Introduction
INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and is spreading further with continuance and increasing incidence in the 21st century. A recent report from population-based cancer registries in India, for 1999-2000 shows that the age-adjusted cancer incidence for males in the urban centres vary from 97.8 to 119.8 per 1,00,000 people, and in females from 109.6 to 126.7. In the rural registry, its incidence is 45.0 and 54.2 per 1,00,000 people in males and females respectively (Sharma, 2005). About 8,00,000 new cases of cancer are estimated to occur every year (Reddy et al., 2005) in India.

Cancer is characterized by uncontrolled abnormal proliferation of cells that invade normal tissue and metastasize into distant organs. Cancer disturbs the cellular activities that are crucial for the development and the maintenance of multicellular organisms, namely growth, differentiation, programmed cell death, and tissue integrity (Mareel and Leroy, 2003). The management of malignant disease with chemotherapeutic drugs has become a reality now. Cytotoxic chemotherapy is routinely used in the treatment of cancer, and has been an important factor in increasing survival rates for some types of this disease. Despite its success, treatment with some of the most effective anticancer drugs provides a traumatic experience for the patients, and there are a number of symptoms of direct toxicity.

Alkylating agents were the first drugs designed to reach clinical practice and they are in use worldwide even today (Ferguson and Pearson, 1996). Cyclophosphamide is a commonly used anticancer and immunosuppressant drug. Although it has some tumour selectivity, it also
possesses a wide spectrum of toxicities (Fraiser et al., 1991). Therapeutic balance and efficacy of antitumour therapy should be optimized, taking the cytotoxic side effects into consideration.

1.1 CYCLOPHOSPHAMIDE

In 1919, the specific action of sulphur mustard on the bone marrow prompted a possible use as an anticancer drug in lymphomas and leukemias. The nitrogen mustards are nitrogen analogues of sulphur mustard. The first anticancer drug to reach clinical use was mechlorethamine, a nitrogen mustard and a bifunctional alkylating agent (Lawley and Philips, 1996). Cyclophosphamide (CP) is a cyclic phosphoramidate ester synthesized first in 1958, as a transport form of chlormethine (mustine, mechlorethamine) (Arnold et al., 1958), belonging to the nitrogen mustard subclass of alkylating agents. The alkylating agents become strong electrophiles and their chemotherapeutic effects are directly related to alkylation of DNA. The 7-nitrogen atom of the guanine residues in DNA is particularly susceptible, which labilizes the imidazole ring and also causes mispairing of bases. Bifunctional alkylators like CP cause interstrand cross linkage of DNA.

1.1.1 CLINICAL SPECTRUM OF ACTIVITY OF CYCLOPHOSPHAMIDE

Since its original production, the clinical use of CP has been extended from neoplastic diseases to organ transplantation and diverse disorders, wherein it is used as an immunosuppressive agent.
1.1.1.1 **Cancer chemotherapy**

Nearly all the chemosensitive tumour types show response to CP. CP is a non-cell-cycle specific alkylating agent (Gharib and Burnett, 2002). However, the effects are seen usually when the cells enter the S phase and progress through the cell cycle is blocked at the G2 phase (premitotic stage). Its potent cytostatic properties makes it effective against

- Acute lymphatic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia
- Ewing’s sarcoma, osteogenic sarcoma, rhabdomyosarcoma, soft tissue sarcoma
- Breast cancer, endometrial cancer, lung cancer, ovarian cancer, testicular cancer
- Hodgkin’s disease, Burkitt’s lymphoma, neuroblastoma, non-Hodgkin’s lymphoma, retinoblastoma, germ cell tumours

1.1.1.2 **Immunosuppression**

The immunosuppressant properties have been exploited successfully in organ transplantations. It forms the nucleus for virtually all preparative regimens for autologous bone marrow transplantation (Ayash *et al.*, 1992). It is used in non-neoplastic settings including

- Rheumatoid arthritis, systemic lupus erythematosis, Wegener’s granulomatosis, psoriatic arthritis
- Glomerulonephritis, idiopathic nephritic syndrome, cryptogenic fibrosing alveolitis
1.1.2 CHEMISTRY AND PROPERTIES

1.1.2.1 Structure

CP was designed as an inert transport derivative that requires metabolic activation by cytochrome P450 mixed functional oxidase in the liver (Ahmed and Hombal, 1984). CP is chemically N,N-Bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine 2-oxide. The biological activity is based on the presence of the bis(-2-chloroethyl) group while the cyclic phosphamide group makes it relatively inert.

![Structure of cyclophosphamide](image)

Figure 1.1 Structure of cyclophosphamide

1.1.2.2 Properties

CP has a melting point of 49.5°C to 53°C and is sensitive to moisture and light. It is soluble in water and ethanol, slightly soluble in benzene, ethylene glycol, carbon tetrachloride and dioxane while sparingly soluble in diethyl ether and acetone. CP is chiral and is administered as a racemate (R and S mixture). It is a fine, white, odourless, crystalline powder prepared by chemical synthesis. It is produced by treating N,N-bis(2-chloroethyl) phosphamide dichloride with preparolamine in the presence of trimethylamine and dioxane (Anderson et al., 1995).
1.1.3 PHARMACOKINETICS

CP can be administered both by oral and intravenous route. Dosage may range from 300 mg/m² body surface area to about 2-3 g/m² over a 3 week period. CP is well absorbed after oral administration with a bioavailability of 75% (Dollery, 1999).

The liver microsomal cytochrome P450 mixed function oxidase system converts the prodrug CP, to 4-hydroxycyclophosphamide, which is in equilibrium with its acyclic tautomeric form, aldophosphamide. 4-hydroxycyclophosphamide is transported to the target tissues by the circulatory system. The final stage of activation, proposed to take place in cells, which are susceptible to cytolysis, involves spontaneous non-enzymatic cleavage of aldophosphamide by a β-elimination reaction to yield N,N-bis(2-chloroethyl)phosphorodiamidic acid or phosphoramidate mustard (PM) and acrolein, both of which are highly cytotoxic and are the active forms of the drug (Chabner et al., 1977). Further hydrolysis of PM yields nor-nitrogen mustard, which does not possess any alkylating activity in physiological conditions (Figure 1.2). The minor alternate pathway of CP metabolism is N-dechloroethylation, which yields dechloroethyl-CP and chloroacetaldehyde. Dechloroethyl-CP seems to be devoid of anticancer or toxic activities while chloroacetaldehyde has been associated with neuro and urinary tract toxicities (Yu et al., 1999).

Serum half-life of CP is 6.5 h while that of 4-hydroxycyclophosphamide and PM are 6 min and 40 min respectively. Protein binding of CP is low (12-25%) but higher values of about 50% are seen for the metabolites (Dollery, 1999).
Figure 1.2 Metabolism of Cyclophosphamide
1.1.3.1 Cytochrome P450 Isoenzymes

CYP2B6 is the major enzyme contributing to ~45% of total metabolic activation of CP, compared with 25 and 12% for CYP3A4 and CYP2C9, respectively in humans (Chen et al., 2004). CYP2C6 and CYP2C11 are the major catalysts responsible for the bioactivation of CP in uninduced rat liver microsomes (Clarke and Waxman, 1989). The liver is the primary organ for oxazaphosphorine activation, but extrahepatic organs such as lungs, intestines, kidneys and in particular tumour tissue may also play a role in the 4-hydroxylation of oxazaphosphorines to a much lesser extent (Zhang et al., 2005).

1.1.3.2 Detoxification and Elimination

Dehydrogenation of 4-hydroxycylophosphamide by aldehyde dehydrogenase forms inactive 4-ketocyclophosphamide while oxidation of aldophosphamide yields inactive carboxyphosphamide. CP is eliminated primarily in the form of metabolites with less than 20% of the drug appearing unchanged in urine (Chabner et al., 1977).

1.1.4 SPECIFICITY OF CYCLOPHOSPHAMIDE

CP targets rapidly dividing cells such as cancer cells, bone marrow cells, stimulated lymphocytes, fetal cells, hair follicle cells and intestinal cells. CP was initially designed to be activated by cleavage of phosphamide ring by high phosphamidase activity in tumour tissue (Chabner et al., 1996). The basis of high cytotoxic specificity may be the reactions of 4-hydroxycylophosphamide with various thiol compounds which enhance the stability of its metabolites in plasma, facilitate their entry into cells, direct
their movement into specific sites inside the cells and delay the release of the alkylating PM (Ahmed and Hombal, 1984). Another hypothesis states that normal cells contain a higher level of oxidative enzymes for detoxification of 4-hydroxycylophosphamide and aldophosphamide than in neoplastic cells. The active metabolites are highly hydrophilic and can permeate tumour cells, which have more hydrated membranes than the normal cells (Brock and Hohorst, 1967).

1.1.5 CYCLOPHOSPHAMIDE TOXICITY

CP presents a cytotoxic action and interferes with normal mitosis and cell division in all rapidly proliferating tissues, which provides the basis for its therapeutic effects and many of its toxicities (Fraiser et al., 1991). CP is known to cause mutagenic, teratogenic and carcinogenic effects (Ahmed and Hombal, 1984). In CP injected rats, the frequency of micronucleated polychromatic erythrocytes increased in the bone marrow and peripheral blood (Selvakumar et al., 2006a). It induces glutathione-S-transferase (GST-Pi) positive single cells and foci expression in rat liver, which can be considered as precursors of preneoplastic foci leading to hepatocarcinogenesis (Devi and Devaraj, 2006).

1.1.5.1 Myelosuppression

Nausea and vomiting are predominant side effects while myelosuppression consisting primarily of leukopenia is noteworthy after CP treatment (Fraiser et al., 1991). CP causes a reduction of rapidly circulating small lymphocytes as well as impairs humoral antibody production and
cellular immune responses (Muruganandam et al., 2005). This results in a decreased ability to tolerate bacterial and viral infections.

1.1.5.2 Urotoxicity

Hemorrhagic cystitis is a distressing complication of CP chemotherapy (Xu et al., 2001). The toxic metabolites of CP react with the urothelium and increase the sensitivity of the bladder to damage. Acrolein specifically is presumed to cause urotoxicity. Epithelial degeneration, hemorrhage, necrosis and severe bladder damage were observed after CP administration (Manesh and Kuttan, 2005). Hyperplasia with eventual bladder fibrosis has also been reported (Austin et al., 1986). The syndrome of inappropriate secretion of antidiuretic hormone has been observed in patients receiving high-dose CP (Defronzo et al., 1973). Mesna, a thiol compound is recommended to prevent urotoxicity (Hensley et al., 1999).

1.1.5.3 Liver and Lung Toxicities

CP causes an early onset pneumonitis that is reversible and a late-onset type with progressive fibrosis associated with lateral pleural thickening (Malik et al., 1996). Venkatesan et al. (1998) have reported that CP-induced lung fibrosis results in alterations in collagen synthesis, accumulation and also in glycoprotein content. Relatively high activity of prostaglandin H synthase may cause toxicity of CP (Smith and Kehrer, 1991). Liver and kidney cytosol contain high levels of detoxifying enzymes and are unaffected by the metabolites of CP (Cox et al., 1975). However CP administration resulted in destructive changes in the subcellular organelles of hepatocytes, which may be mediated by reactive oxygen species (ROS) (Sulkowska et al., 1999).
1.1.5.4 Reproductive toxicity

A significant decrease in cytochrome P450, accompanied by a decrease in the hormone testosterone was noted in male rats after a single dose of CP (Clarke and Waxman, 1989). Selvakumar et al. (2006b) have reported that CP-induced oxidative testicular injury is characterized by oligozoospermia. Le Blanc and Waxman (1990) suggested that acrolein may act at the hypothalamic-pituitary gonadal axis, decreasing testosterone and perturbing the regulation of cytochrome P450. CP causes a dose- and time-dependent depletion of ovarian follicles and ovarian atrophy (Plowchalk and Mattison, 1992).

1.1.6 CARDIOTOXICITY OF CYCLOPHOSPHAMIDE

1.1.6.1 Manifestations

High doses of CP can cause an acute form of cardiotoxicity within 10 days of its administration (Gharib and Burnett, 2002). Administration of intermittent massive dosage of CP has been found to be advantageous in chemotherapy (O’Connel and Benenbaum, 1974). Recently Morandi et al. (2005) have reported that high dose CP-containing chemotherapy regimens have been most commonly associated with cardiac toxicity, with a progressively decreasing incidence over time. The incidence of symptomatic cardiomyopathy is 22% and that of fatal cardiotoxicity is 11% (Kumar et al., 1992). A recent report shows the development of acute cardiotoxicity after CP treatment (Senthilkumar et al., 2006). Evidence of cardiomyopathy with vascular involvement in rats has been reported by Hopkins et al. (1982).
Administration of high doses of CP could cause a lethal cardiotoxicity, which presents as a combination of symptoms and signs of myopericarditis leading to fatal complications such as congestive heart failure, arrhythmias, cardiac tamponade and myocardial depression (Shanholtz, 2001).

1.1.6.2 Clinical Picture

Electrocardiographic and echocardiographic observations

The clinical syndrome of severe congestive heart failure was accompanied by diffuse voltage loss, cardiomegaly, pulmonary vascular congestion and pleural effusions on chest radiograph after CP administration. Abnormal ECG changes may occur with acute depression of QRS complex and left ventricular systolic function (Gottdiener et al., 1981). Echocardiography demonstrated decreased fractional wall shortening, increased end-diastolic volume and pericardial effusions (Goldberg et al., 1986).

Histochemical changes

Pathological examination of the heart after CP treatment reveals hemorrhagic myocardial necrosis, interstitial oedema, thickening of the left ventricular wall, serosanguinous pericardial effusions and fibrinous pericarditis (Cazin et al., 1986; Kumar et al., 1992).

Electron microscopic abnormalities

CP cardiotoxicity may be characterized by multifocal necrosis associated with fibrin microthrombi near areas of capillary endothelial
damage. Loss or lysis of myofilaments and detrimental effect on the mitochondrial cristae and nuclei have been reported (Wutzen, 1992).

**Biochemical findings**

Recent studies have reported the increased activities of serum enzymes such as creatine phosphokinase, lactate dehydrogenase and aminotransferases in CP treated rats, which are well known diagnostic indicators of cardiotoxicity (Al Nasser, 1998; Sudharsan *et al.*, 2005a). CP causes a depression in the activities of enzymes of the respiratory chain and membrane ATPases (Gvozdjakova, 1982).

1.1.6.3 Mechanisms involved in CP cardiotoxicity

Although the mechanism of cardiotoxicity elicited by CP is not fully understood, cellular mechanisms of cardiotoxicity are thought to be mediated by an increase in free oxygen radicals through intracellular phosphoramidate mustard, the principal alkylating metabolite of CP which affects endothelium and ion transport mechanisms (Lee *et al.*, 1996). This is consistent with a previous report where cardiac toxicity of CP was substantially increased by glutathione depletion (Friedman *et al.*, 1990).

Cardiac pathology from CP is by direct endothelial damage with extravasation of proteinaceous fluid, high concentrations of CP and erythrocytes into the myocardial interstitium and muscle cells with resultant toxic damage to these cells and precipitation of fibrin in capillaries, interstitium and damaged muscle cells (Fraiser *et al.*, 1991). Microthrombosis would lead to further ischemic damage (Kumar *et al.*, 1992). Increase in cardiac weight may be caused by myocardial oedema and pericardial effusion.
The clinical findings are consistent with myocardial necrosis following CP induced endothelial damage (Goldberg et al., 1986) (Figure 1.3). CP treatment also induces apoptosis or programmed cell death as reported in other normal tissues including hair follicles, bone marrow, testis, thymus and urothelium (Jezernik et al., 2003; Lopez and Luderer, 2004).

1.1.6.4 Cyclophosphamide induces free radicals and apoptosis

CP induced apoptosis may be through one or both of the two major signaling pathways of apoptosis. It is known to activate the mitochondrial pathway of cell death (Schwartz and Waxman, 2001). The second pathway of Fas/Fas-ligand interactions has also been implicated in the induction of the apoptotic cell death in thymus (Wang and Cai, 1999). Apoptosis is induced by acrolein, a metabolite of CP (Tanel and Averill-Bates, 2005). Increase in the highly reactive free oxygen radicals have been reported to play a role in the pathogenesis of CP cardiotoxicity (Lee et al., 1996). These ROS are known to be potent inducers of apoptosis (Gottlieb et al., 1994). In the dysfunctional myocardium, high nitric oxide levels produced by inducible nitric oxide synthase, contribute to progressive cardiac failure by causing apoptosis (Wildhert et al., 1995).

Extensive production of these highly reactive free radicals may mediate an apoptotic signaling pathway that leads to cell death. Characteristic features of apoptosis include morphological cell shrinkage, chromatin condensation, nuclear fragmentation, non-inflammatory phagocytosis and biochemical internucleosomal cleavage of DNA and proteolytic cleavage of a number of intracellular substrates (Zimmermann et al., 2001). One of the target protein substrates of apoptosis could be the myofilaments. The
Figure 1.3 Mechanism of cyclophosphamide induced cardiotoxicity
ultrastructure of the heart contains large number of myofibrils consisting of bundles of myofilaments, which actually shorten upon cardiac contraction. Free radicals can adversely affect the myofilaments, which may contribute to the progression of heart failure (Giordano, 2005). Loss of myofilaments and decrease in their calcium sensitivity would contribute to altered myocardial function in the failing heart (Hajjar et al., 2000). Apoptosis may also contribute to the proteolysis of myofilaments (Marston and Redwood, 2003).

Pretreatment of cardiac myocytes with glutathione monoethyl ester provided protection against cardiotoxic effects of CP metabolites (Levine et al., 1993). Antioxidant ascorbic acid suppresses CP induced lipid peroxidation to a significant extent in liver homogenate (Ray, 2005). Cardioprotective antioxidants can thus play a pivotal role in preventing the CP induced deterioration of cardiac function.

1.2 LIPOIC ACID

DL-α-lipoic acid is a naturally occurring coenzyme of mitochondrial dehydrogenase multienzyme complexes. It was first isolated by Reed et al. (1951) from bovine liver extracts and was described as a crystalline growth-promoting enzyme cofactor. As lipoamide, it functions as a cofactor in the mutienzyme complexes that catalyse the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate and branched chain α-keto acids. Apart from its role in energy metabolism, recently it is gaining attention as an efficient antioxidant. LA is a unique, effective and safe substance that displays the best possible scenario for natural antioxidant.
1.2.1 STRUCTURE

LA is characterized as a 1,2-dithiolane-3-pentanoic acid, a heterocyclic carboxylic acid with a five-membered cyclic disulphide ring, carrying a chiral center at the C3 carbon centre. It exists as two enantiomers R(+) and S(-)-α-LA with the former being the naturally occurring species. Due to its lipophilicity and acidity, the compound is given the trivial name α-lipoic acid.

![α-Lipoic acid](image1)

![α-Dihydrolipoic acid](image2)

**Figure 1.4 Structure of α-lipoic acid and α-dihydrolipoic acid**

1.2.2 PROPERTIES

LA is not extractable from tissues by hot water or by lipid solvents. It is released only by hydrolysis with acid, alkali or crude proteolytic enzymes indicating that it is tightly bound to protein. The racemate is almost insoluble in water but soluble in lipophilic solvents and alkaline solutions. The five-membered cyclic ring is cleaved via reduction, forming dihydrolipoic acid (DHLA) (Figure 1.4).
1.2.3 SOURCES, BIOSYNTHESIS AND REGENERATION

LA is present in several food products such as meat, in particular liver and heart. After digestion, LA is absorbed as lipoyllysine, as the peptide bond is not cleaved effectively by proteolytic enzymes (Mattulat, 1992). In addition, it can be obtained by de novo biosynthesis from fatty acids and cysteine (Carreau, 1979). Morikawa et al. (2001) have identified and characterized a mouse cDNA, designated mLIP1, which encodes a lipoic acid synthase located in mitochondria. In E. coli, lipoic acid synthase encoded by lipA catalyses the synthesis of LA from octanoic acid by the addition of two sulphur atoms to the octanoyl group bound to the acyl carrier protein (Miller et al., 2000). Octanoate is indicated to serve as the precursor for the synthesis of LA in E. coli, but in rat liver, biosynthesis occurs in the microsomes, where linoleic acid and to a lesser extent oleic acid act as the precursors. Methionine and cysteine have been shown to be effective as sulphur donors in the biosynthesis. However the complete enzyme pathway responsible for its de novo synthesis has not yet been elucidated (Dupre et al., 1980). LA is reduced to DHLA by NADH dependent mitochondrial lipoamide dehydrogenase, cytosolic NADPH-dependent flavoprotein dehydrogenase and glutathione reductase (GR) (Haramaki et al., 1997). LA can also be reduced in an NADPH dependent manner by thioredoxin reductase (Nordberg and Arner, 2001). Mammalian GR catalyses the reduction of S-lipoic acid twice as fast as the R-form reduction, while lipoamide dehydrogenase reduces the R-form 28 times as fast as S-enantiomer (Biewenga et al., 1997).
1.2.4 BIOAVAILABILITY

The half life in plasma is approximately 30 min. The mean total plasma clearance is in the same range as the plasma flow of the liver (about 11-17 ml/min kg). The liver presumably eliminates LA. After oral administration of α-LA, an absolute availability of about 20-25% for the S-enantiomer and about 27-34% for the R-enantiomer have been recorded (Hermann et al., 1996).

1.2.5 METABOLISM AND EXCRETION

The major pathway of metabolism is the β-oxidative degradation of pentanoic side chain leading to CO$_2$ (from the carboxyl end and C-2 carbon) and the bisnorlipoic acid, tetrnorlipoic acid, a keto compound as well as the logical intermediary hydroxybisnorlipoic acid in rats (Harrison and McCormick, 1974). β-oxidation has also been reported in humans (Biewenga et al., 1997). Urinary excretion of LA is maximal 3 to 6 h after administration of LA. Urine is the major route of excretion of metabolites with negligible amounts of unchanged LA (Bustamante et al., 1998). Neither animal nor human studies have shown serious side effects with administration of LA. The LD$_{50}$ is approximately 400-500 mg/kg following intravenous administration in rats (Packer et al., 1995).
1.2.6 UNIVERSAL ANTIOXIDANT-LA

1.2.6.1 Antioxidant potential in a nutshell

- LA scavenges hydroxyl radical, hypochlorous acid, nitric oxide, peroxynitrite, and singlet oxygen while DHLA scavenges superoxide radical and peroxyl radicals (Packer et al., 1995).

- Exogenously supplied lipoate is rapidly taken up by cells and reduced to an even more potent antioxidant DHLA. The redox system, LA/DHLA can regenerate the glutathione system.

- It is referred to as a universal antioxidant as it acts both in the membranous phase and the aqueous phase (Kagan et al., 1992).

- Both LA and DHLA can regenerate other essential cellular antioxidants. DHLA is involved in vitamin E recycling through the reduction of ascorbaryl radicals (Kagan et al., 1992), oxidized glutathione (GSSG) (Bast and Haenan, 1990) or by ubiquinol (Kagan et al., 1990).

- LA effectively chelates metals such as Mn$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Pb$^{2+}$ and Cu$^{2+}$ while DHLA chelates Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Pb$^{2+}$ and Cd$^{2+}$ ions which induce tissue damage and peroxidative changes (Navari-Izzo et al., 2002).

- LA has the ability to repair oxidative damage. It can improve the repair of oxidized methionine residues by supplying peptide methionine sulfoxide reductase with reducing equivalents (Biewenga et al., 1997).
1.2.6.2 Mode of action of the ideal antioxidant “LA”

Free supplemented LA acts as an antioxidant and regulates the redox status of cells (Bustamante et al., 1998). DHLA predominantly interacts with ROS but the oxidized form of LA can also inactivate free radicals (Packer et al., 2001). The LA/DHLA couple approaches the ideal as it satisfies the criteria of specificity of free radical quenching, metal chelation, interaction with other antioxidants, effects on gene expression and bioavailability (Packer et al., 1995). The antioxidant activity is related to chemical reactivity of its 1,2-dithiolane ring. A strain on the oxidized dithiolane ring cyclic structure of LA gives the molecule a high tendency for reduction according to environmental conditions. On the other hand, the low negative redox potential ($E_0 = -0.29$ V) makes the LA/DHLA couple a strong reductant of redox active compounds such as GSSG. Similar to other vicinal thiols, DHLA (which has a high pK value for the -SH, around 10.7) is more easily oxidized in comparison with monothiols, leading to high activity in -SH/S-S- interchange reactions (Bustamante et al., 1998). The sulphur atoms can play a role in metal complexation, but in some cases only the carboxylic group participates (Navari-Izzo et al., 2002).

1.2.7 THERAPEUTIC POTENTIAL OF LA IN DIVERSE PATHOLOGIES

Therapeutically when LA is administered as a racemic mixture, it is expected that the reduction of a racemic mixture would be superior to that of both isomers administered apart (Biewenga et al., 1997). However, in some models such as in acute treatment of obese Zucker rats, R-form increased insulin-mediated glucose transport, when compared with the S form (Packer
et al., 2001). LA has been proposed to be a potential therapeutic agent in the treatment or prevention of different pathologies that may be related to an imbalance of the oxidoreductive cellular status (Figures 1.5a and 1.5b).

Jacob et al. (1996) found that treatment with LA improved insulin mediated 2-deoxyglucose uptake, glucose oxidation, glycogen synthesis, and lowered the plasma levels of insulin and the fatty acids. An increase in glucose uptake was observed in L6 muscle cells and adipocytes after treatment with LA, due to translocation of Glut-1 and Glut-4 transporters from the intracellular pool to the membrane (Estrada et al., 1996). LA can prevent the glycation of protein, thus leading to a dose dependent normalization of nerve blood flow in experimental diabetic neuropathy. High doses of LA are approved in Germany for treatment of diabetic polyneuropathy (Packer et al., 1995).

Tang and Aizenman (1993) demonstrated the neuroprotective properties of DHLA and LA due to redox modulation of the N-methyl-D-aspartate (NMDA) receptor complex. LA attenuated the age related loss in GSH by inducing Nrf2 (nuclear factor erythroid 2-related factor) binding to the antioxidant response element (Suh et al., 2004). Incubation of jurkat T cells with LA was found to inhibit NF-κB which may be effective in blocking the HIV transcription (Suzuki et al., 1992).

Long-term treatment with LA efficiently overcome the development of hypertension, vascular hypertrophy and renal injury in hypertensive rats, possibly through the suppression of endothelin-1 overproduction (Takoaka et al., 2001). LA has anti-obesity effects mediated by the suppression of hypothalamic AMP-activated protein kinase activity (Kim et al., 2004).
Figure 1.5a The redox antioxidant network concept (ROOH) is a hydroperoxide, ROH is an alcohol, ROO• is a peroxyl radical, and RO• is an alkoxyl radical.

Figure 1.5b Protective effects of α-lipoic acid
Inhibition of 15-lipoxygenase oxidative activity and human lipoprotein peroxidation by DHLA could result in specific antioxidant and antiatherogenic effects (Lapenna et al., 2003).

LA remarkably potentiated Fas mediated cell death in leukemic Jurkat cells, but not in healthy peripheral blood lymphocytes (Sen et al., 1999). The differential selectivity of the pro-apoptotic effects of LA for transformed cells supports its potential use in the treatment of neoplastic disorders (Van De Mark et al., 2003). Use of LA in doxorubicin chemotherapy lead to an increase in survival of leukemic mice (Dovinova et al., 1999).

Studies from our laboratory have shown that LA affords protection against calcium oxalate stone formation in rats (Jayanthi and Varalakshmi, 1992). LA prevented Ca^{2+}-induced lipid peroxidation in brain, heart and testes (Sumathi et al., 1994). We have also reported the protective efficacy of LA administered along with gentamycin in rats rendered bacteremic (Varalakshmi et al., 2003). LA acts as a protective agent for hyperlipidemia, associated with ADR induced nephrotic syndrome (Malarkodi et al., 2003). LA in combination with a chelator dimercaptosuccinic acid was effective in maintaining renal integrity in lead treated rats (Sivaprasad et al., 2004). LA has been found to be cytoprotective in experimental models of oxidative testicular injury (Selvakumar et al., 2004; Prahalathan et al., 2005).
Scope of the Present Study
1.4 SCOPE OF THE PRESENT STUDY

CP is a useful chemotherapeutic agent active against a variety of human neoplasms. However, drug toxicity is the principal deterrent to treatment with higher and potentially more effective doses of CP. The potent antitumour drug CP has been associated with cardiotoxicity in patients receiving high doses over a few days, as part of an intensive antineoplastic regimen or in conjunction with bone marrow transplantation procedures to effect bone marrow suppression (Frishman et al., 1997). Two different types of acute cardiac effects from high doses of CP are described; myocarditis which can be asymptomatic and congestive heart failure which may be fatal (Dorr and Lagel, 1994). The highly reactive species produced by CP (Lee et al., 1996) may attack soluble cell components as well as membranes, eventually leading to impairment of cell functioning and cytolysis (Machlin and Bendich, 1987). Antioxidant defense mechanisms can effectively protect cells and tissues from the free radical mediated deleterious effects.

LA, a disulphide by virtue of its antioxidant effects has opened new vistas in the field of experimental therapeutics

LA plays a protective role against cardiac injury elicited by ischemia-reperfusion (Freisleben, 2000). It has also been shown to prevent hypertension and improve vascular reactivity and morphology of vessels. It lowers plasma lipids and is likely to reduce the cardiovascular risk factor (Vasdev et al., 2002). LA may act directly on vascular calcium channels to increase free sulfhydryl groups and normalize calcium transport (Vasdev et al., 2002), which is important for normal contractility of the heart. It also enhances the antioxidant defences and functioning of endothelial cells (Jones
et al., 2002). LA has been found to be beneficial in antineoplastic drug adriamycin induced cardiac damage (Balachandar et al., 2003).

These rationales motivated us to maneuver strategies to modulate cardiotoxicity with the aid of LA. The present study is aimed/designated at investigating the following:

- Evaluation of the efficacy of lipoic acid in countering the cardiotoxicity elicited by CP using in vivo model.
- in vitro studies using H9c2 cardiac cell line to assess the development of apoptosis by CP and to further evaluate the impact of LA on these cardiac changes.
- To isolate cardiac myofilaments and study the effect of LA on CP induced alterations in calcium sensitivity of myofilaments using in vivo model.