INTRODUCTION
Respiration in all aerobic cells is carried out by mitochondria, which vary widely in size, shape, number and location in different cell types. They are spherical in brown fat cells, cylindrical in kidney, oblong in liver and thread like in fibroblasts. The liver mitochondria is 1- 5 \mu in length and 0.5 - 1 \mu in width, with an average dry weight of \(1 \times 10^{-13}\) g. Inner mitochondrial membrane is composed of lipids and proteins, the latter being higher in proportion. Besides they contain nucleic acids, metabolites, cofactors and variety of metal ions. Lipid content of mitochondria is about 20 to 30% of dry weight and over 90% of the lipid is phospholipid. The inner and outer membranes of mitochondria show differences in the lipid composition; cardiolipin is concentrated in inner membrane while cholesterol is associated with outer membrane.

Mitochondria carry out a variety of biochemical processes. The most important among these is oxidative phosphorylation, thus it is called as powerhouse of the cell. In mitochondria energy yielding and energy consuming processes are linked by the way of protonic circuits (Skulachev et al, 1981). The transfer of electrons from reduced substrate to molecular oxygen through the electron transport chain results in vectorial translocation of protons (Mitchell, 1966) and this proton gradient is utilized for the synthesis of ATP, ion transport and the for import of the proteins. The transduction of oxidation energy to a protonic force and conservation of this protonic force, into the phosphoanhydride bond of ATP is catalyzed by a discrete multisubunit enzyme complexes located in the inner membrane (Hatefi and Ragan,1985).

Functionally, mitochondrial respiratory chain is composed of four protein-lipid complexes plus ubiquinone and cytochrome c.

Complex I NADH : Ubiquinone Oxidoreductase.
Complex II Succinate : Ubiquinone Oxidoreductase.
Complex III Ubiquinol: Ferricytochrome c Oxidoreductase.
Complex IV Ferrocytochrome c: oxygen Oxidoreductase.
ATP synthase Constitutes Complex V.

Efficient energy supply is essential for most of the highly differentiated functions of mammalian cells, therefore, disruption of mitochondrial function is a common cause of loss of cell function and cell death. Mitochondrial dysfunctions can occur (1) by agents that affect ATP production, or (2) by agents that effect the membrane potential and osmotic stability, or, (3) by agents that effect the substrate oxidation. As a consequence of these functional alterations, energy dependent processes in the cell decreases significantly leading to cell injury and ultimately to cell death. There are three categories of mitochondrial dysfunctions i.e., (1) nutritional or disease related (2) chemical-induced, and (3) genetic in nature.
Drugs and cytotoxic agents causing Mitochondrial dysfunction:

Chloroethylamines are able to uncouple respiration and oxidative phosphorylation in isolated mitochondria of tumor and normal cells. (Belousava, 1966). Sarcosylsine is similar to the classic uncouplers like 2-4 dinitrophenol. The uncoupling effect is manifested by ( a) the inhibition of ATP synthesis (b ) activation of latent mitochondrial ATPase and, (c) loss in respiratory control (Romanova, 1972). The dipeptides of sarcosylsine exhibits a strong inhibition on respiration and the mechanism of action is similar to that of oligomycin (Belousava, 1978).

Adriamycin inhibited electron transfer through Complex 3 and, the Complex 2 was inhibited to a lesser extent in mitochondria isolated from rat liver, rat heart and bovine heart ( Nicolay and Kruijff, 1987).

Adverse effects of antitumor drug cisplatin (60 mg/ kg i.p) on rat kidney mitochondria, showed decrease in State 3 respiration, respiratory control ratio, Succinatexytochrome c reductase, NADH.xytochrome c reductase and cytochrome c oxidase activities. There was an inhibition on glutathione peroxidase activity with corresponding increase in plasma creatinine and blood urea nitrogen contents compared with the control group ( Sugiyama et al, 1989).

The triaryl methane derivative Victoria Blue-Bo (VB-BO) and the Chalcogenapyrylium (CP) dyes have potential for the use in photochemotherapy as they are taken up by the mitochondria of malignant cells and cause cell death. These compounds tested induced some uncoupling of oxidative phosphorylation. Photoactivation of VB-BO produced selective inhibition of complex I. Photoactivated CP dyes inhibited both complex I and II. Activities of NADH and succinate cytochrome c reductase as well as other membrane bound enzymes were also inhibited ( Josephine et al, 1990).

Administration of mitoxantrone and doxorubicin (15 mg/kg, i.p) to mice resulted in 155%, 73% and 52 % increase in spontaneous chemilumini-scence, malonaldehyde levels and hydroperoxide- initiated chemilumini-scence. There was 50%, 27% and 42% decrease in Cu-Zn dismutase, catalase, glutathione peroxidase activities respectively. Administration of doxorubicin resulted in 51% and 53% increase in spontaneous chemilumini-scence and malonaldehyde levels without having significant effect on other parameters suggesting higher hepatotoxic potential of mitoxantrone when compared to doxorubicin (Llesuy and Arnaiz, 1990).

Administration of paracetamol resulted in the inhibition of cellular respiration, decreased levels of ATP contents and ADP/O ratios before the appearance of plasma membrane damage (Burcham and Harman, 1990). Administration of bis(tributylin) oxide (0.5 ml/kg) to rat resulted in accumulation of the compound in the liver cell mitochondria and disturbed oxidative phosphorylation ( Yoshizuka et al , 1992).
Doxorubicin is shown to influence oxygen consumption of mitochondria. Rats treated with doxorubicin (2.5 mg/kg, i.p body wt once in a week for 8 weeks) showed significant decrease in NADH dehydrogenase, cytochrome c oxidase and Na\(^+\)K\(^+\)ATPase activities (Geeta & Shymala devi, 1992). Psychosine, a cytotoxic agent in micromolar concentration is shown in vitro to inhibit electron transfer through site 1 and site 3, and the electron transfer through site 2 was not affected. (Tapasi et al, 1998)

Administration of 2,2'-azo bis-(2-amidinopropane) dihydrochloride (AAPH) resulted in decreased state 3 respiration, ADP/O ratios and respiratory control ratio in vitro and in vivo. Mitochondria were uncoupled via lipid peroxidation and swelling by long term incubation with AAPH (Kanno et al, 1994). Administration of ethionine (Padma and Setty, 1997a) or thioacetamide (Padma and Setty, 1997b) or chronic alcoholism (Sebastian and Setty, 1998) is reported to induce mitochondrial dysfunctions involving a significant decrease in the rate of respiration and P/O ratio.

Cationic detergent, Cetyltrimethyl ammonium(bromide) when administered to rats accumulated in mitochondrial matrix by membrane potential driven uptake mechanism, which finally dissipates the membrane potential by increasing permeability of mitochondrial membrane (Bragadin and Dell’Antone, 1996). Administration of piroxicam stimulated the respiration in the absence of endogenous ADP and inhibited in the presence of ADP. ADP/O ratio, respiratory control rates and uncoupler stimulated ATPase activity were lower compared to control, suggesting an uncoupler like activity (Salgueiro-Pagadigorria et al, 1996).

The use of combination chemotherapy: Intraneoplastic diversity is the root problem underlying the concept of neoplastic progression, that is acquisition of new characteristics by tumors during their development, so the treatment with a single agent, or one therapeutic modality, may have a far lower chance of eradicating all the subpopulations of cancer cells compared to combination chemotherapy of multiple modalities. Concomitant administration of drugs in association would not only overcome the problem of clonal heterogeneity but also would result in better control over tumor growth, as some of the drugs are to interact synergistically when used in combination. Cancer treatment often involves chemotherapeutic drugs that can have adverse effects on normal body tissue. Alkylating agents such as cyclophosphamide, cisplatin, nitrosourea etc. have been shown to be highly toxic to normal body tissues (Yuhas and Storer, 1969). Cisplatin, Cyclophosphamide, Mitomycin C, Methotrexate and Vinblastine are used in various combination chemotherapy regimens in the treatment of ovarian cancers, lung cancer, salivary gland cancer, mammary cancer etc. In the present study the effect of these drugs in various combinations on mitochondrial energy transduction is studied in normal cells.
CISPLATIN

Cisplatin is a platinum containing antineoplastic agent. The drug is an inorganic complex that contains a platinum atom surrounded in a plane by two chloride atoms and two ammonia molecules in the cis position.

Chemical studies: In aqueous solution, cisplatin loses both chloride ions from the coordination sphere of the Pt(II) ion and water or hydroxide ion becomes bound. Thus, a distribution of species is set up involving the presence of unhydrolyzed and partially hydrolyzed species. However, this equilibrium is labile, so that if the chloride ion concentration is raised to that of isotonic saline, the majority species in solution will be unhydrolyzed cisplatin. Thus under certain conditions of pH and metal ion concentration, hydroxy-bridged polymeric species can be formed. The structures of dimeric and polymeric species have been determined by X-ray crystallography (Lock et al, 1977). Hydrolysis products of cisplatin may be responsible for some of its cytotoxic effects like nephrotoxicity observed in vivo (Broomhead et al, 1980).

The exact mechanism of action of cisplatin has not been conclusively determined but the drug has biochemical properties similar to those of bifunctional alkylating agents. Studies in culture cells have indicated the relevance of platinum-DNA binding to cytotoxicity. Changes in ultra violet absorption spectrum of salmon sperm DNA after reaction with either cis or trans Pt(II) (NH_3)_2Cl_2 provided conclusive evidence that both platinum compounds bind to organic bases of DNA. The clinical kinetics of intact cisplatin, total platinum, and filtered platinum in plasma, have been followed. After the intravenous injection of the commonly used doses of 50-100 mg/m total platinum, level declined in a triphasic manner. The initial distribution phase had half lives of about 20 min and 1 hour and appeared to complete after 2 hr., while long terminal half life was over 24 hr. Where as half lives of intact cisplatin and filterable cisplatin declined in a monophasic manner, and was considerably shorter than for total platinum, being 20-30 min (Himmelstein et al, 1981). Ovarian cancer tend to confine to the peritoneal cavity and near by lymph nodes until late in the disease, which prompted the intraperitoneal route of delivery for chemotherapy. A comparison of pharmacokinetics and toxicity of drug given intraperitoneally and intravenously in dogs showed that the intraperitoneal route of administration resulted in higher drug levels in target peritoneal tissues a corresponding increase in systemic toxicity (Pretorius et al, 1981). Cisplatin reacts with blood and plasma at 37 °C. The empirical formula of the active principal is PtCl_2H_6N_2 and have a molecular weight of 300.1. It can be used by itself or in combination with other chemotherapeutic drugs in tumors like, metastatic testicular tumors, metastatic ovarian tumors, head and neck cancer, bladder cancer and, prostatic cancer. Main adverse effects observed are impaired renal function, manifested by elevations in BUN, creatinine and serum uric acid levels, myelosuppression, nausea and vomiting, ototoxicity, neurotoxicity etc. The gastric distention seen in rats is thought to have some bearing on the gastric nausea often experienced by patients receiving cisplatin. The neurotic convulsive properties of cisplatin was apparently
due to paralysis of gastric emptying (Roos et al., 1981). The impairment of renal function induced by cisplatin in various animal species and man, appears to be due to renal tubular injury (Blachely and Hill, 1981).

**Clinical studies:** It was reported that the administration of cisplatin to rabbits resulted in renal damage to proximal tubules located in the cortex of the kidney (Lee, et al., 1988). Cisplatin-DNA adducts were measured in renal, gonadal and tumor (sarcoma) tissues of sprague-dawley rats following i.v (8 mg/kg) or i.p (30 mg/kg) administration of cisplatin. It was concluded that the route of drug administration, diet and hormonal status are the factors that might effect cisplatin-DNA adduct formation (Eddie Reed et al., 1987). Platinum analogues C1-973 and Cis-diammine(glycolato)platinum(254-S;NSC 375101 D) and others are on clinical trails (Peter et al., 1992)(Yusutsuna Sasaki et al., 1991). Cisplatin induced a decrease of cytochrome P-450, glutathione-S-transferase and some cytochrome P-450 b and P-450 h isoforms. Further more, cisplatin-induced N-glucuronyl transferase and lipid peroxidation which might contribute, atleast in part, in cisplatin induced hepatotoxicity. These results indicate that cisplatin induces toxic effects in an unspecified manner (Bompart, 1990). Administration of cisplatin also resulted in an increase in the total phospholipids in rat liver in the earlier part of the experiment at the expense of the change in the contents of lysofractions as well as phosphotidylcholine, polyglycerophosphates and phosphatidic acids. Suppression of antiradical activity was also observed (Saprykina et al., 1991). The mitochondria prepared from renal cortical slices which has been exposed to cisplatin, resulted in depletion of glutathione (GSH) and increased thiobarbituric acid reactive substances (TBARS) in a time dependent manner, which indicates an increased lipid peroxidation in the mitochondria. Thus cisplatin induced depletion of GSH is an early event and a determinative step in oxidative stress to mitochondria in the kidney and may lead to irreversible cell injury. (Zhang and Lindup, 1993). Administration of cisplatin (5 mg/kg body wt, i.p) in to male wistar rats resulted in loss of body wt. (Ammer et al., 1993).

Administration of cisplatin at LD\textsubscript{50} to mice caused significant changes in hepatic metabolism. There was enhanced lipid peroxidation with accumulation of malonic dialdehydes, diene conjugates, schiffs bases, decreased antiradical activity and alterations in the fractional composition of phospholipids. There was also an increase in the serum activity of transaminases and alkaline phosphatase (Vetoshkina and Dub skaia, 1993) (Dambska et al., 1994). Cisplatin is known to damage normal organs dose des pondently by its toxicity. The mechanism of organ injuries have been studied to prevent and rescue patient. The onset dose to cause organ injuries is as follows. The change in the salivary glands were noted at 15 mg/body wt. Renal injury started at 19.5 mg/body wt, and damages to bone marrow started at 19.7 mg/body wt of CDDP. The minimal dose for liver dysfunction was 21.3 mg/body wt (Chiba and Kano, 1994). Cisplatin treatment in rats results into a significant increase in the activity of Ca\textsuperscript{2+}- independent nitric oxide synthase (NOS) in liver and kidney. Significant enhancement of lipid peroxidation was also observed in gastric mucosa, kidney and liver (Srivastava et al., 1996).
Some of the protective agents used against the toxicity induced of cisplatin are as follows: WR 1065 (2-[ (amino propyl) amino] etanethiol), a free thiol compound of radio protector WR 2721 was reported to be effective in protecting against cisplatin induced mutagenesis and cytotoxicity. The exact mechanism of protection is not known, but it has been suggested that it exerts protective effect by binding to unreacted cisplatin complexes, or by interfering in the formation of bifunctional cross link or by scavenging free radicals (Bieserka Nagi et al, 1986). In mice mesna (sodium-2-mercaptoethane sulfonate), caused significant reduction in the gastrointestinal toxicity of cisplatin (Simon et al, 1986). The administration of dimethylsulfoxide with cisplatin at a mole ratio of 200:1 resulted in a considerable reduction in the nephrotoxicity in rats (Jones et al, 1991). Elastase when administered to rats along with cisplatin resulted in reduced platinum deposits in the renal tissues, particularly in the tubular epithelium, thus protecting the kidneys (Suzuki et al, 1991). In mice, an extract of Crocus sativus stigmas, partially prevented the decrease in body weight, hemoglobin levels and leukocyte counts caused by 2 mg/kg of cisplatin i.p for 5 days (Nair et al, 1991).

Cisplatin induced nephrotoxicity can be blocked by using Acivicin, a non competitive inhibitor of gamma glutamyl transeptidase (GGT). Acivicin acts at the initial step in the metabolism of cisplatin, preventing the formation of mercaptouric acids which are proximal tubule of kidney (Hanigan et al, 1994). Dithiocarbamate derivative, N- methyl D-glucamine dithiocarbamate, could prevent anorexia and weight loss and enhance survival with out decreasing the antitumour efficacy of high dose cisplatin therapy (Yee et al, 1994). Treatment of rat renal cortical slices with 2mM cisplatin resulted in decrease in Na\(^+\) and water content, with concomitant increase in K\(^+\) ion, malonaldehyde (MDA) concentration and lactate dehydrogenase released in to the medium were decreased. Dithiothreitol ameliorated all these toxic effects in a concentration related manner (0.5-2mmole). These results suggest that the protective mechanism of dithiothreitol is by its antioxidant property (Zhang et al, 1994). N- methyl D-glucaminedithiocarbamate (NMGDTC) a derivative of dithiocardamate could produce marked reduction of CDDP-induced ototoxicity and weight loss (Walker, 1994). Gluthathione, N- acetylcysteine or the iron chelator defroxamine or Ginkgo biloba extract or the xanthine derivative torfaylline were shown to inhibit cisplatin induced lipid peroxidation (Inselmann et al, 1995). 4-methylbenzoic acid (MTBA) offered protection against cisplatin induced nephrotoxicity by reducing the plasma creatinine levels and by prevention of gluthathione depletion and lipid peroxidation (Hussain et al, 1996). Hepatic and renal subacute toxicity induced by the antineoplastic drugs chloroambucil, cisplatin, epirubicin and methotrexate and steroid alkylating agent 3-beta-hydroxy-13 alpha-amino-3,17-seco-5 alpha-androst-17-one-13, 17-lactam 9 p-[bis 92-chloroethyl) amino] phenyl acetate was investigated in rats. The results indicate that The overall toxicity impact of the antitumour drugs was Methotrexate < Cisplatin< epirubicin< chlorambucil (Pispirigos et al, 1993).
MITOMYCIN C

Wakakai reported the isolation of mitomycin C from Streptomyces Caespitosus as blue violet crystals (Wakakai et al, 1958). Mitomycin is a FDA approved antitumour agent. It is used in the treatment of sarcoma, leukemia and various types of carcinomas. The structure of mitomycin C has been determined by chemical, physio-chemical and X-ray method (Webb et al, 1962). It is a dark bluish violet compound with visible and UV-absorption maxima at 217, 360 and 560 nm. Mitomycin C has the formula \( \text{C}_{16}\text{H}_2\text{O}_{14}\text{N}_4\text{O}_3 \) and it does not melt or decompose below 360 °C. It is soluble in water and polar organic solvents. The unreconstituted or native product is stable when it is stored in neutral solution.

**Biological activation of mitomycin C (MMC):** Mitomycin C is very effective cross linker of DNA both in vivo, and in vitro, but the effect demands prior activation of the molecule a property that distinguishes mitomycin C from majority of alkylating agents and gives it an edge for efficient treatment of certain tumors. Activation is accompanied by reduction mediated in vivo by NADPH-dependent enzyme systems and in vitro by a variety of reducing agents like dithionate (Tomasz et al, 1974). Mitomycin C may cause toxicity either through alkylation and cross linking of DNA (Iyer and Szybalski, 1964) or through oxygen radical generation (Pritsos and Sartorelli, 1986). The enzymes responsible for the activation of mitomycin C have been well studied and include NADPH: cytochrome c reductase (Bachur et al, 1979), Xanthine oxidase (Pan et al, 1984; Gustafson and Pritsos, 1992), DT-diaphorase (Keyes et al, 1984; Keyes et al, 1989; Siegel et al, 1990), NADH b5 reductase (Hodnick and Sartorelli, 1991) and Xanthine dehydrogenase (Gustafson and Pritsos, 1992). Activation of mitomycin C by NADPH: cytochrome c reductase, Xanthine oxidase, NADH b5 reductase and Xanthine dehydrogenase under aerobic conditions result in the generation of oxygen free radicals (Bachur et al, 1979; Pan et al, 1984; Pritsos and Sartorelli, 1986; Hodnick and Sartotrelli, 1991; Gustafson and Pritsos, 1992). Interaction of the activated mitomycin C with DNA synthesis results in the inhibition of cell division and loss of cell viability. Mutagenic, carcinogenic and lysogenic inductive effects also, occur. The drug is shown to be active against sixteen of twenty tumors against which it has been tested. Toxic effects observed in animals were hypoplasia of bone marrow, lymphoid tissue damage and lesions in the intestinal epithelium. In clinical studies it is used against the neoplasm of breast, colon, stomach and pancreas and oestrogenic sarcoma. The acute toxicity observed in most cases was thrombocytopenia, leukopenia or both. Mitomycin C is useful in treating disseminated breast, gastric, pancreatic or colorectal adenocarcinomas in combination with 5-Fluorouracil and Adriamycin. It is used in combination with cyclophosphamide and adriamycin for lung cancer.

When 0.5 mg/ml mitomycin C was administered with the interferon in to human malignant mesothelioma Xenografts in to nude mice, it was found that the interferon augment the activity of mitomycin C (Sklarin et al, 1988). The cytotoxic effects of mitomycin C have been attributed to bisadducts formed by interstrand crosslinking (Tomasz, et al, 1987). Bisadducts of mitomycin C are formed in much less amounts than monoaadducts, in normal tissues since anaerobic conditions that prevail in solid tumors.
The alkylating antitumour agents mitomycin A, mitomycin C and N 7 analogues were compared in terms of their cardiotoxicity and antitumor activity in vitro. Mitomycin C did not enhance doxorubicin (adriamycin) induced cardiotoxicity in vitro (Dorr et al, 1992). Polyoxyethylene-modified superoxide dismutase (SOD-POE) could prevent the side effects of superoxide generation by mitomycin C and adriamycin, without compromising their antitumor activity either in vitro or in vivo. SOD-POE prevented the decrease of the specific activity of Complex I induced by adriamycin (10 mg/kg), in mice. It also prevented bone marrow suppression induced by mitomycin C in rats (Kawasaki et al, 1992).

CYCLOPHOSPHAMIDE

Cyclophosphamide (2-[bis(2-chloroethyl) amino]tetra 2H-1,3,2-Oxazaphosphorine 2-Oxide), a widely used antineoplastic and immunosuppressive agent is of great interest due to its relatively high oncotoxic specificity (Friedman et al, 1979) and its complexity on activation process. Its carcino-static activity depends on its metabolism by the hepatic microsomal mixed function oxidase-catalyzing C4 hydroxylation. The resulting 4-hydroxycyclophosphamide (4-OHCP) undergoes ring opening to aldo, followed by generation of cytotoxic phosphoramid mustard (PDA) and Acrolein by β-elimination. It is used in the treatment of malignant lymphomas, Hodkins disease, Non-Hodkins lymphoma, multiple-myeloma chronic lymphocyte and granulocytic leukemias, acute lymphoblastic leukemis and solid tumors especially cancers of breast, lung, ovary, testis, neurublastoma and Ewings sarcoma. It is also used in auto immune diseases.

It is well tolerated both locally and systematically. Nausea and Vomiting or headache may be observed with high dose, which can be prevented by the administration of antiemetic agent frequently alopecia is observed but the hair grows after several weeks. Leukopenia is observed but is reversible. Only severe conditions require blood transfusion and administration of y globulin’s. An antibiotic as well as antymycotic therapy is important in particular when massive doses are given. Adverse reactions of the urinary bladder is reported occasionally.

Toxic studies: Temperature above 40.5 °C inhibit the metabolism of cyclophosphamide by microsomes, quite considerably and suggest that this is not a suitable drug to be used with hyperthermia (Clawson et al, 1981). Studies on the role of glutathione in the toxicity of cyclophosphamide in vivo have suggested the sulphahydryl compounds may protect against toxicity of acrolein but not impair the chemotherapeutic activity of cyclophosphamide (Gurtoo et al, 1981). Low doses of cyclophosphamide were effective in reducing bacterial proliferation in case of bacterial pneumonia, high doses exacerbated the bacterial growth (Jacab, and Warr, 1981).

Haemorragic cystitis is a characteristic of the oxazophosphorine mustards and is dose limiting in the case of cyclophosphamide. It is established that the bladder damaging agent is acrolein and this agent can be neutralized by thiols with out reducing the antitumour effectiveness of cyclophosphamide (Connors, 1981). Mesna is the most selective protector of acrolein induced bladder and kidney damage and is used clinically. Thiol reacts with circulating acrolein and a portion
of it is converted to the disulfide. During filtration in kidney, disulfide is converted to free thiol which can then detoxify reactive metabolites in the glomerular filtrate (Brock et al., 1981). The finding that chronic administration of low doses of cyclophosphamide can effect rabbit heart mitochondria (Gvozdjak et al., 1981) may be of importance since there has been a report of cardiotoxicity in patients receiving high doses of the drug (180 mg/kg over 4 days) (Gottinder, 1981).

Cyclophosphamide has often been used in the treatment of autoimmune diseases, and disease of collagen and its effects in mice, which are used as model for lupus syndrome, have been extensively studied (Chai et al., 1981). There are also reports on its value in the treatment of steroid-dependent nephrotic syndrome (Seigel et al., 1981), rheumatoid arthritis (Grimaldi, 1981) and marginal corneal ulcers in young Africans (Connors, 1983). Activated cyclophosphamide such as 4-Sulfoethylthio cyclophosphamide (mafosfamide) are suitable for local intraperitoneal chemotherapy where as cyclophosphamide required a metabolic activation. Mafosfamide administered i.p in mice was less toxic (50% lethal dose, 640 mg/kg) than its i.v application (50% lethal dose, 480 mg/kg). A further remarkable reduction of toxicity (50% lethal dose, 1500 mg/kg) was obtained by simultaneous i.p application of cystine which is accompanied by loss of antitumour activity. These are the results of studies on sarcoma 180 ascites tumour of mice (Thomas Wagner et al., 1986). Under in vivo/in vitro conditions ascorbic acid caused a dose related decrease in cyclophosphamide and mitomycin C induced sister chromatid exchanges up to a dose of 3.34 g/kg. At this concentration approximately 50% inhibition of cyclophosphamide and mitomycin C induced sister chromatid exchanges was observed in bone marrow and spleen cells (Krishna et al., 1986).

Cyclophosphamide has adverse effects on the reproductive system and developing embryo (Padmanabhan and Singh, 1980). Administration of cyclophosphamide to rats (100 mg/kg, i.p.) once a day for 4 consecutive days resulted in mitochondrial dysfunction and a decrease in enzyme activities of the respiratory chain. These findings can be correlated to the cyclophosphamide-induced cardiotoxicity and the changes in the autonomic nervous system. (Hanaki et al., 1990). Administration of cyclophosphamide to rats resulted in multiple interstitial myocardial hemorrhage, multifocal myofibril necrosis, inflammatory reaction, vascular changes, pericarditis and valvulites, mainly in heart ventricles (Kumar et al., 1992). The effects of acrolien on protein thiol content did not correlate with toxicity suggesting that these groups are not the critical targets for cyclophosphamide induced bladder injury (Frasier and Kehrer, 1992). Acrilien and 4-hydroperoxy cyclophosphamide are cytotoxins and a transient depletion in GSH accompanies this toxic effect in cardiac myocytes (Dorr, and Lagel, 1994).

Coadministration of mitomycin C and cyclophosphamide resulted in increase in the frequency of sister chromatid exchange after exposure to both compounds even at lower doses (Pariani et a., 1992). A single dose of cyclophosphamide (100-400 mg/kg. i.p) produced a significant dose dependent increase in weight of urinary bladder within 48 hr of treatment. Disulfiram prevented cyclophosphamide-induced bladder damage in a dose dependent manner in mice when administered orally (Ishikawa, et al., 1994). Mercaptopropionyl glycine and WR-2721 which are being tested at clinical levels
have not only shown to reduce the toxicity of cyclophosphamide but also have shown to selectively protect the normal cells (Bhanumathy et al, 1986).

The effect of the administration of an extract of garlic was studied in mice that were treated with a chronic lethal dose of cyclophosphamide (50 mg/kg body wt, for 14 days). The intraperitoneal administration of garlic extract (50 mg /animal for 14 days ) along with cyclophosphamide, reduced the toxicity (70% increase in the life span ). It also reduced the level of lipid peroxidation induced by the administration of cyclophosphamide in liver with out effecting antitumour activity (Unnikrisnan et al, 1990). Life span of mice treated with a chronic lethal dose of cyclophosphamide (50 mg/kg, i.p. for 14 days) was also increased by the administration turmeric extract or curcumin (20 mg powder, i.p, for 14 days). Increased glutamate-pyruvate transaminase, alkaline phosphates and thiobarbituric acid reacting material in the liver were also reduced by turmeric and curcumin (Soudamini and Ramdasa Kuttan, 1991).

**METHOTREXATE**

Methotrexate (amethopterin,4-amino-4-deoxy N\textsuperscript{1}-methylptroyl-glutamic acid) is a folic acid antagonist introduced in 1948 to treat acute leukemia. It is used extensively in the treatment of several other malignancies and also many non-neoplastic diseases. Methotrexate (MTX), was first used for acute lymphoblastic leukemia in children and chorio-carcinoma ( Bertino,1993). Methotrexate is now used to treat various solid tumors such like osteosarcoma, urothilieal cancer and breast cancer etc. Methotrexate is an important component in the maintenance regimens used in lymphoblastic leukemia with doses ranging from 50 mg/m\textsuperscript{2}/week. The use of leukovorin or 5- formyl tetra hydrofolate, has allowed further dose escalation (i.e. up to 33 g/m\textsuperscript{2}) (Bertino, 1993). The mega doses were shown to have beneficial effects on cancers such as acute lymphoblastic leukemia, lymphoma and oust-sarcoma ( Patte et al, 1991). Plasma concentration of methotrexate have shown to be the best predictor of methotrexate toxicity ' (Masson et al, 1996). The intracellular transport of methotrexate and naturally occurring folates is a consentrative and energy dependent process. It is dependent on membrane associated carrier proteins with high affinity for both drug and vitamin. Conversion of metothorexate to polyglutamate is known to occur in both normal and malignant tissues. It is of considerable interest because polyglutamates appear to be retained in the cell even after the disappearance of parent drug. Studies have confirmed the retention of polyglutamates in both normal and malignant hepatocytes. Balinska et al, 1981, have shown that the rate of efflux of polyglutamates from hepatoma cells (H35) and from normal hepatocyte was 10 to 65 times slower than the efflux of methotrexate.

Mechanism of action: Methotrexate is classified as an antimetabolite due to its antagonistic effect on folic acid metabolism and function. Dietary folate is reduced enzymatically to dihydrofolate, then tetrahydrofolate and other reduced folates, of which tetrahydrofolate appears to be metabolically active (Cline and Haskell, 1980). The enzyme responsible for converting folic acid to metabolically active reduced folates is dihydrofolate reductase (DHFR). Dihydrofolate reductase also
regenerates tetrahydrofolates from dihydrofolate which is the inactive byproduct of
tetrahydrofolate metabolism. Tight but reversible binding of dihydrofolate
reductase results in cessation of biosynthesis of thymidilic acid, inosinic acid and
other purine metabolites (Bleyer, 1978).

Methotrexate effects protein synthesis by inhibiting interconversions of
aminoacids, principally glycine to serine, and homocysteine to methionine. In
human, DNA synthesis is inhibited to a greater extent than RNA or protein
synthesis, suggesting that the inhibition of thymidilate synthesis is the most
important mechanism of the drug induced cytotoxicity (Calabresi and Parke,
1980).

Adverse effects: The most common adverse effects seen with methotrexate
involve the gastrointestinal tract, due to its high proportions of proliferating cells.
These include nausea and vomiting, anorexia, diarrhea and stomatitis. Most severe
side effects of this drug include hepatotoxicity, potential carcinogenicity,
pulmonary toxicity and nephrotoxicity (Goodman and Polisson, 1994). 7-
Hydroxy methotrexate plays a direct role in the toxic effect of methotrexate in kidney and
liver cells (Smeland et al, 1994). Alcohol intake, obesity and diabetes increase the
risk of hepatotoxicity. Methotrexate associated nephrotoxicity is quite common
when large doses are employed. Due to poor solubility in acidic urine, the drug can
precipitate in the renal tubules and cause significant damage.

Chloroquine reduces the bioavailability of methotrexate which explains the
reduction in methotrexate associated liver toxicity (Seideman et al, 1994). Hepatic
and renal subacute toxicity induced by some of the antineoplastic agents was
investigated in rats using biochemical parameters in serum indicated the overall
toxicity impact of the antitumour drugs as follows methotrexate<
cisplatin<epirubicin<chlorambucil (Pspirigos et al, 1993). Methotrexate
increased paracetamol induced toxicity by decreasing the amount of glutathione
which is required for conjugation with reactive metabolites of paracetamol
(Lindenthal et al, 1993). Soybean concentrate offers good protection. It abolishes
the methotrexate induced anorexia and diarrhoea, when included as sole protein
source (Funk and Baker, 1991). The prior administration of solcoseryl significantly
decreases the acute toxicity of MTX (Danysz et al, 1991).

VINCRIStINE

Vincristine is the vinca alkaloid derived from perivinkle plant. It is an
important anticancer drug that is effective against wide variety of neoplasms like
Hodkins and non Hodkins Lymphomas, Acute lymphoblastic leukemia,
embryonal rhabdomyosarcoma, neuroblastoma, Breast carcinoma and Wilms tumor
(Sieber et al, 1976). It is a cell cycle specific drug, which arrests cell growth
exclusively during metaphase by attaching to the growing end of microtubules and
inhibiting their assembly (Owellsen et al, 1972). It has been shown that
liposomal formulations of vincristine can exhibit reduced toxicity when compared
to the free drug. It is also shown that the antitumour activity of vincristine is
strongly dependent on the life of circulating liposomal carrier and the rate of drug
release from the carrier (Vaage et al, 1993). Zotikov and Barbouk (1980) studied
bone marrow cells in rat and noted the ultrastuctral changes in the membranes of mitochondria and nuclear envelope. Inabe et al (1981) reported energy-independent uptake of anthracyclins and vinca alkaloids with shared routes of energy dependent efflux as the reason for cross resistance for antimetimetic agents. Goldstein et al (1981) showed that the multiple doses of Vincriptide causes neurological impairment which results from damage to muscle, spindle and peripheral nerves. Administration of vincristine resulted in neural degeneration and these changes are characteristic to those described in many cells undergoing apoptosis (Muzylak and Maslinska, 1992). Coadministration of vincristine and cyclophosphamide resulted in lesions in the perivascular astrocytes. Other structural elements of the CNS exhibited lesions characteristic of the given drug: Proliferation of the endoplasmic membrane, destruction of microtubules and proliferation of microfilaments due to vincristine administration were reported (Dumbaska and Maslinska, 1992).

Vincristine is used in the combination chemotherapy with cyclophosphamide, mitomycin C, adriamycin, methotrexate and other drugs in the treatment of various types of cancers. Non-ionic surfactant vesicles (niosomes) are promising drug carriers for anticancer drugs. Niosome encapsulated vincristine sulfate prepared by transmembrane pH gradient drug uptake process (remote loading method) was evaluated for toxicity and antitumor activity. The toxicity of vincristine sulfate was reduced after encapsulation and anticancer activity was increased due to encapsulation (Parathsarathy et al, 1994).

**OXIDATIVE STRESS, FREE RADICALS, AND OXIDATIVE MEMBRANE DAMAGE.**

Free radicals are species that are capable of independent existence, that contain one or more unpaired electrons (Halliwell, 1994). Reactive oxygen species (ROS) is a collective term that refers to superoxide, the hydroxyl radical, hydrogen peroxide, singlet oxygen, hypochlorous acid, and ozone. Reactive nitrogen species can be derived from nitric oxide. Free radicals are produced as the result of normal metabolism, and reactive oxygen species such as hydrogen peroxide formed in vivo. Possible free radical damage to cellular targets includes oxidative damage to proteins, membranes (lipid and proteins), and to DNA. Lipid peroxidation is a free radical mediated chain reaction which can be initiated by lipid peroxidation will also damage membrane proteins directly through free radical attack. Protein modifications include oxidation of thiol groups and in particular the generation of carbonyl derivatives of amino acid residues (Oliver et al, 1987).

Lipid peroxidation and reactive oxygen species are likely to be involved in many pathological conditions, including inflammation, radiation damage, metabolic disorders, cellular ageing, and repurfusion damage. Lipid peroxidation is known to occur in three steps which include initiation, propagation and termination. Initiation of peroxidation usually occurs by the attack of any species capable of abstracting hydrogen from polyunsaturated fatty acid- side chain in a membrane (such side chains are more susceptible to free radical attack then are saturated or monounsaturated side chains).
Species able to abstract hydrogen include hydroxyl radical and peroxy radicals and the carbon centered radicals react fast with oxygen. A fatty acid peroxy radical is formed which can attack adjacent fatty acid side chains and propagate lipid peroxidation. The chain reaction thus continues and the lipid peroxides accumulate and destabilize the membrane which make them leaky to ions. Peroxyl radical can attack not only lipids but also membrane proteins and oxidize cholesterol. 80 -90 % of cellular oxygen is normally consumed by the activity of mitochondrial respiratory chain ;Mitochondria represents the main site for their cellular oxygen activation (Chance and Boveris, 1979).

Interaction of cytotoxic reagents results in the generation of reactive oxygen species. These reactive oxygen species can contribute to oxidative damage of mitochondrial lipids, proteins, and DNA. Phospholipids are required for the normal functioning of variety of enzymes. During lipid peroxidation there is a loss of enzymatic activity due to critical alteration of necessary membrane phospholipids. Lipid peroxidation in the membrane increases its permeability leading to mitochondrial swelling, disintegration and haemolysis in RBC and rupture of endoplasmic reticulum etc. Heat stress decreases mitochondrial phosphatidylycholine and phosphatidylethanolamine and increases cardiolipin phosphatidylserine, phosphatidic acids and lysophospholipids (Almatov et al, 1994). Changes of mitochondrial lipids appear to affect the integrity of cellular metabolism via mitochondrial dysfunction during ischemia and recirculation (Nakahara, 1991).

NADH dehydrogenase, NADH oxidase, Succinate dehydrogenase, Succinate oxidase, and ATPase activities were rapidly inactivated by the exposure to hydroxyl radical. Oxygen is a good inactivator of NADH dehydrogenase, NADH oxidase and ATPase and mild inactivator of succinate dehydrogenase and a poor inactivator of succinate oxidase. Hydrogen peroxide partially inactivated NADH dehydrogenase, NADH oxidase and cytochrome c oxidase. Cytochrome c activity was resistant to oxidative inactivation by hydroxyl radical, super oxide radical, or singlet oxygen. The cytochrome c activity was 40% inactivated by hydroxyl radical (Zhang et al, 1990). Majority of studies reported that several anticancer drugs like mitomycin C, bleomycin, etc augment free radical generation and lipid peroxidation process in vitro and in vivo (Sangeeta et al, 1990). Reaction of cytochrome c with hydrogen peroxide promotes membrane oxidation. Ferricytochrome c reacts with mitochondrial hydrogen peroxide to yield site specific mitochondrial lipid peroxidation (Radi et al, 1991). Initiation of lipid peroxidation may results in loss thiols of membrane protein , which leads to hepatocellular injury (Pompella et al, 1991). Degradation of mitochondrial lipids, associated with mitochondrial dysfunction suggested the significance of it in disruption of cellular energy metabolism, during cerebral ischemia (Nakahara et al, 1991).

Cells have defense system to either prevent or control lipid peroxidation . These defense systems are classified as enzymatic and non enzymatic or combination of two . The first category includes the enzymes that control the formation of endogeneous initiators of lipid peroxidation . The second category includes chain breaking antioxidants, or radical scavengers (Scholz et al, 1990). The enzymes identified in controlling lipid peroxidation
are superoxide dismutase, catalase and glutathione peroxidase. They control the concentration of superoxide anion, hydrogen peroxide and lipid hydroperoxides (Flohe, 1982). Antioxidants contribute to non enzymatic cellular defenses against lipid peroxidation by donating hydrogen atoms to free radicals resulting in their inactivation. Water soluble antioxidants like ascorbic acid, uric acid, cysteine and glutathione exist in cell cytosol and prevent lipid peroxidation by scavenging radicals in the aqueous phase (Chow & Khan 1983).

Vitamin E is known to be the major lipid soluble antioxidant of membranes (Burton, et al., 1986). It is present both in the inner and the outer mitochondrial membrane and is significantly more in the inner membrane than in outer membrane. (Thomas et al 1981). The normal level of vitamin E in mitochondria is 0.2 to 0.3 n moles /mg protein. (Thomas et al, 1993). Vitamin E is superior to ubiquinols with respect to its antioxidant activity. Rats were fed with diet containing either Vitamin E alone or in combination with selenium or beta carotene or coenzyme Q for 42 days. Vitamin E exhibited greatest protection against lipid peroxidation in liver heart and spleen. Selenium showed maximum protection in kidney (Leibovitz et al, 1990).

**MEDICINAL PLANTS**

Administration of picroliv (12 mg/kg, p.o) an iridoid glycoside fraction of *Picrorhiza kurroa* for 15 days showed significant protection against toxicity induced due to alcohol administration (Rastogi, et al, 1995). Ethanolic extract of *Euphorbia antisiphilitica* (Saraf et al, 1996), petrol fraction of root bark of *Capparis spinosa* (Shirwaikar, et al, 1996), Ethanolic extract of *Laneneria siceraria* fruit (Shirwaikar and Sreenivasan, 1996), *Solarium lyratum* extract (Choi, et al, 1996), methanol extract of *Paderi foiteda* leaf (De, et al, 1996), *Capparis spinosa* extract (Gadgoli et, al, 1995), Icarin (flavanol glycoside), isolated from aerial parts of *Ep rangeum koreanum* (Lee et al, 1995) and three flavonoids (25 mg/kg), weightone, naringenin and populnin (Kaemferol - glucoside) isolated from ethanolic extract of *Cudrania cochinchinensis* (Lin, et al, 1996) exhibited hepatoprotective effects on CCL4 induced liver injury.

Administration of ethanolic extract of *Picrorhiza kurroa* (100 mg/kg) for 7 days showed marked effects on lipid peroxidation and super oxide dismutase activity in liver and brain of albino rats (Mishra et al, 1996). Andrographaloide, the main active constituent of *Andrographis panniculata*, given orally (3-12 mg/kg), exhibited a dose dependent activity in rats against galactoseamine induced hepatic damage (Saraswat et al, 1995). On incubation of hepatocytes with galactoseamine or tertbutyl hydro-peroxide (TBH) in the presence of the extract of *Meliothera maderaspatana*, a significant protection was observed at concentration of 500 mg/ml (Thabrew et al, 1995).

Studies with powdered rhizomes and aqueous extracts of *Curculigo orchiodes* showed marked hepatoprotective activity (Rao et al, 1996). The extract of *Curcuma xanthorrhiza* (100 mg/kg) when administered p.o significantly reduced the acute elevation of serum transaminases induced by hepatotoxins (Lin et al, 1996). The hepatoprotective effect of taiwaniese herb *Horngtyanwu* (A. sessilis, 300 mg/kg, p.o) was tested against acute hepatitis induced by chemicals such as
CCL₄ (31.25 micro lit/kg, i.p.) or acetaminophen (paracetamol; 600 mg/kg) in mice and D (+)-galactosamine (188 mg/kg, i.p.) in rats showed positive results (Lin, 1994). Perfusion of liver of rats administered with galactosamine or thioacetamide with a 0.02% solution of picroliv (glycoside fraction of P.kurroa 1ml/min, 6 mg/rat) significantly reduced changes induced due to administration of galactosamine or thioacetamide (Dwivedi et al., 1993). Clausinamide is an alkaloid isolated from the leaf of clausena lansium. It inhibited ferrous cystine induced lipid peroxidation (malondialdehyde formation) of microsomes from the rat brain, heart, liver and testis (Lin Tongjun et al., 1992).

The scavenging effects of the flavonoids of Glycyrrhiza (GF), 0.265 to 26.5 mg/ml or 2.58 to 250 mg/ml on O₂ and OH was reported, suggesting antioxidant nature of Glycorriza flavonoid (Ju et al., 1989). Purpurogallin (from nutgall) is a plant phenol, from 0.5 to 2.0 mM, purpurogallin prolongs survival of rat hepatocytes substantially against oxyradicals generated with xanthine oxides and hypoxanthine (Wu et al., 1991).

**PHYLLANTHUS FRATERNUS**

*P.fraternus* is a perennial herb, growing up to 60 cms in height, occurring as a winter weed throughout the hotter parts of India. Fresh leaves and roots are used for various medicinal purposes. The plant is bitter in taste, astringent, stomachic, diuretic and antiseptic. It is used in gastric complaints including dyspepsia, colic, diarrhea dysentery and diseases of urinogenital system. This plant is useful in diabetes. A decoction of the leaves is used as a refrigerant for scalp leaves and roots are made into poultice with rice water for application on oedematous swellings and ulcers. The latex is applied to offensive sores and ulcers, mixed with oil, it is used in the treatment of jaundice.

Chemical studies: Phyllanthin (a bitter constituent) and hydrophyllanthin (a non-bitter constituent) isolated as early as 1946 by Krishnamurty and seshadri from the leaves of *P.fraternus* were later identified as lignans (Row et al., 1964). The hexane extract of the leaves gave three additional extracts viz., niranthin, nirtetralin and phyltetralin. The aerial parts of *phyllanthus* yielded two alkaloids, 4-methoxysecurinine and 4-methoxy norsecurinine. Their structures were established based on spectroscopic studies (Mulchandani and Hasseranjani, 1984). From the methanol extract of *P.niruri* three new alkaloids namely 4-methoxy dihydrosecurinine, 4-methoxy dihydrosecurinine and 4-hydroxysecurinine were extracted (Hasseranjani and Mulchandani, 1990).

Pharmacological studies: Petrol extracts of whole plant and leaves of *phyllanthus fraternus* showed antifungal activity against *Helminthosporium sativum* (Bhatnagar, et al., 1961) and *Alternaria alternata* (Bowmick and chowdhary,1982). The aqueous extract of *P.fraternus* leaves were reported to produce hypoglycemic action in normal as well as alloxan-diabetic rabbits (Ramakrishnan et al., 1982). *Phyllanthus fraternus* has been shown to be effective as an adjunct along with other siddha drugs in the treatment of jaundice due to ineffective hepatitis (Ramanan and Sainani, 1961; Thyagarajan et al., 1977).
The phytochemical tests of species of *Phyllanthus* indicated the presence of alkaloid, saponin, flavonoid, tannin, vitamin C and oxalic acid in the various organs of these plants. Chromatographic studies of root, shoot and fruit revealed the presence of 14 amino acids along with 2-amide aspartic acid, β alanine, cysteine, threonine and serine were exclusively present in all parts. Chromatographic studies indicated the presence of all intermediates of krebs cycle except isocitric acid, aconitic acid, oxalic acid, tartaric acid, tannic acid and few keto acids like, alfa ketoglutaricacid, oxaloacetic acid levulinic acid and ketomalic acid (Bharadwaj, 1994).

Oral administration of aqueous extract of *Phyllanthus emblica* and *Phyllanthus niruri* leaves to laboratory bred albino mice for a week, significantly reduced toxicity induced by lead nitrate and aluminum sulphate. The plant extract was equally effective in modifying the clastogenic effects of both lead nitrate and aluminium sulphate (Dhir et al, 1990). 50% alcoholic extract of *phyllanthus emblica* (100 mg/100g) and quercetin (15 mg/100 g) showed hepatoprotective effect against country made liquor (CML) and paracetamol in rats and mice (Gulati et al, 1995).

**BERBERIS ARISTA TA**

A genus of shrubs and small trees, distributed in the temperate and subtropical parts of Asia, Europe and America. Around 77 species are recorded from India commonly known as Barberry. Berberry roots form a reputed drug in Ayurvedic medicine. The chief source of drug is *B.aristata* which is native to Nepal. The roots are yellowish brown, less knotty, hard and tough. Drug (powdered form) is bright yellow with a slight odor and bitter taste (Uniyal & Issar,1967). The root and stem contain number of alkaloids the chief active alkaloid is berberine, its concentration being higher in plants growing at lower altitudes. Berberine forms yellow needles soluble in water, less soluble in alcohol and is extracted as its hydrochloride by cold percolation method . Berberine hydrochloride and berbering sulfate find application in Cholera, diarrhea, dysentery and eye troubles. It also helps in recognizing latent malaria by releasing the parasites in to the blood stream. The drug is locally prepared in various ways. A thick extract is made from root bark, root and stem wood, by boiling them with water; this is strained and evaporated till dark brown sticky mass of the consistency of opium is obtained It is bitter astringent and fairly soluble in water and partially soluble in alcohol .The drug is regarded as a bitter tonic and is reported to be used as cholagouge, stomachic, laxative, diaphoretic, antipyretic and antiseptic. It is used in the treatment of leprosy (Chopra et al, 1981)

**Chemical composition of Berberis aristata:** The unsaturated hydrocarbons are low and the odd hydrocarbons are considerable. The delta seven sterols are more abundant than delta 5 sterols, stigmasterol derivatives are also more in *B.aristata*. Alcoholic extract of the bark yielded berberine chloride and palmitine chloride. The plant is useful in the treatment of jaundice, enlargement of spleen etc. The dried berries are edible and the decoction is used as a mouth wash and as a treatment for swollen gums and toothache. The alcoholic extract of the roots of *Berberis aristata* showed hypoglycemic effect in rats. The extract of the plant also possesses anti cancer activity (Dhar et al , 1969). Berberine
hydrochloride was found to have significant anti-inflammatory activity on acute, subacute and chronic types of inflammations produced by immunological and non immunological methods. Hepatoprotective action of crude extract of *Berberis aristata* fruits through microsomal drug metabolising enzymes inhibitory action have been indicated (Gilani and Janbaz, 1995).

Extracts of Lycovin capsules, *Piccorrhiza kurroa, Phyllanthus niruri, Cichicorium intybus, Eclipta alba Boer-havia diffusa and Berberis aristata* were tested for their antioxidant activity. All the six plant extracts were found to be potent inhibitor of lipid peroxide formation and scavenger of hydroxyl radicals *in vitro*. *B. diffusa, B. aristata, E. alba, P. kurroa, C. intybus and P. niruri*, showed 50% inhibition on lipid peroxidation (Joy, 1995). Acetaminophen induced liver damage was prevented by *Berberis aristata* leaves. (Gilani and Janbaz, 1992). *Berberis aristata* caused a significant reduction in intestinal fluid accumulation, caused by enterotoxics *E. coli* (Khin-Maung *et al*, 1993).

**STRYCHNOS NUX VOMICA**

This tree is wild one and plentiful through out tropical India, commonly seen in the jungles. Parts used are stem bark, dried ripe seeds are called *nux vomica*. Indian *nux vomica* seeds contains 2.6 to 3% of total alkaloids, (strychine(1.5%), brucine (1.7%), vomicine and igasurine), loganin, a glucoside (which is present also in the pulp of the fruit), proteins 11%, yellow coloring matter, a concrete oil or fat, gum, starch, sugar 6 %, wax, earthy phosphates and 2 % ash. Wood, bark and leaves contain brucine but no strychine. *S. nux vomica* seeds induce intoxication for which they are habitually taken by some as an aprodisiac. *S nux vomica* seeds in powdered form is preferred for administration, especially in the treatment of dyspepsia and diseases of nervous system. It is used as a remedy for chronic dysentery, atonic diarrhoea, paralytic and neuralgic affections, worms, hysteria, mental emotion and epilepsy.

Seed extract of *S nux vomica* maintains the hepatic content of glutathione in a dose and time dependent manner. It also inhibitsthe process of lipid peroxidation even in the presence of toxin. Thus indicating that probably it acts through the scavenging of free radicals (Tripathi, and Chaurasia, 1996). Different fractions of *Rubia cordifolia, Strychnos nux vomica, Moringa olefera, Bacopamanniera, Nardostachys jatmansi, Macuna pruriens and Tamara bhasma* were tested *in vitro and in vivo*, specifically for the generation of free radicals. The mechanism to protect against lipid peroxidation was different in different plants. The chemical composition was further characterized by HPLC fingerprint. *S.nux vomica* inhibited lipid peroxidation by chelating metal ions. It blocks the interconversion of ferrous to ferric ion which is essential for the initiation of lipid peroxidation. *S.nux vomica* chelated both ferrous and ferric ions in a concentration dependent manner (Chaurasia and Tripathy, 1996).

The contents of strychnine and brucine were the same in the decoction of *S. nux vomica* but the content of vomicine, strychnine N-oxide and brucine N-oxide were greater after processing of *nux vomica*. The contents of strychnine and brucine were greater under scalding with hot sand than deep-frying with sesame
oil (Cai et al., 1993). Sand processing of S. nux vomica is good for analgesic potency of nux vomica. It is suggested that the crude alkaloid fraction of nux vomica has distinct antinociceptive potency, even after treatment with licorice, oil, vinegar and sand processing (Cai, et al, 1996).

**CHELIDONIUM MAJUS**

Administration of C. majus along with CCl4 for 3 weeks to rats showed good protection against CCl4 induced hepatic (Mitra et al., 1996). Total hydrolysis of aqueous /methanolic extract obtained from the air dried or lyophilized aerial material was separated using column chromatography on sephadex Lh 20 and subsequent MLCCC, final purification was by HPLC on RP18 yielded 2-(-)caffeoyl-D-glyceric acid, 4-(-)caffeoyl-D-glyceric acid, and 4-(-)caffeoyl-L-threonic acid (Hahn and Nahrstedt, 1991).

The antimycotic activity of ethanol drug extracts of chelidonium majus was reported (Vukusic, 1991). Sanchelin gel (the mixture of sanguine and chelerytrine, chelidonium majus alkaloids , in 0.05% concentration is found to be effective in the treatment of inflammatory periodontal disease (Cerna,1989).

The alcoholic extract of Chelidonium majus (125 mg/kg/day) showed hepatoprotective properties against CCL4 (1mg/kg; twice a week ) treatment (Mitra, et al, 1992) The fraction of quaternary benzophenanthridine alkaloids from the roots of Chelidonium. majus exhibited antimicrobial activity. Sanguinarine and chelerythrine are benzophenantridine alkaloids isolated from the roots of C. Majus. Sanguinarine inhibited cardiac Na+/K+ATpase in vitro and both sanguinarine and chelerythrine inhibited rat liver L-alanine and L-aspartate amino transferases. Sanguinarine, chelerythrine and QBF (containing besides traces of Chelirubine, only chelerythrine and sanguinarine) exhibited anti-inflammatory activity (Linfeild et al, 1981).

Cancer patients were treated with Ukrain (a semisynthetic drug derived from Chelidonium majus) alkaloids conjugated with thiophosphoric acid. The drug was injected intravenously every second day in a dose of 10 mg/injection. The results obtained indicate that it suppresses the growth of cancer cells with out being cytostatic to normal cells (Nowicky et al, 1992). Ukrain, causes a regression of tumors and metastasis in many ontological patients. More than 400 patients with various carcinomas in different stages of development have been treated with Ukrain.

Ukrain can be helpful in improving the general condition and prolonging life by reduction in the tumour progression and its immunomodulating effect on the organism (Lohninger and Hümler, 1992).

*Chelidonium majus* was given as an intravenous Injection every three days-. One course consisted of 10 injections of 10 mg each to patients suffering from lung cancer. The restoration of cellular immunity was accompanied by an improvement in patients who responded further to chemotherapy (Staniszewski et al, 1992).