CHAPTER-I

GENERAL INTRODUCTION
Chapter I

Background:

We are living in a modern era where we can witness the massive development and advancements in the field of science, technology and medicine. This has lead in the eradication and control of a wide spectrum of diseases like plague, cholera, typhoid and polio which were causing collateral deaths in previous centuries. Further, improved technologies in blood transfusion and infection control during post-surgery periods have saved millions of lives during Second World War and are in practice till now. However, the scientific and technological inventions have altered the life style and food habits of people in many ways and have paved the way for the development of diseases and disorders like diabetes mellitus, arthritis, cardiovascular diseases (CVDs) and cancer.

Cancer:

Rapid and uncontrolled cell proliferation with eventual formation of malignant and benign tumors is popularly known as cancer (Hanahan and Weinberg, 2011). There are about 200 various types of cancers and the exact cause of cancer is still unknown. However, several factors are picked up that trigger cancer development such as tobacco consumption, dietary factors, radiations, environmental toxins, obesity and infections. These factors possibly trigger somatic variations (mutations) and eventually transform normal healthy cells to dreadful cancerous cells (Hanahan and Weinberg, 2011). Besides cancer cells hire or take on to the normal cells and architect tumor associated stroma and end up with cancer development. Cancer includes two types of tumor malignant and benign. Malignant tumors spread all over the body through lymphatic and blood circulation systems, whereas benign is restricted to its tumor itself.
### Table 1.1: Different Types of Cancer, Organs Affected and Common Chemotherapeutic Drugs Advised in Combination.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Cancer Type</th>
<th>Organ Affected</th>
<th>Drugs Advised</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leukemia (Blood cancer)</td>
<td>Blood</td>
<td>Cyclophosphamide, Methotrexate, Doxorubicin, Vincristine, Busulfan</td>
</tr>
<tr>
<td>2</td>
<td>Lung cancer</td>
<td>Lung</td>
<td>Cyclophosphamide, Doxorubicin, Vincristine, Methotrexate, Cisplatin, Carboplatin, Paclitaxel</td>
</tr>
<tr>
<td>3</td>
<td>Larynx cancer</td>
<td>Larynx</td>
<td>Cisplatin, 5-fluorouracil, Carboplatin, Paclitaxel, Docetaxel</td>
</tr>
<tr>
<td>4</td>
<td>Testicular cancer</td>
<td>Testis</td>
<td>Bleomycin, Etoposide, Cisplatin</td>
</tr>
<tr>
<td>5</td>
<td>Breast cancer</td>
<td>Breast</td>
<td>Cyclophosphamide, Methotrexate, 5-fluorouracil, Doxorubicin</td>
</tr>
<tr>
<td>6</td>
<td>Gynecological cancer</td>
<td>Female reproductive organs involving ovaries, uterus, vagina, cervix, fallopian tube, vulva</td>
<td>Bleomycin, Etoposide, Cisplatin</td>
</tr>
<tr>
<td>7</td>
<td>Bladder cancer</td>
<td>Urinary bladder</td>
<td>Methotrexate, Vincristine, Doxorubicin, Cisplatin</td>
</tr>
<tr>
<td>8</td>
<td>Colorectal cancer</td>
<td>Colon, rectum</td>
<td>5-fluorouracil, Folinic Acid, Oxaliplatin</td>
</tr>
<tr>
<td>9</td>
<td>Stomach cancer (gastric cancer)</td>
<td>Stomach</td>
<td>Epirubicin, Cisplatin, 5-fluorouracil, Capecitabine</td>
</tr>
<tr>
<td>10</td>
<td>Hodgkin's disease (Hodgkin’s lymphoma)</td>
<td>White blood cells (lymphocytes)</td>
<td>Carmustine, Vincristine, Procarbazine, Prednisolone, Bleomycin, Dacarbazine, Vinblastine, Doxorubicin</td>
</tr>
<tr>
<td>11</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>Any kind of lymphoma except hodgkin’s lymphoma</td>
<td>Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone</td>
</tr>
<tr>
<td>12</td>
<td>Nephroblastoma (Renal cancer)</td>
<td>Kidney</td>
<td>Afinitor (Everolimus), Aldesleukin, Avastin (Bevacizumab), Axitinib</td>
</tr>
<tr>
<td>13</td>
<td>Osteosarcoma (Bone cancer)</td>
<td>Metaphyseal region of tubular long bones</td>
<td>Cyclophosphamide, Methotrexate, Vincristine, Etoposide, Doxorubicin, 5-fluorouracil, Bleomycin</td>
</tr>
<tr>
<td>14</td>
<td>Bone marrow cancer</td>
<td>Shafts of long bones</td>
<td>Vincristine, Ifosfamide, Doxorubicin, Cyclophosphamide, Etoposide, Actinomycin D</td>
</tr>
<tr>
<td>15</td>
<td>Eye cancer</td>
<td>Eye</td>
<td>Methotrexate, Gemcitabine, Treosulfan</td>
</tr>
<tr>
<td>16</td>
<td>Anal cancer</td>
<td>Anus</td>
<td>5-fluorouracil, Cisplatin, Mitomycin</td>
</tr>
<tr>
<td>17</td>
<td>Liver cancer</td>
<td>Liver</td>
<td>Doxorubicin, Cisplatin, 5-fluorouracil</td>
</tr>
</tbody>
</table>
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Cancer is prevalent equally both in men and women, but they are influenced by different types of cancer such as breast cancer in women and prostate cancer in men is predominant. It is reported that adults are more prone for cancer than younger one and at least one in three people develop cancer at certain point in their lifetime (Siegel et al., 2013). Thus, cancer is the second leading cause of death throughout the world that raises a major challenge for physicians, pharmacologists and researchers (Gibellini et al., 2010).

Treatment:

Over the past decades, cancer is being treated by various modalities such as surgery, radiation therapy, chemotherapy, Stem cell transplantation (SCT), biological therapy, hormone therapy, etc, (Amin et al., 2012). Depending on the diagnosis, treatment can be mainly classified into two type’s local and systemic treatment. Local treatment mainly affects the cancer cells in the tumor and its surrounding while in systemic treatment it reaches the cancer cells all over the body through blood stream thereby killing the cancerous cells. Hence, health care professionals mainly choose the mode of treating cancer depending on various properties such as specific characteristics of the cancerous cells, type of cancer, stage of cancer, patient’s general state of mental and physical health and personal wishes during treatment.

Chemotherapy:

The best option to treat malignant solid tumors is surgery, but during re-occurrence of cancer after surgery chemotherapy is advised for advanced malignances. Chemotherapy plays an important role in the treatment regime of cancer with chemotherapeutic agents such as cytotoxic, antineoplastic or anticancer drugs
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(Papageorgiou et al., 2005). Unfortunately, most of the synthetic chemotherapeutic agents accessible today are immunosuppressant, cytotoxic and exert toxic side effects mainly by preventing the rapidly dividing cells (Sheeja and Kuttan, 2006). Further, most of them are not target specific and affects rapidly dividing normal cells of skin, hair, gastrointestinal and bone marrow.

During chemotherapy regimen, drug doses are calculated based on blood counts to avert anemia (red cell deficiency), neutropenia (white cell deficiency) and thrombocytopenia (platelet deficiency). Chemotherapy drugs are occasionally panic because of a patient's anxiety about toxic side effects. Chemotherapy regimen is a gift which adds up the survival rate of cancer patients, but has adverse side effects such as nausea, vomiting, alopecia, infertility, hepatotoxicity, nephrotoxicity, genotoxicity, hematopoetic suppression (Berrigan et al., 1982; Pratheeshkumar and Kuttan 2010), gastrointestinal toxicity, urotoxicity, immunotoxicity, mutagenicity, teratogenicity (Hales, 1982), carcinogenicity, reprotoxicity, hemorrhagic cystitis (HC) and cardiotoxicity (Ray et al., 2010). Chemotherapy might be used alone or in combination with other treatments such as surgery, radiation therapy to increase the antitumor effect. Hence, discovery and development of anticancer drugs with cost-effectiveness and impact on quality of life is one of the boons that have been the center of attention for several pharmaceutical companies as well as health care professionals in the treatment of cancer patients.
Anticancer Drugs:

Anticancer drugs can cause liver damage frequently, thereby leading to hepatosis, which later forces patients to give up chemotherapy. Hence, during chemotherapy regimen it is appropriate to use the combination of two or more drugs which results in the decreased dosage, reduced side effects, and in the progress of antitumor effect (Huang et al., 2013). Frequently used anticancer drugs have three targets to function/work appropriately.

1. DNA damaging of cancer cells
2. Prevent the synthesis of new DNA strands to avoid cell multiplication that leads to tumor growth.
3. Prevention of mitosis of cancer cells where original cell divides into two new cells thereby halting the growth of cancer.

Classification of Antineoplastic Agents / Anticancer Drugs:

Depending on mechanism of action, anticancer drugs are divided into two classes: cycle specific and non-cycle specific. Cycle specific drugs work only at particular points of the cell's replication cycle, such as anaphase or metaphase, while non-cycle specific drugs may work at any point in the cell cycle to achieve highest effect; they are further divided into five groups namely alkylating agents, antimetabolites, natural products, hormones and antagonists and miscellaneous.

1. Alkylating Agents
   - Nitrogen mustards: Melphalan, Cyclophosphamide, Ifosfamide
   - Nitrosoureas
   - Alkylsulfonates
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- Ethyleneimines
- Triazene
- Methyl hydrazines
- Platinum coordination complexes: Cisplatin, Carboplatin, Oxaliplatin

2. Antimetabolites
   - Folate Antagonists: Methotrexate
   - Purine antagonists
   - Pyrimidine antagonists: 5-Fluorouracil, Cytarabine

3. Natural Products
   a. Plant Products
      - Vinca Alkaloids: Vincristine, Vinblastine
      - Taxanes: Paclitaxel, Docetaxel
      - Epipodophyllotoxins: Etoposide
      - Camptothecins: Irinotecan
   b. Microorganism Products
      - Antibiotics: Doxorubicin, Bleomycin
      - Enzymes: L-Asparaginase

4. Hormones and Antagonists
   - Corticosteroids: Prednisone, Dexamethasone
   - Estrogens: Ethinyloestradiol
   - Antiestrogens: Tamoxifen
   - Progesteron derivative: Megestrol Acetate
   - Androgen: Testosterone propionate
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- Antiandrogen: Flutamide, Bicalutamide
- Aromatase inhibitor: Letrozole, Anastrazole
- 5-alpha reductase inhibitor: Finasteride
- GnRH Analogue: Leuprolide, Buserelin
- Growth Hormone, glucagon and insulin inhibitor: Octreotide

5. Miscellaneous

- Hydroxyurea
- Imatinib mesylate
- Rituximab
- Epirubicin
- Bortezomib
- Zoledronic acid
- Geftinib
- Leucovorin
- Pamidronate
- Gemcitabine

(Source: http://www.srspharma.com/antineoplastic-agents.htm)

Mechanism of Action of Anticancer Drugs:

The current anticancer drugs are the result of screening of many compounds with cytotoxic property both in vitro against murine/human cancer cells and in vivo against rodent tumor models. To the best of our knowledge regarding the anticancer drugs their aim is to transform or restrain/arrest the cellular and molecular levels of targets that are essential for the cancer growth. Further, they have diverse mechanisms
of action which may differ depending on the class of drug, concentrations and their impact on the normal and neoplastic cells (Gaspar et al., 2013).

### Table 1.2: Main Groups of Anticancer Drugs with their Mechanism of Action

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Families of Anticancer drugs</th>
<th>Examples of drugs</th>
<th>Mechanism of action</th>
<th>Drug targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkylating agents</td>
<td>Cyclophosphamide, Melphalan, Chlorambucil, Cisplatin</td>
<td>Binding with DNA, breaks and inappropriate links between DNA strands</td>
<td>DNA molecule</td>
</tr>
<tr>
<td>2</td>
<td>Antimetabolites</td>
<td>Methotrexate, Fluorouracil, Cytosar, 5-Azacytosine, 6-Mercaptopurine, Gemcitabine</td>
<td>Inhibition of enzymes participating in DNA and RNA synthesis</td>
<td>Dihydrofolate reductase, thymidylate synthetase,</td>
</tr>
<tr>
<td>3</td>
<td>Antimicrotubles</td>
<td>Vinca alkaloids (vinblastine), Podophyllotoxins (etoposide), Taxanes</td>
<td>Depolymerization of microtubules, damage to mitotic spindle</td>
<td>Cytoplasmic microtubules, mitotic spindle</td>
</tr>
<tr>
<td>4</td>
<td>Topoisomerase inhibitors</td>
<td>Doxorubicin, Camptothecin (Irinotecan and Topotecan)</td>
<td>Topoisomerase inhibition</td>
<td>Topoisomerase I and II</td>
</tr>
<tr>
<td>5</td>
<td>Cytotoxic antibiotics</td>
<td>Anthracycline, Actinomycin, Bleomycin, Mitomycin</td>
<td>DNA intercalation, topoisomerase inhibition, prevention of DNA, RNA synthesis</td>
<td>DNA molecule and Topoisomerase</td>
</tr>
</tbody>
</table>

Source: (Stavrovskaya AA, 2000)
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Alkylating agents are a group of chemotherapeutics having highly reactive molecules with their ability to covalently bind an alkyl group to bimolecular compounds such as RNA, DNA and proteins which is the most important basis for their anticancer effects. Majority of the alkylating agents are bipolar in nature having two groups that are capable of reacting with DNA forming cross links between a single strand (intrastrand) or two separate strands of DNA (interstrand), thereby interrupting with the action of the enzymes that are implicated in DNA replication. Further, if the cell attempts to replicate cross linked DNA during cell division, or tries to correct it, the DNA strands can break. Thus, the cell dies or loses its ability to proliferate and triggers apoptosis. The alkylating agents intercross the DNA during S-phase of the cell cycle, where the cell has very less time to eliminate the damaged fragments (Bhattacharya et al., 2003).

Antimetabolites are group of molecules having similar structure to that of naturally occurring substances such as vitamins, nucleobases, nucleosides and amino acids. Hence, they interfere with the enzymes implicated in the synthesis of DNA and RNA by competing for the catalytic site of essential enzymes with the substrates. Hence, antimetabolites play a culprit role through inhibiting mitosis and causing DNA damage thereby it prevents cell division. Antimetabolites are cell cycle dependent and work particularly during the S-phase of the cell cycle (Zhang et al., 2014).

Antimicrotubules are group of molecules particularly derived from the plants capable of obstructing the cell proliferation by inhibiting the microtubule function. Vinca alkaloids and taxanes are examples for antimicrotubules which causes microtubule dysfunction but with entirely opposite mechanism of action where vinca alkaloids specifically stop the synthesis of microtubules, while the taxanes avert the microtubule disassembly. Hence, antimicrotubules stop the cancer cells to carry out the
mitosis process leading to cell cycle arrest and induces apoptosis. Besides, they also affect blood vessel growth a vital process in which tumors makes use of these blood vessels in order to cultivate and transfer the malignancy (Zhang et al., 2014).

Fig 1.1: Schematic Representation of General Mechanism of Anticancer Drugs

Topoisomerase inhibitors are class of drugs capable of inhibiting the two active enzymes topoisomerase I and II that are concerned with the DNA duplication, transcription and chromatid segregation. Further, these enzymes are required for the maintenance of normal 3D structure of DNA through unwinding, cleaving and rejoining reactions. Thus, topoisomerase inhibitors target the prevention of DNA duplication of cancer cells and prevent it from carrying out mitosis from G2 phase.
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Cytotoxic antibiotics are the derived products of bacterial and fungal cultures with wide variety of mechanism of action which mainly affect the synthesis of nucleic acids. Anthracycline mechanism involves the addition of molecules between the two strands of DNA a process called DNA intercalation, production of highly reactive free radicals that lead to injury to the intercellular molecules and topoisomerase inhibition. Further, DNA gyrase cleaves the DNA helix and rejoin it to overwhelm the torsional forces that would hinder with replication. Thus, anthracyclines stabilize the DNA topoisomerase II complex and avoid rejoining of the strands (Gaspar et al., 2013).

Actinomycins are the molecules that intercalate DNA between guanine and cytosine base pairs and hinder the transcription of DNA thereby inhibit RNA synthesis. Bleomycin, a glycopeptide basis for DNA fragmentation, intercalates DNA, generates free radicals that break DNA by binding to a metal ion that becomes a chemically reduced and reacts with oxygen. Mitomycin, an antibiotic alkylates DNA by producing cross links (King, 2001).

Limitations of Anticancer Drugs:

Majority of the cytotoxic anticancer drugs are prescribed to maximum amount to withstand and to reach highest effect in cell killing. The frequent toxicities of cytotoxic anticancer drugs involve:

- Damage to the growing stem cells thereby leading to bone marrow depression, the most dangerous form of toxicity of anticancer drugs which results in the decreased WBC, RBC and platelet counts. These in turn, could cause vulnerability to infections, hemorrhage and anemia. Besides, certain drugs
cause unique and severe bone damage, for e.g. osteonecrosis of the jaw connected with bisphosphonates.

- Further, injury to growing cells may possibly lead to temporary alopecia, skin problems, changes in the color and texture, or loss of fingernails and toenails but these toxicities are usually reversible.

- In gastrointestinal tract, surface epithelial damage might result in ulcers, stomatitis, complicated swallowing and susceptibility to oral infections such as candidiasis, and changes in saliva secretion. Nausea, vomiting, diarrhea or constipation may also occur usually.

- Kidney damage may occur from some drugs due to widespread cell destruction, purine catabolism and accumulation of urates in the renal tubules. As well, liver damage may also occur since it receives large blood supply. Liver and kidney are the main metabolic sites that are regularly examined for probable involvement of drugs in blood levels and dosage adjustment, and are the major drug elimination sites.

- Drugs, for instance paclitaxel and vincristine, might cause peripheral neuropathy. Likewise, anthracyclines are known for unusual but severe cardiotoxicity (Narang and Desai, 2009).

**Cyclophosphamide: A Historical Perspective**

During, Second World War nitrogen mustards were used as agents in chemical war-fare which made researchers to pay more attention towards their application. The new area of research in the discovery and synthesis of nitrogen mustard derivatives was launched in the late 1950’s and early 1960’s. Arnold and Burseaux headed a research team in 1957 to develop nitrogen mustard derivatives, among which
Cyclophosphamide (CP) is one of the nitrogen mustard derivatives first developed in German laboratories and marketed in Europe by the trade name of Endoxan. Further, Norbert Brock also a German researcher developed and synthesized CP which was approved by FDA in 1959 as the 8th cytotoxic drug. They scrutinized more than 1000 oxazaphosphorine compounds by converting the base nitrogen mustard into a nontoxic form which can be carried to the cancer cells. Once it enters the cell, enzymes present in the cell acts on the prodrug by converting it to active toxic forms. Great contentment was created by the synthesis and discovery of CP which soon came into extensive use (Elangovan et al., 2006). Being aware of the fact that, CP is one such anticancer drug with bifunctional alkylating agent belongs to the class of nitrogen mustard widely used with high therapeutic index and broad spectrum of activity to treat lymphomas, myeloma, solid tumors, chronic lymphocytic leukemia, and as an immunosuppressant in autoimmune disorders and in the bone marrow preparation for transplantation. Further, CP destroys other normal cells which is its major drawback (Pratheeshkumar and Kuttan, 2010; Oboh et al., 2012).

**Fig 1.2**

![Structure of Cyclophosphamide](image)

*Fig 1.2: Structure of Cyclophosphamide*
Mechanism of Action of Cyclophosphamide:

The impact of CP is mainly due to its reactive metabolites phosphoramid mustard and acrolein. These active metabolites produces irreversible crosslink in both intra and inter DNA strands by adding an alkyl group (C_nH_{2n+1}) to the guanine bases of DNA, at the N-7 position of the imidazole ring thereby inhibit DNA replication and repair that lead to cell death through apoptosis. Further, CP put forth its cytotoxic effect equally on resting and dividing lymphocytes. CP works at any point in the cell cycle and is a cell cycle independent drug (Elangovan et al., 2006). Hence, the impact on the cells mainly depends on the dose; the fraction of cells that die is directly proportional to the dose of drugs. Its exact mechanisms in the treatment of autoimmune diseases are not clearly demonstrated. But, in rheumatoid arthritis patients, CP has been shown to suppress T-helper cell functions with prolonged reduction of B-cells due to the slower rate of recovery of B lymphocytes from an alkylating agent. Moreover, reactive metabolites of CP are formed specially when there is low concentration of aldehyde dehydrogenases (ALDHs). But, at relatively large concentration of ALDHs present in the actively proliferating tissues such as bone marrow stem cells, liver and intestinal epithelium protects against the toxic effects of phosphoramid mustard and acrolein by converting aldophosphamid to carboxyphosphamid which averts the formation of toxic metabolites phosphoramid mustard and acrolein (Devine et al., 2012).
Fig 1.3: Schematic representation of Mechanism of Action of CP in Hepatic Cell through Cytochrome P-450 Enzymes to Phosphoramid mustard and Acrolein

**Metabolism of Cyclophosphamide:**

CP itself is not an active prodrug, but the drug administered gets activated to 4-hydroxycyclophosphamide by the action of phase I detoxifying enzymes cytochrome P-450 mixed functional oxidase system extensively in the liver and to a smaller extent in lungs via 4-hydroxylation and N-dechloroethylation (Afsharian *et*
Furthermore, an unstable 4-hydroxycyclophosphamide can cross the cell membrane and gets decomposed into reactive metabolites such as phosphoramide mustard and acrolein. In addition, CP also gets metabolized into reactive metabolites through arachidonic acid metabolism by the action of prostaglandin H synthase and horseradish peroxidase mediated by free radical mechanism (Stankiewicz and Skrzydlewska, 2005). Thus, reactive metabolites formed are dispersed to other tissues by systemic circulation. About 20% of the drug administered is excreted in urine as unchanged compound (Afsharian et al., 2007).

**Reactive Metabolites of CP:**

**Phosphoramide mustard:**

In liver, bio-activation of CP leads to the formation of 4-hydroxycyclophosphamide by the action of cytochrome P-450 2B1 isoenzyme. 4-hydroxycyclophosphamide is not stable; hence it impulsively breaks down to the cytotoxic metabolites phosphoramide mustard and acrolein. These cytotoxic intermediates produced in liver are spread to other tissues by systemic circulation. In addition, phosphoramide mustard is converted to aziridinium ion, which alkylates DNA at the N-7 position of guanine. Guanine present in the other DNA strand reacts with the alkylated DNA making interstrand DNA cross-links. CP and 4-hydroxycyclophosphamide primarily acts on DNA interstrand to produce DNA cross-links (Tripathi and Jena, 2009).
Acrolein:

Another cytotoxic reactive metabolite of CP is acrolein, which is formed in liver by hepatic microsomal cytochrome P-450 enzymes namely CYP-3A4 and CYP-2B6. Thus, acrolein formed is dispersed to other tissues by systemic circulation resulting in the oxidative stress and tissue damage induced by CP. (Manda and Bhatia, 2003; Abraham and Sugumar, 2008). Further, at inflammation site acrolein stimulates endogenous reactive oxygen species (ROS) and nitric oxide (NO) formation resulting in the peroxynitrite (ONOO') generation which is harmful to bladder leading to bladder toxicity (Hamsa and Kuttan, 2011).
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Acrolein mainly reacts with α, β-unsaturated aldehydes and quickly conjugates with glutathione (GSH) thereby reducing cellular GSH. Further, it also interacts with protein residues and nucleophilic sites in DNA. Patients undergoing chemotherapy for solid tumors, show the presence of high concentration of acrolein in urine which is assessed by determining the urinary acrolein one of the key sources for cytotoxicity. Thus, in case of CP induced oxidative stress and organ damage acrolein toxicity has come across the urinary system and is not from direct toxic effects (Korkmaz et al., 2007).

Table 1.3: The Proposed Mechanism of Acrolein-Induced Hemorrhagic Cystitis

1) Acrolein enters rapidly into the uroepithelium because of its chemical nature.
   a. Acrolein causes increased ROS production in the bladder epithelium.
   b. Acrolein causes both directly and/or indirectly iNOS induction leading to NO overproduction.
   c. Acrolein induces some intracellular transcription factors such as NF-κB and AP-1.
   d. Activated NF-κB and AP-1 cause cytokine (TNF-α, IL-1β) gene expression, iNOS induction, and again ROS production. Thus, the production of harmful molecules (cytokines, ROS, NO) increases dramatically.
   e. Cytokines leave the uroepithelium and spread to other uroepithelial cells, detrusor smooth muscle, and bloodstream.

2) ROS and NO form peroxynitrite in both uroepithelium and detrusor smooth muscle.

3) Peroxynitrite attacks cellular macromolecules (lipids, proteins, and DNA) and causes damage.

4) Cellular and tissue integrity are broken and damage appears as edema, hemorrhage, and ulceration.

Source: (Korkmaz et al., 2007)
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Limitations of CP:

Among the existing anticancer drugs CP has been in clinical use for more than 50 years and is often used in combination with other treatments like surgery and radiation therapy (Huang et al., 2013). CP treatment in large number of inter-individuals has been reported to cause varied clinical effects which may be due to variation in metabolism and drug distribution. Yet pharmacokinetics and biological effects of CP are not understood clearly. Nevertheless, use of CP as an effective chemotherapeutic agent is often confined because of its wide adverse side effects that include hepatotoxicity, nephrotoxicity, genotoxicity, hematopoetic suppression (Berrigan et al., 1982; Pratheeshkumar and Kuttan 2010), gastrointestinal toxicity, urotoxicity, immunotoxicity, mutagenicity, teratogenicity (Hales, 1982), carcinogenicity, reprotoxicity, HC and cardiotoxicity (Ray et al., 2010). Prevention of these toxic side effects of CP can cause a better tolerance, more efficiency and comfort in patients undergoing CP regime (Pratheeshkumar and Kuttan 2010; Abraham and Isaac, 2011). Along with these toxic side effects, CP has shown to have pro-oxidant character and its exposure/administration induces oxidative stress in vital organs such as liver, kidney, lungs, brain, spleen and serum of mice and rats thereby resulting in reduced antioxidant defense and elevated lipid peroxidation (LPO) leading to organ toxicity (Rehman et al., 2012). Since cytotoxicity and immunosuppression is the primary dose limiting step towards the normal host tissue in CP regime that declines the survival rate and confines the treatment protocol. Several studies have demonstrated that oxidative stress mediated interruption of pro-oxidant/antioxidant balance after CP administration leads to changes in physiological and biochemical functions (Sekeroğlu et al., 2011; Wei et al., 2011).
Hence chemotherapeutic drugs capable of killing tumor cells and preventing the tumor growth should be selected in such a way that the drugs should cause less injury to normal cells particularly hepatocytes. Therefore, mouse model is suggested to be a very good substitute for humans to carry out the toxicological study of chemotherapeutic drugs associated with hepatotoxicity, genotoxicity and cytotoxicity that are expressed when drugs are metabolically activated (Elangovan et al., 2006; Tripathi and Jena, 2009; Rehman et al., 2012).

**Organ Toxicity Induced by CP:**

**Liver:**

Liver is the only organ that can regenerate itself even after failure by 95%. It has an imperative role in many diverse functions such as metabolism, glycogen storage, detoxification, synthesis of hormones and plasma protein, synthesis of bile, an alkaline compound that helps in digestion through emulsification of lipids and decay of red blood cells. No other vital organs can reimburse the dearth of liver function for a long period (Niemelä and Alatalo, 2010).

CP undergoes bioactivation extensively in liver producing phosphoramide mustard and acrolein by-products of CP where phosphoramide mustard is known for its anticancer effect by producing cross links between DNA that is DNA alkylation, while acrolein is concerned with the toxic side effect by hindering the body antioxidant defense system through the formation of ROS thereby causing injury to both the structure and functions of hepatocyte membranes which was supported by the leakage of liver marker enzymes to the blood stream (Oboh et al., 2012).

Further, current reports suggest that CP administration during chemotherapy will lead to oxidative stress and liver injury through a reactive metabolite acrolein
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which binds to cytochrome enzymes, hepatic biomolecules (proteins, lipids, nucleic acids) preventing hepatic glutathione-S-Transferase (GST) and reducing the cellular GSH content which lead to the accretion of acrolein in liver after exposure to CP. Hence, frequent administration of CP can lead to hepatic toxicity (de Jonge et al., 2006).

Kidney:

Kidney is one of the sensitive target organs for various xenobiotics and toxins. According to the recent reports, the rate of drug-induced nephrotoxicity is high up to 66 % due to recommendation of multifunctional drugs for cancer, CVDs and diabetes that results in the renal failure. On top of that, anticancer drugs and their reactive metabolites are mainly excreted from kidneys through urine, influencing kidney toxicity by altering vital functions of kidney. The hepatic cytochrome P-450 specifically hydroxylates CP yielding nitrogen mustards which interacts with DNA eventually. In addition, they produce cytotoxic intermediates chloroacetaldehyde, a therapeutically inert N-dechloroethylated metabolite which is reported to cause neurotoxicity and nephrotoxicity. In addition, yet another metabolite of CP acrolein is also reported to cause urotoxicity (Rehman et al., 2012).

Further, CP induced nephrotoxicity was evidenced by the proximal tubular damage, reduction in conductance and a marked reversible depolarization (Senthilkumar et al., 2006). CP exposure initially set off oxidative stress in the mitochondria of kidney proximal tubule and epithelial/endothelial cells, followed by increased ROS/ reactive nitrogen species (RNS) production, causing peroxidative damage to kidney thereby worsening the mitochondrial structure and function, an inflammatory response and renal damage by depleting the antioxidant enzyme activity.
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through reducing the endogenous GSH concentration. The toxic reactive metabolites are eliminated by several ALDHs and GSTs by conjugating with GSH (Zhang et al., 2005).

Bladder:

Urinary bladder is an organ that collects and stores urine from kidney. Bladder inflammation or HC is a generally reported and major dose limiting side effects of CP regimen. The prevalence of this adverse effect is reported as 75% high in patients receiving high dose of CP intraperitoneally. Acrolein, a reactive metabolite of CP is mainly responsible for urinary bladder toxicity in humans and animal models (Al-Yahya, 2009; Hughes et al., 2013). Urothelial damage is mainly due to the direct contact of acrolein that exhibits edema, hemorrhage, ulceration, leukocyte infiltration and in severe cases fibrosis and necrosis of the bladder. Since urine is stored in the bladder for many hours acrolein metabolite concentration is more in bladder than in any other areas of the urinary tract which makes the bladder vulnerable to damage. Hence, acrolein mainly acts as the chief culprit and one of the most expected causes of the CP-induced bladder injury, further supported by ROS with other inflammatory mediators, cytokines such as tumor necrosis factor (TNF-\(\alpha\)), interleukins, cyclooxygenase -2 (COX-2) and transcriptional factor nuclear factor kappa B (NF-\(\kappa\)B), activator protein (AP-1) which also contributes to the pathogenesis of CP-induced bladder injury. Thus, acrolein is inactivated by treating with 2-mercaptopoethane sulfonate (Mesna) (Motawi et al., 2010).
Oxidative Stress: A General Overview

Because of aerobic lifestyle of our body cells, ROS are continuously produced as obvious byproducts during the conversion of molecular oxygen to water. Under normal circumstances, the physiological antioxidant system efficiently counteracts the ROS and protects cells from their deleterious actions. However, when this balance is disturbed in favor of ROS, the situation is called ‘oxidative stress’. Oxidative stress has been shown to be associated with many pathological conditions like arthritis, CVDs, cancer, neurodegenerative disorders etc and has been considered as one of the pathomechanism for the progression of above said pathologies (Aktan et al., 2003).

ROS includes both free radicals and non-radical molecules. Free radicals are defined as the groups of atoms with an odd number of electrons and have very short half-life. The free radicals of ROS comprise hydroxyl radical (•OH), superoxide anion radical (O²⁻) and NO. And non-radical molecules include hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl) and ONOO⁻ (Suresh et al., 2012).

Free Radicals:

Superoxide Anion (O²⁻):

Superoxide anion is fairly toxic in nature. It is the chief ROS generated during the course of oxygen metabolism, which is transformed to a less reactive product, H₂O₂ through a family of metalloenzymes known as superoxide dismutase (SOD) which represents a front line defense against ROS mediated injury (Prabhu et al., 2013). Further, it is well documented that, O²⁻ can initiate the production of more potent prooxidants like •OH and singlet oxygen (¹O₂).
Hydroxyl Radical (•OH):

Hydroxyl radical is a highly reactive radical with a half-life of less than 1 ns in an aqueous environment. However, it can be formed in many ways such as photolytic disintegration of alkyl hydroperoxides, ionizing the breakdown of water into •OH and hydrogen atoms. Most of the •OH produced in vivo is mainly through metal catalyzed decomposition of H$_2$O$_2$. Metals are transition metals like iron, copper, cobalt, chromium etc. Further, •OH mainly provokes LPO in biological membranes by summarizing the hydrogen atoms from unsaturated fatty acids and has the capacity to activate mutagenesis, carcinogenesis and cytotoxicity (Badmus et al., 2013).

Nitric Oxide (NO):

NO and nitrite (NO$_2^-$) are reactive nitrogen species with biochemically reactive resulting in the harmful effects to the organism. It is a free radical with a half-life of few seconds (>15 s) in the aqueous state. Its response with ROS results in the generation of ONOO$^-$ which reacts with proteins, lipids, and DNA, through direct oxidative reactions or by indirect, radical mediated pathway, causing cytotoxicity (Souza-Fiho et al., 1997). Even though NO$_2^-$ is considered as an inert molecule, it has shown to be active at physiological conditions as a signaling molecule and to post-translationally alter proteins independent of NO. NO is generated by particular NOS in cells and tissues during the conversion of arginine to citrulline. Surplus RNS production in biological system results in nitrosylation that alters the membrane proteins and lipids leading to a condition termed as ‘nitrosative stress’ thereby preventing the normal functioning of the cells (Abraham et al., 2011). Furthermore, NO being an important oxidative signaling molecule which was equally soluble in
both aqueous and lipid media and quickly move to cytoplasm and plasma membrane thereby involving in various biological processes such as boosting and regulating the immune system, smooth muscle relaxation, maintenance of blood pressure, neurotransmission, etc, (Al-Yahya et al., 2009).

Non-radical Molecule:

**Hydrogen Peroxide (H$_2$O$_2$):**

H$_2$O$_2$ is a non-radical species due to the absence of unpaired electron. It is produced in a large quantity in mitochondria. H$_2$O$_2$ formation is estimated to be approximately 2 % of the total oxygen uptake by the organism under suitable biological conditions (Valko et al., 2006). Further, H$_2$O$_2$ synthesis takes place by other reactions catalyzed by various oxidases involving amino acid oxidase and ascorbate oxidase. Even though, H$_2$O$_2$ is not considered as a radical still it causes cellular damage moderately at a lower concentration (10 µM) and provokes the release of iron from heme proteins, enzymes inactivation, oxidation of DNA, lipids and -SH groups. Further, they provide a source for the production of •OH and HOCl either by reacting with superoxide anion (Haber – Weiss reaction) or with free iron (Fenton reaction) (Sebastian et al., 2013).

\[
\begin{align*}
H_2O_2 + O_2 &\rightarrow O_2 + HO + •OH & \text{(Haber – Weiss reaction)} \\
Fe^{2+} + H_2O_2 &\rightarrow Fe^{3+} + HO + •OH & \text{(Fenton)}
\end{align*}
\]

**Peroxynitrite:**

Excess production of RNS namely NO, ONOO$^-$ during metabolism is termed nitrosative stress. A very close correlation exists between ROS and RNS. To be specific, O$_2^-$ interacts with NO and forms ONOO$^-$ radical which leads to an increase in
Chapter I

the NO toxicity. ONOO- is a potent oxidant and nitrating species which can oxidize and covalently alter biomolecules such as membrane lipids, proteins, thiols and nucleic acids ultimately damaging the host cell (Abraham et al., 2011).

**Hypochlorous Acid (HOCl):**

Hypochlorus acid is formed by the neutrophils through myeloperoxidase mediated peroxidation of chloride ions and is the prime end-product during neutrophils respiratory burst. It is used to kill broad spectrum of pathogens and mainly affects the tissues at the site of inflammation causing damage to the tissues. Further, it is known to activate cell signaling pathways. Besides, it is known to react with many intracellular biomolecules such as nucleotides, DNA, RNA, proteins, lipids, etc. Furthermore, NADH and the NH-groups of pyrimidines, reacts with HOCl that leads to DNA denaturation (Pullar et al., 2000).

$$\text{Cl}_2 + \text{H}_2\text{O} \xleftrightarrow{} \text{HOCl} + \text{HCl}$$

Further, based on source, ROS can also be classified as endogenous ROS and exogenous ROS.

**Endogenous Source of ROS:**

Mitochondria are the foremost place for free radical production, where oxygen is reduced to water under normal circumstances but electrons passing throughout the chain oxygen hastily get reduced to give $\text{O}_2^-$. Other endogenous sources of ROS are macrophages, neutrophils and eosinophils. Activated macrophages set off an increase in oxygen acceptance that gives rise to various ROS comprising of $\text{O}_2^-$, NO and $\text{H}_2\text{O}_2$. Further, cytochrome P-450 has also been anticipated as a reserve of ROS during the stimulation of cytochrome P-450 enzymes. $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ may be formed by the
breakdown or uncoupling of the P-450 catalytic cycle. Besides, microsomes are accountable for the 80 % H\textsubscript{2}O\textsubscript{2} concentration produced \textit{in vivo} at hyperoxia sites. H\textsubscript{2}O\textsubscript{2} is produced by peroxisomes at physiological conditions but not the O\textsuperscript{2-}. Oxidation of fatty acids by peroxisomes has been accepted as a possible source of H\textsubscript{2}O\textsubscript{2} formation as a consequence of malnutrition/starvation for a long period of time (Valko \textit{et al.}, 2006). Several other enzymes like xanthine oxidase, NADPH oxidase, nitric oxide synthases (NOS), LOX and COX generate ROS during their metabolic processes. Further, biomolecules like hemoglobin, catecholamine and flavones undergo auto-oxidation and generate ROS.

**Exogenous Source of ROS:**

In our day-to-day life, our body gets exposed to many drugs, xenobiotics, pollutants, tobacco, smoke, radiations, etc. which are the prime source of exogenous ROS generation. Besides, human body comprises of 60-70 % of water hence chances of undergoing radiolysis is more in the presence of ionizing radiation. Water exposed to radiation dissociates sequentially into •OH, H\textsubscript{2}O\textsubscript{2} and O\textsuperscript{2-} highly reactive intermediates and oxygen through a chain reaction. •OH takes away electrons from any molecules in its path, and changing them into a free radical and proliferate the chain reaction. H\textsubscript{2}O\textsubscript{2} is considered to be more effective towards DNA than •OH because lower reactivity of H\textsubscript{2}O\textsubscript{2} afford sufficient time for the molecule to move into the nucleus of the cell, consequently causing destruction of macromolecules (Valko \textit{et al.}, 2006). Besides, many dietary pro-oxidants like H\textsubscript{2}O\textsubscript{2}, lipid peroxides, aldehydes may also serve as exogenous source of ROS.
Table 1.4: Reactive Oxygen and Nitrogen Species of Biological Interest

<table>
<thead>
<tr>
<th>Reactive species</th>
<th>Symbol</th>
<th>Half life</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reactive oxygen species</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide</td>
<td>O$_2^-$</td>
<td>10$^{-6}$ sec</td>
<td>Generated in mitochondria, less reactive</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>•OH</td>
<td>10$^{-9}$ sec</td>
<td>Highly reactive, generated during iron overload</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H$_2$O$_2$</td>
<td>Stable</td>
<td>Formed during various oxidase mediated reactions, yields potent *OH species</td>
</tr>
<tr>
<td>Peroxyl radical</td>
<td>ROO*</td>
<td>Sec</td>
<td>Reactive and formed from lipids, proteins, sugars during oxidative damage</td>
</tr>
<tr>
<td>Organic hydroperoxide</td>
<td>ROOH</td>
<td>Stable</td>
<td>Reacts with transitional metal ions to yield reactive species</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>¹O$_2$</td>
<td>10$^{-4}$ sec</td>
<td>Highly reactive, formed during photosensitization</td>
</tr>
<tr>
<td><strong>Reactive nitrogen species</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>NO</td>
<td>Sec</td>
<td>Neurotransmitter, blood pressure regulator, prooxidant during pathological states</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>ONOO$^-$</td>
<td>10$^{-3}$ sec</td>
<td>Formed from NO$^*$ and superoxide, highly reactive.</td>
</tr>
<tr>
<td>Peroxynitrous acid</td>
<td>ONOOH</td>
<td>Fairly stable</td>
<td>Protonated form of ONOO-</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>NO$_2$</td>
<td>Sec</td>
<td>Formed during atmospheric pollution</td>
</tr>
</tbody>
</table>

Source: (Devasagayam et al., 2004)
Role of Antioxidants in Preventing Oxidative Stress: An Overview

Antioxidants are vital compounds in living organisms with a characteristic property to counteract the free radicals and alleviate oxidative damage. Antioxidants either delay or stop free radical generation by giving hydrogen atom or scavenging free radicals. Scientifically it’s been proved that antioxidants reduce the risk of chronic disorders such as cancer and heart diseases. Antioxidants scavenge free radicals such as peroxide, hydroperoxide, lipid peroxyl by preventing oxidative damage that results in degenerative diseases (Motawi et al., 2010).

Antioxidants are either endogenous molecules (naturally synthesized within the human body) or exogenous molecules (some plant derived or synthetic molecules taken up through diet). Endogenous source comprise both enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants can be divided into primary or first line enzymes and secondary enzymes. Primary antioxidant enzymes including SOD, catakase (CAT), and Glutathione peroxidase (GPx) can initiate cascade of reactions to convert ROS to more stable molecules such as water and oxygen. However, second line enzymes Glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH) provides co-substrate molecules (GSH and NADPH) for primary enzymes to counterbalance ROS (Karajibani et al., 2009).

Enzymatic Antioxidants:

Superoxide Dismutase:

SOD is a vital intracellular antioxidant enzyme present in all oxygen metabolizing cells. SOD mainly catalyzes the decomposition of superoxide into oxygen and H2O2. It acts as the first line of defense against ROS. There are three
isoforms of SOD in humans depending on their nature of metal center, amino acid composition, cofactors and the number of subunits namely mitochondrial Mn-SOD, cytosolic Cu, Zn-SOD and extracellular Fe-SOD (Devine et al., 2012). Mitochondrial Mn-SOD is a homotetramer having a molecular mass of 96 kDa where as Cu, Zn-SOD is a homodimer with molecular mass of about 32 kDa and more specific in binding the heparin and heparin sulphate a common glycosaminoglycans.

\[ 2O^2^- + 2H^+ \rightarrow O_2 + H_2O \]

**Catalase:**

CAT is another prime antioxidant enzyme which offers protection to the cells from oxidative damage by ROS. It is mainly found in all aerobic cells containing cytochrome system. It catalyzes the degradation of \( \text{H}_2\text{O}_2 \) into oxygen and water (Kim et al., 2012) and is the only enzyme that does not get saturated with the consumption of \( \text{H}_2\text{O}_2 \) at any concentration. Approximately 6 million molecules of \( \text{H}_2\text{O}_2 \) can get converted more efficiently to water and oxygen every minute by one molecule of catalase. It is a tetramer of four polypeptide chains containing four porphyrin heme groups which allow the enzyme to react with \( \text{H}_2\text{O}_2 \). It is greatly effective in preventing various ROS mediated injuries and could safeguard the vital organs from drug induced toxicity (Wang et al., 2007).

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

**Glutathione Peroxidase:**

GPxs are non-hemethiol peroxidases belong to a family of phylogenetically related enzymes. GPxs mainly catalyzes the decomposition of \( \text{H}_2\text{O}_2 \) to water and to the corresponding alcohols, using GSH as reductant. They mainly use selenium or
sulfur at their redox center (Brigelius-Flohé and Maiorino, 2013). GPx is confined to mitochondria, endoplasmic reticulum, chloroplasts, and cytosol. GPx proceed in conjunction with the tripeptide GSH, which is present in cells at higher concentrations. Further, GPx struggle with catalase for H$_2$O$_2$ as a substrate and is the foremost cause of protection against low levels of oxidative stress. In humans, GPx is highly expressed in kidney wherein it scavenges and inactivates the hydrogen and lipid peroxides, and also eliminates peroxides and peroxynitrite that can cause renal damage. Furthermore, GPx serve as peroxynitrite reductase in peroxynitrite mediated oxidations (Prabhu et al., 2013).

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O} \quad \text{(Glutathione Peroxidase mediated reaction)}
\]

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \quad \text{(Catalase mediated reaction)}
\]

**Glutathione-S-Transferase:**

GSTs are set of multi-gene and multifunctional detoxifying enzymes which belong to the phase II isoenzymes family. GST is known to preserve cells against a broad range of lethality from extracellular drugs, carcinogens, xenobiotics and its metabolites, and intracellular products of oxidative stress. Various GST polymorphisms directly have an effect on the activity of enzymes that control the removal of toxic intermediates of DNA, and amplify the susceptibility to oxidative stress (Tang et al., 2013; Huang et al., 2013). GSTs are found in all species and are comprises of higher concentration in cytoplasm constituting about 10 % of the cytosolic protein. There are seven classes of cytosolic GST depending on their structural and functional properties that are named as alpha, mu, pi, sigma, theta, omega, and zeta. Glutathione-S-transferase-mu (GSTM1), glutathione-S-transferase-
Chapter I

theta (GSTT1), and glutathione-S-transferase-pi (GSTP1) are the most common isoenzymes (Bardai et al., 2013).

Further, it aid in the conjugation of GSH with reactive metabolites forming thioether bond that makes the conjugated compound less reactive than the parental compound (Chen et al., 2008).

\[
\text{Xenobiotics + GSH } \xrightarrow{\text{GST}} \text{ Glutathione-S-conjugate}
\]

**Glutathione Reductase:**

GSH plays a key role in maintaining proper function and preventing oxidative stress in human cells. It can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles. GSH reduces the oxidized form of the enzyme GPx, which in turn reduces H$_2$O$_2$, a dangerously reactive species within the cell. In addition, it plays a key role in the metabolism and clearance of xenobiotics, acts as a cofactor in certain detoxifying enzymes, participates in transport, and regenerates antioxidants such Vitamins E and C to their reactive forms. The ratio of GSSH/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell, moreover, it is critical that the cell maintains high levels of the GSH and a low level of the GSSG. This narrow balance is maintained by GR, which catalyzes the reduction of GSSG to GSH. Thereby, plays an important role in the GSH redox cycle by which it maintains adequate levels of reduced GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress (Pai and Schulz, 1983).

\[
\begin{align*}
\text{H}^+ + \text{NADPH}^+ + \text{GR} & \quad \leftrightarrow \quad \text{NADP}^+ + \text{GRH}_2 \\
\text{GRH}_2 + \text{GSSG} & \quad \leftrightarrow \quad 2 \text{GSH}
\end{align*}
\]
Glucose-6-Phosphate Dehydrogenase (G6PDH):

G6PDH a key enzyme catalyses the first step in HMP pathway (also Pentose Phosphate Pathway), generating NADPH which is utilized by GR to reduce GSSG to GSH. Although, G6PDH is not directly involved in GSH synthesis still it plays a vital role in maintaining cellular GSH levels. Hence G6PDH is necessary to develop resistance against oxidative stress thereby, protecting both the human and animal cells from ROS.

Fig 1.6: G6PDH Enzyme Reaction Showing NADPH Formation and Maintenance of GSSG to GSH Ratio
Fig 1.7: Schematic Representation of vital Antioxidant Enzymes Action

**Non-Enzymatic Antioxidants:**

**Endogenous Source:**

In addition to enzymatic antioxidants, several small molecules (GSH, \(\alpha\)-tocopherol, ascorbic acid, \(\beta\)-carotene, Co-Q, dihydrolipoic acid, melatonin, bilirubin, uric acid), large molecules (albumin, transferrin, ceruloplasmin) and metal chelator (lactoferrin) may serve as endogenous sources of antioxidants. Among these molecules, GSH play a pivotal role in reducing oxidative stress.

**Reduced GSH:**

GSH is a tripeptide (glutamyl-cysteinyl-glycine), non-enzymatic antioxidant, play a pivotal role in preserving the cell structure by detoxifying and protecting the cellular constituents from oxidizing and alkylating agents (Hamsa and Kuttan, 2012). About 90% of the total glutathione pool exists in reduced (GSH) form.
and remaining 10% present in oxidized/disulfide (GSSG) form. It acts as the first line of defense against injury by inhibiting damage to biological macromolecules caused by ROS and free radicals. Oxidized glutathione formed can be converted back to reduced form by GSH reductase using an electron donor NADPH. A difference between the ratios of GSSG to GSH within cells is generally used to evaluate cellular toxicity and is measured as a marker of oxidative stress. GSH metabolism is frequently associated with cellular susceptibility to anticancer agents. It reacts and detoxifies the toxic endogenous and exogenous substances, including free radicals and anticancer agents (Pratheeshkumar and Kuttan, 2010).

Some of vital functions of GSH are:

- A major endogenous non-enzymatic antioxidant synthesized by the cells to detoxify ROS, free radicals and to maintain exogenous antioxidants such as vitamin C and vitamin E.
- Nitric oxide cycle regulation which is important for life comes to problem if not regulated.
- Biochemical and metabolic functions utilize glutathione for synthesis and repair of DNA, protein, prostaglandin, amino acid transport, and enzyme activation.
- Depletion of GSH affects immune system, central nervous system, gastrointestinal system and respiratory system.

GSH acts as a coenzyme or cofactor for various enzymes in many metabolic pathways (Kim et al., 2012; Hamsa and Kuttan, 2012).
Exogenous Source:

Exogenous source comprise of antioxidants from plant sources (polyphenolic compounds involving flavonoids, hydroxycinnamic acid) and synthetic antioxidants such as BHA (butylated hydroxyl anisole), BHT (butylated hydroxyl toluene), PG (propyl gallate), TVHQ (tertiary butylated hydroxyl quinone) which were approved by US Food and Drug Administration (Shiv Kumar, 2011). Compare to synthetic antioxidants several natural or plant derived antioxidants like vitamin E and vitamin C are highly studied and have been shown to have protective effects against oxidative stress.

Vitamin E:

Vitamins are organic compound that are in need for the maintenance of normal/good health. Vitamin A, D, E and K are fat/lipid-soluble vitamins present in several forms. Vitamin E is a potent antioxidant present in eight different forms among which α-tocopheral is one of the most active forms of vitamin E. It may be the most imperative non-enzymatic antioxidant that takes part in a wide variety of cellular functions. Cells mainly use vitamin E efficiently as the chief membrane bound antioxidant particularly to prevent LPO both in vitro and in vivo. Vitamin E present in serum is mainly carried as constituent of lipoprotein (Lee et al., 2013). Recent literatures have demonstrated the beneficial effects of vitamin E supplement of 200 IU /day in our daily requirement which would help in preventing the colorectal cancer through free radical scavenging and activating endonucleases (Valko et al., 2006).
**Vitamin C:**

Vitamin C, also known as ascorbic acid is a water soluble vitamin. It is well thought-out to be the vital aqueous phase antioxidant in animals, humans and in *in-vitro* studies. Vitamin C, exhibits its antioxidant property by scavenging the free radicals and ROS before they damage lipids. Apart from the antioxidant property of vitamin C, latest reports reveal the chelating effect of vitamin C against lead toxicity (Sadeghi *et al.*, 2013), hepatoprotective effect against rifampicin induced oxidative stress and liver injury/damage, in humans and animal models (Vahdati-Mashhadian *et al.*, 2013). Vitamin C is an essential nutrient required for many physiological and biochemical functions such as synthesis, secretion, maintenance and degradation of collagen (Ghomian *et al.*, 2013). It is most popularly used as dietary supplement to prevent oxidative stress mediated diseases. But its antioxidant activity is approximately less than 15 % when compared to antioxidant activity of fruits (Lee *et al.*, 2003).

Thus, herbal products having antioxidant property are used extensively as an auxiliary therapy due to less toxicity, to treat various free radical mediated ailments and to develop novel drugs that are having antioxidant/free radical scavenging property as a part of its activity (Manda and Bhatia, 2003). Antioxidants generated endogenously during oxidative stress are insufficient to completely avert the damage; hence food containing antioxidants are important to maintain health. Thus, dietary antioxidants should be supplied through the diet which can enhance cellular resistance and assist to avert oxidative damage to cellular constituents.
CP-induced Oxidative Stress: A General View

Several reports showed that CP used during chemotherapy induces oxidative stress and multiple organs damage (Rehman et al., 2012). CP exposure results in the generation of ROS over the physiological values that upshot in vital damage to cell structures leading to oxidative stress through its reactive metabolites phosphoramidemustard and acrolein. They are the key culprits for the excess production of ROS, thus, ROS plays a vital role in the pathogenesis of CP-induced oxidative stress (Ayhanci et al., 2010). Further, ROS acts as a second messenger to excite NF-kB dependent expression of pro-inflammatory cytokines, which causes more ROS production (Poli, 2000). But lipids, proteins, nucleic acids, carbohydrates altered by ROS are considered as the best markers of oxidative stress due to its lifetime ranging from hours to weeks. MDA being one of the markers of oxidative stress is known to for its elevated level after CP administration which has a destructive effect on cell membranes (Al-Yahya et al., 2009). GSH depletion is noticed due to CP treatment. Thus, depleted GSH level is mainly because of the consumption of GSH to detoxify ROS that is produced in surplus amount. In addition, decrease in antioxidant enzymes is also observed which are necessary for the maintenance of normal structure and functions of the cell. Thus, modulation in the antioxidant enzymes, enhanced LPO, depleted GSH confirms that ROS is generated in excess has disturbed the redox status of a cell leading to a condition termed oxidative stress (De Leve, 1996).
Auxiliary Therapy:

From past decades it is well known fact that CP can induce oxidative stress and organ toxicity in both animals and humans. Hence, there is a need to develop a new therapeutic modality like antioxidant treatment in order to limit the deleterious side effects of CP. Of late, there is an increasing awareness towards the use of home-grown medications in clinical trials. Thus, medicinal plants and their imitative has become a priceless source of curative agents to treat different ailments including cancer. Plant kingdom is the only asset of medicinal resources that is easily accessible naturally. Further, the use of medicinal plants and their plant extracts/metabolites with anti-tumor/antineoplastic properties has been an ancient practice all the way through historic times whose knowledge, that assemble through the practice of many generations. Thus, the study of plant derived phytochemicals against adverse effects of chemotherapy is of great importance as they are economical, reduced side effects, no expiry, room temperature-storage, local availability and easy administration. Several plant extracts are reported to avert inflammation, hemorrhage, hepatotoxicity, nephrotoxicity and neurotoxicity induced by chemotherapy. At present, attempts are being made to neutralize CP induced oxidative stress and organ toxicity by isolated/purified compounds having biological properties from medicinal plants. Table shows the protective effects of plant extracts/isolated compounds against CP induced oxidative stress and organ toxicity.
Table 1.5: Phytochemicals used to Treat CP-induced Various Toxic Effects

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Phytochemicals used</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Andrographis paniculata</em> extract</td>
<td>Ameliorates CP-induced toxicity in mice</td>
<td>Sheeja and Kuttan, 2006</td>
</tr>
<tr>
<td>2</td>
<td><em>Cardiospermum halicacabum</em> L. methonolic extract</td>
<td>Inhibits CP-induced immunosuppression and Oxidative Stress in Mice</td>
<td>Pratheeshkumar and Kuttan, 2010</td>
</tr>
<tr>
<td>3</td>
<td><em>Acacia senegal</em> gum exudates</td>
<td>Protects CP-induced urinary bladder cytotoxicity</td>
<td>Al-Yahya <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>4</td>
<td>Astaxanthin</td>
<td>Inhibits cytotoxic and genotoxic effects of CP in mice germ cells</td>
<td>Tripathi and Jena, 2008</td>
</tr>
<tr>
<td>5</td>
<td>Yellow dye extract from root of Brimstone tree (<em>Morinda lucida</em>)</td>
<td>Attenuation of CP-induced neurotoxicity in rat</td>
<td>Oboha <em>et al.</em>, 2012</td>
</tr>
<tr>
<td>6</td>
<td>Ellagic acid</td>
<td>Protective effect on CP-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice</td>
<td>Rehman <em>et al.</em>, 2012</td>
</tr>
<tr>
<td>7</td>
<td>Hot short pepper (<em>Capsicum frutescens</em> L. var. <em>abbreviatum</em>)</td>
<td>Protective effect on CP-induced oxidative stress in brain</td>
<td>Oboh <em>et al.</em>, 2010</td>
</tr>
<tr>
<td>8</td>
<td>Polar and non-polar extracts of Annatto (<em>Bixa orellana</em> L.) seeds</td>
<td>Inhibition of CP-induced oxidative stress in rat brain</td>
<td>Oboh <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>9</td>
<td>Carboxymethylpachymaran</td>
<td>Inhibitory effect on CP-induced oxidative stress in mice</td>
<td>Wei <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>10</td>
<td>Ethanol extract of the inner bark of <em>Caesalpinia pyramidalis</em> (Tul.)</td>
<td>Reduces urinary bladder damage during CP-induced cystitis in rats</td>
<td>Moraes <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>11</td>
<td><em>Viscum album</em> L. extract and Quercetin</td>
<td>Reduces CP-induced cardiotoxicity, urotoxicity and genotoxicity in mice</td>
<td>Şekeroğlul <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>12</td>
<td><em>Vernonia cinerea</em> L.</td>
<td>Ameliorates CP-induced immunosuppression and oxidative stress in mice</td>
<td>Pratheeshkumar and Kuttan, 2010</td>
</tr>
</tbody>
</table>

Table Contd.,
<table>
<thead>
<tr>
<th></th>
<th>Plant Extract/Component</th>
<th>Protective Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Pine bark extract (Pinus maritima)</td>
<td>Protective effect on CP-induced developmental toxicity in rats</td>
<td>Kim et al., 2012</td>
</tr>
<tr>
<td>14</td>
<td>Ipomoea obscura (L.)</td>
<td>Protective effect on CP-induced uro- and nephrotoxicities by modulating antioxidant status and pro-inflammatory cytokine levels</td>
<td>Hamsa and Kuttan, 2011</td>
</tr>
<tr>
<td>15</td>
<td>Thymoquinone from Negella sativa seeds</td>
<td>Attenuates CP-induced cardiotoxicity in rats</td>
<td>Nagi et al., 2011</td>
</tr>
<tr>
<td>16</td>
<td>Alcoholic extract of Tinospora cordifolia</td>
<td>Ameliorates urotoxic effect of CP by modulating GSH and cytokine levels</td>
<td>Hamsa and Kuttan, 2012</td>
</tr>
<tr>
<td>17</td>
<td>Zataria multiflora ethanolic extract</td>
<td>Hepatoprotective effect on CP-induced liver toxicity in Mice</td>
<td>Shokrzadeh et al., 2014</td>
</tr>
<tr>
<td>18</td>
<td>Ethanol extract of Origanum vulgare</td>
<td>Attenuates CP-induced pulmonary injury and oxidative lung damage in mice</td>
<td>Shokrzadeh et al., 2014</td>
</tr>
<tr>
<td>19</td>
<td>Punica granatum leaf extract</td>
<td>Protective effect was observed against CP-induced DNA damage and inhibition of hepatic LPO, enhancement of GSH, GST, SOD and CAT in mice pretreated with PLE</td>
<td>Dassprakash et al., 2012</td>
</tr>
<tr>
<td>20</td>
<td>Ethanolic extract of Madagascar Harungana (Harungana madagascariensis) Bark</td>
<td>Modulatory effect on CP-induced neurotoxicity in rats</td>
<td>Oboh et al., 2010</td>
</tr>
<tr>
<td>21</td>
<td>Salvia officinalis extract</td>
<td>Protective effects on CP-induced genotoxicity and oxidative stress in rats</td>
<td>Alkan et al., 2012</td>
</tr>
<tr>
<td>22</td>
<td>Citrullus colocynthis (L.) fruit Extract</td>
<td>Mitigating Effect against genotoxicity induced by CP in mice bone marrow cells</td>
<td>Shokrzadeh et al., 2013</td>
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Table Contd.,
<table>
<thead>
<tr>
<th>No.</th>
<th>Extract/Compound</th>
<th>Effect/Protection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Alcoholic root extract of <em>Plumbago zeylanica</em></td>
<td>Protective effect on CP-induced genotoxicity and oxidative stress in Swiss albino mice</td>
<td>SivaKumar et al., 2006</td>
</tr>
<tr>
<td>24</td>
<td><em>Ficus hispidia</em> Linn.</td>
<td>Cardioprotective effect on CP provoked oxidative myocardial injury in a rat model</td>
<td>Shanmugarajan et al., 2008</td>
</tr>
<tr>
<td>25</td>
<td><em>Coccinia indica</em></td>
<td>Chemoprotective potential against CP-induced oxidative stress, genotoxicity, and hepatotoxicity</td>
<td>Nitharwal et al., 2013</td>
</tr>
<tr>
<td>26</td>
<td><em>Saraca indica</em></td>
<td>Cardioprotective effect against CP-induced cardiotoxicity in rats</td>
<td>Viswanatha Swamy et al., 2013</td>
</tr>
<tr>
<td>27</td>
<td><em>Syzygium cumini</em> extract</td>
<td>Antigenotoxic potential on CP-induced genotoxicity and oxidative stress in mice</td>
<td>Tripathi et al., 2013</td>
</tr>
<tr>
<td>28</td>
<td>The root extract of <em>Decalepis hamiltonii</em></td>
<td>Amelioration of CP-induced hepatotoxicity in mice</td>
<td>Zarei and, Shivanandappa, 2013</td>
</tr>
<tr>
<td>29</td>
<td>Lycopene and Melatonin</td>
<td>Synergistic effect against the genesis of oxidative stress induced by CP in rats</td>
<td>Al-Malki, 2012</td>
</tr>
<tr>
<td>30</td>
<td><em>Buchanania lanzan</em> Spreng. Bark extract</td>
<td>Protective effect on CP-induced genotoxicity and oxidative stress in mice</td>
<td>Jain and Jain, 2012</td>
</tr>
<tr>
<td>31</td>
<td>Cinnamic acid from <em>Cinnamomum cassia</em></td>
<td>Amelioration of CP-induced myelosuppression and oxidative stress</td>
<td>Patra et al., 2012</td>
</tr>
<tr>
<td>32</td>
<td>Egyptian sweet marjoram leaves (<em>Origanum majorana</em>)</td>
<td>Protect against genotoxicity, immunosuppression and other complications induced by CP in albino rats</td>
<td>Ramadan et al., 2012</td>
</tr>
<tr>
<td>33</td>
<td>Hydroalcoholic extract of <em>Phyllanthus niruri</em> and its isolated compounds</td>
<td>Alleviated CP-induced hemorrhagic cystitis in mouse</td>
<td>Boeira et al., 2011</td>
</tr>
<tr>
<td>34</td>
<td>Red dye extracts from sorghum stem (<em>Sorghum bicolor</em>)</td>
<td>Inhibition of CP-induced oxidative stress in brain</td>
<td>Oboh et al., 2010</td>
</tr>
</tbody>
</table>

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| 35 | Lycopene and ellagic acid | Antiperoxidative and anti-apoptotic effects on CP-induced testicular lipid peroxidation and apoptosis | Türk et al., 2010 |
| 36 | Hawthorn fruit extract (*Crataegus microphylla*) | Protective effect against CP-induced genotoxicity in mouse bone marrow cells | Hosseinimehr et al., 2008 |
| 37 | Cruciferous vegetable mustard leaf (*Brassica campestris*) | Protective effect against in vivo chromosomal damage and oxidative stress induced by gamma-radiation, CP and urethane | Tiku et al., 2008 |
| 38 | Alpha-tocopherol, beta-carotene and melatonin | Protects CP-induced oxidative damage to bladder tissue in rats | Sadir et al., 2007 |
| 39 | Linseed oil (*Linum usitatissimum*) | Prevented the CP-induced oxidative stress in mouse brain | Bhatia et al., 2006 |
| 40 | Saffron (*Crocus sativus* Linn.) | Protective effects on genotoxins-induced oxidative stress in Swiss albino mice | Premkumar et al., 2003 |
| 41 | Aqueous extract of walnut (*Juglans regia* L.) | Protects mice against CP-induced biochemical toxicity | Haque et al., 2003 |
| 42 | Curcumin and chlorophyllin | Protective effect against CP-induced DNA mutation | Ibrahim et al., 2007 |
| 43 | Ascorbic acid | Protective effect on CP-induced testicular gametogenic and androgenic disorders in male rats | Das et al., 2002 |
Crocin:

Kingdom : Plantae  
Division : Angiosperms  
Class : Monocots  
Order : Asparagales  
Family : Iridaceae  
Genus : Crocus  
Species : C.sativus  

Fig 1.8: Pictorial Representation of Crocus sativus plant and its Stigma (Adopted from Google images)

Crocin a glycosyl esters of crocetin/di-gentibiose esters of crocetin is derived from the plant species Crocus sativus L commonly known as saffron belongs to the Iridaceae family. Saffron comprises of four major bioactive components namely picrocin responsible for taste, safranal for aroma, crocin and crocetin for colour. Crocin is one of the pharmacologically active constituent of saffron with exceptional/special water soluble carotenoids that are liable for its antioxidant
property and characteristic colour. It has been in use from ancient times as coloring or flavoring agent as well as in folklore medicine (Mohamadpour et al., 2013). Besides, its antioxidant property, there are literatures suggesting its pharmacological properties such as anticonvulsant, antidepressant, anticancer, improvement of memory power, aphrodisiac, antidote activities, ant-ischemia, etc (Hosseinzadeh et al., 2005). Due to its antioxidant and anticancer property it prevents the DNA and RNA damage from various chemicals and toxins (Hosseinzadeh et al., 2014).

**Table 1.6: Therapeutic use of Crocin**

<table>
<thead>
<tr>
<th>Properties</th>
<th>References</th>
<th>Properties</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesic</td>
<td>Amin and Hosseinzadeh, 2012; Tamaddonfard and Hamzeh-Gooshchi, 2010;</td>
<td>Anticancer</td>
<td>Li et al., 2012; Noureini et al., 2012; Aung et al., 2007</td>
</tr>
<tr>
<td>Anti-arthritic</td>
<td>Ding et al., 2013; Hemshekhar et al., 2012</td>
<td>Antidepressant</td>
<td>Wang et al., 2010</td>
</tr>
<tr>
<td>Antiinflammation</td>
<td>Sebastin et al., 2013; Nam et al., 2010; Xu et al., 2009;</td>
<td>Antioxidant</td>
<td>Sebastin et al., 2013; Thushara et al., 2013; Ghadredoost et al., 2011</td>
</tr>
<tr>
<td>Anti-diabetic</td>
<td>Shirali et al., 2012;</td>
<td>Anticonvulsant</td>
<td>Tamaddonfard et al., 2012; Vakili et al., 2012</td>
</tr>
<tr>
<td>Cardioprotective</td>
<td>Goyal et al., 2010; Thushara et al., 2013</td>
<td>Hypolipidemic</td>
<td>Sheng et al., 2006</td>
</tr>
<tr>
<td>Anxiolytic</td>
<td>Hosseinzadeh and Noraei, 2009.</td>
<td>Anti ischemic</td>
<td>Hosseinzadeh et al., 2009</td>
</tr>
</tbody>
</table>

*Source: (Sebastin et al., 2013)*
Fig 1.9: Structure of Crocin, Picrocin and Safranal
Sesamol:

- **Kingdom**: Plantae
- **Division**: Angiosperms
- **Class**: Eudicots
- **Order**: Lamiales
- **Family**: Pedaliaceae
- **Genus**: Sesamum
- **Species**: S. indicum

**Fig 1.10**

![Image of Sesamum indicum plant and sesame seeds](Adopted from Google images)

**Fig 1.10: Pictorial Representation of Sesamum indicum plant and Sesame seeds**

(Adopted from Google images)

In the recent years, an escalating concentration has been centered on a budding molecule Sesamol (3,4-methylenedioxyphenol), one of the prime components of sesame seed (*Sesamum indicum* L.) belongs to the family Pedaliaceae. Sesame seed is available in various forms for consumption worldwide. Sesamol, produced from
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sesamolin during thermal processing or storage of the oil. Hence it is a thermally stable phenolic compound with its unique solubility in both aqueous and organic phases which enhances its local concentration in cell membranes and makes it chain breaking antioxidant (Kumar et al., 2009). Apart from its antioxidant property, it has been reported in literatures, to have various other pharmacological properties namely, anti-mutagenic, anti-inflammatory, anti-ageing chondroprotective, chemoprevention and antihepatotoxic effects (Kumar et al., 2011).

Fig 1.11

![Structure of Sesamol](image)

**Fig 1.11: Structure of Sesamol**

In folklore practice, sesame seed oil was applied to eradicate wrinkles and prevent ageing during facial massage to the skin. Recent literatures have reported/supported the beneficial effects of sesamol *in vivo*, e.g. the rising molecule sesamol was found to provoke growth arrest and apoptosis in cells of cancer and cardiovascular diseases. Further, it also found to improve overall vascular fibrinolytic capacity by controlling gene expression of plasminogen activator and nitric oxide release in endothelial cells (Chang *et al.*, 2011). It is worth mentioning that promising molecule sesamol contributes to the protection of liver/multiple organ damage by various chemical compounds such as acetaminophen (Chandrasekaran *et al.*, 2009), carbon tetrachloride (Zhao *et al.*, 1996) and heavy metals (Chandrasekaran *et al.*, 2013) suggesting its importance towards hepatoprotection. The radioprotective
activity of sesamol is mainly due to free radicals scavenging property, activating the endogenous antioxidant enzymes, inhibiting the DNA damage and to safeguard the hematopoietic system which protects the cells from free radical mediated injury. In addition it also enhances cells resistance by inhibiting hydroxyl, lipid peroxyl radicals and thereby decreasing radical-induced deoxyribose degradation and DNA damage (Jan et al., 2008). Furthermore, sesamol prevents many steps in the generation of neoplasia and mutagenesis (Galano et al., 2011).

**Table 1.7: Therapeutic use of Sesamol**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Reference</th>
<th>Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant</td>
<td>Kanimozhi et al., 2009; Geetha et al., 2009</td>
<td>Anti-diabetic</td>
<td>Kuhad et al., 2008</td>
</tr>
<tr>
<td>Anti-mutagenic</td>
<td>Kaur and Saini, 2000</td>
<td>Anticlastogenic and wound healing property in rodents.</td>
<td>Shenoy et al., 2011; Parihar et al., 2006</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Shimizu et al., 2014</td>
<td>Reduces oxidative stress and multiple organ injury in septic and endotoxemic rats</td>
<td>Hsu et al., 2006</td>
</tr>
<tr>
<td>Anti-ageing</td>
<td>Geetha et al., 2009</td>
<td>Chemopreventive</td>
<td>Kapadia et al., 2002</td>
</tr>
<tr>
<td>Anti-hepatoxic</td>
<td>Chandrasekaran et al., 2011</td>
<td>Chondroprotection</td>
<td>Lu et al., 2011</td>
</tr>
<tr>
<td>Anti-arthritis</td>
<td>Hemshekhar et al., 2013</td>
<td>Renoprotective</td>
<td>Gupta et al., 2009</td>
</tr>
</tbody>
</table>
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Silymarin:

- **Kingdom**: Plantae
- **Division**: Angiosperms
- **Class**: Eudicots
- **Order**: Asterales
- **Family**: Asteraceae
- **Genus**: Silybum
- **Species**: S. marianum

Fig 1.12

![Pictorial Representation of Silybum marianum plant](Adopted from Google images)
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Plant products have been considered as an important source for treating various disorders/ailments with no/less side effects. Silymarin is one such product to treat hepatic ailments. It is a mixture of flavonoids and lignans isolated from milk thistle plant (*Silybum marianum*). It is known for its antioxidant property which helps in scavenging free radicals and ROS (Galal *et al.*, 2012; Prabhu *et al.*, 2013).

Fig 1.13

![Structure of Silymarin](image)

**Fig 1.13: Structure of Silymarin**

The present study has made an effort to explore and highlight the beneficial effects of Crocin and Sesamol against CP-induced oxidative stress and organ damage using Silymarin as standard hepatoprotective drug. Further, they are expected to continue with the hope for more effective and less toxic therapeutic options. The experimental end points involve biochemical estimation of oxidative stress markers namely, non-enzymatic and enzymatic antioxidants, liver function parameters, pro-inflammatory cytokines and histology of the liver and kidney tissues which supports the morphology.