Chapter-2

Review of Literature
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Dimensions of cancer

Cancer grows out of normal cells in the body. Normal cells multiply when the body needs them, and die when the body doesn't need them. Cancer appears to occur when the growth of cells in the body is out of control and cells divide too quickly (IARC factsheet, WHO). It can also occur when cells forget how to die. There are many different kinds of cancers. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones, or nerve tissue (IARC factsheet, WHO).

Genesis of cancer is associated with various causative agents. These causes ultimately lead to generation of cancer. In normally dividing cells, any type of alteration in the environment or metabolism of the cell may trigger uncontrolled growth of cells which is initiation of cancer.

The chemical, biological and other environmental entities are responsible for uncontrolled and unorganized proliferation of cells. Basically, under special circumstances carcinogens interact with DNA of the normal cells resulting into a series of complex multistep processes responsible for uncontrolled cell proliferation or tumors (Carmaea, 1993). A significant variation of cancer has
been reported due to life styles and food habits (Helbock et al, 1998). In the present scenario cancer has taken a monstrous size in terms incidence of cancer and death related to cancer. In India cancer is responsible for 7% of death. Table 2.1 gives an idea about the scenario of cancer at present and in the near future (NCRP-ICMR, 2008).

Table 2.1 - Cancer cases in India by site and sex in 2001 and projection for 2016

<table>
<thead>
<tr>
<th>Site of Cancer in the body</th>
<th>Male 2001</th>
<th>Female 2001</th>
<th>Male 2016</th>
<th>Female 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Cavity</td>
<td>42,725</td>
<td>22,080</td>
<td>65,205</td>
<td>35,088</td>
</tr>
<tr>
<td>Pharynx and Larynx</td>
<td>49,331</td>
<td>9,251</td>
<td>75,901</td>
<td>14,550</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>24,936</td>
<td>17,511</td>
<td>38,536</td>
<td>28,165</td>
</tr>
<tr>
<td>Stomach</td>
<td>20,537</td>
<td>11,162</td>
<td>31,538</td>
<td>17,699</td>
</tr>
<tr>
<td>Lung</td>
<td>39,262</td>
<td>9,525</td>
<td>60,730</td>
<td>15,191</td>
</tr>
<tr>
<td>Breast</td>
<td>....</td>
<td>89,914</td>
<td>....</td>
<td>140,975</td>
</tr>
<tr>
<td>Cervix Uteri</td>
<td>....</td>
<td>79,827</td>
<td>....</td>
<td>125,821</td>
</tr>
<tr>
<td>Others</td>
<td>214,967</td>
<td>166,629</td>
<td>315,840</td>
<td>254,410</td>
</tr>
<tr>
<td>Total</td>
<td>391,758</td>
<td>405,899</td>
<td>587,750</td>
<td>631,899</td>
</tr>
</tbody>
</table>

Source: (Murthy et al., 2008)
The following chart (Figure 2.1) shows common causes of cancer. However, the cause of many cancers remains unknown (source of information 'A.D.A.M. Medical Encyclopedia').

![Diagram of Major Causes of Cancer]

In normal tissues and organs, the activities of the constituent cells are strictly restricted to the tasks assigned to them during development. In addition, they (with the exception of leukocytes) remain inflexibly confined to their territorial domains by regulatory interactions with their neighbors. This creates specialized local micro-environments in which structure and function are
orderly, stable and tightly controlled by feedback loops, within interacting regulatory networks. This system has considerable ability to adapt to changing conditions. In contrast, the microenvironment in regions where tumors are forming and expanding is characterized by progressive loss of specialized or differentiated cellular functions, disorderly molecular signals, degeneration of microscopical organ structure. This, coupled with the traffic of cells into and out of the tumor, often culminating in local invasion and metastasis to other organs (Tarin, 2012).

Oxidatively induced DNA damage is repaired in living cells by different pathways that involve a large number of proteins. Unrepaired and accumulated DNA lesions may lead to disease processes including carcinogenesis. Mutations also occur in DNA repair genes, destabilizing the DNA repair system. A majority of cancer cell lines have somatic mutations in their DNA repair genes. In addition, polymorphisms in these genes constitute a risk factor for cancer (Dizdaroglu, 2012).

Cancers are classified by the type of cell that resembles the tumor as follows (ATSDR, CDC, Cancer Fact Sheet, 2012):

1. **Carcinoma:** These cancers originate in the epithelium. The epithelium is the lining cells of an organ. Carcinomas are the most common type of cancer. Common sites of carcinomas are the skin, mouth, lung, breast, stomach, colon and uterus.
2. **Sarcoma:** Sarcomas are cancers of connective and supportive tissue (soft tissues) or mesenchymal cells of all kinds. Sarcomas can be found anywhere in the body, and they often form secondary growths in the lungs.

3. **Lymphoma and leukemia:** Malignancies derived from hematopoietic cells.

4. **Germ cell tumor:** Tumors derived from totipotent cells (in adults most often found in the testicle and ovary).

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**Figure 2.2 a – Key advances in history of cancer chemotherapy (1900-1960)**
Cancer treatment

Choice of cancer therapy depends upon the location and grade of the tumor and the stage of the disease. The modern era of cancer therapy began following World War II. Figure 2.2a-b shows the development of different treatment strategies for cancer. Important advance that aided in the development of the modern era of cancer treatment was the development of animal systems that were to be predictive for antitumor drugs (Zubrod et al., 1966). In 1929, Berenblum while studying carcinogenesis, observed that sulfur mustard was "anti-carcinogenic" (Berenblum and Riley-Smith, 1929). This observation stimulated research in cancer chemotherapy.
Chemotherapeutic drugs are classified into ten major groups as listed below (Spiridon and Maria, 2004).

1. **Antimetabolites** act as non-functional analogues of essential metabolites in the cell, thus blocking physiological functions of the tumor. (e.g. Purines, Pyrimidines, Para-amino benzoic acid etc.)

2. **Alkylating agents** chemically bond with DNA through alkyl groups, thus disrupting gene structure and function, or with proteins, thus inhibiting enzymes. (e.g. Cyclophosphamide, Ifosfamide, Streptozocine etc.)

3. **Topoisomerase inhibitors** inhibit DNA replication in rapidly dividing cells, as in the case of tumors. (Etoposide, Doxorubicin, Camtothecin etc.)

4. **Plant alkaloids** also inhibit tumor cell division by blocking microtubule depolymerization, an essential step for chromosome detachment during mitosis. However, novel plant alkaloids act through other mechanisms as well, which will be analyzed further in this book. (e.g. Vincristine, Vinblastin, Quercetin etc.)

5. **Antibiotics** are derived from diverse groups of microorganisms or synthesized and block DNA replication and protein synthesis. (e.g. Bleomycin, Doxorubicin etc.)

6. ** Anthracyclins** are a subgroup of antibiotics, associated with considerable toxic side effects on the heart and bone marrow. (e.g. Epirubicin, Danarubiccin etc.)
7. **Enzymes**, in particular proteolytic and fibrinolytic ones, as well as tyrosinase inhibitors, such as *Gleevec*, a new cytotoxic drug used for treating chronic myeloid leukemia. (e.g. Tamoxifen, Amagdalyn etc.)

8. **Hormones** are substances interfering with other chemotherapeutic agents by regulating the endocrine system. They find specific application against carcinomas of breast, prostate and endometrium. (e.g. Estrogen, Letrozole, Androgen etc.)

9. **Immunomodulators** act by inhibiting tumor proliferation through the stimulation of the host's immune system. (Glucocorticoid, Cyclophosphamide etc.)

10. Various other substances used in chemotherapy not falling in any of the above categories. (Electrochemotherapy, Nanoparticles etc.)

**Alkylating anticancer agents**

Alkylating agents are one of the most commonly used agents in chemotherapy of cancer. The alkylating agents and the platinum antitumor compounds form strong chemical bonds with electron-rich atoms (nucleophiles), such as sulfur in proteins and nitrogen in DNA (Quintal et al., 2005). Although these compounds react with many biologic molecules, the primary cytotoxic actions of both classes of agents appear to be the inhibition of DNA replication and cell division produced by their reactions with DNA (Feuerhahn et al., 2011). Other alkylating agents are also associated with treatment of cancer. Closely related
to the nitrogen mustards are the aziridines, which are represented in current therapy by thiotepa, mitomycin C, and diaziquone (Greenspan, 1968). Thiotepa (triethylene thiophosphoramide) has been used particularly in the treatment of carcinomas of the ovary and breast and for the intrathecal therapy of meningeal carcinomatosis (Greenspan, 1968; Gutin et al., 1977; Perloff et al., 1978). The alkyl alkane sulfonate busulfan was one of the earliest alkylating agents (Haddow and Timmis, 1953). Nitrosoureas also demonstrate significant activity against tumor (Schabel et al., 1963) and is used for the treatment of primary brain tumors (Walker et al., 1978) and in the treatment of multiple myeloma (Presant and Klahr, 1978).

**Toxicity of anticancer drugs**

All major anticancer chemotherapeutic drugs such as cyclophosphamide, doxorubicin, cisplatin, bleomycin etc. are associated with toxic side effects (Seiber, 1977; Wheler et al., 2012). Cisplatin (cis-dichloro-diammineplatinum(II)) is a widely used chemotherapeutic drug toxic to kidney in rat (Vickers et al., 2004). It increases blood urea nitrogen and serum creatinin levels and decrease in serum total lipids (Safirstein et al., 1981). Doxorubicin is also one of the known antineoplastic drugs inducing cardiotoxicity and increase in serum lactate dehydrogenase, aspartate transaminase, Na\(^+\)-K\(^+\) levels increase in cardiac tissue LPO, decrease in serum and tissue glutathione and tissue catalase activity in rats (Shan et al., 1996). L-buthionine-SR-sulfoximine treatment in chemotherapy associated urotoxicity is a troublesome
complication and it was also found that L-buthionine-SR-sulfoximine treatment exerted an additive toxic effect on the CP-treated animals (Rouissi et al., 2011).

Tamoxifen (TAM) is used in the treatment of estrogen receptor-positive breast cancer and also for chemoprevention for patients at high risk for developing breast cancer. It is metabolised via the cytochrome P450 pathway to form several metabolites, including endoxifen and 4-OH tamoxifen which are the most potent. Endoxifen is present at a much higher plasma concentration than 4-OH-tamoxifen and is thought to be largely responsible for the therapeutic effect of tamoxifen (Stearns et al., 2003). In more than 30% of breast cancer patients given adjuvant TAM treatment induced fatty liver (Elefsiniotis et al., 2004). TAM is reported to cause oxidative liver damage and it has been revealed to reason hepato-carcinogenicity in rodents (Karki et al., 2000). Bleomycin (BLM) is a glycopeptide antibiotic used in cancer treatment along with cisplatin and other chemotherapeutic drugs (Calabro et al., 2012). BLM is reported to cause pulmonary interstitial fibrosis and impaired lung function in rats (Zhang et al., 2011). Many of the chemotherapeutic drugs discussed here are given in combination in a treatment regimen and singly as well.
Cyclophosphamide

CP is a chemotherapeutic drug usually given to treat lymphomas, leukaeemias, lung cancer and breast cancer. It may also be used to treat many other types of cancer. It is a crystalline solid in anhydrous form details in Figure 2.3. CP belongs to oxazaphosphorine class of drugs. CP is a DNA-alkylating agent which is most commonly used in cancer chemotherapy (Sladek, 1988). It is also used as multifunctional alkylating agent and also known as a potent immunosuppressor (Kumari and Sahoo, 2005). Because of the immunosuppressive effect of CP, it is used in the patients with autoimmune disease and organ transplant (Konopacki et al., 2012).

CP does not act in a cell-cycle specific manner, i.e. exclusively on proliferating cells. It also kills non-proliferating cells, as shown by application of CP to

\[ \text{Cyclophosphamide} \]


Chem. Abstr. Name: 2H-1,3,2-Oxazaphosphorin-2-amine, N,N-bis(2-chloroethyl)tetrahydro-, 2-oxide

IUPAC Systematic Name: N,N-Bis(2-chloroethyl)-1-oxo-6-oxa-2-aza-1λ5-phosphacyclohexan-1-amine

Figure 2.3 – Classification of cyclophosphamide
L1210 ascites tumour-bearing mice during plateau phase growth of the tumour (Schafer and Maurer-Schultz, 1986). Moreover, treatment with CP of L 1210 ascites tumour cells, double-labelled with [3H] and [14C]-thymidine, suggests that CP is not cell cycle phase dependent, but kills cells out of all cycle phases.

There is also an extensive cytocidal effect of CP (300 mg/kg) on the jejunal crypt cells of the mouse, which is even more pronounced than that of cisplatinum (13 mg/kg). However, rapid regeneration of crypt cells occurs after treatment with the drugs (Schafer and Maurer-Schultz, 1986).

**Metabolism of cyclophosphamide**

CP is administered as prodrug that is activated by liver cytochrome P450-catalyzed 4-hydroxylation reaction that yields active, cytotoxic metabolites. Several liver-expressed cytochrome P450 enzymes catalyze this activation reaction; the phenobarbital-inducible rat cytochrome P450 enzyme 2B1 (Clarke and Waxman, 1989) and its human counterpart 2B6 (Chang et al., 1993; Huang et al., 2000) show particularly high cyclophosphamide 4-hydroxylase activity compared with other cytochrome P450 forms. The primary metabolite, 4-hydroxycyclophosphamide, equilibrates with the ring-open aldophosphamide and undergoes α-elimination to yield the therapeutically active, DNA cross-linking phosphoramide mustard and the byproduct acrolein (4-hydroxylation pathway). CP is subject to an alternative, P450-catalyzed side chain oxidation that generates therapeutically inactive N-dechloroethylated metabolites and the
neurotoxic and nephrotoxic byproduct chloroacetaldehyde (N-dechloroethylation pathway) (Furlanut and Franceschi, 2003). Figure 2.4 shows an outline of metabolic pathway of CP.

**Figure 2.4 - Metabolic pathway of cyclophosphamide**

**Cyclophosphamide toxicity**

The side effects associated with most of the useful chemotherapeutic agents including CP, especially in high-dose treatment regimens and continuous use for a longer duration, on the non-target tissues are a major deterrent in their widespread frequent use (Haque et al., 2003, Bhatia et al., 2006a). Therefore,
in recent past very extensive studies were being done to discover various protective agents that may detoxify and help to achieve reduced or negligible undesirable side effects of CP if cannot be eliminated completely to make the life of patient comfortable (Arafa, 2009, Al-yahya, 2009; Popov et al., 2011). The toxic side effects of CP that are widely reported include nausea, vomiting, alopecia, hematopoietic suppression, nephrotoxicity, mutagenicity, carcinogenicity and teratogenicity (Fleming, 1997; Mirkes, 1985). CP treatment also leads to lung injury and decreased efficiency of lung function beside cardiac toxicity, which are mediated by the ROS and lipid peroxide formation (Patel, 1987; Sulkowska et al., 1998).

CP-induced immunosuppression is reported to prompt various types of infection (Wijesundera, 1980; Angulo et al., 2002). CP is converted to therapeutically active DNA-alkylating metabolites by the hepatic cytochrome P450 enzymes (Yu et al. 1999). It has been reported by Cohen et al. (1992) that CP acts as a carcinogen in therapy related to leukemia and bladder cancer. The deleterious effects of CP in the urinary bladder include mucosal edema, hemorrhage, ulceration, subendothelial telangiectasia and fibrosis (Levine and Richie, 1989). In most of the toxic manifestations of CP including urotoxicity, reactive oxygen species (ROS) have been implicated to play a major role (Korkmaz et al., 2007). The incidence of urotoxic effect of CP varies from 2 to 40% in patients taking low doses of drug for long term. Mortality with massive bladder hemorrhage in up to 2–4% patients is reported under high
dose intravenous CP treatment regimen (Topal et al., 2005; Korkmaz et al., 2007).

CP is reported to decrease the cellularity and size of various compartments and inhibited germinal center development in Peyer's patches of young rats, mice or rabbits (Cozon et al., 1991). The immunosuppressive effect of CP on humoral immunity is of major concern in the cancer patients (Pratheeshkumar and Kuttan, 2010). Hou et al. (2007) reported that rats exposed to 10 mg/kg CP orally for 30 days, induced decreases in body weight, body weight gain, relative weight of spleen and thymus, antibody plaque-forming cells, delayed-type hypersensitivity reaction, natural killer cell activity, lipopolysaccharide-induced B-cell proliferation, and Concanavalin A-induced T-cell proliferation. In the histopathological examination of spleen and thymus revealed decreased cellularity of these organs.

CP also induces oxidative stress in various tissues resulting from the production of ROS (Sulkowska et al., 1998; Il'yasova et al., 2011). Production of ROS leads to decrease in the antioxidant enzyme level in the cells (Sun, 1990; Abraham et al., 2011). Moreover, decrease of the tissue and serum levels of GSH and activities of GR, SOD and CAT of rats and mice has also been reported in case of CP treatment (Kaya et al., 1999; Rekha et al., 2001). CP is used in stem cell transplantation therapy conditioning regimen and cardiotoxicity has been reported after introduction of CP in such treatment regime (Santos et al., 1971; Storb et al., 2001). Several reports have indicated a
positive correlation between severity of cardiotoxicity and CP dose (Goldberg et al., 1986). Symptoms of cardiotoxicity generally become noticeable after 10 to 20 years of transplantation in patients who survive long enough (Tichelli et al., 2008). However, death due to cardiac failure has been reported within few weeks of CP exposure (Ayash et al., 1992).

**Cytochrome P450 and its role in metabolism of toxic drugs**

Cytochromes P450 are a superfamily of haemeproteins containing monooxygenases, called (CYP and P450), as essential protein catalysts for the oxidative metabolism of many xeno- and endobiotics (Estabrook, 2003). These P450s play key roles in steroid hormone biosynthesis, the activation and detoxication of many drugs and environmentally contaminating chemicals, the metabolism of polyunsaturated fatty acids (such as arachidonic acid and prostaglandins), the synthesis of a vast array of secondary metabolites in plants and insects metabolism of contaminating environmental chemicals to toxic and carcinogenic agents (Estabrook, 2003).
Cytochrome P450 is mainly concentrated in liver of the organism but it is present in other organs of the body, other than liver. It is very important to have knowledge of the effect of drugs on P450 activity to avoid drug interactions and improve therapeutic efficacy (Park et al., 1995). Reduced P450 forms a complex with carbon monoxide to produce a unique 450 nm absorption peak. This spectral property was used for the specific estimation of P450 content (Omura and Sato, 1964). Figure 2.6 shows the catalytic cycle of cytochrome enzymes.

Figure 2.5 - Structure of cytochrome P450 hemoprotein
In patients with cancer, CP is primarily activated in the liver, a tissue rich in P450 activity, followed by transport of the activated metabolites to the tumor via blood flow. Though, these activated metabolites induce host toxicity after going inside the normal body tissues (Chen et al., 2004).

**Herbal drugs in traditional system of medicine**

In India, several complementary and alternative systems of medicine such as Ayurveda, Homeopathy, Siddha and Unani are practiced. Plants and plant-based formulations are employed vigorously in these systems for healthcare and disease treatments (Mukherjee and Wahile, 2006). These systems have rightfully existed side by side with so called modern medicine system (Allopathy) (Vaidya and Devasagayam, 2007). The traditional system of
medicines are not only folklore or traditional herbal practices but there are basic axioms of these systems leading to a rational and efficient structure of pathogenesis and diagnosis of disease and related problems, paving the way for proper therapy and prescription (Vaidya 1992). In India pluralistic healthcare system existed since a long time, which offers a complimentary choice for the hunt of new clinical effects of medicinal plants used traditionally (Patwardhan, 2000). Ayurveda system of medicine is based on experience from the time immemorial, some of which has been proven experimentally. Formulations and dosage forms have great importance in Ayurveda. Generally Ayurvedic formulations are multi-component mixtures, containing plant and animal-derived products, minerals and metals.

The principle of Homoeopathy has been known since the time of Hippocrates from Greece, the founder of medicine, around 450 B.C. Homoeopathy as it is practiced today was evolved by the German physician, Dr. Samuel Hahnemann (1755–1843). The word ‘Homoeopathy’ is derived from two Greek words, ‘Homois’ meaning similar and ‘pathos’ meaning suffering. Homoeopathy simply means treating diseases with remedies, which are capable of producing symptoms similar to the disease when taken by healthy people. For instance, cinchona bark, which contains quinine, is taken by a healthy person, it produces symptoms that exactly mimicked intermittent fever (now called malaria) (Mukherjee and Wahile, 2006).
The principles and concepts of Siddha system of medicine are closely similar to those of Ayurveda, with specialization in iatro-chemistry (Sathyanarayana and Kameswara, 1993). The Siddha system is a psychosomatic system, where attention is given to minerals and metals along with plant constituents (Mukherjee, 2001). As in Ayurveda, this system also considers the human body as a conglomeration of three humors, seven basic tissues and the waste products. The equilibrium of humors is considered as health and its disturbance or imbalance leads to disease or sickness. The system describes 96 principal constituents of a human being which include physical, physiological, moral and intellectual components. When there is any change or disturbance in functioning of these principal constituents, body as a system deviates towards the cause of disease (Pillai, 1998).

In India, Arabs introduced the Unani system of medicine, which was developed and blended with the Indian culture under the Mughal Emperors. The Unani system of medicine is used by a large portion of the population in India. Unani considers the human body to be made up of seven components. Arkan (elements), Mizaj (temperaments), Aklath (humors), Anza (organs), Arawh (spirits), Quo (faculties) and Afal (functions) each of which has a close relationship with the state of health of an individual. A physician takes into account all these factors before diagnosing and prescribing treatment.

In Unani medicine, single drugs or their combinations in raw form are preferred over compound formulations. The naturally occurring drugs used in this
system are symbolic of life and generally free from side effects. Such drugs, which are toxic in crude form, are processed and purified in many ways before use (Mukherjee and Wahile, 2006). This system believes that every person has a unique humor constitution, which represents his healthy state (Siddiqui, 1981; Ansari et al., 2010). In this traditional system of medicine a single drug or combinations in raw form are preferred over compound formulations. The system offers excellent remedies for gastrointestinal, cardiovascular and nervous disorders tested through many centuries (Mukherjee, 2002b; Khan et al., 2011).

Rasheed and Gupta (2012) have reported use of modern technique for standardization of Unani herb Qurs-e-luk. Unani polyherbal formulations are also preferred and research is going on exploring their physicochemical, and phytochemical properties are going on. Ajazuddin and Saraf (2010) have reported the properties of Suffof-e-sana a polyherbal Unani formulations. Kalim et al. (2010) have explained the preventive effect of Unani herbs in oxidative DNA damage. Khan et al. (2011) have recently highlighted the anti-inflammatory and analgesic role of betel nut used in traditional Unani system of medicine. Sometime back cardio protective and cardiac tonic role of khamiras has been highlighted by Ahmad et al. (2010).
Figure 2.7: Number of plants used in different systems of medicines in India

In all the traditional system of medicines one fundamental similarity was that almost all the systems uses herbal products or herbs as medicine for the treatment of disease and together they are largely called as complementary and alternative medicine. There is a long history related with the use of plants and its products for the treatment of cancer (Hartwell, 1982). Natural product secondary metabolites from plants and microbes in particular play a very important role in the amelioration of this group of diseases and the toxicity caused due to other drugs in the course of chemotherapy (Gurib-Fakim, 2006). Most of the synthetic chemotherapeutic agents available today are immunosuppressants, cytotoxic, and exert variety of side effects (Adidala et al., 2011).
Concept of adaptogens in traditionally used drugs for stress and toxicity prevention

The substances which is generally a plant product causing "a state of non-specifically increased resistance" of the organism were named "adaptogens" (Brekhman and Dardimov, 1969). Adaptogens are used as curative agents in treating some neurologic and psychiatric disorders, such as asthenia, neurosis, depression, and alcoholism, and in a number of other conditions, as well as being prescribed as adjuvants to other medicines in diseases such as tuberculosis and in conventional cancer therapy. The concept of "one drug for one disease" does not apply in the use of adaptogens in actual practice (Rege et al., 1999; Seely and Singh, 2007). Evidence are found that indicates that the adaptogens display their greatest efficacy in the form of extracts containing a combination of several active substances from a single plant species (Panossia, 2003).

Many herbs and herbal products have been evaluated in clinical studies and are currently being investigated phytochemically to understand their antitumour actions against various cancers (Olaku and White, 2011; Kado et al., 2012; Wang et al., 2012a). Cancer patients who already got crippled with this disease, who are further burdened by drug-induced toxic side effects, have now turned to seek help from the complementary and alternative medicine hoping for a better cure (Rao et al., 2008; Chan et al., 2012; Schultz et al., 2012).
Use of natural products to modulate various biological responses has become a subject of intense scientific investigations all over the world. Plant derived extracts containing antioxidant principles have shown cytotoxicity towards tumor cells (Jiau-Jian and Larry, 1977) and antitumor activity in experimental animals (Ruby et al., 1995). Antioxidants with free radical scavenging capacity can play an important role in biological system and may exert their beneficial effects by different mechanisms, such as suppressing the formation of active species by reducing hydroperoxides (ROO-) and $\text{H}_2\text{O}_2$, and also by sequestering metal ions, scavenging free radicals, repairing or removing damage (Tiwari, 2001). Plant derived and natural antioxidants are reported to protect DNA and other macromolecules in the cells against damages caused by generation of ROS, leading to lipid peroxidation, damage to protein resulting in membrane damage, and DNA strand breakage (Rai, 2010, Wang et al., 2012a). Natural products have also been shown to modulate immune functions in normal and tumour bearing mice. Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers (Kumaran and Karunakaran, 2007). These natural products can be used against cancer and for mitigating the toxic effects of anticancer drugs.

**Herbal drugs used for toxicity prevention of cyclophosphamide**

Herbal extracts not only have been used for the prevention of toxicity of CP and other drugs but their use is also subject matter of study from cancer treatment point of view. Some of the most extensively studied plants for their anticancer
activities are *Brassica aristata*, *Brassica oleracea*, *Cichorium intybus*, *Crocus sativus*, *Ferula galbaniflua*, *Paris polyphylla* Smith, *Rosa damacena* (Ahmad et al., 1986; Ahmed and Farooqi, 1991; Falodun et al., 2012; Li et al., 2012). The constituent plant extracts of *Ocimum sanctum* and *Tinospora cordifolia* have also been reported to ameliorate the radiation or cyclophosphamide-induced damage in mice (Uma Devi et al., 1995; Mathew and Kuttan 1997). A number of different plant extracts and biologically derived compounds have been used to overcome CP-induced toxicity (Sharma et al., 1999; Haque et al., 2001; Bhatia et al., 2006a, 2008).

Aqueous extract of walnut (*Juglans regia* L.) has been reported to protect mice against CP-induced biochemical toxicity (Haque et al., 2003). Walnut provided protection by inducing reduced glutathione levels in liver and kidney. This approach can prove to be very promising as CP is reported to deplete GSH levels in all the major tissues (Haque et al., 2003). Similarly, the GSH content in bladder and liver was drastically reduced after administration of CP (Davis and Kuttan, 2000). It was further shown that administration of *Withania somnifera* enhanced the GSH content in both liver and bladder. Haque et al. (2003) also reported a significant increase in GSH, GST and GP levels in mouse liver when treated with *J. regia* extract.

CP and other chemotherapeutic agents also cause immunosuppression in the subjects undergoing treatment (Bin Hafeez et al., 2001). Cytoprotective agents are supposed to reduce or prevent the toxicities related to chemotherapy (Jeong
et al., 2012). These agents should ideally be selective for normal cells versus cancer cells, be effective in reducing or preventing toxicity, should have no negative impact on anticancer therapy, and have minimal adverse effects. None of the agents currently under development fulfills these criteria completely (Hoekman et al., 1999).

*W. somnifera* has been reported as an effective immunostimulator in immunosuppressed animal (Ziauddin et al., 1996). Davis and Kuttan (1998) has reported that treatment of *W. somnifera* extract was found to significantly reduce leukopenia induced by CP treatment, indicating *W. somnifera* could reduce the CP-induced toxicity and its usefulness in cancer therapy. *T. cardifolia* is a herb whose stems are used traditionally for its various immunopharmacological activities, e.g. anti-oxidant properties and reducing toxic side effects of CP-induced toxicity (Mathew and Kuttan, 1997), anticomplementary and immunomodulatory activities (Kapil and Sharma, 1997). Previously, the protective effects of *Asparagus racemosus* and *T. cordifolia* against myelosuppression induced by single doses of CP have been reported (Diwanay et al., 2004). Leukopenia produced by CP was prevented, to varying degrees suggestive of their potent immunostimulatory activity (Thatte and Dahanukar, 1988).

It has been observed that compounds drugs have greater efficiency. Drugs used in the natural system of medicines such as Unani-tibb and Ayurvedic system of Medicine are generally compound drugs or extracts of a plant/herbal
product. A herbal product Immune-21 (I-21) containing extracts of *Ocimum sanctum*, *W. somnifera*, *E. officinalis* and *T. cardifolia* has been shown to possess immunopotentiating and immunoprophylactic activities (Jena et al., 2003). It showed significant protection against UV-ray, cyclosporine and CP-induced immunosuppression and provided 50% protection against *E. coli* induced abdominal peritonitis (De et al., 1998). It is suggested that these natural products and drugs containing natural products, especially herbs can play an important role in reducing toxicity associated with the use of anticancer drugs such as CP. However, these claims have not been validated with scientific investigations. Most of the compound herbal drugs show adaptogenic effects to prevent from stress and toxicity to the physiology (Seely and Singh, 2007). Other than herbs and their products natural agents like vitamins and even amino acids have also exhibited potential adaptogenic activity (Gupta et al., 2005; Seely and Singh, 2007).

In a tumour bearing mice excessive production of free radicals results in oxidative stress, which leads to damage of macromolecules such as lipids leading to LPO in vivo (Yagi, 1991; Bhattacharya et al., 2011). Increased LPO would cause degeneration of tissues. Lipid peroxide formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of LPO (Sinclair et al., 1990). LPO was reported to be higher in carcinomatous tissue than in non-diseased organs (Yagi, 1991; Chaiswing et al., 2011).
A large number of single natural products and herbal extracts containing these products have shown protective effects against CP-induced toxicity (Sharma et al., 2001; Haque et al., 2003; Bhatia et al., 2006a, 2008). However, there is limited knowledge about the efficacy of such natural products and extracts in the animals exposed to CP and also concurrently challenged with tumour cells. Such effects of protection therapy may be desirable, as it will not only enhance the therapeutic index but also be a rational approach to minimize the toxic manifestations of anticancer drugs in already immunosuppressed/immunocompromised host.

Earlier, it has been shown that aqueous gooseberry (E. officinalis) extract protected mice from CP toxicity (Sharma et al., 2000a). E. officinalis is widely used as an antioxidant constituent of a large number of traditional and folklore herbal formulations (Krishnaveni and Mirunalini, 2010). It has been reported that during tumorogenesis there is a severe crunch of antioxidant (Gupta et al., 2004). Therefore, antioxidants are invariably associated with anticancer as well as tumour burden-reducing activities (Bonmassar et al., 1968; Sannigrahi et al., 2010). Glutathione supplementation in particular has shown to elicit response which leads to increased apoptosis of tumour cells (Navarro et al., 1999). A majority of herbal extracts which has shown protection against cytotoxicity induced by chemotherapeutic drug acts by modulating the antioxidant status of blood and tissues including GSH (Bhattacharya et al., 2003; Li et al., 2008; Patra et al., 2012).
Myelosuppression and anemia are major problems in cancer chemotherapy (Price and Greenfield, 1958; Maseki et al., 1981). The anemia encountered in tumor-bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Fenninger and Mider, 1954). Gupta et al. (2004) has reported antitumor activity and antioxidant role of Bauhinia racemosa extract given orally in Swiss albino mice with Ehrlich ascites carcinoma.

Unani formulations as protective agents against toxicity

Unani system identifies and attributes diseases to a weak immune and digestive system (Singh et al., 2011). Protective effect of Unani formulations used for treatment traditionally has been reported to be some protective effects which were not reported earlier (Najmi et al., 2005; Nazmi et al., 2011). Cardioprotective and nephroprotective effect of Khameera abresham Hakim Arshad wala has been reported against doxorubicin-induced cardiotoxicity (Nazmi et al., 2011). Singh et al. (2011) has reported antiarthritic effect of Majoon suranjan, a Unani polyherbal formulation. Yousuf et al. (2005) evaluated the effect of Khamira abresham and Majun baladar against cerebral ischemia-induced oxidative damage in discrete brain part and also reported a protective role of these Unani formulations by the synergistic modulation of its various antioxidant compounds. Goyal et al. (2010) has reported the efficacy of Khamira abresham in isoproterenol-induced myocardial necrosis in rats.

Diuretic and nephroprotective effect of a polyherbal unani formulation
Jawarish zarooni sada has been reported by Afzal et al. (2004). These drugs protect against the toxicity of drugs and other xenobiotics and pathogens by strengthening the antioxidant defence system of the body including the improvement in the various antioxidants (Yousuf et al., 2005, 2010).

**Animal tumour models**

Several tumour models are used for the assessment of anticancer activities of drugs and herbs in solid form or in ascetic form, Daltons lymphoma (Mallick et al., 2010), Lewis lung sarcoma (Lewis et al., 2012) and Ehrlich's ascites tumour (Ehrlich and Apolant, 1905). These tumours are capable of very fast growth and viability is high.

Ehrlich ascites Tumour is a spontaneous murine mammary adenocarcinoma (Ehrlich and Apolant, 1905) adapted to ascites form and carried in outbred mice by serial intraperitoneal (i.p) passages. Tumor growth causes antioxidant disturbances in different tissues of tumor hosts. Evidenced by in vitro experiments where livers of tumor-hosts were found to undergo significantly greater lipid peroxidation reactions than livers of normal animals (Baglei and Burlakova, 1968; Burlakova and Molochkina, 1973). These observations were confirmed under in vivo conditions with several different tumor systems, including EAT (Burbina and Nejilakh, 1970). The in vivo studies showed that tumor growth causes an increase in vitamin E turnover, resulting in a decrease in vitamin E content of spleen and liver. Losses as high as 66% were reported.
in the case of liver. Growth of EAT in Swiss webster mice is associated with an increase in the turnover rate of selenium in liver and kidney. Losses of selenium-75 from these tissues were accompanied by an increase in radioactivity in tumors (Baumgartner et al., 1978).

It has been demonstrated that EAT growth leads to suppression of immune responses (Pal et al., 2005; Torello et al., 2012). Tumour challenge if not handled by immune surveillance of host may cause immunosuppression (Wheatley and Easty, 1964; Pal et al., 2005). EAT caused disruption of antioxidants including depletion of reduced glutathione. It has been reported that EAT cells lack H-2 histocompatibility antigens (Chen and Watkins, 1970), which apparently is the reason for their rapid proliferation in almost any mouse host (Patt and Straube, 1956). Pal et al. (2005) have reported that curcumin, which is an active ingredient of Curcuma longa a drug used in the traditional system of medicines viz., Unani system of medicine and a dietary constituent, administration to tumor-bearing mice decreased EAT cell number significantly in a dose-dependent manner. In tumor-bearing mice it inhibits hematopoietic toxicity, and acts as hepatoprotective agent and also ameliorates depressed anti-oxidant and detoxification systems.

From the review of the literature of previously published work it is clear that the Unani herbal formulations which are part of the traditional system of medicine in India largely exploit the therapeutic potential of plants and their constituents. These formulations may have a good potential against the
toxicity of drugs and xenobiotics. It is also evident that some of the herbal drugs have potential to decrease the spread of cancer. It is also proved that CP and other anticancer drugs provide protection against the spread of cancer but are associated with the deleterious side effects which makes the life of the cancer patient quite miserable. However, with the use of the herbal drugs the toxic effects can be minimized and the life expectancy of the cancer patient can be increased by easing out the side effects.

In case of higher tumour load the condition worsened and all the system of the body get severely compromised. The antioxidant defense of the body become ineffective practically. The immune system also becomes compromised making the patient vulnerable to infections. CP is used for the treatment of cancer and it causes severe toxicity after getting metabolized in the liver by cytochrome P450 dependent enzymes. The same tumour bearing animals when treated with CP become highly vulnerable to the toxic side effects of the anticancer drug. In addition to antioxidant system the immune system of the subject gets severely depressed as CP is a known immunosuppresor which causes an additive effect in the tumour bearing animal condition. Urotoxicity and hematotoxicity of CP also become a cause of concern. Compound and single drugs of herbal origin are being used now a days for mitigating the toxic side effects of the anticancer drugs and to make the life of the cancer patient a little easier.
In the present study we have studied the modulatory effect of two Unani compound formulations *Jawarish amla sada* and *Habbe-khabsul hadid* on the toxicity caused by CP in Ehrlich's ascites tumour bearing mice. The two drugs have been used in the traditional system of Unani medicine for a long time. The modulatory effect of the two drugs has been studied for their preventive role against the CP-induced antioxidant depletion, immunosupression and the survival of animals.