PROTECTIVE EFFECT OF THE MEDICINAL PLANT EXTRACT AGAINST MITOCHONDRIAL DYSFUNCTIONS INDUCED BY THE ANTICANCER DRUGS

A
Thesis
submitted
for the degree of
Doctor of Philosophy

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DECLARATION

I, Y.KAVITA KUMARI, declare that the work presented in my thesis has been carried out by me under the supervision of Dr. O.H.Setty, and has not been submitted for any degree or diploma of any other University.

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CERTIFICATE

This is to certify that Y.KAVITA KUMARI, has carried out the research work embodied in the present thesis entitled " PROTECTIVE EFFECT OF THE MEDICINAL PLANT EXTRACT AGAINST MITOCHONDRIAL DYSFUNCTIONS INDUCED BY THE ANTICANCER DRUGS" under my supervision and guidance for the full period prescribed under the Ph.D ordinance of this University. I recommend her thesis for the submission for the degree of Doctor of Philosophy of this University.

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DEDICATED TO MY

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ABBREVIATIONS

AAPH:	2,2'- azobis-(2-amidino propane) dihydrochloride.
ADP:	Adenosine 5'- Di phosphate.
ATP:	Adenosine 5'- Tri phosphate.
ANSA:	1- amino-2-napthol-4-sulphonic acid.
BSA :	Bovine serum albumin.
CCL_4 :	Carbon tetrachloride.
Cis, CDDP:	Cisplatin.
CTAB :	Cetyl trimethyl ammonium (bromide).
CY :	Cyclophosphamide.
DMSO:	Dimethysulphoxide.
DCPIP:	2,6 Dichlrophenol indophenol.
DTT :	Dithiotreitol.
EDTA :	Ethylene diamine tetra acetic acid.
FDA :	Food and drugs administration.
FAD:	Flavin adenine dinucleotide.
FMN :	Flavin mononucleotide.
$FMNH_2$:	Flavin mononucleotide (reduced).
GGT:	Gamma glutamyl transpeptidase.
HEPES:	N-2-Hydroxy piperazine- N-2- ethane sulphonic acid.
i.p :	Intraperitonially.
KCl :	Pottasium chloride.
KCN:	Potassium cyanide.
LPO :	Lipid peroxidation.
MDA:	Malondialdehyde.
MMC:	Mitomycin C.
MTX :	Methotrexate.
MTBA :	4- methyl benzoic acid.
NAD(P):	Nicotinamide adenine di nucleotide (phosphate)
NAD(P)H:	Nicotinamide adenine di nucleotide (phosphate) (reduced).
NMGDTC:	N-methyl 1-d-glucamine dithio carbamate.
NOS:	Nitric oxide synthase
OHCP:	4- hydroxyl cyclophosphamide.
PDA :	Phsphoramide mustard.
P/O:	Phosphate to Oxygen ratio.
PMS :	Phenazine methosulphate.
PUFA :	Polyunsaturated fatty acids
PC :	Phosphatidyl choline.
PS :	Phosphatidyl serine.
PE :	Phosphatidyl ethanolamine.
RCR:	Respiratory control ratio.
SOD:	Superoxide dismutase. ,
SDH :	Succinate dehydrogenase.

TBARS :	Thio barbituric acid reactive substance.
TCA :	Trichloroacetic acid.
TLC :	Thin layer chromatography.
TMPD :	N,N,N',N' Tetramethyl ethylene diamine.
TRIS :	Tris (hydroxy methyl) amino methane 2- Amino-2-(hydroxy methyl-Amino-2- (hydroxy methyl) propane- 1,3-diol.
Vin :	Vincristine.
WR 2721 :	(S-2-(3- amino propylamino) ethyl phosphorothioic acid).

CONTENTS

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 μ in length and 0.5 - 1 μ in width, with an average dry weight of 1×10^{-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 power house 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 Oxidoredutase.
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). Sarcolysine 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 sarcolysine 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 1and site 3, and the electron tranfer 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 , 1997_a,) or thioacetamide (Padma and Setty , 1997_b) 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 dessipates 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 chemotheraputic 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 Vincristine 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

Cisplatinum 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 losses both chloride ions from the coordination sphere of the Pt (11) ion and water or hydroxide ion becomes bound. Thus a distribution of species is set up involving the presence of unhydrolysed and partially hydrolysed 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 unhydrolysed 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 cisplatinum 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 ofPlatinum-DNA binding, to cytotoxicity. Changes in ultra violet absorption spectrum of salmon sperm DNA after reaction with either *cis or trains* Pt (11) $(NH_3)_2Cl_2$, 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 pharmocokinetics 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 Pt Cl_2 H_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, myelosuppresion, 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 convulsent 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-dawly 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, polyglycerohosphates 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 determinant 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_{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 Dubskaia, 1993) (Dambska *et al,* 1994). Cisplatin is known to damage normal organs dose despondently 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 $Ca^{(2+)}$- 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 transpeptidase (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 1 -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 - methy 1 -d-glucaminedithiocarbamate (NMGDTC) a derivative of dithiocarbamate could produce marked reduction of CDDP-induced ototoxicity and weight loss (Walker, 1994). Glutathione, N- acetylcysteine or the iron chelator defroxamine or *Ginkgo biloba* extract or the xanthine derivative torbafylline 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 glutathione 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-androstan-17-oic-13, 17-lactam 9 p-[bis 92-chlroethyl) 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 have 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 $C_{16}H_20\ N_4\ O_5$ 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 b_5 reductase (Hodnick and Sartorelli, 1991) and Xanthine dehydrogenase (Gustafson and Pritsos, 1992). Activation of mitomycin C by NADPH: cytochrome *c* reductase, Xanthine oxidase, NADH b_5 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 Sartotrelli, 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 dessimnated breast,gastric,pancreatic or colerectal adenocarcinomas in combination with 5-Flurouracil 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 monoadducts, 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 super-oxide 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-chloroetyl) amino]tetra 2H-1,3,2-Oxazo-phosphorine 2-Oxide), a widely used antineoplastic and immunosuppresive 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 C_4 hydroxylation. The resulting 4-hydrooxycyclophosphamide (4-OHCP) undergoes ring opening to aldo, followed by generation of cytotoxic phosphoramide 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 antimycotic 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 ascities 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). Acrolien 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 intraperitonial 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^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. Methoterxate (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 /week. The use of leukovorin or 5- formyl tetra hydrofolate, has allowed further dose escalation (i.e. up to 33 g/m^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 nerhrotoxicity 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 (Pispirigos *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 rabdomyosarcoma, 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 (Owellen *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 antimemitic agents. Goldstein *et al* (1981) showed that the multiple doses of Vincrictine 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, hypochlorus 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 poly unsaturated 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 peroxyl 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 phosphatidylcholine and phosphatidylethanolamine and increases cardiolipin phospatidylserine, 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 irioid 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 antisyphilitica* (Saraf *et al,* 1996), petrol fraction of root bark of *Capparis spinosa* (Shirwaikar, *et al,* 1996), ethanolic extract of *Langenaria siceraria* fruit (Shirwaikar and Sreenivasan, 1996), *Solarium lyratum* extract (Choi, Cu *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 *Epideum koreanum* (Lee *et al,* 1995) and three flavonoides (25 mg/kg), weighteone, naringenin and populnin (Kaemfterol - glucoside) isolated from ethanol extract of *Cudrania cochinchinensis* (Lin, *et al,* 1996) exhibited hepatoprotective effects on CCL4 induced liver injury.

Administration of ethanolic extract of *Picrorhiza kurrooa* (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 hapatic 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 a/,* 1995).

Studies with powdered rhizomes and aqueous extracts of *Curculigo orchiodes* showed marked hepatoprotective activity (Rao *et al,* 1996), The extract of *curcuma xanthorhiza* (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

CCL_4 (31.25 micro lit/kg, *i.p*) or acetaminophen (paracetamol ; 600 mg/kg) in mice and D (+) - galactoseamine (188 mg/kg, *i.p)* in rats showed positive results (Lin, 1994). Perfusion of liver of rats administered with galactoseammine 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 galactoseamine 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 O_2 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 through out 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 in to poultice with rice water for application on oedamatous 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 ; Thygarajan *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, aconotic 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 accumilation, caused by enterotoxic 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 inhibits the 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 CCl_4 for 3 weeks to rats showed good protection against CCl_4 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 sangurine 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 Hamler, 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).

OBJECTIVES

OBJECTIVES AND SCOPE OF THE PRESENT STUDY.

Mitochondria are present in all the eukaryotic cells. They are important subcellular organelles of the cell. The main physiological function of these organelles is oxidative phosphorylation. Cellular ATP is generated via oxidative phosphorylation.

Impaired energy metabolism is reported in both cancer and normal cells that are exposed to anticancer drugs (Gvozdjak *et al,* 1981; Sugiyama *et al,* 1989; Hanaki *et al,* 1990; Geeta and Shymala Devi, 1992). Cisplatin, methotrexate, cyclophosphamide, mitomycin C and vincristine are anticancer drugs that are widely used in the chemotherapy of malignant diseases either alone or in combination. The dose dependent side effects appears to be increased in the case of combination chemotherapy. Most common adverse effects observed with chemotherapy are, primarily nausea, vomiting and fever; However hepatotoxicity and nephrotoxicity has remained a concern. Renal and hepatic dysfunction has been confirmed biochemically, but the mechanism of toxicity has not been worked out fully.

There are number of herbs employed in traditional system of medicine for liver and kidney disorders. They either stimulate liver or kidney cells or selectively protect them from the damage.

Objectives of the study:

1) To study the effect of anticancer drugs that are widely used for the treatment of tumors. Five combinations were selected for this study based on their clinical use. The effect was studied on, oxidative phosphorylation, various enzymes involved in the transfer of electrons to electron transport chain, lipid peroxidation and the phospholipid composition of the mitochondria.

2) The important objective of this study is not merely to establish the mitochondrial dysfunctions due to the administration of these anticancer drugs but to prevent or minimize these effects by prior administration of some selected plant extracts. Four medicinal plants i.e. *Berberis aristata, Phyllanthus fraternus, Chelidonium majus and Strychonus nux vomica* were selected to study the protective effect against the dysfunctions induced by anticancer drugs.

MATERIALS AND METHODS

Animals : All the experiments were done with colony bred rats of Wistar strain, raised in the animal house facility, University of Hyderabad, Hyderabad. Rats weighing around 100 gm body weight were used for the present study and were given water and food *ad libitum.*

Chemicals: Mitomycin C, Cisplatin, Cyclophosphamide, Vincristine and Methotrexate were purchased from local medical stores. Succinate, glutamate, malate, ADP, NADH were purchased from Sigma chemical company, St Louis MO. All other chemicals were purchased from reputed Indian companies and were of Analytical grade.

Glassware and Solutions : All the glassware used for the preparation of mitochondria were routinely cleaned by immersion in a hot chromic acid bath. They were thoroughly washed with tap water, distilled water and finally rinsed with double distilled water. The double distilled water was boiled and cooled before using for the preparation of isolation medium, which was made fresh and adjusted to pH 7.4. Sterile saline and glassware were used for the reconstitution of mitomycin C. Commercially reconstituted solutions were used for cisplatin, vincristine, methotrexate. Cyclophosphamide solution was prepared by dissolving tablets in sterile double distilled water.

Preparation of aqueous extract of *Phyllanthus fraternus*: The whole plant of *P.fraternus* including roots were homogenized in water (5 gm/ 12.5 ml) using a motor and a pestle. The homogenate was filtered through a cheese cloth. **An** aqueous extract equivalent to 10 mg dry powder of the plant / 100 g body wt was administered daily to each rat. The dry wt content of the plant was determined after drying the plant in an oven and it was found to be 25% of the wet tissue.

Alcoholic extracts of *Berberis.aristata, Strychnos.nux vomica and chelidonium majus* were obtained from local medical stores. The dry wt was determined after drying the alcoholic extract in an oven. Optimal dose of plant extract for the future studies was decided from the preliminary studies.

The dose and the duration for drug treatment was also decided from the preliminary studies.

TREATMENT SCHEDULE

Protective effect of *Berberis aristata*

1) Protective effect of *Berberis aristata* against cisplatin and cyclophosphamide.

Group 1:	Rats which received saline .
Group 2:	Single dose of cisplatin (12 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral) was given and were sacrificed 24hr after the administration of these drugs.
Group 3:	Alcoholic extract of *B. aristata* (10 mg /100g bd wt) was administered for three days.
Group 4:	Alcoholic extract of *B. aristata* (10 mg/100g bd wt) was given for three days and then single dose of cisplatin (12 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral) was given. They were sacrificed 24 hr after the administration of these drugs.

2) Protective effect of *Berberis aristata* against mitomycin *C* and cyclophosphamide.

Group 1:	Rats which received saline .
Group 2:	Single dose of mitomycin C (2 mg/kg ,*i.p)* and cyclophosphamide (150 mg/kg, oral) was given and were sacrificed 48 hr after the administration of these drugs.
Group 3:	Alcoholic extract of *B. aristata* (10 mg /100g bd wt) was administered for three days.
Group 4:	Alcoholic extract of *B. aristata* (10 mg/kg bd wt) for three days along with single dose on mitomycin C(2 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg,oral) was given. They were sacrificed 48 hr after the administration of these drugs.

3) Protective effect of *Berberis aristata* against mitomycin *C* and cisplatin

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin C (2 mg/kg ,*i.p)* and cisplatin(12 mg/kg, *i.p)* was given and were sacrificed 48 hr after the administration of these drugs.

Group 3: Alcoholic extract of *B.aristata* (10 mg /100g bd wt) was given for three days.

Group 4: Alcoholic extract of *B. aristata* (10 mg/100g bd wt) for three days and then single dose of mitomycin C (2 mg/kg ,*i.p)* and cisplatin (12 mg/kg, *i.p)* was given. They were sacrificed 48 hr after the administration of these drugs.

4) Protective effect of *Berberis aristata* against methotrexate and vincristine

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg, *i.p)* and vincristine (1 mg/kg, *i.p)* was given and were sacrificed 72 hr after the administration of these drugs.

Group 3: Alcoholic extract of *B. aristata* (10 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *B.aristata* (10 mg/lOOg bd wt) was administered for three days and then a single dose of methotrexate (2 mg/kg, *i.p)* and vincristine (1 mg/kg, *i.p)* was given. They were sacrificed 72 hr after the administration of these drugs.

5) Protective effect of *Berberis aristata* against methotrexate and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg ,*i.p)* and cyclophosphamide (150 mg/kg, oral) was given and were sacrificed 24 hr after the administration of these drugs.

Group 3: Alcoholic extract of *B. aristata* (10 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *B. aristata* (10 mg/1 OOg bd wt) was administered for three days and then single dose of methotrexate(2mg/kg , *i.p)* and cyclophosphamide(150 mg/kg, oral). They were sacrificed 24 hr after the administration of these drugs.

Protective effect of *Phyllanth us fraternus.*

1 Protective effect of *Phyllanthus fraternus* against cisplatin and cyclophosphamide

Group 1: Rats which received saline .

Group 2: Single dose of cisplatin (12 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral) was given, and were sacrificed 24 hr after the administration of these drugs.

Group 3: Aqueous extract *of P.fraternus* (10 mg/lOOg bd wt) was administered for seven days.

Group 4: Aqueous extract of *P.fraternus* (10 mg/lOOg bd wt) was administered for seven days and then a single dose of cisplatin (12 mg/kg ,*i.p)* and cyclophosphamide(150 mg/kg,oral). They were sacrificed 24 hr after **the** administration of these drugs.

2) Protective effect of *Phyllanthus fraternus* against mitomycin C and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin C (2 mg/kg ,*i.p)* and cyclophosphamide (150 mg/kg,oral) was given and were sacrificed 48 hr after the administration of these drugs.

Group 3: Aqueous extract of *P.fraternus* (10 mg/100g bd wt) was administered for seven days.

Group 4: Aqueous extract of *P.fraternus* (10 mg/g bd wt) was given for seven days and then a single dose of mitomycin C (2 mg/kg ,*i.p)* and cyclophosphamide (150 mg/kg, oral). They were sacrificed 48 hr after the administration of these drugs.

3) Protective effect of *Phyllanthus fraternus* against mitomycin C and cisplatin

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin C (2 mg/kg ,*i.p)* and cisplatin (12 mg/kg, *ip)* was given and were sacrificed 48 hr after the administration of these drugs.

Group 3: Aqueous extract of *P. fraternus* (10 mg/100g bd wt) was administered for seven days.

Group 4: Aqueous extract of *P.fraternus* (10 mg/100g bd wt) was administered for seven days and then a single dose of mitomycin C (2 mg/kg, *i.p)* and cisplatin (12 mg/kg, *ip)* .They were sacrificed 48 hr after the administration of these drugs.

4) Protective effect of *Phyllanthus fraternus* against methotrexate and vincristine

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg ,*i.p*) and vincristine (1 mg/kg, *ip)* was given and were sacrificed 72 hr after the administration of these drugs.

Group 3: Aqueous extract of *P.fraternus* (10 mg/lOOg bd wt) was administered for seven days.

Group 4: Aqueous extract *of P.fraternus* (10 mg/100g bd wt) was administered given for seven days and then single dose of methotrexate (2 mg/kg, *i.p)* and vincristine (1 mg/kg, *ip).* They were sacrificed 72 hr after the administration of these drugs.

5) Protective effect of *Phyllanthus fraternus* against methotrexate and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral) was given and were sacrificed 24 hr after the administration of these drugs

Group 3: Aqueous extract of *P.fraternus* (10 mg/100g bd wt) was administered for seven days.

Group 4: Aqueous extract of *P.fraternus* (10 mg/lOOg bd wt) was administered for seven days and then single dose of methotrexate (2mg/kg ,*i.p)* and cyclophosphamide (150mg/kg, oral).They were sacrificed 24 hr after the administration of these drugs.

Protective effect of *Chelidonium majus.*

1) Protective effect of *Chelidonium majus* against cisplatin and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of cisplatin (12 mg/kg ,*i.p)* and cyclophosphamide (150 mg/kg, oral) was administered and were sacrificed 24 hr after the administration of these drugs.

Group 3: Alcoholic extract of *C.majus* (12.5 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *C.majus* (12.5 mg/kg bd wt) was administered for three days and then a single dose of cisplatin(12 mg/kg ,*i.p)* and cyclophosphamide(150 mg/kg, oral) .They were sacrificed 24 hr after the administration of these drugs.

2) Protective effect of *Chelidonium majus* against mitomycin *C* and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin C (2 mg/kg ,*i.p)* and cyclophosphamide(150 mg/kg, oral) was administered and were sacrificed 48 hr after the administration of these drugs.

Group 3: Alcoholic extract of *C.majus* (12.5 mg/100g bd wt) was given for three days.

Group 4: Alcoholic extract *of C.majus* (12.5 mg/kg bd wt) was given for three days and then a single dose of mitomycin C (2 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral). They were sacrificed 48 hr after the administration of these drugs.

3) Protective effect of *Chelidonium majus* against mitomycin *C* and cisplatin

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin C (2 mg/kg ,*i.p)* and cisplatin (12 mg/kg, oral) was administered and were sacrificed 48 hr after the administration of these drugs.

Group 3: Alcoholic extract of *C.majus* (12.5 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *C.majus* (12.5 mg/kg bd wt) was administered for three days and a single dose of mitomycin C (2 mg/kg, *i.p)* and cisplatin (12 mg/kg, *i.p)* and were sacrificed 48 hr after the administration of these drugs.

4) Protective effect of *Chelidonium majus* against methotrexate and vincristine

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg ,*i.p)* and vincristine (1 mg/kg, oral) was administered and were sacrificed 72 hr after the administration of these drugs.

Group 3: Alcoholic extract of *C.majus* (12.5 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *C.majus* (12.5 mg/100 g bd wt) was administered for three days and a single dose of methotrexate (2 mg/kg ,*i.p)* and vincristine (1 mg/kg, oral). They were sacrificed 72 hr after the administration of these drugs.

5) Protective effect of *Chelidonium majus* against methotrexate and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg ,*i.p*) and cyclophosphamide (150 mg/kg, oral) was administered and were sacrificed 24 hr after the administration of these drugs.

Group 3: Alcoholic extract of *C.majus* (12.5 mg/100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *C.majus* (12.5 mg/100 gm bd wt) was administered for three days along with single dose of methotrexate (2 mg/kg ,*i.p)* and cyclophosphamide (150 mg/kg, oral) .They were sacrificed 24 hr after the administration of these drugs

Protective effect of *Strychnos.nux vomica*

1) Protective effect of *S.nux vomica* against cisplatin and cyclophosphamide

Group 1: Rats which received saline .

Group 2: Single dose of cisplatin (12 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral) was given and were sacrificed 24 hr after the administration of these drugs.

Group 3: Alcoholic extract of *S.nux vomica* (10 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *S.nux vomica* (10 mg/kg bd wt) was administered for three days and then a single dose **of** cisplatin (12 mg/kg, *i.p)* and cyclophosphamide(150 mg/kg, **oral)** was given. They were sacrificed 24 hr after the administration of these drugs.

2) Protective effect of *S.nux vomica* against mitomycin C and cyclophosphamide.

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin C (2 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral) was given and were sacrificed 48 hr after the administration of these drugs.

Group 3: Alcoholic extract *of S.nux vomica* (10 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *S.nux vomica* (10 mg/100g bd wt) was administered for three days and then single dose of mitomycin C (2 mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral).They were sacrificed 48 hr after the administration of these drugs

3) Protective effect of *S.nux vomica* against mitomycin C and cisplatin

Group 1: Rats which received saline .

Group 2: Single dose of mitomycin c (2 mg/kg, *i.p*) and cisplatin (12 mg/kg, oral) was given and were sacrificed 48 hr after the administration of these drugs.

Group 3: Alcoholic extract of *S.nux vomica* (10 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *S.nux vomica* (10 mg/100g bd wt) was administered for three days and a single dose of mitomycin C (2 mg/kg, *i.p)* and cisplatin (12 mg/kg, *i.p).* They were sacrificed 48 hr after the administration of drugs.

4) Protective effect of *S.nux vomica* against methotrexate and vincristine

Group 1: Rats which received saline .

Group 2: Single dose of methotrexate (2 mg/kg ,*i.p*) and vincristine (1 mg/kg, oral) was administered and were sacrificed 72 hr after the administration of these drugs.

Group 3: Alcoholic extract of *S.nux vomica* (10 mg /100g bd wt) was given for three days.

Group 4: Alcoholic extract of *S.nux vomica* (10 mg/100g bd wt) was given for three days and then a single dose of methotrexate (2 mg/kg, *i.p)* and vincristine (1 mg/kg, *i.p)* .They were sacrificed 72 hr after the administration of these drugs.

5) Protective effect of *S.nux vomica* against methotrexate and cyclophosphamide.

Group 1: Rats which received saline.

Group 2: Single dose of methotrexate (2 mg/kg, *i.p)* and cyclophosphamide(150 mg/kg, oral) was administered and were sacrificed 24 hr after the administration of these drugs.

Group 3: Alcoholic extract of *S.nux vomica* (10 mg /100g bd wt) was administered for three days.

Group 4: Alcoholic extract of *S.nux vomica* (10 mg/100 gm bd wt) was administered for three days and then a single dose of methotrexate(2mg/kg,*i.p*) and cyclophosphamide(150mg/kg, oral).They were sacrificed 24 hr after the administration of these drugs.

Isolation of Mitochondria: Mitochondria were isolated from liver and kidney as described by Laurence and Davis (1986) with slight modifications. According to this procedure the isolation buffer consisted of medium 'a'(70 mM sucrose, 220 mM mannitol, 2 mM Hepes, 0.2 mM EDTA, BSA 36% and pH was adjusted to 7.4) or medium 'b'(sucrose 70 mM and mannitol 220 mM, and pH was adjusted to 7.4). Final mitochondrial pellet was suspended in known volume of medium 'b'.

Preparation of Liver Mitochondria: Rats were sacrificed and the liver was removed quickly and placed in ice cold medium 'a'. All subsequent operations were carried out at 4° c. Liver was washed with cold medium 'a', 2-3 times to remove the blood and then weighed. The tissue was finally minced and made 10% homogenate by using Potter Elevehjem homogenizer with a teflon pestle, by 3-4 strokes up and down for complete homogenization.. The pestle was driven by an electric motor at 3,500 rpm. The 10 % homogenate was centrifuged at 700 x g for 10 minutes in Beckman J2-21 M/E refrigerated centrifuge using JA 20 rotor. The pellet was discarded and the supernatant was centrifuged at 7000 x g for 10 min. The supernatant thus obtained contains a white fat layer which was removed with a cotton swab. The supernatant was discarded and the pellet containing mitochondria was suspended in medium 'a' by gentle homogenization by hand using a pestle and centrifuged at 7000 x g (9250 rpm). This step was repeated thrice to wash the mitochondria. The final pellet was suspended in medium 'b' to a protein conc of 20- 30 mg protein/ml and immediately used for polarographic studies, and the rest was stored in liquid nitrogen for further studies.

Preparation of kidney Mitochondria: Killing of animals and removal of tissue was same as described for liver .The kidney capsules were removed by gently squeezing it in between the thumb and fore finger .The kidney was cut and the medullary portion was discarded. Mitochondria were then prepared from the cortex portion by the method described for liver.

Protein estimation: The protein was estimated by biuret method (Gornall *et al* ,1949) with bovine serum albumen as standard. Deoxycholate was used for solubilisation. To 100 µl of mitochondrial suspension, 0.2 ml of 1 % deoxycholate was added. The volume was then made up to 1ml with double distilled water. 4ml of biuret reagent was added to this and mixed well. Simultaneously a blank and a standard were run in duplicates. The tubes were left at room temp for 30 min and the optical density was measured at 540 run.

Assay of Oxidative phosphorylation: Polarographic determination of oxidative Phosphorylation was carried out according to Estrabrook (1967) with a Gilson 5/6 oxygraph fitted with a Clark type of electrode. After obtaining a steady baseline 3 mg protein was added to the reaction chamber containing 1.7 ml of buffer (contains 50 mM Tris-Cl, 20 mM Potassium phosphate, 2mM EDTA, 7mM $Mgcl_2$,

50 mM sucrose, pH 7.4). Respiration was initiated by addition of either glutmate + malate or succinate. State 3 respiration was obtained by addition of ADP and the ADP exhausted respiration was state 4 respiration. Respiratory control ratio was calculated from the ratio of state 3 to state 4 respiration. P/O ratio was calculated from the amount of ADP added and the amount of oxygen utilized during the rapid state 3 respiration.

Succinate oxidase: Succinate oxidase was measured polarographically with a clark type electrode using 1.7 ml reaction medium (containing 50 mM Tris-cl,20 mM potassium phosphate, 2 mM EDTA, 7 mM $Mgcl_2$, 50 mM sucrose, pH 7.4) and 9mM succinate. State 3 respiration was measured by adding 150 n moles of ADP .

NADH oxidase: NADH cannot be transported through the inner mitochondrial membrane if the mitochondria is tightly coupled. Since externally added NADH cannot be oxidized, glutamate and malate were used to reduce NAD^+ in the matrix to NADH, which then gets oxidized through electron transport chain. NADH oxidase was measured polarographically using glutamate and malate as substrates. The reaction medium was the same as used for the succinate oxidase except that 2 mM glutamate and 4 mM malate were used instead of 9 mM succinate as substrate. State 3 respiration was obtained by adding 200 n moles ADP.

Cytochrome *c* oxidase: The enzyme was assayed by following the decrease in the absorbance of ferrocytochrome ***c*** at 550 nm as described by Copperstein and Liazarow,(1951).The reaction was initiated by the addition of 1µg mitochondrial protein (E_{mM}=19.1).

Preparation of reduced Cytochrome *c*. 17 mg of cytochrome *c* was dissolved in 20 ml of 30 mM potassium phosphate buffer, pH 7.4. It was then reduced by addition of small amounts of sodium dithionate (Yonetani,1967), excess sodium dithionate was removed by dialysis against 30 mM phosphate buffer, pH 7.4 for 10-20 hr with three to four changes of buffer.

NADH dehydrogenase : [NADH:(acceptor) Oxidoreductase, E.C.1.6.99.3]
NADH dehydrogenase was measured using potassium ferricyanide as electron acceptor (King and Robert, 1967). The reaction system contained 250 mM sucrose, 30 mM Tris -Cl, 10 mM potassium phosphate, 5 mM $Mgcl_2$, 1mM KCN, 1 mM Potasium ferricyanide, pH 7.4 and 20 µg of mitochondrial protein in a reaction volume of 1 ml. The reaction was initiated by the addition of NADH (1.5 mM) and the rate of reduction of ferricyanide was followed at 420 nm (E_{mM}=1.0).

Succinate dehydrogenase: [succinate;(acceptor) Oxidoreductase, E.C.1.3.99.1]

Succinate dehydrogenase was assayed using DCPIP as electron acceptor (King 1967). The reaction system was same as for NADH dehydrogenase assay except that potassium ferricynide was substituted by 1mM PMS and 70 uM DCPIP. The rate of reduction of DCPIP was followed at 600 run. 10 µg of mitochondrial protein was incubated with 10 ul of 0.5 M sodium succinate (pH 7.4) at room temp for 10 min before assaying SDH activity (E_{mM}=16.9).

Succinate:cytochrome *c* reductase:[succinate:Ubiquinone Oxidoreductase EC.1.9.3.1]

The activity of succinatexytochrome ***c*** reductase was determined by the modified method of Tisdale (1967). The reaction was carried out in a total volume of 1 ml and consisted of buffer (10 mM Potassium phosphate, 0.8 mM EDTA, 0.25 M sucrose, pH 7.4), 5 mM succinate, 1 mM KCN. 2 n moles of rotenone and 130 n moles of ferricytochrome *c*. The reaction was initiated by adding 20 ug of mitochondrial protien. The increase in the absorbance at 550 nm was followed. (E_{mM}=19.1)

NADH:cytochrome c reductase :[NADH: Ubiquinone Oxidoreductase.EC 1.6.99.3]

NADHxytochrome C reductase activity was determined by a modified method of Hatefi and Rieske (1967). The reaction mixture consisted of (1.0 M Potassium phosphate-HCl buffer, 0.1 M NaN_3, 1 mM. EDTA ,1 % deoxycholate, pH 8.0 and 1% ferricytochrome *c*. 20 µg of mitochondrial suspension was taken and the reaction was initiated by the addition of 10 mM NADH. After 15 sec incubation at 30 ° C. The reaction rate was followed for one min by recording the increase in absorbance of cytochrome ***c*** at 550 nm. The activity of NADHxytochrome *c* was deduced from the increasing rate of absorbance. (E_{mM}=19.1)

Swelling of Mitochondria : About 20-40 µ g of mitochondrial protein was suspended in a buffer containing 10 m M Tris, 50 mM $MgCl_2$,10 mM Kcl and 0.25 mM ADP. The reaction was started by the addition of 5 mM Succinate. Swelling was followed by change in absorbance at 520 nm in **a** Shimadzu-160A spectrophotometer (Packer, 1967).

Detection of Free radical by electron spin resonance(ESR) Spectroscopy:.

Liver and kidney were collected in ice cold containers from both control and experimental rats . These organs were minced lyophilized to powder form. 20 mg of lyophilized samples were used for ESR spectrophotometry, for the detection of free radicals. The operational conditions for ESR spectrometer (JEOL Model FE-3X, Japan) were as follows;

Microwave Frequency : 9.220 GH_2

Modulation amplitude :4x 10^3

Times constant : 1 sec

Scanning Time : 4 min

Signal intensity was determined by calculating the peak area of the signal and g value of the free electron was calculated using the formula:

$h v = g \beta H$

Where ,

h= Planks constant

v= Frequency, 9.220 GH_2

g= splitting factor

P= Bohr magneton, 9.273 x 10^{-24} Joules/Tesla

H= Applied magnetic Field , 3280 gauss or 0.3280 Tesla.

Assay of **lipid peroxides** by **thiobarbituric** acid reaction:
Lipid peroxide level was determined in the liver and kidney homogenates and mitochondria of control and experimental rats (Ohkawa *et al*, 1979). According to this procedure a 10 % homogenate was prepared in 1.15% KCl using potter elevehjem homogenizer . Mitochondria were washed with 1.15% KCl and suspended in the same medium. Protein estimation was done by biuret method. To 5 mg protein, 0.2 ml of 8.1 % SDS, 1.5 ml of 20 % acetic acid (adjusted to pH 3.5 with NaOH) and 1.5 ml of 0.67% (W/V) aqueous solution of thiobarbituric acid were added. The total volume was made up to 4.0 ml with distilled water and the tubes were heated in a water bath at 95°C for 60 min using marble as condenser. A blank was also run simultaneously and tetramethoxypropane was used as an external standard. After cooling, 1 ml of distilled water and 5 ml of n - butanol were added, vortexed and then centrifuged at 4000 rpm for 10 min at room temp.

The absorbance of organic layer was measured at 535 nm. The level of lipid peroxides are expressed as n moles /100mg protein

Separation of mitochondrial phospholipids:

Thin layer chromatography was used for the separation of Phospholipids.

Preparation of TLC plates: TLC plates were prepared with Silica Gel G(E Merck).50 gm of silica Gel was used for 100 ml of distilled water, mixed rigorously and the slurry was spread on glass plates with an applicator. The plates were dried at room temperature and activated at 110 °C for one hour before use. Mitochondrial lipids were extracted by the procedure of Bligh and Dyer (1959). About 6-8 mg of mitochondrial protein was taken in a stoppered tube to which 4 ml of methanol, 2 ml of chloroform and 2 ml of water were added, mixed and centrifuged at 1000 rpm for 10 min. The chloroform layer at the bottom was used as a source of phospholipid. It is applied as a streak on TLC plates. The phospholipids were separated by using a solvent, containing Chloroform:methanol:water (65:25:4). When the solvent front reaches to the top, the plates were taken out, dried and exposed to iodine vapor. To detect the various phospholipids, authentic standards were also applied along with the sample. For the estimation of individual phospholipids, corresponding area on the TLC plate was marked and scraped in to a boiling tube and digested with 1ml of 60% perchloric acid at 170-180^0 C (until the sample became colorless).

Estimation of inorganic phosphorus: After digestion as described above, tubes were cooled to room temperature and 7.5 ml of double distilled water, 0.4 ml of ammonium molybdate (2.5% dissolved in 3 N H_2SO_4), 0.2 ml of ANSA (0.2%) were added and mixed well. The tube were heated in a boiling water bath for 10 min cooled and the total volume was adjusted to 10 ml with distilled water. Now the tubes were centrifuged to sediment silica gel and the color in the supernatant was measured at 640 nm suitable phosphorus were also run. It is expressed as Phospholipid phosphorus /gm tissue. (Fiske and Subba row, 1925).

Statistical analysis:

Statistical analysis were conducted by student's t- test and *p* value less than 0.05 was considered as significant. All the data were expressed as mean + SD .

TABLE 1: Effect of anticancer drugs on NADH oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1. Control	100 ±11	100 ± 14	100 + .4.4
2. Cisplatin + Cyclophosphamide	60 + 4.8***	68 + 7.4**	62 + .3.1***
3. Mitomycin C+ Cyclophosphamide	55 + 3.4***	72 ± 4.4*	68 ± 3.1 **
4. Mitomycin C+ Cisplatin	72 + 4.0*	68 + 6.4**	72 + 2.2*
5. Methotrexate + Vincristine	39 + 44***	56 + 3.2***	49 + 3.15***
6. Methotrexate + Cyclophosphamide	66 + 5.3**	76 + 3.5	64 + 3.8***

Experimental details

1. Control group recieved saline.

2. Cisplatin (Cis) and Cyclophosphamide (Cy) group received cisplatin (12 mg/kg, *i.p)* and Cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs.

3. Mitomycin C (MMC) and Cyclophosphamide (Cy) group received Mitomycin C (2 mg/kg, *i.p)* and Cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 48 hr afterthe administration of the drugs.

4. Mitomycin C (MMC) and Cisplatin (Cy) group received Mitomycin C (2 mg/kg, *i.p)* and Cisplatin (12 mg/kg, *i.p).* Rats were sacrificed 48 hr after the administration of the drugs.

5. Methotrexate (Mtx) and Vincristine (Vin) group received Methotrexate (2 mg/ kg *i.p)* and Vincristine (1 mg /kg, *i.p).* Rats were sacrificed 72 hr after the administration of the drugs.

6. Methotrexate (Mtx) and Cyclophosphamide (Cy) group received (2 mg/kg, *i.p)* and Cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs. (P T O)

2.5 mg protein was used for each assay. 2 mM glutamate and 4 mM malate were used as substrates to reduce matrix NAD * to NADH. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/ mg protein.

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/0 = ADP/O.

All the values are expressed relative to the control, which is taken as 100. The control value for state 3_{ADP} respiration, RCR and P/O ratio was 72 $\pm$ **7.9,** 4.32 $\pm$ 0.60 **and** 2.90 + 0.13 respectively.

Values are mean + SD for atleast 13 animals. *p 0.05, **p 0.01, ***p 0.001

TABLE 2 : Effect of anticancer drugs on Succinate oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1. Control.	100 + 9.1	100 + 7.5	100 + 3.0
2. Cisplatin + Cyclophosphamide.	59 + 2.0***	68 +_1.8**	60 + 3.0***
3. Mitomycin C + Cyclophosphamide.	56 ± 5.0***	67 + 10**	70 ± 5.0**
4. Mitomycin C + Cisplatin.	74 + 7.2	70 ± 4.2**	67 + 4.0**
5. Methotrexate + Vincristine.	52 +.2.6***	55 ± 2.5***	56 + 5.0***
6. Methotrexate + Cyclophosphamide.	88 + 5.1	82 + 2.6	72 + 5.0*

All the experimental details are same as given for TABLE 1.

2.5 mg protein was used for each assay. 9 m M succinate was used as substrate. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/mg protein.

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = ADP/O.

All the values are expressed relative to the control, which is taken as 100. The State 3 $_{ADP}$ respiration, RCR and ADP/O ratio were 120 $\pm$ 11, 4.1 + 0.31 and 1.85 + 0.056 respectively.
Values are mean $\pm$ SD for atleast 13 animals. *$p<0.05$, **$p< 0.01$, ***$p<0.00J$.

TABLE 3: Effect of anticancer drugs on the activities of NADH dehydrogenase and Succinate dehydrogenase in liver miochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1. Control.	100 +_ 8.8	100+ 13.6
2. Cisplatin + Cyclophosphamide.	135 ± 6.1***	160+ 15***
3. Mitomycin C + Cyclophosphamide.	136 ± 3.4*****	52+ 3.2***
4. Mitomycin C+ Cisplatin.	160 + 4.7***	58 + 5.0.***
5. Methotrexate + Vincristine.	58 ± 5.3***	54 + 5,0.***
6. Methotrexate + Cyclophosphamide.	70 + 1.6**	152+ 4.1***

All the experimental details are same as given for TABLE 1.

NADH Dehydrogenase assay : 20 μg of mitochondrial protein was used for each **assay** NADH (10 m M) was used as substrate. NADH dehydrogenase activity is expressed as NADH units / min / mg protein. (One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein)
Succinate Dehydrogenase assay 30 μg of mitochondrial protein was used for each assay Succinate (9 mM) was used as substrate SDH activity is expressed as SDH units / min / mg protein (One SDH Unit = One n mole of DCPIP reduced / min / mg protein)

Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2750 ± 243 and 146 + 20 respectively in liver mitochondria.

Values are mean ±_SD for atleast 9 animals. * *p 0.05,** p 0.0J,***p 0.00/.*

TABLE 4 : Effect of anticancer drugs on the activities of NADH dehydrogenase and Succinate dehydrogenase in kidney mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1 Control.	100 + 10.5	100+ 13
2. Cisplatin + Cyclophosphamide.	127 + 7.6*	130 + 12**
3. Mitomycin C + Cyclophosphamide	155 ± 7.7***	90 + 8.2.
4. Mitomycin C + Cisplatin.	142 + 3.9***	70 + 6.3**
5. Methotrexate + Vincristine.	70 ± 4.7**	67 + 5.1**
6 Methotrexate + Cyclophosphamide.	90 ± 4 1.	54 + 4 0. ***

All the experimental details are same as given for TABLE 1.

NADH Dehydrogenase assay: 20 μg mitochondrial protein was used for each assay NADH (10 m M) was used as substrate NADH dehydrogenase activity is expressed as NADH units / min / mg protein.(One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein).
Succinate Dehydrogenase assay: 30 μg was used for each assay. Succinate (5 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein (One **SDH** Unit = One n mole of DCPIP reduced / min / mg protein).
Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2030 ± 214 and 107 + 14 respectively in kidney mitochondria.

Values are mean ± SD for atleast 7 animals. * *p 0.05*, * * *p 0.01*,** **p 0.001*

TABLE 5: Effect of anticancer drugs on respiratory chain enzymes of liver mitochondria.

Group	NADHxytochrome c reductase	Succinatexytochrome c reductase	Cytochrome *c* Oxidase
1. Control.	100 ± 9.4	100 +_13	100 + 12.5
2. Cisplatin + Cyclophosphamide.	66+_5.1**	50 ± 5.7***	47+ 3.2***
3. Mitomycin C + Cyclophosphamide	69+ 6.7**	55 ± 4.0***	52+ 2.9***
4. Mitomycin C + Cisplatin.	70 + 5.5**	65+ 10**	64+ 3.1***
5. Methotrexate + Vincristine.	65 ± 5.5***	46 + 12***	57+ 5.8***
6. Methotrexate + Cyclophosphamide.	68 ± 6.3**	72 + 8.5*	152 + 5.2***

All the experimental details are same as given for TABLE 1.

NADH:Cytochrome *c* reductase : 20 ug of mitochondrial protein was used for each assay. Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 254 ± 24.

Succinate:Cytochrome *c* reductase: 20 ug of mitochondrial protein was used for each assay . Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which is taken as 100 . The control value is 49 ± 6.4.

Cytochrome *c* Oxidase: 1 ug of mitochondrial protein was used for each assay . Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of Cytochrome *c* oxidised / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 1138 + 134.

Values are mean + SD for atleast 6 animals. *$p< 0.05$, **$p<0.01$, ***$p<0.001$

TABLE 6: Effect of anticancer drugs on respiratory chain enzymes of kidney mitochondria.

Group	NADH: cytochrome c reductase	Succinate: cytochrome *c* reductase	Cytochrome *c* Oxidase
1. Control.	100 + 4.9	100 ± 13	100 + 9.7
2. Cisplatin + Cyclophosphamide.	74 + 6.9*	54 + 8.6***	46 + 5.8***
3. Mitomycin C + Cvclophosphamide.	73 + 4.2*	53 ± 16***	59 + 2.4***
4 Mitomycin C + Cisplatin.	63 + 1,4***	71 + 14*	66 + 5.4***
5. Methotrexate + Vincristine.	79 + 4.9	58 ± 11***	75 + 3.6*
6. Methotrexate + Cyclophosphamide	82 + 4.1	74 + 8.6*	134 ± 8.8***

All the experimental details are same as given for TABLE 1.

NADH:Cytochrome *c* reductase: 20 µg of mitochondrial protein was used for each assay . Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 262 ± 13

Succinate:Cytochrome *c* reductase: 20 µg of mitochondria! protein was used for each assay. Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken as 100 The control value is 33 + 4.0.

Cytochrome *c* Oxidase: 1 µg of mitochondrial protein was used for each assay Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of Cytochrome *c* oxidised / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 884 + 86.

Values are mean ± SD for atleast 8 animals. *p 0.05. **p 0.01, ***p 0.001

TABLE 7: Effect of anticancer drugs on free radical production.

Group	Liver homogenate	kidney homogenate
1.Control.	100 + 8.0	100 + 7.4
2. Cisplatin + Cyclophosphamide.	148 + 4.0***	143 + 4.8**
3. Mitomycin C +Cyclophosphamide.	145 ± 4.0***	135 + 6.3*
4. Mitomycin C+ Cisplatin.	175 + 6.9***	168 + 8.0***
5. Methotrexate +Vincristine.	256 + 14***	209 + 8.0***
6 Methotrexate + Cyclophosphamide.	176 ± 10***	145 ± 6.4***

All the experimental details are same as given for TABLE 1.
20 mg of lyopilized samples of liver and kidney were **used** for **ESR** spectrophotometry, for the detection of free radicals.
Signal intensity was determinined by calculating the area under the peak. Results are expressed relative to control which is taken as 100.

Values are mean ± SD for 8 animals. *p 0.05, **p 0.01, ***p 0.001

TABLE 8 : Effect of anticancer drugs on the lipid peroxide levels

Group	Liver homogenate	Kidney homogenate	Liver mitochondria
1.Control.	100 + 8.4	100 ± 12.7	100 +2.7
2. Cisplatin + Cyclophosphamide.	144 + 5.1**	136 + 5.2	135 ± 9.1
3. Mitomycin C + Cyclophosphamide.	183 + 8.6**	123 ± 4.3	178 + 9.8***
4. Mitomycin C + Cisplatin.	134 + 7.5*	134+ 7.8**	148 + 6.0*
5. Methotrexate + Vincristine.	236 + 11.5**	230 + 8.3**	231 + 18***
6. Methotrexate + Cyclophosphamide.	191 + 17.0***	132 + 15.4**	171 + 13*

All the experimental details are same as given for TABLE 1.
2 mg of protein was used for each assay Tetramethoxypropane was used as a external standard Lipid peroxide level is expressed as n moles MDA formed/ 100 mg protein Results are expressed relative to control which is taken as 100. The control **values** are 139 ± 11.7, 149 ± 19 and 76 + 2.1 for liver homogenate, **kidney homogenate and liver** mitochondria respectively

Values are mean ±_SD for atleast 10 animals. *p 0.05, **p 0.01, ***p 0.001

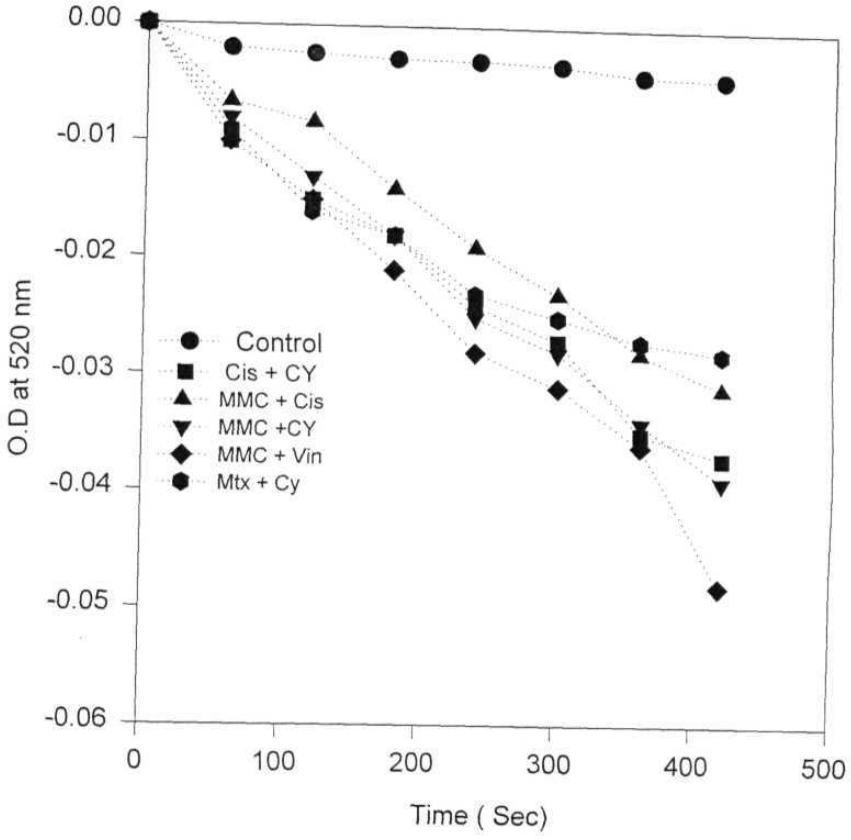
TABLE 9 : Effect of anticancer drugs on the phospholipid composition of liver mitochondria.

Group	Total Phospholipids	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Cardiolipin
1. Control.	100 + 7.5	100 ± 6.25	100 + 4.6	100 + 5.2
2. Cisplatin + Cyclophosphamide.	87 ± 5.0	68 ± 2.8**	69 ± 3**	69 ± 4.7**
3. Mitomycin C + Cyclophosphamide.	70 + 3.7*	60 ± 6.2**	79 + 8.0	64 ± 3.2***
4. Mitomycin C + Cisplatin.	55 + 3.0***	71 + 12	100 ± 4.2	52 ± 5.2***
5. Methotrexate + Vincristine.	163 ± 5.4***	146 ± 9.3**	111 ± 4.5	42 ± 9.2***
6. Methotrexate + Cyclophosphamide.	62 ± 3.7***	56 ± 3.1***	86 ± 4.3	65 ± 10***

All the experimental details are same as given for TABLE 1.
Phospholipids were separated by TLC using chloroform : methanol : water as solvent. 200 ul of the lipid extract was applied as a streak . The separated phospholipids were scraped out with gel from TLC plate, and then digested with 60% perchloric acid The inorganic phosphorus was estimated according to Fiske and Subba row method. Phospholipids are expressed as µg phospholipid Phosphorus /gm tissue. Results are expressed relative to control which is taken as 100. The control values are 24 ± 1.8, 32 ± 2.0, 26 + 1.2 and 19 ± 1.0 for total phospholipids, phosphatidylcholine, phosphatidylethnolamine and cardiolipin content respectively.

Values are mean + SD for atleast 10 animals. *$p < 0.05$, **$p<0.01$, ***$p<0.001$

Fig 1: Effect of anticancer drugs on swelling of mitochondria.



Swelling of mitochondria:
All the experimental details are same as given for TABLE 1
Mitochondria was suspended (40 mg /ml) in a buffered medium Respiration was initiated by addition of 50 m mol succinate and the decrease in OD was followed at 520 nm.

TABLE 10: Protective effect of *B.aristata* against the effects induced by **anticancer** drugs on NADH oxidase of liver mitochondria

Group	State 3	RCR	P/O ratio
1 Control.	100 + 11	100 ± 14	100 ± 4 4
2 *B.aristata.*	107 ± 7.6	103 + 10	104 + 3 7
3 *B.aristata* + (Cisplatin + Cyclophosphamide).	101 + 10.7	112 + 12	84 + 2 4
4 *B.aristata* + (Mitomycin C+ Cyclophosphamide).	92 + 12	100 + 7.8	87 + 7
s *B.aristata* +(Mitomycin C + Cisplatin).	90 + 11.2	93 + 9.2	96 + 1 5
B.aristata + (Methotrexate Vincristine).	82 + 4.4	90 + 8.5	98 + 1 6
7 *B.aristata* + (Methotrexate + Cyciophosphamide).	92 + 5.7	100+ 14	90 + 3 4

1. Control group recieved saline.
2. *B.aristata* group received alcoholic extract of *B.aristata* (10 mg / 100 g body wt. oral) **for** three days
3. *B.aristata* + (Cisplatin + Cyclophosphamide) group received the alcoholic extract of *B.aristata* for three days and then received a single dose of cisplatin (12mg/kg. *i.p)* and cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs.
4. ***B.aristata*** + (Mitomycin C + Cyclophosphamide) group received the alcoholic extract of *B.aristata* for three days and then received a single dose **of mitomycin** (' (2 mg/kg. *i.p)* and cyclophosphamide (150 mg /kg, oral). Rats were sacrificed 48 hr after the administration of the drugs
5. ***B.aristata*** + (Mitomycin C+ Cisplatin) group received the alcoholic extract of *B.aristata* for three days and then received a single dose of mitomycin C (2 mg/kg. *i.p)* and cisplatin (12 mg/kg, *i.p).* Rats were sacrificed 48 hr after the administration of the drugs.
6. *B.ansiata* + (Methotrexate + Vincristine) group received the alcoholic extract of *B.ansiata* for three days and then received a single dose of methotrexate (2mg/kg. *i.p)* and vincristine (1 mg/kg, *i.p).* Rats were sacrificed 72 hr after the administration of the drugs.
7. *B.aristata* + (Methotrexate + Cyclophosphamide) group received the **alcoholic** extract of *B.aristata* for three days and then received a single dose **of methotrexate** (2mg/kg, ***i.p)*** and cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs. (P.T 05)

2.5 mg protein was used for each assay. 2 mM glutamate and 4 raM malate were used as substrates to reduce matrix NAD^+ to NADH. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/ mg protein.

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = ADP/O.

All the values are expressed relative to the control, which is taken as 100. The control value for state 3_{ADP} respiration, RCR and P/O ratio was 72 ± 7.9, 4.32 ± 0.60 and 2.9 + **0.13** respectively.

Values are mean + SD for atleast 13 animals.

TABLE 11 : Protective effect of *B.aristata* against the effects induced by anticancer drugs on succinate oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1 Control.	100 + 9.1	100 +.7.5	100 + 3.0
2. *B.aristata.*	107 + 5.9	105 + 5.7	105 + 5.7
3. *B.aristata* + (Cisplatin + Cyclophosphamide).	94 + 4.2	109 ± 13	87+ 2.10
4. *B.aristata* + (Mitomycin C+ Cyclophosphamide).	95 + 6.6	105 + 10.7	92 + 1.9
5. *B.aristata* +(Mitomycin C + Cisplatin)	92 + 10	94 + 8.5	84 + 1 1
6 *B.aristata* + (Methotrexate+ Vincristine).	93 + 7.4 .	9 2 + 1 1	105 + 2 16
7. *B.aristata* + (Methotrexate + Cyclophosphamide).	90 +2.4	95+ 6.8	93 + 5.2

All the experimental details are same as given for TABLE 10

2.5 mg protein was used for each assay. 9 m M succinate was used as substrate. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/mg protein.

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = ADP/O

All the values are expressed relative to the control, which is taken as 100. The State 3 ADP respiration. RCR and ADP/O ratio were 120 ± 11, 4.1 + 0.31 and **1.85 ± 0.056** respectively.

Values are mean + SD for atleast 8 animals.

TABLE 12 : Protective effect of *B.aristata* against the effects induced by **anticancer** drugs on NADH dehydrogenase and succinate dehydrogenase of liver mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1 Control.	100 +.8.8	100+ 13.6
2. *B.aristata.*	106 + 3.8	108 + 7.3
3. *B.aristata* + (Cisplatin + Cyclophosphamide).	110 +.5.2	120 + 14
4. *B.aristata* + (Mitomycin C + Cyclophosphamide).	95 + 3.1	106 + 12
5.*B.aristata*+(Mitomycin C + Cisplatin).	125 ± 4.0	**94 ± 6.8**
6.*B.aristata*+ (Methotrexate Vincristine).	82 + 4.0	113 + 4.2
7. *B.aristata* + (Methotrexate + Cyclophosphamide).	88+ 1.1	105 ± 2.0

All the experimental details are same as given for TABLE 10

NADH Dehydrogenase assay : 20 µg of mitochondrial protein was used for each assay NADH (10 mM) was used as substrate. NADH dehydrogenase activity is expressed as NADH units / min / mg protein. (One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein).
Succinate Dehydrogenase assay : 30 |ig of mitochondrial protein was used **for** each assay Succinate (9 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein. (One SDH Unit = One n mole of DCPIP reduced / **min** / mg protein)

Results are expressed relative to control which is taken as 100 The control values of NADH dehydrogenase and Succinate dehydrogenase are 2750 ± 243 and 146 + 20 respectively in liver mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 13 : Protective effect of ***B.aristata*** against the effects induced by anticancer drugs on NADH dehydrogenase and succinate dehydrogenase of kidney mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1. Control.	100 + 10.5	100 ± 13
2 *B.aristata*.	105 ± 3.7	99 + 10
3. *B.aristata* + (Cisplatin + Cyclophosphamide).	107 ± 11	88 + 19
4. ***B.aristata*** + (Mitomycin C + Cyclophosphamide).	91 + 4.1	98 + 2.0
5.*B.aristata* + (Mitomycin C + Cisplatin).	112 + 7.3	93 + 5.6
6. *B.aristata* + (Methotrexate + Vincristine).	91 ± 4.5	112 + 6.0
7. *B.aristata* + (Methotrexate + Cyclophosphamide).	95 + 2.3	93 + 3.7

All the experimental details are same as given for TABLE 10.

NADH Dehydrogenase assay: 20 μg mitochondrial protein was used for each assay NADH (10 m M) was used as substrate . NADH dehydrogenase activity is expressed as NADH units / min / mg protein.(One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein)
Succinate Dehydrogenase assay: 30 μg was used for each assay. Succinate (5 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein (One SDH Unit = One **n** mole of DCPIP reduced / min / mg protein).
Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2030 ± 214 and 107 ± 14 respectively in kidney mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 14: Protective effect of *B.aristata* **against the effects induced by anticancer** drugs on respiratory chain enzymes of liver mitochondria.

Group	NADH: cytochrome *c* reductase	Succinate:cytochrome *C* reductase	Cytochrome *c* **Oxidase**
1. Control.	100 ± 9.4	100 + 13	100 + 12.5
2. B.aristata	95 + 9.0	112 + 10	108 ± 7.6
3. *B.aristata* + (Cisplatin + Cyclophosphamide).	88 + 2.9	104 +_ 5.9	81 + 14
4. *B.aristata* + (Mitomycin C + Cyclophosphamide).	95 + **5.7**	88 + 4.0	95 + 2.0
5. *B.aristata* + (Mitomycin C + **Cisplatin).**	**95 + 4.7**	116+ 8.1	92 + 8.6
6. fi.nWsMM+(Metliotrexate Vincristine).	93 + 9.8	88 + 13	91 + 6.0
7.*B.aristata* + (Methotrexate + Cyclophosphamide).	93 + **5.12**	83 + 3.2	120 + 3.4

All the experimental details are same as **given** for TABLE 10.

NADH:C'ytoehrome *c* reductase : 20 µg of mitochondrial protein was used for each assay. Activity is expressed as **n** moles of Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken **as** 100. The control **value** is 254 ±24.

Succinate:Cytochrome *c* reductase: 20 µg of mitochondrial protein was used for each assay Acti\ity is expressed as **n** moles of Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken as 100 The control **value** is **4"** + **6.4.**

Cytochrome *c* Oxidase: 1 µg of mitochondrial protein was used for each assav **Reduced** Cytochrome *c* was used as substrate Cvtochrome *c* Oxidase activity is expressed as **n** moles of cvtochrome *c* oxidised / **min** / mg protein Results are expressed **relative** to control which is taken as 100. The control value is 1070 + 134
Values are mean + SD for atleast 6 animals.

TABLE 15: Protective effect of ***B.aristata*** against the effects induced by anticancer drugs on respiratory chain enzymes of kidney mitochondria.

Group	NADH: cytochrome c reductase	Succinate:cytochrome *c* reductase	Cytochrome *C* Oxidase
1. Control.	100 + 4.9	100 ± 13	100 + 9.7
2. *B.aristata.*	97 + 4.9	101 ± 13	108 ± 7.0
3. ***B.aristata*** **+ (Cisplatin** + Cyclophosphamide).	109+ 9.1	85 ± 9.1	85 ± 4.8
4. *B.aristata* + (Mitomycin C + Cyclophosphamide).	88+ 3.6	92 + 12	88 + 5.0
5.B.nrislata +(Mitomycin C + Cisplatin).	91 + 9.9	88 + 10	96 + 10.8
6. *B.aristata* + (Methotrexate + Vincristine).	91 + 5.7	117 + 3.4	96 + 7.5
7. *B.aristata*+ (Methotrexate + Cyclophosphamide).	85 + 5.7	81 + 5.8	108 ± 4.0

All the experimental details are same as given for TABLE 10.

NADH Cytochrome *c* reductase: 20 µg of mitochondrial protein was used **for** each assay Activity is expressed as n moles **of** Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken as 100. The control value is 262 ± 13

Succinate:Cytochrome *c* reductase: 20 µg of mitochondria! protein was used **for** each assay. Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken as 100 The control value is 33 ± 4.0.

Cytochrome *c* Oxidase: 1µg of mitochondrial protein was used for each assay Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of Cytochrome *c* oxidised / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 884 + 86.

Values are mean + SD for atleast 8 animals.

TABLE 16: Protective effect of *B.aristata* against the lipid peroxides induced by the anticancer drugs.

Group	Liver homogenate	Kidney homogenate	Liver **mitochondria**
1.Control.	100 + 8.4	100 ± 12.7	100 **+2.7**
2. *B.aristata.*	96 + 5.7	117 + 6.7	117 ± 9.2
3. *B.aristata* + (Cisplatin + Cyclophosphamide).	122 +5.0	114 + 3.6	113 + 6 3
4. *B.aristata* + (Mitomycin C + Cyclophosphamide).	112 + 5.0	107 + 7.4	132+ 8.7
5. *B.aristata* + (Mitomycin C + Cisplatin).	93 + 9.6	110 + 4.8	107 + 6 6
6. *B.aristata* + (Methotrexate **+ Vincristine).**	128 + 4.3	127 + 4.0	142 + 17
7 *B.aristata* + (Methotrexate + Cyclophosphamide).	127 ± 2.4	100 + 4.6	107 *+12*

All the experimental details are same as given for TABLE 10.

2 mg of protein was used for each assay Tetramethoxypropane was used as a external standard Lipid peroxide level is expressed as n moles MDA formed/ 100 mg protein Results are expressed relative to control which is taken as 100. The control values are 139 ± 11.7, 149 ± 19 and 76 + 2.1 for liver homogenate . kidney homogenate and liver mitochondria respectively.

Values are mean + SD for atleast 10 animals

TABLE 17: Protective effect of *B.aristata* against the effects induced by the **anticancer** drugs on phospholipid composition of liver mitochondria.

Group	Total Phospholipids	Phosphatidyl-choline	Phosphatidyl ethanolamine	Cardiolipin
1 Control.	100 + 7.5	100 ± 6.25	100 + 4.6	100 + 5.2
2 ***B.aristata,***	100 + 12.5	103 + 3.7	88 **+.4.5**	92 + 10.5
3 *B.aristata* + (Cisplatin + Cyclophosphamide).	97 ± 7.5	118 + 5.9	113 + 4.0	115 + 3.8
4.*B.aristata* + (Mitomvcin C + Cyclophosphamide).	87 + 5.0	104 + 5.6	107 + 3.8	88 + 6.2
5.*B.aristata* + (Mitomvcin C **+ Cisplatin).**	98 + 6.2	94 + 5.64	94 + 5.4	96 ± 6.3
6 *B. aristata* + (Methotrexate + Vincristine).	85 ± 8.3	102 ± 6.0	99 + 6.0	88 + 3.1
7 *B.aristata* + (Methotrexate + Cyclophosphamide).	100 + 7.5	**87** ± 5.7	96 + 4.6	78 + 3.64

All the experimental details are same as given for TABLE 10.
Phospholipids were separated by TLC using chloroform : methanol : water as solvent 200 ul of the lipid extract was applied as a streak . The separated phospholipids were scraped out with gel from TLC plate, and then digested with 60% perchloric acid The inorganic phosphorus was estimated according to Fiske and Subba row method Phospholipids are expressed as |ig phospholipid Phosphorus /gm tissue. Results are expressed relative to control which is taken as 100 The control values are 24 ± 1.8, 32 ± 2 0, 26 + 1.2 and 19 ± 1.0 for total phospholipids, phosphatidylcholine, phosphatidylethnolamine and cardiolipin content respectively.

Values are mean + SD for atleast 10 animals

TABLE 18: Protective effect of *P.fratermus* against the effects induced by **anticancer** drugs on NADH oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1 Control	100 + 11	100 ± 14	100 ±4 4
2 *P.fratenms.*	108 + 7.3	102 + 6.4	100 ± 3 3
3 *P.fratermus* + (Cisplatin + Cyclophosphamide).	96 + 4.0	97 + 6.2	92 + 4
4 ***P.fratenms*** +(Mitomycin C + Cyclophosphamide).	78 + 3.6	101 + 1.4	86 ± 1 0
5 ***P.fratenms*** + (Mitomycin C + Cisplatin)	92 + 2.7	108 + 7.35	116 ± 6 6
6 ***P.fratenms*** + (Methotrexate + Vincristine).	82 + **4.16**	102 + 10	90 + 1 4
7 *P. fratermus* + (Methotrexate + Cyclophosphamide).	103 + 16	97 + 1.65	90 ± 3 8

1 Control group recieved saline.

2. *P.fratermus*group received **aqueous** extract of *P.fratermus*(10 mg / 100 g body wt, oral) for three days.

3. ***P.fratenms*** + (Cisplatin + Cyclophosphamide) group received the aqueous extract of *P.fratermus* for three days and then received a single dose of cisplatin (12 mg/kg, *(i.p)* and Cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr **after the** administration of the drugs

4 ***P.fratenms*** + (Mitomycin C + Cyclophosphamide) group received the aqueous extract of ***P.fratenms*** for three days and then received a single dose of mitomycin **C** (2 mg/kg, ***i.p)*** and cyclophosphamide (150 mg /kg, oral). Rats were sacrificed 48 hr after the administration of the drugs

5. ***P.fratenms*** + (Mitomycin C+ Cisplatin) group received the aqueous extract **of** ***P.fratenms*** for three days and then received a single dose of mitomycin C (2mg/kg, *i.p)* and cisplatin (12 mg/kg, *i.p).* Rats were sacrificed 48 hr after the administration of the drugs.

6. ***P.fratenms*** + (Methotrexate + Vincristine) group received the aqueous extract of *P.fratermus* for three days and and then received a single dose of methotrexate (2mg/kg, ***i.p)*** and vincristine (1 mg/kg, *i.p*) Rats were sacrificed 72 hr after the administration of the drugs (f T·O)

7. *P.fraternus* + (Methotrexate + Cyclophosphamide) group received the aqueous extract of *P.fraternus* for three days then received a single dose **of** methotrexate (2mg/kg, I.P.) and cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs.

2.5 mg protein was used for each assay. 2 mM glutamate and 4 mM malate were used as substrates to reduce matrix NAD^+ to NADH. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/ mg proteia

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = ADP/O

All the values are expressed relative to the control, which is taken as 100. The control value for state 3_{ADP} respiration, RCR and P/O ratio was 72 $\pm$ 7.9, 4.32 $\pm$ 0.60 **and** 2.9 + **0.13** respectively.

Values are mean + SD for atleast 8 animals.

TABLE 19: Protective effect of ***P.fratermis*** against the effects induced by anticancer drugs on Succinate oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1. Control	100 + 9 1	100 ± 7.5	100 + 3.0
2. *P.fratermis.*	97 ± 6.7	96 + 7.6	108 ± 3.0
3. *P.fratermis* + (Cisplatin + Cyclophosphamide).	89 + 3.2	102 ± 16	102 ± 3.7
4 *P.fratermis*+ (Mitomycin C + Cyclophosphamide).	98 + 1.6	97 + 8.2	99 + 2.9
5. *P.fratermis* + (Mitomycin C + Cisplatin).	108 + **6.5**	94 + **1.03**	116 + **5.5**
6. ***P.fratermis* +** (Methotrexate + Vincristine).	77 + 9.0	92 + 1.24	84 + **2.16**
7. ***P.fratermis*** +(Methotrexate + Cyclophosphamide).	105 ± 8.0	92 ± 2.0	89 + 1 84

All the experimental details are same as given **for** TABLE 18.

2.5 mg protein was used for each assay. 9 m M succinate was used as substrate. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/mg proteia

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) **respiration** and P/O = ADP/O.
All the values are expressed relative to the control, which is taken as 100. The State 3 and respiration. RCR and ADP/O ratio were 120 ± 11, 4.1 ± 0.3 1 and 1 85 + **0.056** respectively.
Values are mean + SD for atleast 8 animals.

TABLE 20 : Protective effect of ***P.fratermis*** against the effects induced by **anticancer** drugs on NADH dehydrogenase and Succinate dehydrogenase of liver mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
!. Control.	100 + . 8.8	100 + 13.6
2. *P.fratermis.*	107 + 6.0	108 + 8.2
3. ***P.fratermis*** + (Cisplatin + Cyclophosphamide).	91 +_ 3.4	93 +_ 7.5
4. *P.fratermis*+ (Mitomycin C + Cyclophosphamide).	% + 3.2	109 +_ 1.1
5. *P.fratermis* + (Mitomycin C + Cisplatin).	92 +_ 2.6	96 **+.7.5**
6. *P.fratermis* + (Methotrexate + Vincristine).	82 + 3.2	113 **+.6.2**
7. ***P.fratermis*** + (Methotrexate Cyclophosphamide)	88 + 3.2	112 + 8.2

All the experimental details are same as given **for** TABLE 18.

NADH Dehydrogenase assay : 20 μg of mitochondrial protein was used **for** each **assay** NADH (10 m M) was used as substrate. NADH dehydrogenase activity is expressed as NADH units / min mg protein. (One NADH Unit = One n mole of Potassium ferricyanide reduced **min** / mg protein).

Succinate Dehydrogenase assay : 30 μg of mitochondrial protein was used **for** each assay. Succinate (9 mM) was used as substrate. SDH activity is expressed as SDH units / **min** / mg protein. (One SDH Unit = One n mole of DCPIP reduced / min / mg protein)

Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2750 +_ 243 and 146 + 20 respectively in liver mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 21 Protective effect of ***P.fratermis*** against the effects induced by anticancer drugs on NADH dehydrogenase and Succinate dehydrogenase of kidney mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1 Control.	100 + 10.5	100 + 13
2. P.fratermis.	108+ 4.7	93 + 10.2
3. ***P.fratermis*** + (Cisplatin + Cyclophosphamide).	72 +_ 2.9	86 + 7.4
4. *P.fratermis*+ (Mitomycin C + Cyclophosphamide).	101 + 2.6	96 + 9.4
5. ***P.fratermis*** + (Mitomycin C + Cisplatin).	105 +_ 2.6	90 + 9.3
6. ***P.fratermis*** **+ (Methotrexate** + Vincristine).	91 + 8.3	112 ± **5.8**
7. ***P.fratermis*** + (Methotrexate Cvclophosphamide).	106+ 6.15	83 + 2.7

Ail the experimental details are same as given for TABLE 18.

NADH Dehydrogenase assay: 20 |ig mitochondrial protein was used for each assay NADH (10 m M) was used as substrate . NADH dehydrogenase activity is expressed as NADH units / min / mg protein.(One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein)

Succinate Dehydrogenase assay: 30 μg was used for each assay. Succinate (5 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein (One SDH **Unit** = One **n** mole of DCP1P reduced / **min** / mg protein).

Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2030 +_ 214 and 107 + 14 respectively in kidney mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 22: Protective effect of *P.fraternus* against the effects induced by **anticancer** drugs on respiratory chain enzymes of liver mitochondria.

Group	NADHxytochrome c reductase	Succinatexytochrome . c reductase	Cytochrome c **Oxidase**
1. Control	100 + 9.4	100 ± 13	100 + 12.5
2. *P.fraternus*	111 + 4.3	101 ± 9.6	**109 + 7.i**
3. *P.fraternus* + (Cisplatin + Cyclophosphamide)	116 + 7.3	90 ± 9.1	**90 + 8.0**
4. *P.fraternus* + (Mitomycin C + Cyclophosphamide)	87 + 5.3	94 + 12	95 + 6.2
5. *P.fraternus* + (Mitomycin C + Cisplatin)	107 + 4.2	119 + 8.0	110 ± 3.0
6.*P.fraternus* + (Methotrexate + Vincristine)	85 ± 8.0	94 + 7.0	98 + 6
7.*P.fraternus* + (Methotrexate + Cyclophosphamide)	86 + 3.7	79 + 5.4	103 +4.0

All the experimental details are same as given for TABLE 18.

NADH:Cytochrome *c* reductase : 20 μg of mitochondrial protein was used for each assay Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken as 100. The control value is 254 + 24.

Succinate:Cytochrome *c* reductase: 20 ug of mitochondrial protein was used for each assay . Activity is expressed as n moles of Cytochrome c reduced / min / mg protein Results are expressed relative to control which is taken as 100 The control value is 49 + 6.4.

Cytochrome *c* Oxidase: 1 |ig of mitochondrial protein was used for each assay Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of cytochrome *c* oxidised / min / mg protein Results are expressed relative to control which is taken as 100. The control value is 1070 + 134

Values are mean ± SD for atleast 6 animals.

TABLE 23: Protective effect of *P.fraternus* against the effects induced by anticancer drugs on respiratory chain enzymes of kidney mitochondria.

Group	NADHxytochrome *c* reductase	Succinatexytochrome *c* reductase	Cytochrome *c* Oxidase
1. Control.	100 + 4.9	100 ± 13	100 + 9.7
2. *P.fraternus.*	91 + 4.0	101 + 9.1	110 ± 4.0
3. *P.fraternus* + (Cisplatin + Cyclophosphamide).	95 + 7.3	85 ± 9.1	90 + 12
4. *P.fraternus*+(Mitomycin C + Cyclophosphamide).	93 + 5.3	89 + 12	88 + 4.4
5. *P.fraternus* + Mitomycin C + Cisplatin).	83 + 4.2	115 + 12	119 + 3.2
6.*P.fraternus* + (Methotrexate + Vincristine).	91 + 8.0	117 + 5.4	96 + 7.6
7. *P.fraternus* + (Methotrexate + Cyclophosphamide).	103 + 3.7	89 + 6.4	114 +2.8

All the experimental details are same as given for TABLE 18

NADH:Cytochrome *c* Reductase: 20 ug of mitochondrial protein was used for each assay . Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 262 ± 13.

Succinate:Cytochrome *c* Reductase: 20 ug of mitochondrial protein was used for each assay. Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which is taken as 100 .The control value is 33 + 4.0.

Cytochrome *c* Oxidase: 1μg of mitochondrial protein was used for each assay . Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of Cytochrome *c* oxidised / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 884 + 86.

Values are mean + SD for atleast 8 animals.

TABLE 24: Protective effect of ***P.fratemus*** against free radical generation induced by anticancer drugs.

Group	Liver homogenate	Kidney homogenate
1. Control	100 ±1 6.2	100 ± 5.4
2. *P.fratemus*	89 4- 7.9	88 +, 8.2
3. *P.fratemus* + (Cisplatin + Cyclophosphamide)	110 j 9.9	118 ± 13
4. *P.fratemus*+ (Mitomycin C+ Cyclophosphamide)	118 ± 9.2	123 + 11
5. *P.fratemus* + (Mitomycin C + Cisplatin)	109 +- 6.8	108 + 10
6. *P.fratemus* +(Methotrexate + Vincristine)	145 ± 14	123 + 14
7. *P.fratemus* + (Methotrexate + **Cyclophosphamide)**	117 j 13	118 + 13

All the experimental details are same as given for TABLE 18
20 mg of lyopilized samples of liver and kidney were used for ESR spectrophotometry, for the detection of free radicals.
Signal intensity was determinined by calculating the area under the peak. Results are expressed relative to control which is taken as 100.

Values are mean + SD for 8 animals.

TABLE 25: Protective effect of ***P.fraternus*** against lipid peroxidation induced by anticancer drugs.

Group	Liver homogenate	Kidney homogenate	Liver mitochondria
1. Control.	100 + 8.4	100 ± 12.7	100 + 2.7
2. *P.fraternus.*	85 + 2.8	93 + 9.4	99 ± 17
3. ***P.fraternus*** + (Cisplatin + Cyclophosphamide).	116 + 2.8	90 ± 2.1	90 + 6.7
4. *P.fraternus*+ (Mitomycin C + Cyclophosphamide)	122 + 8.6	98 + 6.7	140 +6.6
5. ***P.fraternus*** + (Mitomycin C + Cisplatin).	113 + 10	101 + 8.1	108 + 6.5
6. *P.fraternus* + (Methotrexate + Vincristine).	130 + 6.5	110 **+.2.6**	111 + 11
7. ***P.fraternus*** + (Methotrexate Cyclophosphamide).	120 + 10.8	94 + 6.7	112 **+_6.5**

All the experimental details are same as given for TABLE 18.
2 mg of protein was used for each assay Tetramethoxypropane was used as a **external** standard. Lipid peroxide level is expressed as n moles MDA formed/ 100 mg protein. Results are expressed relative to control which is taken as 100. The control **values are** 139 ± 11.7, 149+ 19 and 76 + 2.1 for liver homogenate , kidney homogenate and liver mitochondria respectively.

Values are mean + SD for atleast 10 animals

TABLE 26: Protective effect of *P.fraternus* against the effects induced by anticancer drugs on phospholipid composition of liver mitochondria

Group	Total Phospholipids	Phosphatidyl - choline	Phosphatidyl- ethanolamine	Cardiolipin
1. Control.	100 j: 7.5	100 +.6.2	100 + 4.6	100 + 5.2
2. *P.fraternus.*	88 +: 5.2	81 + 5.0	73 + 3.7	84 ± 40
3. *P.fraternus* + (Cisplatin + Cyclophosphamide).	117 +- 4.3	123 ± 5.1	103 + 5.0	131 + 6 5
4. *P.fraternus*+(Mitomycin C + Cvcloplіosphamide).	87 j: 7.1	87+ 4.3	93 + 8.3	93 + 4.0
5. *P.fraternus* + (Mitomycin C + Cisplatin)	79 +: 5.5	103 + 6.0	100 + 7.5	89 ı s s
6.*P.fraternus*+(Methotrexate + Vmcristine).	111 i: 4.6	106 + 2.1	98 + 9.5	63 + 5 0
7. *P.fraternus* + (Methotrexate + Cvcloplіosphamide).	87 ±: 4.7	87 ±53	98 ± 9.3	84 ± 4 8

All the experimental details are same as given for TABLE 18.
Phospholipids were separated by TLC using chloroform : methanol : water as solvent 200 ul of the lipid extract was applied as a streak . The separated phospholipids were scraped out with gel from TLC plate, and then digested with 60% perchloric acid The inorganic phosphorus was estimated according to Fiske and Subba row method Phospholipids are expressed as ug phospholipid Phosphorus /gm tissue Results **are** expressed relative to control which is taken as 100 The control values are 24 ± 1.8, 32 ± 2.0, 26+ 1.2 and 19 ± 1.0 for total phospholipids, phosphatidylcholine, phosphatidylethnolamine and cardiolipin content respectively.

Values are mean ± SD for atleast 10 animals.

TABLE 27: Protective effect of ***C.majus*** against the effects induced by anticancer drugs on NADH oxidase of liver mitochondria.

Group.	State 3	RCR	P/O ratio
1 Control	100 + 11	100 ± 14	100 ± 4.4
2. ***C.majus.***	108 ± 6.4	102 + 6.2	100 + 8.9
3. *C.majus* + (Cisplatin + Cyclophosphamide).	100 + 7.6	112 + 11	93 **+3.4**
4 *C.majus* + (Mitomycin C + Cyclophosphamide).	**105** + 5.3	89 + 2.0	122 + 2.0
5. *C.majus* + (Mitomycin C + Cisplatin).	99 + 3	83 + 7.3	89 + 9.5
6 *C.majus* + (Methotrexate + Vincristine).	**93 +** 4.9	82 + 6.9	90 + 9.6
7 *C.majus* + (Methotrexate + Cvclophosphamide).	88 + 3.0	95 + **5.5**	80 + 3.0

1. Control group recieved saline
2. *C.majus* group received alcoholic extract of *C.majus* (**12.5** mg / 100 **g body** wt. oral) for three days.
3. *C.majus* + (Cisplatin + Cyclophosphamide) group received the alcoholic extract of *C.majus* for three days and then received a single dose of cisplatin (12mg/kg. *i.p)* and Cyclophosphamide (150 mg/kg, oral) Rats were sacrificed 24 hr after the administration of the drugs.
4. *C.majus* + (Mitomycin C + Cyclophosphamide) group received the alcoholic extract of *C.majus* for three days and then received a single dose of mitomycin C (2 mg/kg. *i.p)* and cyclophosphamide (150 mg /kg, oral). Rats were sacrificed 48 hr after the administration of the drugs
5. *C.majus* + (Mitomycin C+ Cisplatin) group received the alcoholic extract of *C.majus* for three days and then received a single dose of mitomycin C (2 mg/kg, *i.p)* and cisplatin (12 mg/kg, *i.p*). Rats were sacrificed 48 hr after the administration of the drugs.
6. *C.majus* + (Methotrexate + Vincristine) group received the alcoholic extract of *C.majus* for three days and and then received a single dose of methotrexate (2mg/kg. *i.p)* and vincristine (1 mg/kg, *i.p*). Rats were sacrificed 72 hr after the administration of the drugs. (P.T.O)

C.majus + (Methotrexate + Cyclophosphamide) group received the alcoholic extract of *C.majus* for three days then received a single dose of methotrexate (2mg/kg, *i.p*) and cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs.

2.5 mg protein was used for each assay. 2 mM glutamate and 4 mM malate were used as substrates to reduce matrix NAD $^{+}$ to NADH State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/ mg proteia

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = ADP/O

All the values are expressed relative to the control, which is taken as 100. The control value for state 3_{ADP} respiration, RCR and P/O ratio was **72** +_ 7.9, 4.32 $\pm$ 0.60 and **2.9** + **0.13** respectively.

Values are mean + SD for atleast 8 animals

TABLE 28 : Protective effect ***of C.majus*** against the effects induced by anticancer drugs on Succinate oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1 Control.	100 +_ 9.1	100 ± 7.5	100 ± 3 0
2 *C.majus.*	97 ± 3.0	97 + 4.0	108 + 3
3 ***C.majus*** + (Cisplatin + Cyclophosphamide)	86 + 5.5	81 ± 8.8	101 + 3 0
4. ***C.majus*** + (MitomycinC + Cyclophosphamide).	93 + 4.3	104+ **8.5**	117+ 2 38
5. *C.majus* + (Mitomycin C + Cisplatin).	90 + 2.8	100+ 5.7	94 + 2 7
6 *C.majus* + (Methotrexate + Vincristine)	103 + 3.0	108 + 7.3	92 + 1 6
7. ***C.majus*** + (Methotrexate + Cyclophosphamide).	97 ± 2 5	**115** + 2.9	103 ±_:.3

All the experimental details are same as given for TABLE 27.

2.5 mg protein was used for each assay. 9 m M succinate was used as substrate. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/mg proteia

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = AD P/O.
All the values are expressed relative to the control, which is taken as 100. The State 3 ADP respiration, RCR and ADP/O ratio were 120 ± 11, 4.1 + 0.31 and 1.85 + **0.056** respectively.

Values are mean + SD for atleast 8 animals.

TABLE 29: Protective effect of *C.majus* against the effects induced by anticancer drugs on NADH dehydrogenase and Succinate dehydrogenase of liver mitochondria..

Group	NADH dehydrogenase	Succinate dehydrogenase
1. Control.	100 ± 8 8	100+ 13.6
2. *C.majus.*	107 +_ 3.4	106+ 6.3
3 *C.majus*+ (Cisplatin + Cyclophosphamide).	108 ± 7.8	90+ 5.1
4. *C.majus*+ (Mitomycin C + Cyclophosphamide).	117 + 3.2	100 + 5.9
5. *C.majus*+(Mitomycin C + Cisplatin).	107 +_ 4.1	85 +4.5
6. *C.majus*+(Methotrexate + Vincristine).	86 + 2.8	88 + 2.0
7. *C.majus*+(Methotrexate + Cyclophosphamide).	83 + 2.8	97 +4.6

All the experimental details are same as given for TABLE 27.

NADH Dehydrogenase assay : 20 μg of mitochondrial protein was used for **each assay** NADH (10 m M) was used as substrate. NADH dehydrogenase activity is expressed as NADH units / min / mg protein. (One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein)

Succinate Dehydrogenase assay : 30 μg of mitochondrial protein was used **for** each assay. Succinate (9 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein. (One SDH Unit = One n mole **of** DCPIP reduced / min / mg protein)

Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2750 ± 243 and 146 + 20 respectively in liver mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 30: Protective effect of *C.majus* against the effects induced by anticancer drugs on NADH dehydrogenase and Succinate dehydrogenase of kidney mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1. Control	100 + 10.5	100 + 13
2. *C.majus*	108 + 3.0	106 + 4.5
3 *C.majiis* + (Cisplatin + Cyclophosphamide).	108 ± 8.4	94+ 3.3
4 *C.majus*+ (Mitomycin C + Cyclophosphamide).	122 + 4	94+ 7.1
5. *C.majiis* + (Mitomycin C + Cisplatin)	108 ± 2.3	87+ 1.4
6. *C.majiis* + (Methotrexate + Vincristine).	110 + 2.1	96+ 3.8
7 *C.majiis* + (Methotrexate + Cyclophosphamide)	105 + 1.52	95+ 10

All the experimental details are same as given for TABLE 27.

NADH Dehydrogenase assay; 20 µg mitochondrial protein was used **for** each assay NADH (**10** m **M) was used as** substrate . **NADH dehydrogenase** activity **is expressed** as NADH units / min / mg protein.(One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein).

Succinate Dehydrogenase assay: 30 µg was used for each assay. Succinate (5 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein (One SDH Unit = One n mole of DCPIP reduced / min / mg protein).

Results are expressed relative **to** control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2030 ± 214 and 107 - 14 respectively **in** kidney mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 3] : Protective effect of *C.majus* against the effects induced by anticancer drugs on respiratory chain enzymes of liver mitochondria

Group	NADHxytochrome *c* reductase	Succinatexytochrome . **c** reductase	Cytochrome c Oxidase
1. Control.	100 + 9.4	100 +_ 13	100 + 12.5
2 *C.majus.*	93 +_ 6.1	95 ± 4.1	96 **+.3.4**
3 *C.majus* +(Cisplatin + Cyclophosphamide).	95 + 3.4	84 ± 2.2	77 + 5.1
4 ***C.majus*** + (Mitomycin C + Cyclophosphamide).	89 + 3.1	84 + 5.5	88 + 7.4
5 *C.majus* +(Mitomycin C + Cisplatin).	89 + 3.0	88 + 4.0	92 + 4.1
6 *C.majus* + (Methotrexate + Vincristine).	93 + 2.3	88+13	91 +.6.6
7 *C.majus* + (Methotrexate + Cyclophosphamide).	91 + 2.3	87 + 1.20	112 + 2.0

All the experimental details are same as given for TABLE 27.

NADH:Cytochrome *c* reductase : 20 μg of mitochondrial protein was used for each assay Activity is expressed as n moles of Cytochrome **c** reduced / min / mg protein Results are expressed relative to control which is taken as 100. The control value is 254 ±24.

Succinate:Cytochrome *c* reductase: 20 μg of mitochondrial protein was used for each assay . Activity is expressed as n moles of Cytochrome **c** reduced / min / mg protein Results are expressed relative to control which is taken as 100 .The control value is 40 + 6.4.

Cytochrome ***c*** Oxidase: 1 μg of mitochondrial protein was used for each assay Reduced Cytochrome ***c*** was used as substrate Cytochrome ***c*** Oxidase activity is expressed as n moles of cytochrome **c** oxidised / min / mg protein Results are expressed relative to control which is taken as 100 The control value is 1070 + 134

Values are mean ±_SD for atleast 6 animals.

TABLE 32: Protective effect of *C.majus* against the effects induced by anticancer drugs on respiratory chain enzymes of kidney mitochondria.

Group	NADH: Cytochrome *c* reductase	Succinate:cytochrome c reductase	Cytochrome *c* Oxidase
1. Control	100 + 4.9	100 + 13	100 + 9 7
2. *C.majus.*	100 + 6.0	110 + 6.1	99 ± 4.0
3. *C.majus* + (Cisplatin + Cvclopliosphamide).	97 + 3.2	100 +.3.2	87 + 5.7
4. *C.majus* + (Mitomycin C + Cvcloplios phamide).	104 ± 2.6	84 + 12	86 + 4.5
5. *C.majus* + (Mitomycin C + Cisplatin)	100 ± 3.0	98 +6.1	93 + 5 0
6. *C.majus* + (Methotrexate + Vincristine).	96 + 2.6	97 + 6.9	95 + 4.1
7. *C.majus* + (Methotrexate + Cyclophosphamide).	95 + 5.3	90 + 7.4	99 + 10

All the experimental details are same as given for TABLE 27.

NADH: Cytochrome c reductase: 20 µg of mitochondrial protein was used for each assay . Activity is expressed as n moles **of** Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which **is** taken as 100 The control value is 262 ± 13

Succinate:Cytochrome c reductase: 20 µg of mitochondrial protein was used **for** each assay. Activity is expressed as n moles **of** Cytochrome *c* reduced / min / mg protein. Results are expressed relative to control which is taken as 100 The control value is 33 + 4.0

Cytochrome *c* Oxidase: 1µg of mitochondrial protein was used for each assay Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of Cytochrome *c* oxidised / **min** / mg protein. Results are expressed relative to control which is taken as 100. The control value is 884 + 86.

Values are mean + SD for atleast 8 animals.

TABLE 33 : Protective effect of *C.majus* against the lipid peroxidation induced by anticancer drugs.

Group	Liver homogenate	Kidney homogenate	Liver mitochondria
1 Control	100 + 8.4	100 ± 12.7	100 ± 2.7
:. *C.ma/us.*	96 + 8.6	117 ± 6.7	117 + 11.4
› ***C.majus*** + (Cisplatin + Cyclophosphamide)	106 + 7.0	1 16 ± 6.2	113 + 6.0
4 ***C.majus*** + (MitomycinC + Cyclophosphamide)..	135 + 5.7	106 + 4.7	132 + 10
s *C.majus* + (Mitomycin C + Cisplatin).	93 + 3.7	110 + 4.8	107 + 9.6
(» *C.majus* + (Methotrexate + Vincristine).	121 + 7.2	131 ± 6.1	132 + II
7 *C.majus* + (Methotrexate + Cyclophosphamide)	127 + 11	106 + 5.0	112 + 12

All the experimental details are same as given for TABLE 27
2 mg of protein was used for each assay. Tetramethoxypropane was used as a external standard Lipid peroxide level is expressed as n moles MDA formed/ 100 mg protein Results are expressed relative to control which is taken as 100 The control values are 139± 11.7, **149** ± 19 and 76 + 2.1 for liver homogenate , kidney homogenate and **liver** mitochondria respectively.

Values are mean + SD for atleast 10 animals

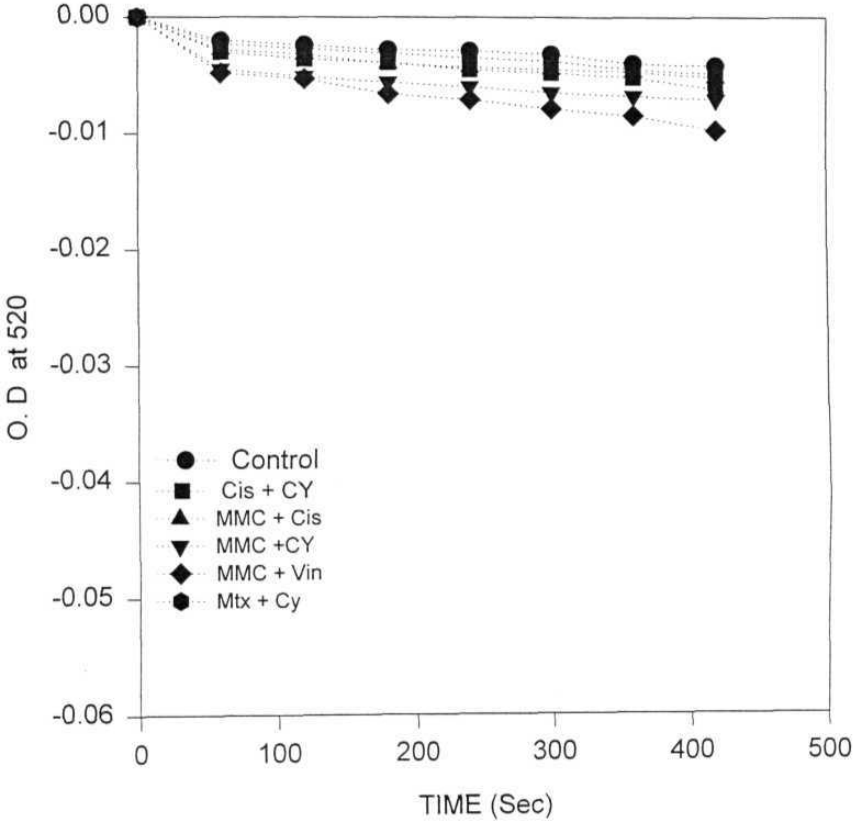
TABLE 34: Protective effect *of C.majus* against the effects induced by anticancer drugs on phospholipid composition of liver mitochondria.

Group	Total Phospholipids	Phosphatidyl - choline	Phosphatidyl-ethanolamine	Cardiolipin
1. Control	100 + 7.5	100 ± 6.25	100 + 4.6	100 ± **5.2**
2. *C.majus*	100 + 8.3	93 ± **8.9**	80 + 4.6	94 + 6.2
3. *C.majus* + (Cisplatin + Cyclophosphamide)	115 ± 12	93 ± **8.1**	92 ± 6.5	100 + 6.3
4. *C.majus* + (Mitomycin C + Cyclophosphamide)	90 + 10	88 ± **8.1**	92 ± 7.6	84 ± **6.8**
5. *C.majus* +(Mitomycin C + Cisplatin)	94 ± 6.9	94 ± **9.4**	112 + 8.4	90 ± 10
6. *C.majus*+(Methotrexate + Vincristine)	83 ± 8.3	105 ± 7.7	96 + 7.68	90 ± 10
7. *C.majus* +(Methotrexate + Cyclophosphamide)	87 + 6.2	102 + 9.8	84 + 7.2	84 + 6.3

All the experimental details are same as given for TABLE 27.
Phospholipids were separated by TLC using chloroform : methanol : water as solvent. 200 ul of the lipid extract was applied as a streak . The separated phospholipids were scraped out with gel from TLC plate, and then digested with 60% perchloric acid The inorganic phosphorus was estimated according to Fiske and Subba row method. Phospholipids are expressed as µg phospholipid Phosphorus /gm tissue. Results are expressed relative to control which is taken as 100. The control values are 24 ± 1.8, 32 ± 2.0, 26+1.2 and 19 ± 1.0 for total phospholipids, phosphatidylcholine, phosphatidylethnolamine and cardiolipin content respectively.

Values are mean ± SD for atleast 10 animals

Fig- 2: Protective effect of *C.majus* against mitochondrial swelling induced by anticancer drugs.



Swelling of mitochondria:

All the experimental details are same as given for TABLE 27

Mitochondria was suspended (40 mg /ml) in a buffered medium. Respiration was initiated by addition of 50 m mol succinate and the decrease in OD was followed at 520 nm

TABLE 35: Protective effect of *S.nux vomica* against the effects induced by anticancer drugs on NADH oxidase of liver mitochondria..

Group	State 3	RCR	P/O ratio
1.Control	100 + 11	100 ± 14	100 ± 4.4
1. S.nux vomica	101 + 3.2	104 + 10	104 ± 11
3. *S.nux vomica* + (Cisplatin + Cyclophosphamide)	106 + 3.0	119 + 2.1	103 + 1.5
4. *S.nux vomica* + (Mitomycin C + cyclophosphamide)	99 + 5.9	89 + 5.0	94 + 2.5
5. *S.nux vomica* + (Mitomycin C + Cisplatin)	95 + 5.7	95 + 1.4	89 + 2.5
6. *S.nux vomica* + (Methotrexate +Vincristine.	73 ± 8.7	97 + 2.0	87 + 1.47
7. *S.nux vomica* + (Methotrexate + Cyclophosphamide)	100 + 5.3	94 + 10	93 + 4.7

1. Control group recieved saline.
2. *S.nux vomica* group received alcoholic extract of *S.nux vomica* (10 mg / 100 g body wt, oral) for three days.
3. *S.nux vomica* + (Cisplatin + Cyclophosphamide) group received the alcoholic extract *of S.nux vomica* for three days and then received a single dose of cisplatin (12mg/kg, *i.p)* and Cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs.
4. *S.nux vomica* + (Mitomycin C + Cyclophosphamide) group received the alcoholic extract of *S.nux vomica* for three days and then received a single dose of mitomycin C (2 mg/kg, *i.p)* and cyclophosphamide (150 mg /kg, oral). Rats were sacrificed 48 hr after the administration of the drugs.
5. *S.nux vomica* + (Mitomycin C+ Cisplatin) group received the alcoholic extract of *S.nux vomica* for three days and then received a single dose of mitomycin C (2 mg/kg, *i.p)* and cisplatin (12 mg/kg, *i.p).* Rats were sacrificed 48 hr after the administration of the drugs.
6. *S.nux vomica* + (Methotrexate + Vincristine) group received the alcoholic extract of *S.nux vomica* for three days and and then received a single dose of methotrexate (2mg/kg, *i.p*) and vincristine (1 mg/kg, *i.p).* Rats were sacrificed 72 hr after the administration of the drugs.
7. *S.nux vomica* + (Methotrexate + Cyclophosphamide) group received the alcoholic extract of *S.nux vomica* for three days then received a single dose of methotrexate (2mg/kg, *i.p)* and cyclophosphamide (150 mg/kg, oral). Rats were sacrificed 24 hr after the administration of the drugs. (P.T.O)

2.5 mg protein was used for each assay. 2 mM glutamate and 4 mM malate were used as substrates to reduce matrix NAD^+ to NADH. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/ mg proteia

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/0 = ADP/O.

All the values are expressed relative to the control, which is taken as 100. The control value for state 3_{ADP} respiration, RCR and P/O ratio was **72 ± 7.9,** 4.32 ± 0.60 **and** 2.90 + 0.13 respectively.

Values are mean ± SD for atleast 13 animals. $*p\ 0.05, **p\ 0.01, ***p\ 0.001$

TABLE 36: Protective effect *of S.nux vomica* against the effects induced by anticancer drugs on Succinate oxidase of liver mitochondria.

Group	State 3	RCR	P/O ratio
1.Control	100 ± 9.1	100 ± 7.5	100 + 3.0
2 *S.nux vomica.*	98 ± **3.5**	95 j 6.8	104 + 1.8
3. ***S.nux*** *vomica* + (Cisplatin + Cyclophosphamide).	91 + **6.9**	77 i 7.4	97 + 4.4
4. *S.nux vomica* + (Mitomycin C + Cyclophosphamide).	**99** + 6.7	108 + **11.2**	96 + 2.7
5 *S.nux vomica* + (Mitomycin C + Cisplatin).	93 + **3.4**	93 + 1.5	93 + 4.0
6 *S.nux vomica* + (Methotrexate + Viincristine.	82 + 4.08	80 + 6.09	82 + 4.8
7. *S.nux vomica* + (Methotrexate + Cyclophosphamide).	97 + 1.3	117 + 3.4	93 + 1.36

All the experimental details are same as given for TABLE 35.

2.5 mg protein was used for each assay 9 m M succinate was used as substrate. State 3 (ADP stimulated) respiration is expressed as ng atom of oxygen / min/mg proteia

Respiratory control ratio is the ratio of State 3 to State 4 (ADP exhausted) respiration and P/O = ADP/O

All the values are expressed relative to the control, which is taken as 100. The State 3 ADP respiration, RCR and ADP/O ratio were 120 ± 1 **1,4.1** ± **0.31** and 1.85 + 0.056 respectively.

Values are mean + SD for 8 animals.

TABLE 37: Protective effect of *S.nux vomica* against the effects induced by anticancer drugs on NADH dehydrogenase and Succinate dehydrogenase of liver mitochondria.

Group	NADH Dehydrogenase	Succinate dehydrogenase
1. Control	100 ± 8.8	100 + 13.6
2. *S.nux voinica.*	100 + 3.1	99+ 4.7
3. *S.nux vomica* + (Cisplatin + Cyclophosphamide).	101 +4.5	91 + 4.6
4. *S.nux vomica* + (Mitomycin C + Cyclophosphamide).	103 + 4.7	90+ 8.9
5. *S.nux vomiea* + (Mitomycin C + Cisplatin).	104 +.2.8	97 + 3.9
6 *S.nux vomiea* +(Methotrexate + Viincristine).	82 + 5.7	83 + 6.5
7. *S.nux vomiea* +(Methotrexate + Cyclophosphamide).	% + 2.6	107 + 5.8

All the experimental details are same as given for TABLE 35.

NADH Dehydrogenase assay : 20 µg of mitochondrial protein was used for each assay. NADH (10 m M) was used as substrate. NADH dehydrogenase activity is expressed as NADH units / min / mg protein (One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein)
Succinate Dehydrogenase assay : 30 µg of mitochondrial protein was used for each assay. Succinate (9 mM) was used as substrate. SDH activity is expressed as SDH units / min / mg protein. (One SDH Unit = One n mole of DCPIP reduced / min / mg protein)

Results are expressed relative to control which is taken as 100. The control values of NADH dehydrogenase and Succinate dehydrogenase are 2750 ± 243 and 146 + 20 respectively in liver mitochondria.

Values are mean + SD for atleast 8 animals..

TABLE 38 Protective effect of *S.nux vomica* against the effects induced by **anticancer** drugs on NADH dehydrogenase and Succinate dehydrogenase of kidney mitochondria.

Group	NADH dehydrogenase	Succinate dehydrogenase
1. Control.	100 + 10.5	100 + 13
2. *S.nux vomica.*	104 ± 2.4	97 + 6.14
3. *S.nux vomica* + (Cisplatin + Cyclophosphamide).	89 + 2.0	93 + 3.7
4 *S.nux vomica* + (Mitomycin C + Cyclophosphamide).	91 + 4.5	116 **+.5.6**
5 *S.nux vomica* + (Mitomycin C + Cisplatin).	102 +.4.8	82 ± 4.5
6. *S.nux vomica* +(Methotrexate + Viincristine.	88 + 4.8	91 + 3.0
7. *S.nux vomica* + (Methotrexate + Cyclophosphamide).	96 + 5.5	95 + 3.5

All the experimental details are same as given for TABLE 35.

NADH Dehydrogenase assay: 20 µg mitochondrial protein was used for each assay. NADH (10 m M) was used as substrate NADH dehydrogenase activity is expressed as NADH units / min / mg protein.(One NADH Unit = One n mole of Potassium ferricyanide reduced / min / mg protein).

Succinate Dehydrogenase assay: 30 µg was used for each assay. Succinate (5 mM) was used as substrate SDH activity is expressed as SDH units / min / mg protein (One SDH Unit = One n mole of DCPIP reduced / min / mg protein)

Results are expressed relative to control which is taken as 100 The control values of NADH dehydrogenase and Succinate dehydrogenase are 2030 ± 214 and 107 + 14 respectively in kidney mitochondria.

Values are mean + SD for atleast 8 animals.

TABLE 39: Protective effect of *S.nux vomica* against the effects induced by anticancer drugs on respiratory chain enzymes in liver mitochondria

Group	NADH:cytochrome *c* reductase	Succinate:cytochrome *c* reductase	Cytochrome **c Oxidase**
1.Control	100 ± 9.4	100 + 13	**100** + 12.5
2. *S.nux vomica*	97 + 5.7	102 + **5.5**	**95 + 6 . 8**
3. *S.nux vomica* + (Cisplatin + Cyclophosphamide)	90 + 4.7	78 + **4.3**	**74 ± 4 4**
4. *S.nux vomica* + (Mitomycin C + Cyclophosphamide)	**89 + 9.2**	**90 + 6.3**	82 + **4.2**
5. *S.nux vomica* +(Mitomycin C + Cisplatin)	91 + 3.4	109 + **5.5**	94 + **5.0**
6. *S.nux vomica* + (Methotrexate + VIincristine	97 + 5.1	88 + **5.5**	83 + **5.0**
7. *S.nux voniica* +(Methotrexate + Cyclophosphamide)	**92 ± 4.0**	**92 + 6.1**	98 + 12

All the experimental details are same as given for TABLE 35.

NADH:Cytochrome ***c*** Reductase : 20 μg of mitochondrial protein was used for each assay. Activity is expressed as n moles of Cytochrome *c* reduced / min / mg protein Results are expressed relative to control which is taken as 100. The control value is **254** + 24.

Succinate:Cytochrome **c** Reductase: 20 μg of mitochondrial protein was used **for** each assay. Activity is expressed as n moles of Cytochrome ***c*** reduced / min / mg protein Results are expressed relative to control which is taken as 100 The control value is 49 ±6.4.

Cytochrome *c* Oxidase: 1 μg of mitochondrial protein was used for each assay. Reduced Cytochrome *c* was used as substrate . Cytochrome *c* Oxidase activity is expressed as n moles of cytochrome *c* oxidised / min / mg protein. Results are expressed relative to control which is taken as 100 The control value is 1070 + 134
Values are mean + SD for atleast 6 animals.

TABLE 40: Protective effect of *S.nux vomica* against the effects induced by **anticancer** drugs on respiratory chain enzymes of kidney mitochondria.

Group	NADH: cytochrome *c* reductase	Succinate: cytochrome *c* reductase	Cytochrome *C* Oxidase
1.Control	100 + 4.9	100 ± 13	100 + 97
2. *S.nux vomica*	90 + 9.5	109 ± 9.2	112 ± 5.0
3. *S.nux vomica* + (Cisplatin + Cyclophosphamide)	93 + 2.0	86 +_4.6	93 + **6.3**
4. *S.nux vomica* + (Mitomycin C + Cyclophosphamide)	91 + 2.6	89 + 4.2	82 + **8.4**
5 .*S.nux vomica* + (Mitomycin C + Cisplatin)	100+ 3.2	109 + 6.0	94 + 6 9
6. *S.mix* *vomica* + (Methotrexate + Vlincristine	86 + 3.0	83 + 7.7	88 + 8 S
7. *S.nux vomica* + (Methotrexate + Cyclophosphamide)	83 + 3.0	100 + 12	93 ± 5.1

All the experimental details are same as given for TABLE 35.

NADH: Cytochrome *c* Reductase: 20 ug of mitochondria! protein was used for each assay . Activity is expressed as n moles of Cytochrome **c** reduced / min / mg protein. Results are expressed relative to control which is taken as 100. The control value is 262 + 13.

Succinate:Cytochrome **c** Reductase: 20 ug **of** mitochondrial protein was used **for** each assay. Activity is expressed as n moles of Cytochrome ***c*** reduced / min / mg protein. Results are expressed relative to control which is taken as 100 .The control value is 33 + 4.0.

Cytochrome *c* Oxidase: 1µg of mitochondrial protein was used for each assay Reduced Cytochrome ***c*** was used as substrate Cytochrome *c* Oxidase activity is expressed as n moles of Cytochrome **c** oxidised / min / mg protein Results are expressed relative to control which is taken as 100. The control value is 884 + 86.

Values are mean + SD for atleast 8 animals.

TABLE 41: Protective effect of *S.nux vamica* against the lipid peroxidation induced by anticancer drugs.

Group	Liver homogenate	Kidney homogenate	**Liver** mitochondria
1 Control	100 ± 8.4	100 ± 12.7	100 ± 2.7
2 *S.nux vomica.*	95 + 5.5	96 + 4.1	91 ± 11
3. *S.nux vomica* +(Cisplatin + Cyclophosphamide).	106 + 9.3	114 + 4.0	113 + **0.0**
4 *S.nux vomica* + (Mitomycin C + Cyclophosphamide).	124 + 2.3	100 + 2.6	152 ± 10
5 *S.nux vomica* +(Mitomycin C + Cisplatin)	102 + 2.0	102 + 1.23	102 + 8.4
(. ***S.nux*** *vomica*- (Methotrexate + Vincristine.	163 + 7.2	152 +_6.0	131 + 7.8
7 *S.nux vomica* +(Methotrexate + Cyclophosphamide)	135+ 10	123+ 5.4	116 + 12

All the experimental details are same as given for TABLE 35.
2 mg of protein was used for each assay Tetramethoxypropane was used as a external standard. Lipid peroxide level is expressed as n moles MDA formed/ 100 mg protein Results are expressed relative to control which is taken as 100. The control values are 139 ± 11.7, 149 ± 19 and 76 + 2.1 for liver homogenate, kidney homogenate and **liver** mitochondria respectively.

Values are mean + SD **for** atleast 10 animals

TABLE 42: Protective effect of *S.nuxvomica* against the effects induced by anticancer drugs on phospholipid composition of liver mitochondria.

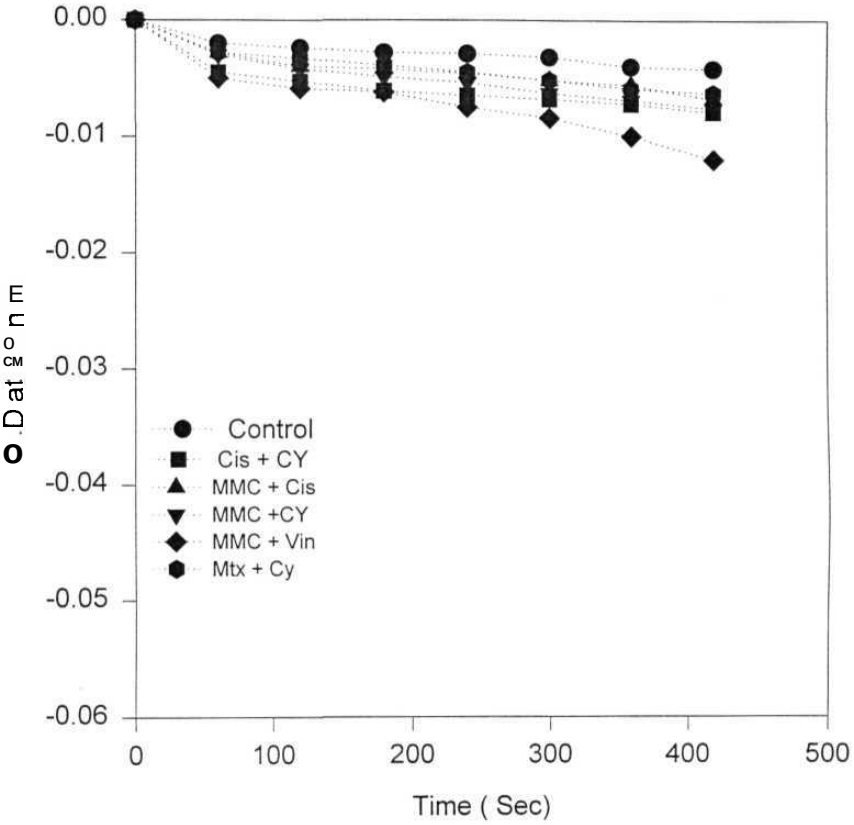
Group.	Total Phospholipids	Phosphotidyl-choline	Phosphatidyl-ethanolamine	Cardiolipin
1.Control.	100 + 7.5	100 +.6.25	100 ± 4.6	100 + 5.2
2. S.nux vomica.	95 + 8.2	89 ± 6.9	86 ± 4.0	94+ 5.2
3 *S.nux vomica* + (Cisplatin + Cyclophosphamide).	116 ± 6.6	100 ± 7.5	111 ± 7.6	110+ 6.2
4. *S.nux vomica* + (Mitomycin C + Cyclophosphamide).	95 + 7.8	112 + 7.4	102 + 4.6	80 + 5.2
5.*S.nux vomica* + (Mitomycin C + Cisplatin)	95 + 8.2	100 + 6.25	112 ± 3.8	100 ± 6.3
6. *S.nux vomica* +(Methotrexate +VIincristine	87 + 7.4	102 + 6.2	84 + 4.5	84 + 5.8
7.*S.nux vomica* + (Methotrexate + Cyclophosphamide)	79 + 4.9	81+ 3.1	80 +3.8	73 ± 6.2

All the experimental details are same as given for TABLE 35.

Phospholipids were separated by TLC using chloroform : methanol : water as solvent. 200 ul of the lipid extract was applied as a streak . The separated phospholipids were scraped out with gel from TLC plate and then digested with 60 % perchloric acid. The inorganic phosphorus was estimated according to Fiske and Subbarow method. Phospholipids are expressed as µg phospholipid Phosphorus /gm tissue. Results are expressed relative to control which is taken as 100. The control values are 24 ± 1.8, 32 ± 2.0, 26+1.2 and 19 ± 1.0 for total phospholipids, phosphatidyl choline, phosphatidylethnolamine and cardiolipin content respectively.

Values are mean + SD for atleast 8 animals.

Fig 3: Protective effect of *S.nux vomica* against **mitochondrial** swelling induced by anticancer drugs.



Swelling of mitochondria:

All the experimental details are same as given for TABLE 35.

Mitochondria was suspended (40 mg /ml) in a buffered medium. Respiration was initiated by addition of 50 m mol succinate and the decrease in OD was followed at 520 nm.

RESULTS

The effects of administration of anticancer drugs and a protective effect to reverse these effects by the prior administration of plant extracts, were studied in the present investigation .The effect was studied on the ability of transfer of electron though different segments of the respiratory chain, Respiratory control ratio, P/O ratio, enzymes involved in the transfer of electrons to electron transport chain, extent of lipid peroxidation and the phospholipid profile in mitochondria. The different anticancer drugs that were used in the present investigation are Methotrexate, Vincristine, Cisplatin, Mitomycin C, and Cyclophosphamide. The drugs were used in various combinations.
1. Cisplatin and Cyclophosphamide 2. Mitomycin C and cyclophosphamide
3. Mitomycin C and Cisplatin 4. Methotrexate and Vincristine 5. Methotrexate and Cyclophosphamide. The plant extracts used are *P.fraternus, S.nux vomica, B.aristata and C. majus.*

Results are expressed relative to control, which is taken as 100 (i.e., percent control). For example: The activity of succinate:cytochrome c reductase is 46 (Table 5) when compared to control, in rats treated with methotrexate plus vincristine i.e., it was inhibited by (100-46) 54% by the drug treatment . Treatment with *P.fraternus* prior to methotrexate plus vincristine treatment has shown protective effects. The inhibition on succinatexytochrome *c* reductase was only 6 % (100-94) (Table 22) when compared to 54% with methotrexate plus vincristine alone. So the inhibition caused due to drug treatment is relieved by *P.fraternus* almost completely. When the inhibition totally disappears, then it is called as 100% relief or recovery. The incomplete inhibition was calculated as follows. The amount of inhibition that was to be relieved in *P.fraternus* + methotrexate plus vincristine group was 54%. The amount of inhibition that was observed in *P.fraternus* + methotrexate plus vincristine group was 6% and the relief was 48%. The percent relief was calculated as 100/54 x 48 = 89% (Table 22). That means *P.fraternus* can relieve 89% of the methotrexate and vincristine induced inhibition on Succinate: cytochrome *c* reductase. The percent relief was calculated to compare the ability of different protective agents in relieving the inhibition.

NADH oxidase: The externally added NADH can not penetrate tightly coupled mitochondria so, glutamate plus malate were used to reduce the matrix NAD^+ pool and generate NADH, which donates electrons. This study gives the information on the transfer of electrons through all the three sites.
Succinate Oxidase: The study of this enzyme gives information on the efficiency of the transfer of electrons from succinate to molecular oxygen through site 2 and site 3. Respiratory control ratio (RCR) which is an index of the membrane integrity of the mitochondria, was determined from the ratio of State 3 to State 4. P/O ratio was determined by measuring the amount of oxygen consumed during active respiration.

NADH dehydrogenase: Studies on NADH dehydrogenase gives the information on the efficiency of transfer of electrons from NADH to prosthetic group of flavoprotein (FP_1) [E-FMN to $E\text{-}FMNH_2$] at site 1.

Succinate dehydrogenase: Studies on Succinate dehydrogenase gives the information about the efficiency of transfer of electrons from succinate to (FP_2) [E-FAD to $E\text{-}FADH_2$] at site 2.

NADH:cytochrome *c* reductase: Studies on NADH:cytochrome *c* reductase gives the information on the ability of transfer of electrons from NADH to cyctochrome *c* (site 1 and site 2).

Succinatexytochrome *c* reductase: Studies on Succinate:cytochrome *c* reductase gives the information on the efficiency of transfer of electrons from succinate to cytochrome *c* (site 2).

Cytochrome *c* oxidase: Studies on cytochrome *c* oxidase gives the information about the efficiency of transfer of electrons from cytochrome c, to oxygen (site 3).

Measurement of lipid peroxidation: Lipid peroxide level in liver homogenate, liver mitochondria and kidney homogenate was measured, as it is an index for several pathological conditions.

Studies on phospholipid composition of liver mitochondria: Effect of administration of drugs on the phospholipid composition was studied. The changes in the membrane phospholipids under certain stress conditions, are shown to affect the cellular metabolism, via mitochondial dysfunction (Nakahara, 1990).

The effects were studied on the rate of mitochondrial swelling as this would also indicate integrity of the mitochondrial membrane.

Effect of coadministration of Cisplatin (12 mg/ Kg, *i.p)* and Cyclophosphamide (150 mg/kg, oral).

The effect of coadministration of cisplatin and cyclophosphamide was studied on rat liver and kidney mitochondria, as a function of time. The results showed that the effects are significant at 24 h. So all the experiments using cisplatin and cyclophosphamide combination were carried out for 24 h after administration of these drugs. The results are as follows: State 3, RCR and P/O ratio were decreased by 40%, 32% and 38% respectively using glutamate plus malate as substrate, when compared to controls (Table 1). State 3, RCR and P/O

ratio were decreased by 41% ,32% and 40 % respectively using succinate as substrate (Table 2). NADH dehydrogenase and succinate dehydrogenase activity was increased in liver (35%, and 60% respectively) and kidney mitochondria (27% and 30% respectively) (Table 3 and Table 4). Activities of NADH:cytochrome *c* reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase were decreased significantly in liver (34%,50% and 53% respectively) (Table-5) and kidney mitochondria (26%, 46% and 54% respectively) (Table 6) compared to controls. Swelling of mitochondria was observed due to treatment with cisplatin plus cyclophosphamide (Fig 1). There was 48% and 43% increase in the amount of free radicals formed in liver and kidney homogenate (Table 7). A significant increase in the level of lipid peroxides was observed in liver homogenate, kidney homogenate and liver mitochondria (44 %, 36% and 35 % respectively) due to administration of these drugs (Table 8). Phospholipid composition of liver mitochondria was significantly different when compared to controls. 32%, 31% and 31% decrease was seen in phosphatidylcholine, phosphatidylethanolamine and cardiolipin content respectively. The total phospholipid content was also decreased (13%) when compared to controls (Table 9).

Effect of coadministration of Mitomycin C (**2mg/kg, *i.p* and Cyclophosphamide (150 mg/kg, oral).**

The effects of coadministration of mitomycin c and cyclophosphamide was studied on rat liver and kidney mitochondria a function of time. The results showed that the effects are significant at 48 h. So all the experiments using mitomycin c and cyclophosphamide combination were carried out for 48 h after administration of these drugs. The results are as follows: State 3, RCR and P/O ratio were decreased by 45%, 28% and 32% respectively using glutamate plus malate as substrate. when compared to controls (Table 1). State 3. RCR and P/O ratio were decreased by 44%, 33% and 30 % respectively using succinate as substrate (Table 2). NADH dehydrogenase activity was stimulated by 36%, and 55% respectively in liver and kidney mitochondria (Table 3 and Table 4), where as succinate dehydrogenase activity was inhibited by 48% in liver mitochondria (Table 3). There was no significant effect on succinate dehydrogenase activity of kidney mitochondria (Table 4). Activities of NADHxytochrome *c* reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase were decreased significantly in liver (31%, 45% and 48% respectively) (Table 5) and kidney mitochondria (27%, 47% and 41% respectively) (Table 6) when compared to controls. Significant swelling of mitochondria was observed due to administration of mitomycin c and cyclophosphamide when compared to controls (Fig 1). There was 45 % and 35 % increase in amount of free radicals formed in liver and kidney homogenate respectively (Table 7). There was **a** significant increase in the level of lipid peroxides formed in liver homogenate,

kidney homogenate and liver mitochondria (83 %, 23% and 78 % respectively) (Table 8). Phospholipid composition of liver mitochondria was significantly different when compared to controls. 40%, 21% and 36% decrease was seen in phosphatidylcholine, phosphatidylethnolamine and cardiolipin content respectively. The total phospholipid content was decreased by 30% as compared to controls (Table 9).

Effect of coadministration of Mitomycin C (2 **mg/kg,** *i.p)* and Cisplatin (12 mg/kg, ***i.p***)

The effect of coadministration of Mitomycin C and Cisplatin was studied on rat liver and kidney mitochondria as a function of time. The results showed that the effects are significant at 48 h. So all the experiments using mitomycin c and cisplatin combination were carried out for 48 h after administration of these drugs. The results are as follows: State 3, RCR and P/O ratio were decreased by 28%, 32% and 28% respectively using glutamate plus malate as substrate, when compared to controls (Table 1). State 3 , RCR and P/O ratio were decreased by 26%, 30% and 33 % respectively using succinate as substrate (Table 2). NADH dehydrogenase activity was stimulated by 60%. and 42% respectively in liver and kidney mitochondria (Table 3 and Table 4). Succinate dehydrogenase activity was inhibited by 42% and 30% respectively in liver and kidney mitochondria, when compared to controls (Table 3 and Table 4). Activities of NADHxytochrome c reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase were decreased significantly in liver (30%, 35% and 36% respectively) (Table 5) and kidney mitochondria (37%, 29% and 34% respectively) (Table 6). Significant swelling of mitochondria was observed due to administration of mitomycin c and cisplatin when compared to controls (Fig-1). There was 75% and 68% increase in the amount of free radicals formed in liver and kidney homogenate (Table 7). There was significant increase in the level of lipid peroxides formed in liver homogenate, kidney homogenate and liver mitochondria (34 %, 34% and 48 % respectively) (Table 8). A significant difference in the phospholipid composition of liver mitochondria was observed when compared to controls. 29% and 48% decrease was seen in phosphotidylcholine, and cardiolipin content. The total phospholipid content was decreased by 45% as compared to controls. There was no effect on phosphatidylethnolamine content (Table 9).

Effect of coadministration of Methotrexate (2 **mg/kg,** *Lp)* **and** Vincristine **(1** mg/kg, *Lp).*

The effect of coadministration of methotrexate and vincristine was studied as a function of time and the results showed that the effects are significant at 72 h. So all the experiments using methotrexate and vincristine combination were carried out

for 72 h after administration of these drugs. The effects are as follows : State 3, RCR and P/O ratio were decreased by 61%, 44% and 51% respectively using glutamate and malate as substrate, when compared to controls (Table 1). There was 48%, 45% and 44% decrease in State 3, RCR and P/O ratio when succinate was used as substrate (Table 2). NADH dehydrogenase and Succinate dehydrogenase activity was decreased by 42% and 46% respectively in liver, 30% and 33% respectively in kidney mitochondria (Table 3 and Table 4). Activities of NADH:cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase were decreased by 35%, 54% and 43% respectively in liver (Table 5) and by 21%, 42% and 25% respectively in kidney mitochondria (Table 6). Significant swelling of mitochondria was observed due to the administration of methotrexate and vincristine(Fig 1). There was 156 % and 109% increase in the amount of free radicals formed compared to controls in liver and kidney homogenate (Table 7). There was significant increase in the level of lipid peroxides formed in liver homogenate, kidney homogenate and liver mitochondria (136 %, 130%, and 131 % respectively) (Table 8). Phospholipid composition of liver mitochondria was significantly different when compared to controls. 46% increase was seen in phosphatidylcholine and 68% decrease was seen in cardiolipin content. The total phospholipid content was increased by 63% as compared to controls. There was no significant effects **on** phosphatidylethanolamine content (Table 9).

Effect of coadministration of Methotrexate (2 mg/kg, i p) and Cyclophosphamide (150 mg/kg, oral).

The effect of coadministration of methotrexate and cyclophosphamide was studied on rat liver and kidney mitochondria. as a function of time. The results showed that significant effects are seen at 24 h. So all the experiments using methotrexate and cyclophosphamide combination were carried out for 24 h after the administration of these drugs. The results are as follows: State 3, RCR and P/O ratio were decreased by 33%, 24% and 36% using glutamate plus malate as substrate, when compared to controls (Table 1). There was 28% decrease in P/O ratio when succinate was used as substrate, with no significant effects on State 3 and RCR (Table 2). NADH dehydrogenase activity was decreased by 30%, while succinate dehydrogenase activity was increased by 52% in liver mitochondria (Table 3). There was 46% decrease in the activity of Succinate dehydrogenase, with out affecting NADH dehydrogenase activity of kidney mitochondria (Table 4). NADHxytochrome *c* reductase and Succinatexytochrome *c* reductase activity was decreased by 32%, and 28% respectively in liver mitochondria (Table 5). No significant effects was observed on NADHxytochrome *c* reductase but, Succinatexytochrome *c* reductase showed 26% inhibition in kidney mitochondria (Table 6). The activity of cytochrome c oxidase was significantly increased (52%) in liver (Table 5) and decreased (34%) in kidney mitochondria, when compared to controls (Table 6). Significant swelling of mitochondria was observed due to administration of methotrexate and cyclophosphamide (Fig-1). There was 76 % and 45% increase in the amount of free

radicals formed in liver and kidney homogenate (Table 7). There was significant increase in the level of lipid peroxides formed in liver homogenate, kidney homogenate and liver mitochondria (91 %, 32% and 71% respectively) compared to controls (Table 8). Phosphatidylcholine and cardiolipin were decreased by 44% and 35% respectively when compared to controls. There was no significant effect on Phosphatidylethnolamine. The total phospholipid content was decreased (38%) compared to controls (Table 9).

Protective effect of *Berberis. aristata.*

Protective effect of *B.aristata* against the effects induced by coadministration of Cisplatin (2 mg/kg, i.p) and Cyclophosphamide (150 mg/kg, oral).

The protective effect of prior administration of alcoholic extract *of B.aristata* for three days was studied. In general *B.aristata* offered protection on almost all the parameters that are affected by the coadministration of cisplatin and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 100% when glutamate plus malate was used as substrate (Table 10). The inhibition, using succinate as substrate was relieved by 83%, 100% and 60% on State 3, RCR and P/O ratio respectively (Table 11). Increased activity of NADH dehydrogenase was relieved by 71% and 60 % respectively in liver and kidney mitochondria (Table 12 and Table 13). The increased activity of Succinate dehydrogenase was brought down by 66% and 73% respectively in liver and kidney mitochondria (Table 12 and Table 13). The inhibition on NADH:cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome c oxidase was relieved by 64%, 100% and 80% respectively in liver (Table 14) and by 100%, 67% and 72% respectively in kidney mitochondria (Table 15). The increased levels of lipid peroxides in liver homogenate, **liver** mitochondria and kidney homogenate **were** brought down to control level by 63%, 54% **and 61% respectively (Table 16).** Administration of alcoholic extract of *B.aristata* resulted in complete recovery **over** the decreased levels of total phospholipids phosphatidylcholine, phosphatidylethanolamine and cardiolopin content (Table 17).
Administration of *B.aristata* alone did not show any significant effects **when** compared to control on any of the parameters studied.

Protective effect of *B.aristata* against the effects induced by coadministration of **Mitomycin** C (2 **mg/kg,** *i.p)* and **Cyclophosphamide (150 mg/kg,** oral).

The protective effect of prior administration of alcoholic extract of *B.aristata* for three days was studied. In general *B.aristata* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 71%, 100% and 75% respectively when glutamate plus malate was used as substrate (Table 10). The inhibition , using succinate as substrate was relieved by 89%, 100% and 83% on State 3, RCR and P/O ratio respectively (Table 11). The increased activity of NADH dehydrogenase was relieved by 86% and 84 % in liver and kidney mitochondria respectively (Table 12 and Table 13). The inhibition on Succinate dehydrogenase was relieved by 100% and 92% respectively in liver and kidney mitochondria (Table 12 and Table 13). The inhibition on NADHxytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 88%, 73% and 89% respectively in liver (Table 14) and by 58%, 83% and 63% respectively in kidney mitochondria (Table 15). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria was brought down, showing a relief of 86%, 83% and 54% respectively when compared to controls (Table 16). Administration of alcoholic extract of *B.aristata* resulted in relief 56% and 66% respectively against the decreased levels of total phospholipids and cardiolipin content. Complete recovery on the decreased levels of phosphotidylcholine and phosphatidylethnolamine content was observed (Table 17).

Administration of *B.aristata* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect *of B.aristata* against the effects induced by the coadministration of Mitomycin C (2mg/kg, *i.p*) and Cisplatin **(12** mg/kg, *i.p)*

The protective effect of prior administration of alcoholic extract of *B.aristata* for three days was studied. In general *B.aristata* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cisplatin. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 70%, 79% and 86% respectively, when glutamate plus malate was used as substrate (Table 10). The inhibition on State 3, RCR and P/O ratio using succinate as substrate was relieved by 77%, 77% and 70% respectively (Table 11). NADH dehydrogenase was relieved by 58% and 83 % in liver mitochondria and kidney mitochondria (Table 12 and Tablel3). The inhibition on succinate dehydrogenase was relieved by 85% and 76% respectively in liver and kidney mitochondria (Table 12 and Table 13). The inhibition on NADHxytochrome *c* reductase, succinate:cytochrome c reductase and cytochrome c oxidase activities were relieved by 86%, 100% and 79%

respectively in liver (Table 14) and by 70% , 77% and 91% respectively in kidney mitochondria (Table 15). The increased levels of lipid peroxides in liver homogenate, liver mitochondria and kidney homogenate were brought down showing a relief of 100%, 70% and 85% respectively with the administration of *B.aristata* (Table 16). Administration of alcoholic extract of *B.aristata* resulted in 79% and 91% recovery over the decreased levels of phosphatidylcholine and cardiolipin content. There was 95% recovery over the decreased levels of total phospholipids (Table 17).
Administration of *B.aristata* alone did not show any significant effects compared to control on any of the parameters studied.

Protective effects of *B.aristata* against effects induced by the coadministration of Methotrexate (2mg/ kg, *i.p)* and Vincristine (1mg/kg, *i.p).*

The protective effects of prior administration of alcoholic extract of *B.aristata* for three days was studied . In general *B.aristata* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and vincristine. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 61 %, 62%, and 92% respectively when glutamate plus malate was used as substrate (Table 10). The inhibition using succinate as substrate was relieved by 90%, 77% and 100 % on State 3, RCR and P/O ratio respectively (Table 11). The inhibition on NADH dehydrogenase was relieved by 90% and 69% in liver (Table 12) and kidney mitochondria respectively (Table 13). The inhibition on Succinate dehydrogenase was relieved completely in liver and kidney mitochondria (Table 12 and 13). The inhibition on NADH:cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 81 %, 78% and 66 % respectively in liver (Table 14) and by 95%, 100% and 78% respectively in kidney mitochondria (Table 15). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down by 78%, 75% and 67% respectively when compared to controls (Table 16). Administration of *B.aristata* resulted in complete recovery in the increased levels of total phospholipids and phosphatidylcholine content. There was 83% protection over the decreased cardiolipin content (Table 17).
Administration of *B.aristata* alone did not show any significant effect compared to control on any of the parameters studied

Protective effects of *B.aristata* against the effects induced by the coadministration of Methotrexate (2mg/ kg, *i.p*) and Cyclophosphamide (150 mg/kg, oral) .

The protective effects of prior administration of alcoholic extract of *B.aristata* for three days was studied. In general, *B.aristata* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and

cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 75 %, 100%, 72% respectively when glutamate plus malate was used as substrate (Table 10). The inhibition using succinate as substrate was relieved by 75%, on P/O ratio (Table 11). The inhibition on NADH dehydrogenase was relieved by 73% in liver mitochondria (Table 12).The inhibition on Succinate dehydrogenase was relieved by 100% and 86 % respectively in liver and kidney mitochondria (Table 12 and Table 13). The inhibition on NADHxytochrome *c* reductase, succinate:cytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 73%, 65% and 70 % respectively in liver (Table 14) and by 61%, 58% and 76% in kidney mitochondria (Table 15).The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down, showing 71%, 100%, and 81% relief with the administration of *B.*aristata (Table 16). Administration of *B. aristata* resulted in 60 %, 100% and 54% recovery against the decreased levels of total phospholipids, phosphatidylcholine and cardiolipin content (Table 17).
Administration of *B.aristata* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *Phyllanthus fraternus*.

Protective effect of *P.fraternus* against the effects by coadministration of Cisplatin ((12 **mg/kg,** i.p) and Cyclophosphamide **(150mg/kg,** oral).

The protective effects of prior administration of aqueous extract of *P.fraternus* for seven days, was studied. In general *P.fraternus* offered protection on almost all the parameters that are affected by the coadministration of cisplatin and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 90%, 89% and 78% respectively when glutamate plus malate was used as substrate (Table 18). The inhibition, using succinate as substrate was relieved by 75%, 100% and 100 % respectively on State 3 , RCR and P/O ratio (Table 19). Increased activity of NADH dehydrogenase and succinate dehydrogenase was brought down by 86% and 89% respectively in liver mitochondria (Table 20), while the inhibition on NADH dehydrogenase and Succinate dehydrogenase was relieved by 64% and 46% respectively in kidney mitochondria (Table 21). The inhibition on NADHxytochrome c reductase , succinatexytochrome *c* reductase and cytochrome *c* oxidase was relieved by 100%, 81% and 82% respectively in liver mitochondria (Table 22). The inhibition on NADHxytochrome c reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 83%, 60% and 80% respectively in kidney mitochondria (Table 23).The increased levels of free radicals were brought down by 79% and 58% respectively in liver homogenate and kidney homogenate (Table 24).The increased levels of lipid peroxides in liver homogenate, liver mitochondria and kidney homogenate were brought down showing 100% relief with the administration of *P.fraternus* (Table 25). Administration of aqueous extract of *P.fraternus* gave complete protection against

the decreased levels of total phospholipids and phosphatidylcholine, phosphatidylethanolamine and cardiolipin content. (Table 26).
Administration of *P.fraternus* alone did not show any significant effect compared **to** control on any of the parameters studied.

Protective effects of ***P.fraternus*** **against the effects induced by coadministration of Mitomycin C (2 mg/kg, *up*) and Cyclophosphamide (150 mg/kg, oral).**

The protective effects of prior administration of aqueous extract of *P.fraternus* for seven days was studied. In general *P.fraternus* offered protection on almost all the parameters that are affected by the coadministration of mitomycin **c** and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 69%, 100% and 63% respectively when glutamate plus malate was used as substrate (Table 18). The inhibition, using succinate as substrate was relieved by 80%, 90% and 96% respectively on State 3, RCR and P/O ratio (Table 19). Increased activity of NADH dehydrogenase was brought down showing a relief of 90% and 100 % respectively in liver and kidney mitochondria (Table 21 and Table 21). The inhibition on Succinate dehydrogenase was relieved completely liver mitochondria (Table 20). The inhibition on NADH:cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 60%, 86% and 89% respectively in liver (Table 22) and by 75%, 77% and 70% respectively in kidney mitochondria (Table 23). The increased levels of free radicals were brought down by 62% and 91% respectively in liver homogenate and kidney homogenate (Table 24). The increased levels of lipid peroxides in liver homogenate, liver mitochondria and kidney homogenate were brought down, giving a relief of 69%, 92% and 44% respectively, when compared to controls (Table 25). Administration of aqueous extract of *P.fraternus* resulted in 56%, 67%, 66% and 80% respectively, recovery over the decreased levels of total phospholipids, phosphatidylcholine, phosphatidylethanolamine and cardiolipin content (Table 26).
Administration *of P.fraternus* alone did not show any significant effects compared to control on any of the parameters studied.

Protective effects of *P. fraternus* against the effects induced by the coadministration of **Mitomycin C (2 mg/kg, *i.p*) and Cisplatin (12 mg/kg, *i.p).***

The protective effects of prior administration of aqueous extract of *P.fraternus* for seven days was studied in general *P.fraternus* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cisplatin. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by more than 85 %, when glutamate plus malate or succinate was used as substrate (Table 18 and Table 19). NADH dehydrogenase was relieved by 60% and 88 % in liver and kidney mitochondria (Table 20 and Table 21). The inhibition on Succinate dehydrogenase was relieved by 91% and 80% in liver and kidney

mitochondria (Table 20 and Table 21). The inhibition on NADHxytochrome *c* reductase and cytochrome *c* oxidase was relieved completely in liver and kidney mitochondria (Table 22 and Table 23). The inhibition on Succinate:cytochrome *c* reductase was relieved completely in liver, while the relief was 81% in kidney mitochondria (Table 22 and Table 23). The increased levels of free radicals were brought down to control levels showing 88% relief (Table 24). The increased levels of lipid peroxides in liver homogenate, liver mitochondria and kidney homogenate were brought down giving a relief of 60%, 100% and 76% respectively with the administration *of P.fraternus* (Table 25). Administration of aqueous extract of *P.fraternus* resulted in 53 %, 100% and 77% recovery over the decreased levels of total phospholipids, phosphatidylcholine and cardiolipin content respectively (Table 26). Administration of *P.fraternus* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *P. fraternus* against the effects induced by the coadministration of Methotrexate (2 mg/kg, *i.p*) and Vincristine (1 mg/kg, *i.p*).

The protective effect of prior administration of aqueous extract of *P.fraternus* for seven days was studied . In general *P.fraternus* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and vincristine . The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 70 %, 100% and 62% respectively when glutamate plus malate was used as substrate (Table 18). The inhibition using succinate as substrate was relieved by 61%, 81% and 63% respectively on State 3, RCR and P/O ratio (Table 19). The inhibition on NADH dehydrogenase was relieved by 57% in liver (Table 20) and (72%) in kidney mitochondria (Table 21). The inhibition on succinate dehydrogenase was relieved completely in liver and kidney mitochondria (Table 20 and Table 21). The inhibition on NADHxytochrome *c* reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase was relieved by 75%, 89% and 95 % respectively in liver (Table 22) and by 60%, 100% and 84% in kidney mitochondria (Table 23). The increased levels of free radicals were brought down, showing atleast 77% relief (Table 24). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down to showing a **relief of 77** %, 90% and 91% respectively (Table 25). The significant increase in total phospholipids, phosphatidylcholine and a decrease in cardiolipin content was relieved by 82%, 86% and 50% respectively (Table 26).
Administration of *P.fraternus* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *P.fraternus* against the effects induced by the coadministration of Methotrexate (2 mg/kg, *up*) and Cyclophosphamide (150 mg/kg, oral).

The protective effect of prior administration of aqueous extract of *P.fraternus* for seven days was studied. In general *P.fraternus* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 100 %, 81% and 73% respectively when glutamate plus malate was used as substrate (Table 18). The inhibition using succinate as substrate was relieved by 63% on P/O ratio (Table 19). The inhibition on NADH dehydrogenase was relieved by 73% in liver mitochondria and completely in kidney mitochondria (Table 20 and Table 21). The inhibition on Succinate dehydrogenase was relieved by 70% and 86 % respectively in liver and kidney mitochondria (Table 20 and Table 21). The inhibition on NADH:cytochrome *c* reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 64%, 64% and 100 % respectively in liver (Table 22) and by atleast 78% in kidney mitochondria (Table 23). The increased levels of free radicals were brought down by 77% and 60% respectively in liver homogenate and kidney homogenate (Table 24). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down to control levels, showing atleast 70% relief by the administration of *P.fraternus* (Table 25). The significant decrease in total phospholipids. phosphatidylcholine and cardiolipin were prevented by 85%, 71% and 70% respectively (Table 26).

Administration of *P.fraternus* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *Chelidonium majus*

Protective effect of *C.majus* against the effects induced by coadministration of Cisplatin (12 mg/kg, *i.p)* and Cyclophosphamide (150 mg/kg, oral).

The protective effect of prior administration of alcoholic extract of *C.majus* for three days was studied, in general *C.majus* offered protection on almost all the parameters that are affected by the coadministration of cisplatin **and** cyclophosphamide. The results are as follows: The inhibition on State 3 and RCR was relieved completely when glutamate plus malate were used as substrate, where as the inhibition on P/O ratios was relieved by 82% (Table 27). The inhibition , using succinate as substrate was relieved by 68%, 55% and 100% respectively in State 3, RCR and P/O ratio (Table 28). The increased activities of NADH dehydrogenase was brought down by 77% and 70% respectively in liver and kidney mitochondria (Table 29 and Table 30). The stimulation on succinate dehydrogenase was relieved by 100% and 81% respectively in liver and kidney mitochondria (Table 29 and

Table30). The inhibition on NADH:cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 82%, 66% and 66% respectively in liver (Table 31) and by 84%, 100% and 75% respectively in kidney mitochondria (Table 32). The extent of swelling decreased with the administration of plant extract (Fig 2). The increased level of free radicals were brought down to almost control levels, showing 80% relief (Table 33). The increased levels of lipid peroxides in liver homogenate , kidney homogenate and liver mitochondria were brought down showing relief of 85%, 61% and 62% respectively when compared to controls (Table 33). Administration of alcoholic extract of *C.majus* resulted in complete recovery over the decreased levels of total phospholipids and cardiolipin content. There was 78% and 74% recovery in decreased content of phosphatidylcholine and phosphatidylethanolamine content. (Table 34).
Administration of *C.majus* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *C.majus* against the effects induced by coadministration of Mitomycin C (2 mg/kg, *up*) and Cyclophosphamide 150 mg/kg, oral).

The protective effects of prior administration of alcoholic extract of *C.majus* for three days was studied, in general *C.majus* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cyclophosphamide. The results are as follows: Inhibition on State 3, RCR and P/O ratios were relieved by 100%, 60% and 78% respectively, when glutamate plus malate was used as substrate (Table 28). The inhibition, on State 3, RCR and P/O ratio using succinate as substrate was relieved by 89%, 100% and 100% respectively (Table 28). Increased activity of NADH dehydrogenase was brought down by 56% and 57 % in liver and kidney mitochondria (Table 30 and Table 31). The inhibition on Succinate dehydrogenase was relieved completely in liver and kidney mitochondria (Table 30 and Table 31). The inhibition on NADHxytochrome *c* reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 60%. 64% and 75% respectively in liver (Table 31) and by 100%,63% and 57% respectively in kidney mitochondria (Table 32). The extent of swelling decreased with the administration of plant extract (Fig 2). The increased levels of lipid peroxides in liver homogenate , kidney homogenate and liver mitochondria was brought down showing a relief of 52%, 76% and 50% respectively when compared to controls (Table 33). Administration of *C.majus* resulted in 86%, 79% and 80% recovery over the decreased levels of total phospholipids, phosphatidylcholine and cardiolipin content (Table 34).
Administration of *C.majus* alone did not show any significant effect compared to control on any of the parameters studied

Protective effect of *C.majus* against the effects induced by the coadministration of Mitomycin C (2 mg/kg, *up*) and **Cisplatin** (12 **mg/kg,** *up).*

The protective effect of prior administration of alcoholic extract of *C.majus* for three days was studied. In general *C.majus* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cisplatin. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 64%, 100% and 60% respectively when glutamate plus malate was used as substrate (Table 27). The inhibition, on State 3 , RCR and P/O ratio using succinate as substrate was relieved by 100%, 50% and 81% respectively (Table 28). The increased levels of NADH dehydrogenase was brought down by 88% and 80 % respectively in liver and kidney mitochondria (Table 29 and Table 30). The inhibition on Succinate dehydrogenase was relieved by 62% and 63% respectively in liver and kidney mitochondria (Table 29 and Table 30). The inhibition on NADH:cytochrome *c* reductase, succinatexytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 64%, 57% and 77% respectively in liver (Table 31) and by 100%, 93% and 68% respectively in kidney mitochondria (Table 32). The extent of swelling decreased with the administration of plant extract (Fig 2). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down showing relief of 79%, 70% and 85% respectively when compared to controls (Table 33). Administration of alcoholic extract of *C.majus* gave 88%,79% and 80% relief over the decreased levels of total phospholipids phosphatidylcholine and cardiolipin content respectively (Table 34).
Administration of *C.majus* alone did not show any significant effect compared to control on any of the parameters studied

Protective effect of ***C.majus*** against effects induced by the coadministration of Methotrexate (2 **mg/kg,** *up*) and Vincristine(1 **mg/kg,** *up).*

The protective effects of prior administration of alcoholic extract of *C.majus* for three days was studied. In general *C.majus* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and vincristine . The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 65 %, 60% and 69% respectively when glutamate plus malate was used as substrate. (Table 27). The inhibition using succinate as substrate was relieved by 100%, 100% and 81% on State 3 , RCR and P/O ratio respectively (Table 28). The inhibition on NADH dehydrogenase was relieved by 76% and 100% in liver and kidney mitochondria respectively (Table 29 and Table 30). The inhibition on succinate dehydrogenase was relieved by 73% and 86% in liver and kidney mitochondria (Table 29 and Table 30). The inhibition on NADHxytochrome *c* reductase, Succinatexytochrome *c* reductase and cytochrome *c* oxidase was relieved by 70%, 77% and 76 % respectively in liver mitochondria (Table 31) and by 82% , 92% and 68 % respectively in kidney mitochondria (Table 32). The extent of swelling

decreased with the administration of plant extract (Fig 2). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down, showing a relief of 55%, 68% and 70% respectively when compared to controls (Table 33). Administration *of C.majus* resulted in 45% and 80% recovery in total phospholipids and cardiolipin content . Complete protection over decreased levels of phosphotidylcholine was obtained (Table 34).

Administration of *C.majus* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effects of *C.majus* against the effects induced by the **coadministration** of Methotrexate(2 mg/kg, *i.p*) and Cyclophosphamide **(150 mg/kg,** oral).

The protective effects of prior administration of alcoholic extract of *C.majus* for three days was studied. In general *C.majus* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 64 %, 77%, and 41% respectively when glutamate plus malate was used as substrate (Table 27). The inhibition using succinate as substrate was relieved by 100% on P/O ratio (Table 28). The inhibition on NADH dehydrogenase was relieved by 76% in liver mitochondria (Table 29). The inhibition on succinate dehydrogenase was relieved by 89% and 89 % respectively in liver and kidney mitochondria (Table 29 and Table 30). The inhibition on NADH:cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase was relieved by 67%. 60% and 70 % respectively in liver (Table 31) and atleast by 70% in kidney mitochondria (Table 32). The extent of swelling decreased with the administration of plant extract (Fig 2). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria, were brought down showing a relief of 70%, 91% and 62% respectively (Table 33). Administration of *C.majus* resulted in 65, 60%. 66% recovery over the decreased levels of, total phospholipids, phosphatidylcholine and cardiolipin content (Table 34). Administration of *C.majus* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect **of *Strychnos nux vomica.***

Protective effect **of *S.nux vomica*** against the effects induced by coadministration of Cisplatin (12 **mg/kg,** *i.p)* and Cyclophosphamide **(150 mg/kg , oral).**

The protective effects of prior administration of alcoholic extract of *S.nux vomica* for three days was studied. In general *S.nux vomica* offered protection on almost all the parameters that were affected by the coadministration of cisplatin and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved completely when glutamate plus malate was used as substrate

(Table 35). The inhibition on State 3, RCR and P/O ratio, using succinate as substrate was relieved by 79%, 46% and 89% respectively (Table 36). The increased activity of NADH dehydrogenase was brought down by 100% and 66% respectively in liver and kidney mitochondria (Table 37 and Table 38). The increased activity of Succinate dehydrogenase was brought down by 85% and 76% in liver and kidney mitochondria (Table 37 and Table 38). The inhibition on NADH: cytochrome *c* reductase, Succinate:cytochrome *c* reductase and cytochrome *c* oxidase was relieved by 82%,54% and 62% respectively in liver (Table 39) and by 63%,63% and 87% respectively in kidney mitochondria (Table 40). The extent of swelling decreased with the administration of plant extract (Fig 3). The increased levels of lipid peroxides in liver homogenate, liver mitochondria and kidney homogenate were brought down showing relief of 86%, 61% and 62% respectively (Table 41). Administration of alcoholic extract of *S.nux vomica* resulted in complete recovery over the decreased levels of total phospholipids and phosphatidylcholine phosphatidylethanolamine and cardiolopin content (Table 42).
Administration *of S.nux vomica* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *S.nux vomica* against the effects induced by **coadministration** of **Mitomycin** C (2 **mg/kg,** *up*) and **Cyclophosphamide (150** mg/kg, **oral).**

The protective effect of prior administration of alcoholic extract of *S.nux vomica* for three days was studied. In general *S.nux vomica* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 96%,78% and 81% respectively when glutamate plus malate was used as substrate (Table 35). The inhibition , using succinate as substrate was relieved by 97%, 100% and 86% on State 3, RCR and P/O ratio respectively (Table 36). Increased activity of NADH dehydrogenase was brought down by 91% and 60 % in liver mitochondria and kidney mitochondria (Table 37 and Table 38). The inhibition on Succinate dehydrogenase was relieved by 79% in liver mitochondria (Table 37). The inhibition on NADHxytochrome c reductase, succinatexytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 65%,77% and 65% respectively in liver (Table 39) and by 62%, 56% and 45% respectively in kidney mitochondria (Table 40). The extent of swelling decreased to a great extent with the administration of plant extract (Fig 3). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria was brought down showing a relief of 67%, 100 % and 60% respectively (Table 41). Administration of *S.nux vomica* resulted in complete recovery over the decreased levels of phosphatidylcholine content, while total phospholipids and cardiolipin content was brought up by 83% and 44% respectively (Table 42).
Administration of *S.nux vomica* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effects of *S.nux vomica* against the effects induced by the coadministration of Mitomycin C (2 mg/kg, *i.p*) and Cisplatin (12 mg/kg, *up).*

The protective effects of prior administration of alcoholic extract of *S.nux vomica* for three days was studied. In general *S.nux vomica* offered protection on almost all the parameters that are affected by the coadministration of mitomycin c and cisplatin. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 75%,84% and 60% respectively, when glutamate plus malate was used as substrate (Table 35). The inhibition using succinate as substrate was relieved by 70%, 75% and 78% respectively (Table 36). Increased activity of NADH dehydrogenase was brought down completely in liver and kidney mitochondria (Table 37 and Table 38). The inhibition on succinate dehydrogenase was relieved by 92% and 58% in liver and kidney mitochondria (Table 37 and Table 38). The inhibition on NADH:cytochrome *c* reductase, succinate:cytochrome c reductase and cytochrome *c* oxidase activities were relieved by 70%, 100% , and 83% respectively in liver (Table 39) and by 100% , 100% and 83% respectively in kidney mitochondria (Table 40). The extent of swelling decreased with the administration of plant extract (Fig 3). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria were brought down to control levels, showing 100% relief by the administration of *S.nux vomica* (Table 41). A decrease in phosphotidylcholine and cardiolipin was completely relieved bringing upto the control levels. The decrease in total phospholipid was relieved only by 88% (Table 42). Administration of *S.nux vomica* alone did not show any significant effect compared to control on any of the parameters studied.

Protective **effect** of *S.nux vomica* against effects **induced** by **the coadministration** of **Methotrexate (2 mg/kg, *i.p*) and Vincristine (1 mg/kg, *i.p*).**

The protective effect of prior administration of alcoholic extract of *S.nux vomica* for three days was studied. In general *S.nux vomica* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and vincristine . The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 57 %, 91% and 60% respectively when glutamate plus malate was used as substrate(Table 35). The inhibition using succinate as substrate was relieved by 61%, 57% and 60% on State 3, RCR and P/O ratio respectively (Table 36). The inhibition on NADH dehydrogenase was relieved partially by 51% and 52% in liver and kidney mitochondria respectively (Table 37 and Table 38). The inhibition on Succinate dehydrogenase was relieved by 63% and 80% respectively in liver and kidney mitochondria (Table 37 and Table 38). The **inhibition** on NADHxytochrome c reductase, Succinatexytochrome c reductase and cytochrome *c* oxidase activities were relieved by 94%, 77% and 56 % respectively in liver (Table 39) and by 89%,

69% and 53% respectively in kidney mitochondria (Table 40). The extent of swelling decreased to a great extent with the administration of plant extract (Fig 3). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria, were brought down showing **a** relief of 55%, 50% and 69% respectively (Table 41). The increase in total phospholipids, and decrease in cardiolipin due to these drugs was relieved by 66% and 69% respectively. The decreased content **of** phosphatidylcholine was brought up completely (Table 42).

Administration *of S. nux vomica* alone did not show any significant effect compared to control on any of the parameters studied.

Protective effect of *S.nux vomica* against the effects induced by the coadministration of Methotrexate(2 mg/kg, *up*) and Cyclophosphamide (150 mg/kg, oral).

The protective effect of prior administration of alcoholic extract of *S.nux vomica* for three days was studied. In general *S.mix vomica* offered protection on almost all the parameters that are affected by the coadministration of methotrexate and cyclophosphamide. The results are as follows: The inhibition on State 3, RCR and P/O ratios were relieved by 100 %, 72% and 76% respectively when glutamate plus malate was used as substrate (Table 35). The inhibition using succinate as substrate was relieved by 77% **on** P/O ratio respectively (Table 36). The inhibition on NADH dehydrogenase was relieved by 86% and 71% respectively in liver and kidney mitochondria (Table 37 and Table 38). The inhibition on Succinate dehydrogenase was relieved by 86% and 90 % respectively in liver and kidney mitochondria (Table 37 and Table 38). The inhibition on NADH:cytochrome c reductase, succinate xytochrome *c* reductase and cytochrome *c* oxidase activities were relieved by 72%. 70% and 96 % respectively in liver (Table 39) and by 70%, 100% and 81% respectively on all these parameters in kidney mitochondria (Table 40). The extent of swelling decreased to a great extent with the administration of plant extract (Fig 3). The increased levels of lipid peroxides in liver homogenate, kidney homogenate and liver mitochondria showed about 60% relief by the administration of *S.nux vomica* (Table 41). The significant decrease in total phospholipids, phosphatidylcholine and cardiolipin content was relieved by 75%, 65% and 40% respectively (Table 42).

Administration ***of S.nux** vomica* alone did not show any significant effect compared to control on any of the parameters studied.

DISCUSSION

Various potent antitumor drugs have been developed, but cancer chemotherapy has not produced good results due to various reasons of which the most important is clonal heterogeneity. Combination chemotherapy appears to be the key to overcome the problem of clonal heterogeneity, but dose dependent side effects appears to be increased in case of combination chemotherapy. Mitochondria represents 15- 50 % of total cytoplasmic volume of most cells and participate in more metabolic functions than any other organelles and it is also known as power house of the cell. It is apparent that their proper functioning is essential for maintaining a normal cellular metabolism. Any condition altering the mitochondrial homeostasis will, therefore have drastic consequences to the cell (Josephine *et al,* 1990). Anticancer agents like cisplatin (Sugiyama *et al,* 1989), cyclophosphamide (Govdzdjak *et al* 1981; Hanaki *et al,* 1990), doxorubicin (Geeta and Shymala devi, 1992), mitoxantrone (Llesuy and Arnaiz, 1990), piroxicam (Salgueiro- pagadigorria, *et al*,1996) etc. are known to effect normal cells. In the present study the effect of cisplatin, cyclophosphamide, methotrexate, vincristinc and mitomycin C in five different combinations was investigated on liver and kidney mitochondria.

Rosenberg *et al* (1965), developed a novel anti- tumour drug, cisplatin. It is most effective antineoplastic agent used in the treatment of patients suffering from various malignancies. The side effects are primarily, nausea and vomiting, which can be treated with the administration of anti-emetic drugs. The mechanism of the cisplatin induced renal and hepatotoxicity is yet to be worked out.

. Cyclophosphamide, a widely used antineoplastic and immunosupressive agent is of great interest due to its high oncotoxic specificity (Freidman *et al,* 1979). Metabolism is initiated by hydroxylation of mixed function oxidase. The main adverse effects due to administration of cyclophosphamide are bladder toxicity (Connors. 1981), cardiotoxicity (Hanaki *et al,* 1992), nephrotoxicity (Sugiyama *et al,* 1987) and hepatotoxicity (Brock *et al,* 1981). There is a report indicating mitochondrial dysfunction as the primary cause of cardiotoxicity (Hanaki *et al* 1990) . This drug is used in combination chemotherapy along with other drugs in the treatment of various cancers like metastatic urotehlial cancer (Fujji *et al,* 1991), and salivary gland carcinoma (Dimery, 1990). Coadministration of cyclophosphamide with methotexate (Klubes and Cerna, 1981) or vincristine (Connors, *et al,* 1981) is reported.

Mitomycin is a FDA approved antitumour agent. It is used in the treatment of sarcoma, leukemia and various other types of carcinomas. Mitomycin C is very effective cross linker of DNA both *in vivo, and in vitro,* but the effect requires prior activation of the molecule, a property that distinguishes it from other alkylating agents. Toxic effects observed in animals were hypoplasia of bone marrow, lymphoid tissue damage and lesions in the intestinal epithelium. Mitomycin C is useful in treating dessimnated breast, gastric, pancreatic or colorectal adenocarcinomas in combination with 5-Flurouracil and Adriamycin . It is used in combination with cyclophosphamide and adriamycin for lung cancer.

Methotrexate (amethopterin, 4-amino-4-deoxy N^{10}-methylptroyl-glutamic acid) is a folic acid antagonist introduced in 1948 to treat acute leukemia. It is used extensively in the treatment of several neoplastic and also many non-neoplastic diseases. Plasma concentration of methotrexate have shown to be the best predictor of methotrexate toxicity (Masson *et al*, 1996). 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 both normal and malignant hepatocytes even after the disappearance of the parent drug (Baliniska *et al,* 1981). The most common adverse effects seen with methotrexate is on gastrointestinal tract. These effects include nausea, vomiting, anorexia , diarrhea and stomatitis. Most severe side effects of this drug include hepatotoxicity, carcinogenicity, pulmonary toxicity and nephrotoxicity (Goodman and Polisson, 1994). 7- Hydroxy methotrexate plays a direct role in inducing the toxic effect of methotrexate in kidney and liver cells (Smeland *et al,* 1994).

Vincristine is the vinca alkaloid derived from periwinkle plant. It is an important anticancer drug that is effective against wide variety of neoplasm's like Hodkins and non Hodkins Lymphomas, Acute lymphoblastic leukemia, embryonal rabdomyosarcoma, 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 (Owellen, 1972). Zotikov and Barbouk (1980) studied bone marrow cells in rat and noted the ultrastructral changes in the membranes of mitochondria and nuclear envelope. Vincristine is used in the combination chemotherapy with cyclophosphamide, mitomycin C, adriamycin, methotrexate and other drugs in the treatment of various types of cancers.

The present study clearly shows that there is a significant decrease in the State 3 respiration, RCR and P/O ratio using either NADH or succinate as substrates with all the five drug combinations studied. (Table 1 and 2). These changes in the ADP/O ratio appear to be small but, they imply that if all this additional oxygen were generating free radical generation, it would be enough to cause significant damage to mitochondria energy metabolism. In mitochondria, the respiratory chain is stoichiometrically related to that of ATP synthase (Pozzan *et al,* 1979). If the substrate is oxidized throught out all three sites of electron transport chain, three ATP / atom of oxygen is generated (example NADH oxidation), on the other hand it is oxidised only through two sites (site 2 Plus site 3), only 2 ATP / atom of oxygen (example succinate oxidation) oxidation of ferrocytochrome c involves only one site (site 3) and thus generates only one ATP/ atom of oxygen. The mitochondrial respiration is tightly coupled to oxidative phosphorylation in intact cells in normal tissue (Tzagoloff and Meyers 1986).

Tightly coupled mitochondria always have high RCR and ADP/O ratios. Decreased RCR and ADP/O ratio indicates damage of mitochondrial membrane, there by rendering the membrane leaky to their ions that cause uncoupling of mitochondria. Increased permeability of mitochondrial membrane leads to uncoupling of oxidative phosphorylation (Sonussi *et al*, 1996). Impairment could result from the alterations of the very function of the normally synthesized respiratory subunits by a deleterious cellular or mitochondrial event that would occur in the course of drug treatment.

In the present study administration of all the combinations of drugs showed significant decrease in the ADP/O ratios . Under these conditions most of the energy that is liberated by substrate oxidation could not be utilized for phosphorylation . So it is dissipated in the form of heat and results in the production of excess heat which would stimulate the body to lose heat in the form of sweating. The low P/O ratios will also affect vital reactions in the cell. These are the reasons for some of the side effects observed due to the administration of these drugs in cancer chemotherapy. The decrease in P/O ratio may be due to site specific defect (electron leak) in the respiratory chain. This type of electron leak would lead to univalent electron transfer to oxygen at a sites other than cytochrome aa_3 and would be expected to result in free radical generation.

NADH dehydrogenase introduces electrons to electron transport chain at site I level . In the present study the activity in the liver mitochondria was significantly increased (Cisplatin plus cyclophosphamide, mitomycin C plus cyclophosphamide and mitomycin C plus cisplatin) or significantly decreased (Methotrexate plus vincristine and methotrexate plus cyclophosphodphamide) depending upon the drug combination used. NADH dehydrogenase activity of kidney mitochondria also showed a similar pattern (Table 3 and Table 4).

Inhibition of NADH dehydrogenase activity means, the transfer of electrons from NADH to the prosthetic group of flavoprotein (E-FMN to E-FMNH2) is interrupted. Thus electron transfer is effected even before the electrons enter the electron transport chain. Complex 1 catalyzes the transfer of reducing equivalents from NADH to Ubiquinone. Complex 1 is the largest of the five major complexes in the inner membrane. This complex contains approximately 40 protein subunits and number of non protein components including FMN, non heme iron, acid labile sulfure, ubiquinone and phospholipids. The relativly high frequency of occurrence of structural / functional defects in the complex may be due to large size of the complex, large size implying a large target or a large number of potential sites for structural disruption and / or biogenic difficulties . Super oxide that is known to be generated by Complex I (Turrens and Boveris, 1980) has a good chance of attacking this macromolecule in a way that impairs its function.

Succinate dehydrogenase introduces electron to ETC at site II (Complex II) of the respiratory chain. Significant increase (Cisplatin plus cyclophosphamide and methotrexate and cyclophosphamide) and significant decreases (Mitomycin C plus cyclophosphamide, mitomycin C plus cyclophosphamide and methotrexate plus vincristine) in the activity of SDH was observed in liver mitochondria depending upon the drug combination used (Table 3). Kidney mitochondria also

showed a significant increase (Cisplatin plus cyclophosphamide and Methotrexate plus cyclophosphamide) and significantly decrease (Methotrexate plus vincristine and mitomycin c plus cisplatin) in the activity of SDH depending upon the drug combination used. Mitomycin C plus cyclophosphamide did not show a significant change. (Table 4).

Inhibition in succinate dehydrogenase activity means the inhibition of the transfer of electrons from succinate to the prosthetic group of the flavoprotein. Decrease in succinate dehydrogenase activity could be due to a complex formation of mitomycin C with FAD as reported earlier (Okuda *et al,* 1989). Succinate dehydrogenase is sensitive to thiol binding reagents. Inhibition of the enzyme by this kind of reagents results from the modification of a sulfahydryl group located at the active site.(Jay *et al,* 1991). This thiol, although not essential for binding of substrate or catalysis, could influence the binding of dicarboxylates, probably by steric hindrance when a larger group or a charged group is attached to it (Schroder *et al,* 1991). The inhibition of this enzyme by histidine specific reagents has also been reported, and the participation of an imidazole ring in the initial step of succinate oxidation has been suggested (Vik and Hatefi, 1981). NADH: cytochrome *c* reductase activity is a measure of the transfer of electrons from complex I through coenzyme Q to complex III. Succinatexytochrome c reductase activity is a measure of the transfer of electrons from Complex II through coenzyme Q to complex III.

The present study shows that there is a significant decrease in the activity of NADHxytochrome c reductase and succinate: cytochrome c reductase with all five drug combinations using either liver or kidney mitochondria (Table 5 and 6). Cytochrome *c* oxidase was also significantly decreased both in liver and kidney mitochondria for all drug combinations except methotrexate and cyclophosphamide, in which there is a significant decrease in the activity of the enzyme (Table 5 and 6).

It is likely that the decreased activity of NADHxytochrome *c* reductase and succinatexytochrome c reductase is responsible for the significant decrease in the State 3 respiration. The cytochromes contain iron which are highly vulnerable for reactive oxygen species which may induce metal catalyzed oxidation with in the active centers of respiratory chain complexes. Subsequently a chain reaction involving the protein peroxides may occur with in the enzyme complexes, finally leading to inactivation of proteins (Simpson *et al* , 1992 and Davis and Dean, 1995). Inhibition in the activity of cytochrome *c* oxidase indicates that the transfer of electrons through complex IV is hampered.

There was a significant increase in the level of free radicals in liver homogenate and kidney homogenate respectively with all the drug combinations studied (Table 7).

Measurement of MDA is a useful method for determination of LPO products (Sawicki *et al,* 1963; Placer *et al,* 1966). The level of lipid peroxides formed in liver homogenate, kidney homogenate and liver mitochondria were significantly higher due to the drug treatment (in all the five combinations) when, compared to controls (Table 8).

Recent advances in medicine showed that oxygen radicals and hydrogen peroxides are linked to the development of several pathological processes including adverse effects of antitumor drugs. Free radicals induce denaturation of proteins, peroxidation of membrane lipids and generation of chemotactic factors, eventually destroying cellular integrity .

Otani *et al,* (1984) demonstrated that incubation of ischaemic mitochondria with superoxide dismutase, and catalase recovered adenosine triphosphate production under aerobic conditions. Shlafer *et al,* 1987 reported that decrease in glutathione peroxidase and superoxide dismutase in mitochondria are closely related to ischaemia - induced damage. These results suggests that mitochondria are vulnerable targets of free radical - mediated damage. The adverse effects of some of the antitumor drugs might be ascribed to the generation of oxygen radicals (Dorshow, 1983). Many anticancer drugs are known to bring about tumoricidal actions by a free radical dependent mechanism. Anticancer drugs like adriamycin, mitomycin C, bleomycin etc. augment free radical production *in vitro and in vivo* (Sangeeta *et al,* 1990). Cardiotoxicity of cyclophosphamide and nephrotoxicity of cisplatin were reported to be due to mitochondrial dysfunction (Hanaki *et al,* 1990; Sugiyama *et al*, 1989)

Role of mitochondria in cisplatin - induced oxidative damage was reported earlier(Zhang and Lindup 1993). Thus mitochondrial dysfunction may contribute to complications by producing potentially toxic free radicals and diffusible prooxidants. These include for example reactive lipid peroxidation by products, such as malonaldehyde and the hydroxy alkenals (Esterbauer *et al,* 1991). Increased mitochondrial production of reactive oxygen species by mitochondria may consume antioxidants or deplete them at sites where they may be needed.

There is a interdependency between reactive oxygen species and lipid peroxidation. Reactive oxygen species initiate the reactions of lipid peroxidation and are also produced in these reactions as intermediates (Wilhelm, 1990). Following initiation of lipid peroxidation membrane protein thiols can be attacked by lipid derived radicals and or reactive, lipid- soluble aldehydes like 4-hydroxynoneal and other hydroxyalkenals originated within the lipid core of cell membranes, resulting in a membrane protein thiol loss which in turn is associated with the development of hepatocellular injury (Pompella *et al,* 1991). This appears to be the mechanism of hepatotoxicity or neprhotoxicity caused by the anticancer drugs studied in the present investigation. Lipid peroxidation have been implicated in doxorubicin -induced cardiotoxicity (Ogawa *et al,* 1987). Cisplatin is known to enhance production of free radical species in bladder cancer cells (Miyajima *et al,* 1997). Cisplatin induced nephrotoxicity appears to be due to a decrease in mitochondrial protein sulphahydryl concentration and calcium uptake by mitochondria . It was also observed that mitochondrial concentration of glutathione is an important factor in relation to oxidative stress to mitochondria and cytotoxicity (Zhang and lindup, 1994).

The reaction of cytochrome *c* with H_2O_2 promotes membrane oxidation by more than one chemical mechanism. The formation of high oxidation states of iron at the cytochrome heme and also by release of heme iron at higher H_2O_2 concentrations. Cytochrome c^{3+} could react with mitochondrial H_2O_2 to yield site specific mitochondrial membrane lipid peroxidation during tissue oxidant stress (Radi *et al,* 1991).

The present study shows that the administration of anticancer drug (any of the five combinations studied) significantly decreases the cardiolipin content of the liver mitochondria . The decrease in cardiolipin was much more compared to other phospholipid for any drug combinations studied (Table 9).

Studies by Moller and Dargal (1984) have shown that phospholipids are essential for providing proper polar and spatial arrangement necessary for optimal activity of membrane bound enzymes and carriers. A small change in lipid components may cause marked changes in the activity and efficiency of enzymes or carriers. Phosphatidylcholine and phosphatidylethanolamine have been shown to be essential for the normal functioning of cytochrome *c* oxidase in ethanol fed baboons (Aria *et al,* 1984).

Psychosine, a cytotoxic agent in micromolar concentrations inhibits electron transfer through site I and site III and the transfer through site II was not affected. These results are correlated to a difference in the requirement of these three sites for cardiolipin (Tapasi, *et al,* 1998).

The interaction of positively charged cisplatin species with negatively charged phospholipids appears to play important role *in vivo* (Speelmans *et al,* 1996). Cytochrome *c* oxidase activity is dependent upon cardiolipin located in the inner mitochondrial membrane and under conditions of high oxidative stress, it is prone to damage by lipid peroxidation due to the high degree of unsaturation . It has also been reported that cardiolipin activates the cytochrome chain and influences several mitochondrial carrier systems including ATP synthesis.(Awasthi *et al,* 1971, Spencer *et al* 1976, Dekruijff *et al,* 1981). The decrease in cytochrome oxidase activity was due to a decrease in cardiolipin content and its role in controlling membrane fluidity was suggested (Paradies *et al,* 1994).

The decrease in cytochrome oxidase activity was associated with changes in lipid composition of the mitochondrial membranes accompanied by decrease in the concentration of cytochromes (Arai *et al,* 1984). Cisplatin is known to form complex with phosphatidyl serine *in vitro* and *in vivo.* Since phosphatidyl serine is essential in many cellular processes , its interaction with cisplatin may have important implications (Speelmans *et al,* 1997).

A correlation for a decreased rate of respiration increased oxidative modification of mitochondrial protein, and increased lipid peroxidation in isolated mitochondria by hypoxia / reoxygenated is reported recently (Schild *et al,* 1997). . They further showed that the changes were largely prevented by water soluble antioxidants which diminish the formation of ROS by mitochondria.

An impairment in oxidative phosphorylation would cause decreased levels of ATP, which in turn would affect the high turn over of phospholipids (Ellinngson *et al*, 1988). Degradation of mitochondrial phospholipids during ischaemia associated with the deterioration of mitochondrial respiratory functions suggested the importance of phospholipid in cellular energy metabolism during cerebral ischaemia.(Nakahara *et al* 1991).

The production of reactive oxygen species may result in oxidative inactivation of respiratory complexes. (Complex I and Complex II where the superoxide is mostly generated during respiration). This would result in autocatalytic inhibition of mitochondrial chain components. First, the mechanism of enzyme inactivation would reside in the alteration of amino acid residue highly sensitive to oxidation (Lippe *et al,* 1993). These amino acids include methionine , histidine and tryptophan located at strategic sites in enzymes (Johnson and Travis, 1979). The other reason which is more probable is, several enzymes such as dehydratase and tricarboxylic acid enzymes, aconitase are extremely sensitive to superoxide (Gardner *et al,* 1991). The common structural point between these proteins is the existence of prosthetic groups iron sulphur clusters in their structure. It has been assumed that superoxide is electrostatically attracted to the active site region of these clusters where it interacts with and oxidizes the iron atoms, resulting in the rapid loss of enzyme activity (Gardener, 1991). Complex 1 and complex 111 also contains such iron sulphur clusters (Nelson, 1987). Biotier *et al* (1995) concluded that the damaging effect of superoxide was due to the oxidation of iron sulphur clusters. Superoxide was not catabolised by an impaired antioxidant pathway in the course of diethylnitrosamine - induced hepatocarcinogenesis.

Mitochondrial membrane contains large number of Poly unsaturated fatty acids (PUFAS) in their phospholipids and are prime sites of lipid peroxidation (Fleischer and Rouser, 1965). In the present study swelling of mitochondria was observed with the administration of any of these combination of anticancer drugs that was used .(Fig -1). Role of lipid peroxidation in swelling and lysis leading to disintegration of mitochondria is reported (Hunter *et al* 1964; Mcknight *et al* , 1965). //: *vitro,* lipid peroxidation of isolated rat liver brought about mitiohondrial swelling, a decrease in membrane potential and alteration in membrane proteins (Castilo *et al,* 1994).

It is interesting and important to know about the agents which either abolish or minimize the toxicity that is induced by the anticancer drugs, without effecting their antitumor activity. In the present study the protective effect of the extracts from four plants i.e *B.aristata, P.fraternus, C. majus amd S.nux vomica* was studied, against the mitochondrial dysfunctions. The protective effect was studied on State 3 respiration, RCR and P/O ratio using NADH or succinate as substrates. It was also studies on , the transfer of electrons through the different segments of Electron transport chain, lipid peroxidation, and phospholipid distribution in mitochondria. The results indicate that these plant extracts showed protection on all the parameters studied, but extent of protection was varied depending on the plant used, and the parameter studied. For example :- Compare Table 1 with Table 10 or 18 or 27 or 35. The significant effects seen on NADH oxidase in Table 1 due to the administration of anticancer drugs is not seen because

of the protective effects of *B. aristata* (Table 10) or *P. fraternus* (Table 18) or C.majus (Table 27) or *S.nux vomica* { Table 35). Similar protection was observed on all the parameters studied . These plant extracts not only relieved the inhibition on a particular parameter but also abolished stimulation . For example :- A significant inhibition was seen on cytochrome *c* oxidase activity in liver mitochondria (Table 5) with all the drug combinations studied except wth methotrexate and cyclophosphamide, in which there was a significant increase in the activity of this enzyme. Administration of plant extract, increased (when the activity was inhibited) or decreased (when the activity was stimulated) the activity (compared Table 5 with Table 14, 22, 31 and 44) to a level that is insignificant campared to controls . Same thing is true for other parameters like succinate dehydrogenase and NADH dehydrogenases.

Glutathione has been shown to protect against the toxicty induced by cyclophosphammide (Gurtoo *et al,* 1981) or cisplatin (Inselmann *et al,* 1995). Administartion of a lethal dose of cyclophosphamide along with garlic extract *(i.p*) has been shown to reduce the toxicity by reducing the lipid peroxidation (Unnikrishnan *et al,* 1990). Administration of dimethylsulphoxide (DMSO) or elastase protected against the cisplatin induced nephrotoxicity (Jones *et al,* 1991 and Suzuki *et al,* 1991). Dithiothreitol (DTT) Showed a concentration dependent (0.5 - 2 mmole) protective effect on cisplatin induced toxicity (Zhang *et al,* 1994). Methotrexate increased paracetamol induced toxicity by decreasing the level of glutathione (Lindenthal *et al,* 1993). Methotrexate induced anorexia and diarrhea were abolished when soyabean concentrate was used as a sole protein source (Funk and Baker, 1991). Polyoxy- ethylene modified superoxide dismutase could prevent the side effects of mitomycin C plus adriamycin with out effecting the antitumor activity either *in vivo* or *in vitro.* (Kawasaki *et al* , 1992).

Hepatotoxicity induced by ethionine or thioacetamide or chronic alcoholism could be prevented by co- administration of vitamin. E (Padma and Setty , 1997_a, Padma and Setty , 1998)or an aqueous extract of *P fratermis* (Padma, and Setty, 1997_b; Sebastian and Setty, 1998) or *B. aristata* (Sebastian and Setty, 1997). The co adminitration of these protective agents significantly decreased the hepatotoxin induced lipid peroxidation .

Several anticancer drugs like mitomycin C bleomycin etc. increased the free radical production and thus increased lipid peroxidation both *in vivo* and *in vitro* (Sangeeta *et al,* 1990). The enzymes indentified for the control of lipid peroxidation are, superoxide dismutase , catalase ,and glutathione peroxidase (Flohe *et al,* 1982). Antioxidants reduce lipid peroxidation by donating hydrogen atom to a free radical, which then gets inactivated. Water soluble anti- oxidants like ascorbic acid, cysteine, uric acid and glutathione are present in cytosol and prevent lipid peroxidation in aqueous phase (Chow and Khan, 1983) Antioxidants that are present in membrane are vitamin E and ubiquinone, which are known to minimise lipid peroxidation . Inner membrane of mitochondria contain more vitamin E than outer membrane (Thomas *et al,*\ 981) and vitamin E is a better antioxidant than ubiquinol (Thomas *et al,* 1993).

Administration of aqueous extract of *P.fraternus* leaves were reported to produce hypoglycemic action in normal as well as alloxan diabetic rabbits (Ramakrishnan *et al*, 1982). This plant has been shown to be effective along with other siddha drugs in the treatment of jaundice due to infective hepatitis (Ramanan and Sainani, 1961, Thygarajan *et al*, 1977).

The alcoholic extract of *B.aristata* is useful in the treatment of jaundice and enlargement of spleen . The extract of the plant also showed anticancer activity (Dhar *et al*, 1969) . It has been shown that the extract of *P. fraternus* and *B. aristata* scavenge hydroxyl radical and reduce lipid peroxidation (Joy, 1995) . The extract of *S. nux vomica* inhibited lipid peroxidation by chelating metal ions (Chaurasia and Tripathi, 1996). Alcoholic extract of *C. **majus*** showed antimycotic activity (Vukusic, 1991) and it also prevented the CCL_4 induced hepatotoxicity (Mitra *et al*, 1992). Ukrain , which is a semisynthetic derivative of C. majus , is reported to have antitumor activity (Lohninger and Hamler, 1992; Nowicky *et al*, 1992). Recently it has been shown that a herbal formulation (Liv 100 from Himalaya drug company) reduced the peroxidation effect of hydrogen peroxide *in vitro* and this effect was due to the increased supply of reduced glutathione, which inhibits the lipid peroxidation (Suja *et al*, 1997).

From all thes studies it appears that the hepatotoxicity induced by anticancer drugs is mainly attributed to the free radical production which is responsible for oxidation of membrane proteins or/ and increased lipid peroxidation . In the present study the effect of administration of the plant extracts on the antitumor activity of anticancer drugs (all the five combinations studied) was not studied. It is unlikely that these extracts can decrease the antitumor activity because, some of the plant extracts (*B.aristata* and *C.majus*) themselves are shown to have antitumor activity (Dhar *et al*, 1969: Lohninger and Hamler, 1992: Nowicky *et al*, 1992.) A future work will be focussed more in these lines.

This work clearly shows that the administration of anticancer drugs significantly effect the oxidative phosphorylation and these effects could be prevented by prior administration of the extract of *P.fraternus or B.aristata or C.majus or S.nux vomica*. Apart from the antioxidant property, the extracts contains novel pharmacological agents (which are yet to be worked out) that, is responsible for the protective effect on all the parameters studied.

SUMMARY

SUMMARY

1. Effect of five combinations of anticancer drugs (Methotrexate plus vincristine, mitomycin C plus cyclophosphamide , mitomycin C plus cisplatin, cisplatin plus cyclophosphamide and methotrexate plus cyclophosphamude) on various parameters in mitochondria is studied. The results indicate that there is a significant decrease in the State 3 respiration, RCR and P/O ratio using either glutamate plus malate or succinate as substrates. A similar results were obtained for all the five drug combinations, when studied independently.

2. Administration of anticancer drugs (all five different combinations) significantly affected succinate dehydrogenase, NADH dehydrogenase, NADH:cytochrome *c* reductase, succinatexytochrome *c* reductase and cytochrome c oxidase both in liver and kidney mitochondria.

3. Administration of anticancer drugs induced free radical production and thus increased lipid peroxidation. Cardiolipin fraction of the phospholipid was most affected and a significant decrease was seen for all the drug combinations studied.

4. All these effects on mitochondria which are induced due to the administration of anticancer drugs, could be, partly or completely prevented by prior administration of the extract of *P.fraternus* or *B.aristata* or *C.majus* or *S.nux vomica.* The extent of protection varied depending upon the plant used and the parameter studied. The administation of anticancer drugs induced free radical production which is responsible for oxidation of membrane proteins or/ and increased lipid peroxidation. which is responsible for the observed mitochondrial dysfunctions. Apart from the antioxidant property the extract of these plants contain novel pharmacological agents (which are yet to be worked out) that could show protective effect on all the parameters studied.

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