in the cytoplasm-Associated with colon and gastric cancer

*NF-1*- Codes for protein that inhibits the stimulatory Ras protein-Associated with Neurofibroma, leukemia and pheochromacytoma

*MTS1*- Codes for $p^{16}$ protein, a cyclin-dependent kinase inhibitor-Associated with wide range of cancers

$p^{53}$ – Codes for the $p^{53}$ protein, which can halt cell division and induce apoptosis-Involved in wide range of cancers

### 1.5.3 Principle of Tumor Growth:

The growth of most tumors is illustrated by the Gompertzian tumor growth curve.[38] In the early stages, tumor growth is exponential, which means that the tumor takes a constant amount of time to double its size, during this early phase, a large portion of the tumor is actively dividing. The population of cells is called as growth fraction. Most anticancer drugs have greater effect on rapidly dividing cells. Tumors are most sensitive to the effects of chemotherapy when the tumor is small and the growth factor is high. However as the tumor grows the doubling time is slowed. According to Gompertz hypothesis though a percentage of cancer cells are killed with each course of chemotherapy, the existence of tumor never reach zero.

**Invasion and metastasis:**

Metastasis is the spread of neoplastic cells from the primary tumor site to the distant sites. The two primary pathways of metastasis are haematogenous and lymphatic. After neoplastic transformation, the malignant cells and surrounding host tissue secrete substances that stimulate the formation of new blood vessels to provide oxygen and nutrients. This process is known as angiogenesis or neovascularization. [39-40] Because angiogenesis has been recognized as a critical element in primary tumor growth as well as metastasis, it has become a target for development of new anticancer agents. Tumor characteristics and the classification of tumor according to tissue type are enlisted in the **Table1.5 and Table 1.6.**

**Table 1. 5 Tumor characteristics**

| Benign | Malignant |
|---|---|
| 1. Non cancerous growths, often encapsulated, localized and indolent | 1. Genetically unstable Loss of normal cell architecture |
| 2. Cells of benign tumor resemble the cells from which they developed | 2. Cells loss the ability to perform the usual functions. |
| 3. Seldom metastasize, once removed rarely Recur | 3. Prone to metastasize, recurrences are common |

**Table 1.6 Tumor classification according to tissue origin**

| Tissue origin | Benign | Malignant |
|---|---|---|
| **Epithelial tissue** | | |
| Surface epithelium | Papiloma | Carcinoma (squamous,epidermoid) |
| Glandular tissue | Adenoma | Aden carcinoma |
| **Connective tissue** | | |
| Fibrous tissue | Fibroma | Fibro sarcoma |
| Bone | Osteoma | Osteosarcoma |
| Smooth muscle | Leiomyoma | Leiomyosarcoma |
| Striated muscle | Rhabdomyoma | Rhabdomyosarcoma |
| Fat | Lipoma | Liposarcoma |
| **Lymphoid tissue and hematopoietic cells** | | |
| Bone marrow elements | _____ | Leukemia |
| Lymphoid tissue | _____ | Hodgkin's and non-Hodgkin's lymphoma |
| Plasma cell | _____ | Multiple myeloma |
| **Nervous tissue** | | |
| Glial tissue | Benign gliomas | Glioblastoma multiforme, astrocytoma |
| | | Neurofibro sarcoma |
| Nerve sheath | Neuro fibroma | Pigmented melanoma |
| Melanocyte | Pigmented nevus (mole) | |
| **Mixed tumors** | | |
| Gonadal tissue | Teratoma | Teratocarcinoma |

### 1.5.4 Diagnosis and Stages of Cancer:

**Diagnosis:**

The diagnosis of cancer varies depending upon the type of cancer, in adults warning signs as well as loss of appetite may have predictive role. [41]

**Cancer's warning signs:**

- Changes in bowel or bladder habits
- A sore that does not heal
- Unusual bleeding or discharge
- Thickening or lump in breast or elsewhere
- Indigestion or difficulty in swallowing
- Obvious change in wart or mole
- Nagging cough or hoarseness

The definitive diagnosis of cancer relies on the procurement of a sample of the tissue or cells suspected of malignancy and pathologic assessment of this sample. This sample can be obtained by numerous methods, including biopsy, exfoliative cytology, or fine needle aspiration.

**Staging of Tumors:**

Staging provides information on prognosis and guides treatment selection. After treatment is implemented the staging work up is usually repeated to evaluate the effectiveness of the treatment. [42] The various stages of tumor are given in **Table 1.7.**

**Table 1.7 Stages of tumors**

| |
|---|
| **Primary tumor (T)** |
| $T_x$ - Primary tumor cannot be assessed |
| $T_0$ - No evidence of primary tumor |
| $T_{is}$ - Carcinoma in situ: Intra epithelial or invasion of Lamina propria |
| $T_1 - T_4$ - Increasing size of tumor |
| **Regional lymph nodes (N)** |
| $N_x$ - Regional lymph nodes cannot be assessed |
| $N_0$ - No evidence that the tumor has metastasized to lymph nodes in the region of the primary tumor |
| $N_1$ - $N_3$ - Progressive involvement of regional lymph nodes |
| **Distant Metastasis (M)** |
| $M_x$ - Distant metastasis cannot be assessed |
| $M_0$ - No evidence of distant metastasis |
| $M_1 - M_4$ - Single or multiple sites of metastasis have been located |

### 1.5.5 Treatment of Cancer:

The four primary treatment approaches for cancer are

- ✓ Surgery
- ✓ Radiation
- ✓ Chemotherapy
- ✓ Biologic therapy

Surgery remains the treatment of choice for most solid tumors diagnosed in the early stages. Radiation therapy was first used for cancer treatment in the late 1800s and remains main stay in the management of cancer. These modalities are likely to produce a cure in patients with truly localized disease. But most of the patient with cancer has metastatic disease at diagnosis; localized therapy often fails to completely eliminate the

cancer. Chemotherapy (including hormonal therapy) can treat the primary tumor and the disease in metastatic state. Biologic therapy like immunotherapy includes interferons and interleukins boost the host immune system against cancer.

### 1.5.6 Principles of Chemotherapy:

Cancer chemotherapy may be indicated under primary, palliative, adjuvant or neo adjuvant modality. Treatment with cytotoxic drugs is the primary curative modality for a few diseases including leukemia, lymphomas, choriocarcinomas, and testicular cancer. Most solid tumors are not curable with the chemotherapy alone. The response to chemotherapy and other treatment modalities may be described as a cure, complete response, partial response and stable disease.

A cure implies that the patient is entirely free from disease and has the same life expectancy as that of a cancer free individual. Complete response means complete disappearance of all cancer without evidence of new disease for at least one month after treatment. A partial response is defined as a 50% or a greater decrease in the tumor size or other objective disease markers and no evidence of any new disease for at least one month. A patient whose tumor size neither grows nor shrinks by more than 25% has stable disease.

### 1.5.7 Classification of Anti-neoplastic Agents:

**1. Anti metabolites:**

Azacitidine, Cytarabine, Fludarabine, 5Flurouracil (5FU), 6-Mercaptopurine (6MP), Methotrexate

Mechanism of action: Inhibits DNA, RNA and Protein synthesis

ADR: Myelosuppression, diarrhoea, vomiting, immunosuppression, flu-like syndrome are common

**2. Anti microtubule agents:**

Docetaxel, Paclitaxel, Vinblastine, Vincristine

Mechanism of Action: Either induces polymerization of microtubules or disrupts formation of microtubules

ADR: Myelosuppression, hypersensitivity reactions, neurotoxicity, vomiting are more common

**3. Topoisomerase-active agents:**

Daunorubicin, Doxorubicin, Topotecan, Etoposide, Mitoxantrone

Mechanism of action: Inhibit either Topoisomerase I or II and inhibits DNA synthesis or intercalates DNA

ADR: Myelosuppression, vomiting cardiac toxicity, vomiting

**4. Alkylating agents:**

Busulfan, Carboplatin, Chlorambucil, Cisplatin, Cyclophosphamide, Dacarbazine, Carmustine, Thiotepa

Mechanism of action: cross links DNA strand and inhibit DNA, RNA and Protein synthesis

ADR: Myelosuppression, vomiting, nephrotoxicity, thrombocytopenia, neutropenia

**5. Miscellaneous agents:**

Asparginase, Bleomycin, MitomycinC

Mechanism of action: hydrolyses L-aspargine and inhibit DNA, RNA synthesis, cross-links DNA

ADR: Myelosuppression, hypersensitivity reactions

**6. Immune therapies:**

Interferon alfa, Aldesleukin

Mechanism of action: stimulates immune system against tumor cells

ADR: Flu-like syndrome, myelosuppression, thrombocytopenia, neutropenia

**7. Endocrine agents:**

Flutamide, Anastrazole, Tamoxifen, Leuprolide

Mechanism of action: Anti androgenic or anti estrogenic or LHRH agonistic action

ADR: Gynecomastia, hot flashes, Psychological depression, dizziness, hypotension

**8. Biologically directed agents:** Bexarotene, Gefitinib, Imatinib mesylate, Cetuximab, Rituximab

Mechanism of action: Specific molecular targets like proteosomes, Tyrosine kinase inhibition which inhibits cell cycle progression or monoclonal antibodies reacts with CD 20 and causing cytotoxicity.

ADR: GIT related, thrombocytopenia, neutropenia, headache, hypersensitivity reactions

The target sites of anti-neoplastic drugs are given in the **Figure 1.3.**

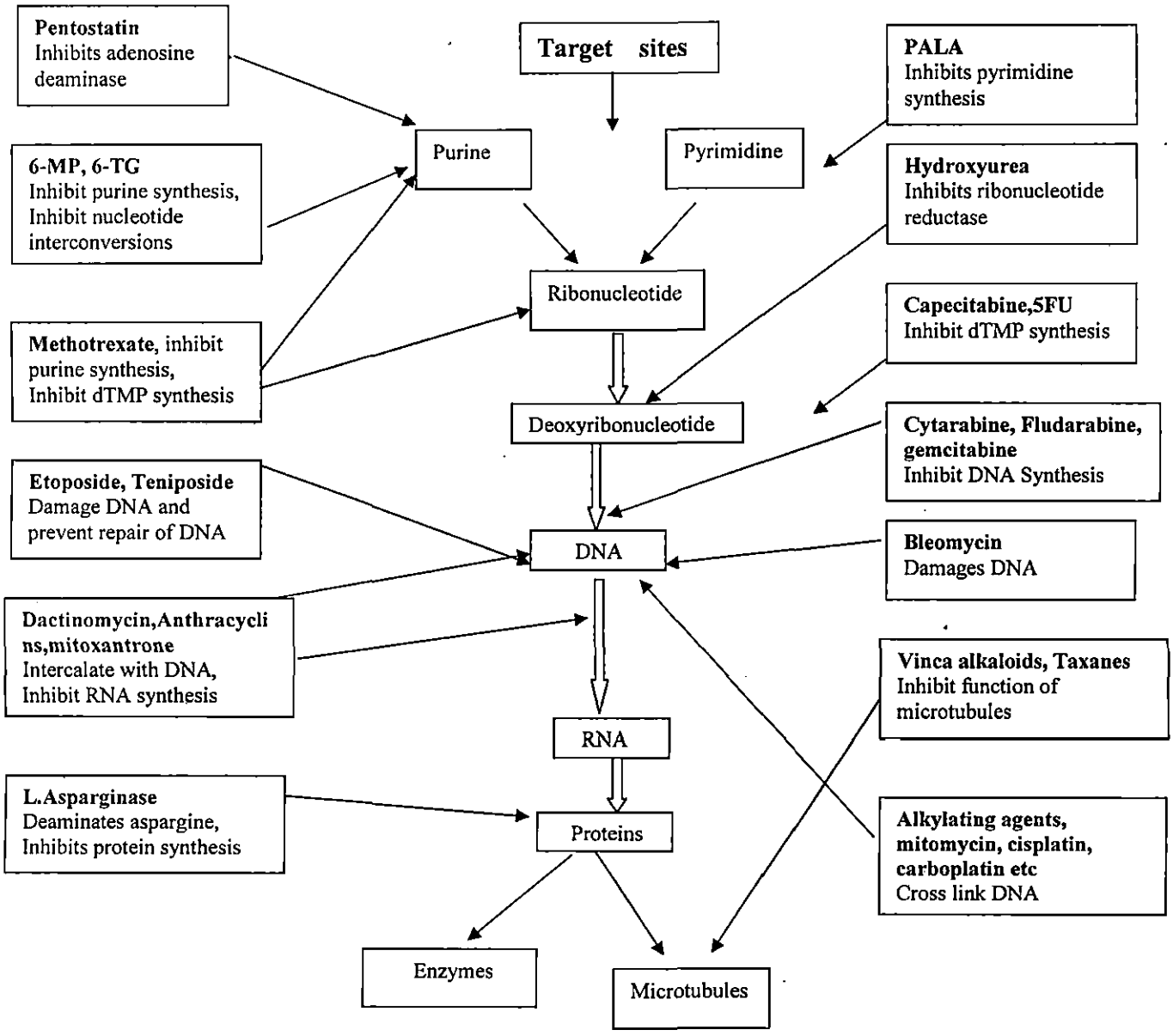


**Figure 1.3 Target site of anti-neoplastic drugs**

### 1.5.8 Prevention of Cancer:

The precautions are follows

1. Eating a variety of healthy foods with an emphasis on plant sources [43]
   - Five or more servings of a variety of vegetables and fruits should be taken each day
   - Preference to be given in changing whole grains than the processed grains and sugars
   - Consumption of red meats, especially high fat and processed meats should be limited
   - Foods that help to maintain a healthful weight should be chosen

2. Adopting a physically active lifestyle

   Adults should engage in at least moderate activity for 30 minutes or more on five or more days of the week; 45 minutes or more of moderate to vigorous activity on more days per week may further enhance reductions in risk of breast and colon cancer.

3. Abstaining from chewing and smoking tobacco

4. Minimizing exposure to the sun and by applying strong sunscreens and sun blocks to sun exposed areas

### 1.6 Role of Plants in Cancer Therapy:

Plant materials have been employed in the ailment of cancer for centuries. There are around 1400 genera used in this category. Recent phytochemical investigation of plants that have a history of use in folklore for the treatment of cancer has resulted in the isolation of phytoconstituents responsible for anti tumor activity. Vinblastine and Vincristine are two important alkaloids, which have been isolated from *Catharanthus roseus* the Madagascan periwinkle during 1955-1960. [44] Vinblastine is used in the treatment of generalized Hodgkin's disease, non Hodgkins lymphomas.Vincristine is used principally in the treatment of acute lymphocytic leukemia in children. Chinese anti

tumor plants *Podophyllum hexandrum, Podophyllum peltatum* have yielded a number of lignans and their glycosides having anti tumor activity. Podophyllotoxin, peltatins are the main phytoconstituent found in the above plants, are unsuitable for systemic use. But the synthetic analogs of etoposide and teniposide are producing more promising action against cancer in clinical trials. Etoposide is currently used in the treatment of small cell lung cancer and testicular cancer and teniposide is used in pediatric cancers. [45] **Table 1.8** listed some plant sources with their phytoconstituents used in cancer treatment.

**Table 1.8 Plant sources and their phytoconstitutent responsible for anticancer action**

| Class | Compound | Plant Source | Family |
|---|---|---|---|
| Monoterpene | Allamandin | *Allamanda cathartica* | Apocynaceae |
| Sesquiterpene | Baccharin<br><br>Phyllanthoside | *Baccharis megapotamica*<br>*Phyllanthus acuminatus* | Compositae<br><br>Euphorbiaceous |
| Triterpenoid, steroid etc | Cucurbitacin E<br>Acer saponin P<br>Withaferin A | *Marah oreganos*<br>*Acer negundo*<br>*Acnistus arborescens* | Cucurbitaceae<br>Aceraceae<br>Solanaceae |
| Alkaloid-Pyrazolidine | Monocrotaline | *Crotalaria spectabilis* | Leguminoseae |
| Alkaloid-isoquinoline | Emetine | *Cephaelis acuminate* | Rubiaceae |
| Alkaloid-pyrroloquinoline | Camptothecin | *Camptotheca accuminata* | Nyssaceae |
| Alkaloid-bisindole | Vinblastine, Vincristine | *Catharanthus roseus* | Apocyanaceae |
| Alkaloid-Non heterocyclic | Colchicines | *Colchicum speciosum* | Liliaceae |
| Peptide | Bouvardin | *Bouvardia ternifolia* | Rubiaceae |
| Stilbene | Combretasin A-4 | *Combretum caffrum* | Combretaceae |
| Lignans | Podophyllotoxins | *P.hexandrum* | Berberidaceae |

### 1.7 Cross Road between Inflammation and Cancer:

Numerous experimental, epidemiological and clinical studies have revealed that NSAIDs are having promising anticancer activities. [46] The mechanism responsible for the anti-tumor activity of NSAIDs is still unknown. It is commonly inhibiting the inducible COX-2, which is over expressed in many epithelial tumors (e.g. in colon tumors). But antineoplastic effects of NSAIDs may also include activation of apoptosis, inhibition of angiogenesis or direct inhibition of cancer cell growth by blocking signal transduction pathways responsible for cell proliferation. [47] Malignancy is thought to develop from chronic inflammation, where uncontrolled cell proliferation occurs in milieu rich with pro-inflammatory cytokines, mediators and growth factors normally involved in chronic and unresolved inflammation. Together with primary DNA alterations by carcinogenic or mutagenic factors, chronic inflammation or factors released during inflammation can promote cancer growth. Inflammatory cells capable to induce genotoxicity, DNA strand break, chromatid changes and of promoting neoplastic transformation in the near by cells. [48-49] In view of these it is expected that anti-inflammatory agents possess anticancer activity and vice versa.

### 1.8 Role of Free Radicals in Inflammation and Cancer:

Free radical induced cellular damage has been implicated in many pathological conditions, like malignancy, ageing process, inflammation and degenerating diseases. [50-51] Free radicals include superoxide anion radical, hydrogen peroxide, peroxynitrite, hypochlorous acid/hypochlorite and the extremely reactive hydroxyl radicals. It has been reported that reactive oxygen species such as superoxide anion, hydroxyl radical and peroxynitrite participate in the process of inflammation in various tissues including the skin. [52] Synovial fluid from the knee joints of human rheumatoid patients contains increased levels of diene conjugates and TBA-reactive material, suggestive of increased lipid peroxidation *in vivo*. The possibility that NSAIDs and other anti-inflammatory drugs having multiple mechanisms of action led to search whether they could have anti-oxidant effects. They might directly scavenge such reactive oxidants as hydroxyl radical (OH) and hypochlorous acid (OHCl). [53] The important process of DNA damage occurs in carcinogenesis; it is conceivable that any agent

capable of reacting with DNA and chemically modifying it could be carcinogenic. In radiation-induced carcinogenesis mediated by ionization of water and formation of highly reactive species such as hydroxyl radical, hydroxyl radical attack upon DNA generates a whole series of modified purine and pyrimidine bases. [54] Attack of OH upon deoxyribose also yields a multiplicity of products. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cancer. [55]

**1.9 Leukemia - An Overview:**

The leukemia is heterogeneous hematological malignancies characterized by unregulated proliferation of the blood forming cells of the bone marrow. The term leukemia was coined by Virchow to describe the white blood of some patients that seen under the microscope. The normal leukocyte count in peripheral blood is 5,500-11,000/cm. An average adult of 70 kg has approx 40-70 billion leukocytes. Normal granulocytopoiesis is initiated at the primitive haematopoietic stem cell and progress from the myeloblast to the metamyelocyte. The metamyelocyte does not undergo further cell division and matures to the PMN. Normal human leukocyte may undergo a maximum of 50 stem cell divisions. In leukemia usually progress from the mature well differentiated leukocytes to the more aggressive immature cell type, which proliferate advantage over its corresponding normal cell. The single clone of leukemia cell therefore soon dominates the hematopoietic system, interfering with the proper normal function. In leukemia granulocytopoiesis, the metamyelocytes ignore the maturation signal and divide in incessantly; therefore the population of leukemia cells increase. [56] Haematopoietic system is presented in the **Figure1.4.**

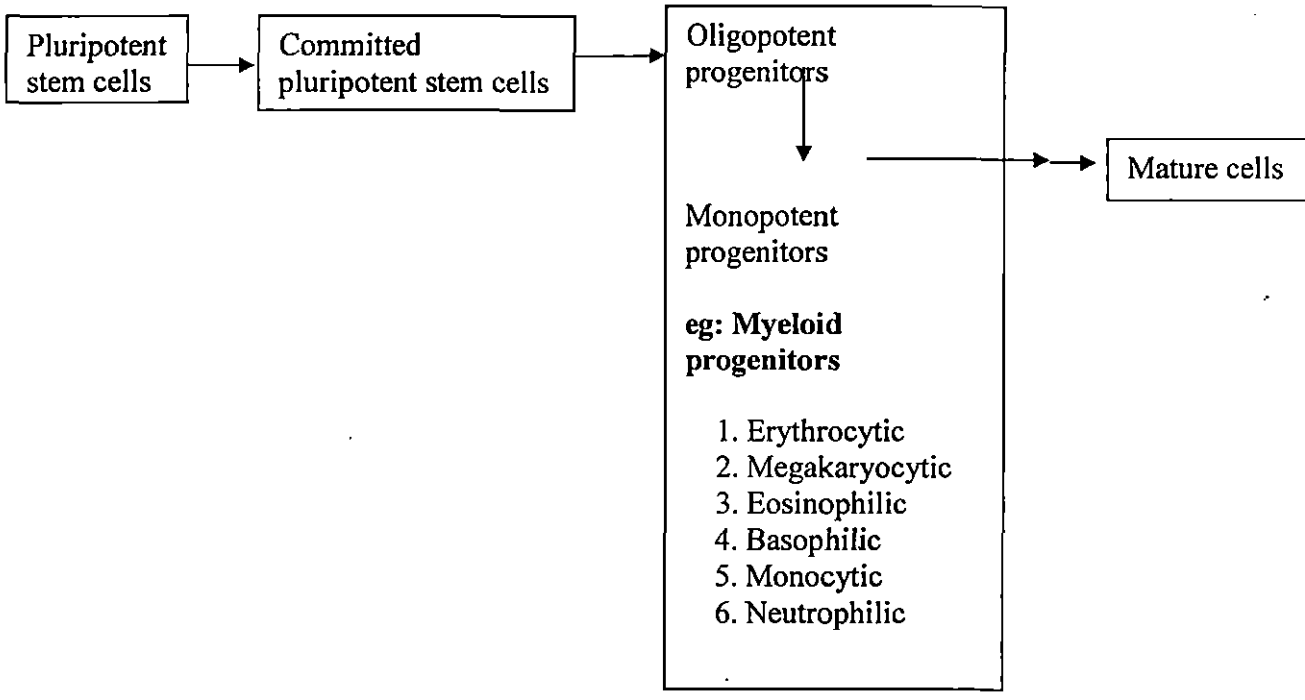


**Figure 1.4 Haematopoietic system**

### 1.9.1 Types of Leukemia:

The four major types of leukemia are given below

Acute lymphocytic leukemia (ALL)

Acute myeloid leukemia (AML)

Chronic lymphocytic leukemia (CLL)

Chronic myelogenous leukemia (CML

Acute lymphocytic leukemia (ALL) is predominantly a disease of childhood with 75% of all cases occurring in patients younger than 15 years of age. [57] In contrast, acute myeloid leukemia (AML) is more common in adults with a chance that increases with age. Chronic Myeloid Leukemia (CML) found in US is 15% of total leukemia in 2005. The exact cause of the acute leukemia is unknown. A multifunctional process involving genetics, environmental and socioeconomic factors, toxins and viral exposure are the likely causes. [58] Both AML and ALL are arise from a single cell that expands and acquires additional mutations, culminating in a monoclonal population of leukemia cells. Secondly, there is failure to maintain balance between proliferation and differentiation.

Proliferation, differentiation and apoptosis are under genetic control and leukemia can occur when the balance between these processes is altered .New anti-leukemia therapies are targeted to the process involved in proliferation and differentiation. The types of genetic alterations involved in leukemia are-

1. Activation of proto-oncogene to create an oncogene that produces a protein product that signals increased proliferation
2. Loss of signals for the blood cell to differentiate
3. Loss of tumor suppressor gene that controls normal proliferation
4. Loss of signals for apoptosis

One example of a genetic defect leading to acute leukemia is abnormal activation of ras gene. [59] CML is mainly due to bcr-abl fusion gene, which produces a mutant tyrosine kinase.

**1.9.2 Symptoms:** weight loss, malaise, fatigue, dyspnea, fever, chills, gum hypertrophy, seizures

**1.9.3 Laboratory tests:** complete blood cell count, anemia, thrombocytopenia, uric acid elevation, and electrolytes like Potassium, and phosphate elevation, and prothrombin time elevation

**1.9.4 Treatment:**

For ALL the therapy includes vincristine, corticosteroid, and asparginase with or without anthracyclins. To prevent CNS diseases the choice of therapy includes a cranial radiation and single agent intrathecal chemotherapy, triple drug intrathecal chemotherapy or high dose systemic therapy. Maintenance therapy includes pulse doses of vincristine, oral methotrexate, and oral mercaptopurine. For AML the therapy is anthracyclins and cytarabine. CML patients were treated with Interferon alpha, imatinib mesylate. [60] Medicinal plants and their phytoconstituents have always a better choice for leukemia, the experimental studies on nutraceuticals proven antileukemic activity. [61]

### 1.10 Apoptosis Pathway:

Apoptosis in Greek "the falling of leaves from a tree". Apoptosis is the one form of Programmed cell death (PCD) an important mechanism in both development and homeostasis in adult tissues for the removal of superfluous, infected, transformed or damaged cells by activation of an intrinsic suicide program. Apoptosis is characterized by maintenance of intact cell membranes during the suicide process so as to allow adjacent cells to engulf the dying cell so that it does not release its contents and trigger a local inflammatory reaction. Cells undergoing apoptosis usually exhibit fragmentation of the cell into membrane-bound apoptotic bodies, nuclear and cytoplasm condensation and endolytic cleavage of the DNA into small oligonucleosomal fragments. [62] The cells or fragments are then phagocytosed by macrophages. Signals that can trigger apoptosis can include lineage information, damage due to ionizing radiation or viral infection or extra cellular signals. Extrinsic signals may either suppress or promote apoptosis, and the same signals may promote survival in one cell type and invoke the suicide program in others. Invocation of the suicide program involves the synthesis of specific messenger RNA molecules and their translation. PCD can sometimes be suppressed by inhibiting transcription or translation, which provides evidence that cell death is mediated by intrinsic cellular mechanisms. The differences between apoptosis and necrosis is represented in the **Table 1.9.**

**Table 1.9 Differences between apoptosis and necrosis**

| Characteristics | Apoptosis | Necrosis |
|---|---|---|
| Cell size | Shrinkage, Fragmentation | Swelling |
| Plasma membrane | Preserved continuity blebbed, Phosphatidyl serine on surface | Smoothing, Early lyses |
| Mitochondria | Increased membrane permeability, Contents released into cytoplasm | Swelling, Disordered structure |
| Organelle shape | Contracted "Apoptotic bodies" | Swelling, Disruption |
| Nuclei | Chromatin: Clumps & Fragmented | Membrane disruption |
| DNA degradation | Fragmented inter nucleosomal cleavage, Free3' ends laddering on electrophoresis DNA appears in cytoplasm | Diffuse and Random |
| Cell degradation | Phagocytosis, No inflammation | Inflammation, Macrophage invasion |
| General stimuli | Developmental programs, Endogenous signals, Intercellular signals disease processes | Disease processes |
| Specific stimuli | Growth factor deprivation: NGF; IL-2<br>Death activators: Bind to surface receptors<br>Cytokines: TNF-α, Lymphotoxin, Fas ligand<br>Toxic: Hormones, Radiation, Mild ischemia<br>Oxidants in cell: Increased DNA damage | Toxic,<br>Severe ischemia,<br>Radiation |
| Cellular processes | Programmed cascade of Reactions. Caspase activation , Internucleosomal endonucleases, Transglutaminase activation requires new RNA transcription and Protein synthesis | No protein synthesis,<br>No RNA transcription,<br>Energy independent ATP depletion |
| Apoptosis Inhibitors | Protease inhibitors NAIP ; crmA; p35<br>Human IAP-1, IAP-2& IAP3<br>Bcl-2 family (Some):Bcl-2; Mcl-1; Bcl-w ; Bcl-xL | Not clear |
| Apoptosis Promoters | Bcl-2 family (Some):<br>Bax; Bcl-xS; Hrk ; Bak; Bid; Bik ; Bad | Not clear |

Caspases (Cyteinyl aspartic acid protease) are a family of proteins which is one of the main effectors of apoptosis. Induction of apoptosis via death receptors results in the activation of an initiator caspase such as caspase 8 or caspase 10. These caspases can then activate other caspases in a cascade. This cascade eventually leads to the activation of the effector caspases, such as caspase 3 and caspase 6. These caspases are responsible for the cleavage of the key cellular proteins that leads to the typical morphological changes observed in cells undergoing apoptosis.

There are a number of mechanisms through which apoptosis can be induced in cells. The sensitivity of cells to any of these stimuli can vary depending on a number of factors such as the expression of pro- and anti-apoptotic proteins (eg, the Bcl-2 proteins or the Inhibitor of Apoptosis Proteins), the severity of the stimulus and the stage of the cell cycle. Mitochondria play an important role in the regulation of cell death. For example, anti-apoptotic members of the Bcl-2 family proteins such as Bcl-2 and Bcl-XL are located in the outer mitochondrial membrane and act to promote cell survival. Many of the pro-apoptotic members of the Bcl-2 family, such as Bad and Bax also mediate their effects though the mitochondria, either by interacting with Bcl-2 and Bcl-XL, or through direct interactions with the mitochondrial membrane. The chemotherapeutic agents that have been identified as being apoptosis-inducing include etoposide, dexamethasone, vincristine, cis-platinum, cyclophosphamide, paclitaxel, 5FU and adriamycin. [63-64]

### 1.11 Aims and Objectives:

Some medicinal plants of Sikkim region were screened for their anti-inflammatory, anticancer and antioxidant properties. In the initial screening of the crude extract of the leaves of two plants namely *Bischofia javanica* and *Fraxinus floribunda* have shown significant properties mentioned above. The literature surveyed also does not provide any report of such study on those plants. It was therefore thought to be worthy to undertake a study to explore those plants whether such potentialities are containing by these plant.

The following objectives have been put forward to undertake this study

- Selection and collection of medicinal plants ethno-medicinally used for inflammation and cancer
- Preparation of crude extract from the plant products
- Anti-inflammatory, anticancer and antioxidant evaluation of crude extract
- Isolation, purification and structure elucidation of phytoconstitutents
- Anti-inflammatory, anticancer and antioxidant evaluation of isolated phytoconstituents
- Draw a conclusion on the isolated phytoconstituents and pharmacological activity of the compounds

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