I. Cancer

A major feature of all higher eukaryotes is the defined life span of the organism, a property that extends to the individual somatic cell, whose growth and division are highly regulated. A notable exception is provided by cancer cells, which arise as variants that have lost their usual growth control. These cancer cells form a mass of tissue called tumor. The cells in malignant (cancerous) tumors are abnormal and divide without control or order. They can invade and damage nearby tissues. Also, cancer cells can break away from a malignant tumor and spread to other parts of the body by a process called metastasis. Genetic alterations in two types of genes can contribute to the cancer process. Proto-oncogenes are normal genes that are involved in cell growth and division. Change in regulation of these genes lead to the development of oncogenes, which can promote excessive cell growth and division. Tumor suppressor genes are involved in controlling cell division. When tumor suppressor genes dysfunction, cells grow and divide abnormally, which leads to tumor growth. De regulation of genes or genetic changes that are not corrected by the cell can lead to the production of abnormal proteins. Damaged proteins may not respond to normal signals, may over-respond to normal signals, or otherwise fail to carry out their normal functions. These malfunctions of proteins lead to disruption of normal crosstalk
between the signaling components of cell division machinery. Normal cell growth and division are largely under the control of a network of chemical and molecular signals. Disruption of the signaling process results in abnormal growth and division of cells. This condition of abnormal growth and uncontrolled division of cells is called cancer, which is one of the major causes of death world wide, including India.

In India 9.5% deaths are due to cancer and with an estimated increase to 14.7% by 2030 (Fig. 1).

Fig. 1. Major causes of death in India (Source: www.who.int)
In 2005, 7.6 million people died of cancer out of 58 million deaths worldwide. More than 70% of all cancer deaths occur in low and middle income countries, where resources available for prevention, diagnosis and treatment of cancer are limited or nonexistent. Based on projections, cancer deaths will continue to rise with an estimated 9 million people dying from cancer in 2015, and 11.4 million dying in 2030.

**Fig. 2. Estimates of new cancer cases in India (Source: www.who.int)**
In India, lung and oral cancers are most common types of cancer occurring in males whereas uteri and breast cancer are common in females (Fig. 2).

II. Cancer types

Histologically there are hundreds of different cancers, which are grouped into five major categories: carcinoma, sarcoma, myeloma, leukemia, and lymphoma. In addition, there are also some cancers of mixed types.

Carcinoma refers to cancer of epithelial origin or of the internal or external lining of the body. Carcinomas account for 80 to 90 percent of all cancer cases.

Sarcoma originates in supportive and connective tissues such as bone, tendon, cartilage, muscle, and fat. Sarcoma tumors usually resemble the tissue in which they grow.

Myeloma is cancer that originates in the plasma cells of bone marrow. Leukemias ("liquid cancers" or "blood cancers") are cancers of the bone marrow (the site of blood cell production).

Lymphomas develop in the glands or nodes of the lymphatic system, a network of vessels, nodes, and organs (specifically the spleen, tonsils, and thymus) that purify bodily fluids and produce infection-fighting white blood cells, or lymphocytes. Unlike the leukemias which are sometimes called "liquid
cancers," lymphomas are "solid cancers." Lymphomas may also occur in specific organs such as the stomach, breast or brain.

### III. Cancer and treatment

Treatment for cancer may involve chemotherapy, radiation therapy, surgery, hormonal therapy, biological therapy or some combination of these. Chemotherapy is the use of anti-cancer drugs that destroy cancer cells by stopping growth or multiplication at some point in their life cycles. Chemotherapy is often given in cycles of alternating treatment and rest periods. Radiation therapy is with ionizing radiation, which destroys cells or the genetic material of cells in the area being treated, thereby making it impossible for these cells to grow. Surgery involves removal of the tumor. Sometimes, surrounding tissue and lymph nodes are also removed. Hormone therapy is the use of hormones, to change the way hormones help cancers to grow in the body. Biological therapy (Immunotherapy) makes use of the body’s immune system, either directly or indirectly, to fight cancer. The most advanced forms of treatment may produce a 5-year survival rate of 75% or more for certain types of cancer, e.g. cancer of the uterine corpus, breast, testis, and melanoma. By contrast, survival rates in cancer of the pancreas, liver, stomach, and lung are generally less than 15%.
IV. Chemotherapy

Chemotherapy is the most effective and widely used form of cancer treatment till date. The most well studied and effective chemotherapy agents are Cisplatin, Doxorubicin, Etoposide, Hydroxyurea, Imitinab, Methotrexate, Paclitaxel and Vinblastine. Most anti-cancer drugs act by inhibiting DNA synthesis or some other process in the cell cycle. While chemotherapy can be quite effective in treating cancer, these agents do not differentiate normal healthy cells from cancer cells and as a result leading to various side effects. The way in which the other cells are affected determines the side-effects of the individual drugs. Other cells affected include blood cells, hair follicles and cells that line the digestive tract. As a result, side effects may include loss of hair, poor appetite, nausea and vomiting, diarrhea, or mouth and lip sores. Moreover, continuous exposure to these chemotherapeutic agents leads to the development of drug resistance, because of which the patients fail to respond to chemotherapy.

V. Chemotherapy and Drug resistance

Drug resistance in cells may be due to three major mechanisms: first, decreased uptake of drugs; second, various changes in the cells that effect the capacity of cytotoxic drugs to kill cells, including alterations in cell cycle, increased repair of DNA damage, reduced apoptosis and altered metabolism of
drugs; and third, increased energy-dependent efflux of drugs. Of these mechanisms, the one that is most commonly encountered in drug resistant conditions is the increased efflux of a broad class of cytotoxic drugs that is mediated by a family of energy dependent transporters, known as ATP-binding cassette (ABC) transporters.

VI. ABC Transporters

ATP binding cassette (ABC) family transporters are characterized by the presence of an ATP-binding site, ABC signature motif and requirement of ATP hydrolysis for their transport action. The human genome contains 48 genes that encode ABC transporters, which have been divided into seven sub families labeled A to G. Diverse substrates are translocated by ABC transporters, ranging from chemotherapeutic drugs to naturally occurring biological compounds. Although several members of the transporters family have dedicated functions involving the transport of substrates, it is becoming evident that the complex physiological network of ABC transporters has a pivotal role to play in host detoxification and protection of body against xenobiotics. This role is revealed by the tissue distribution of ABC transporters, which are highly expressed in pharmacological barriers, such as the brush border membrane of intestinal cells,
the biliary canalicular membrane of hepatocytes, the luminal membrane in the proximal tubules of the kidney and the epithelium of the blood-brain barrier.

VII. MDR1

Among mammalian ABC transporters, P-glycoprotein (P-gp) family and multidrug resistance associated protein (MRP) family have a major role in drug transport. They can generate profound drug resistance through reduced accumulation of substrates (drugs). P-glycoprotein family consists of two classes: Class I (MDR1 in humans, MDR1a and MDR1b in rodents) and class II (MDR 2 or 3 in humans and MDR 2 in rodents). Class I P-gps are present in various normal tissues such as liver, brain, kidney and intestine, whereas class II P-gps are essentially expressed in the liver on the canalicular membrane of hepatocytes.

Fig. 3. Domain organization of MDR1
(NBF- Nuclear binding fold; Barrels indicate twelve transmembrane domains)
MRP family consists of at least six members, known as MRP1-MRP6. Typically MRP's are larger (190-200 kDa) than P-gp (170 kDa), containing 250 additional amino acids at the amino - terminal region. MDR1 contains twelve transmembrane domains and two nucleotide binding domains.

Expression of multidrug resistance genes seen in most of the organs that have a role in absorption and elimination, signifies their role in the efflux of drugs and xenobiotics. Expression of MDR1 was shown in the apical membranes of the liver, kidney, gut and at the blood-brain barrier (Thiebaut et al., 1987; Cordono Cardo et al., 1989). MDR1 and MDR3 mRNA expression was observed in the gastrointestinal tract, cerebral cortex, cerebellum, kidney, lung, and liver of rodents (Brady et al., 2002). Transcripts of multidrug resistance genes were observed in a wide variety of conditions and malignancies. Over expression of MDR1 mRNA in isolated hepatocytes of endotoxin-treated rats (Vos et al., 1998) and in adenocarcinomas derived from adrenal, kidney, liver and bowel (Fojo et al., 1987), breast (Filipits et al., 1996) and in prostate (Bhangal et al., 2000) was observed.

Multidrug resistance is the primary impediment in the cancer chemotherapy. MDR1 transports drugs that are central to most chemotherapeutic regimens, including doxorubicin, daunorubicin, vincristine, actinomycin-D,
paclitaxel, docetaxel, etoposide, teniposide, bisantrene etc. Due to wide range of drugs transported by MDR1, the expression of MDR1 is one of the major factors explaining chemotherapy failure in cancer patients.

**VIII. MDR1 inhibitors**

Due to the significance of MDR1 inhibition in successful chemotherapy, a plethora of agents have been developed that modify, modulate, or reverse the MDR phenotype. Many natural and synthetic products of various structures, including calcium channel blockers [e.g., verapamil, nifedipine], calmodulin antagonists (e.g., trifluoperazine, chlorpromazine), various steroids (e.g., progesterone, tamoxifen), quinolines (e.g., chloroquine, quinidine), immunosuppressive drugs (e.g., cyclosporine, rapamycin), antibiotics (e.g., rifapicin, tetracyclines), surfactants (e.g., Tween 80, Cremophor-EL), and alkaloids (e.g., reserpine, yohimbine) have been shown to block the function of MDR1. New, more potent MDR1 inhibitors such as PSC388, GF120918, dexverapamil and XR9576 are now being evaluated in clinical trials. In most cases MDR reversal agent may expose the patient to unacceptable side effects or toxicity at doses required for effectiveness (Ross et al., 1994; Malayeri et al., 1996). These limitations have spurred efforts to search for new approaches and more effective compounds.
IX. Regulation of MDR1 expression

The complex regulation of MDR1, the important member of ABC drug transporters, has been studied to an extent but our understanding of the MDR1 transcription may still be in its infancy. Recently it became evident that the altered expression of several growth and death controlling proteins can adversely affect drug therapy. The first evidence that tumor suppressor protein could influence the expression of drug resistance gene came from the observation that wild type p53 repressed the transcription of MDR1 gene (Chin et al., 1992). Elevated levels of c-Fos have been demonstrated in a number of drug resistant cell lines when compared to their drug-sensitive counterparts (Bhusan et al., 1992). The MDR1 gene also is target of the ras/raf-signaling pathway. Given the role of MDR1 in the protection against environmental adversity, MDR1 is highly responsive to stress signals. MDR1 inducers include heat shock, cytokines, oxygen free radicals, partial hepatectomy, inflammation, exposure to carcinogens including chemotherapeutics, hypoxia and UV and X- irradiation. In many cell lines and metastatic sarcomas it is generally accepted that MDR1 expression is increased through the upregulation of MDR1 mRNA levels (Abolhoda et al., 1999). Several transcription factors including, GC, HSF1, Sp1, AP-1, NF-IL6, NF-Y, NF-κB, EGR1, YB-1 and MEF-1 up-regulate MDR1 gene transcription.
To date, efforts to combat the overexpression of MDR1 have involved the use of functional modulators or reversal agents that block the MDR1 mediated efflux of anti cancer drugs. Intervention to prevent the transcription of drug transporters rather than block their function subsequent to their overexpression is important. Nevertheless studies on the transcription of MDR1 may provide a therapeutic target in our quest to prevent resistance to cancer therapeutics.

Among many different regulators of MDR1 transcription, Reactive Oxygen Species (ROS) and Cyclooxygenase-2 (COX-2) were shown to be key players, but the mechanisms underlying are not well elucidated. Using experimental and in silico analysis, an attempt was made in the present study to elucidate the role of ROS and COX-2 in the regulation of MDR1 expression and study effect of antioxidant (C-Phycocyanin) and COX-2 inhibitor (Celecoxib) treatment on the regulation of MDR1 expression.