SUMMARY

CP is an alkylating antineoplastic agent widely used in the treatment of acute and chronic lymphocytic leukemias, Hodgkin's disease, multiple myeloma and soft tissue sarcomas. High dose CP therapy is associated with cardiac toxicity apparent in the form of acute cardiomyopathy, myocarditis, myocardial depression, malignant arrhythmias and congestive heart failure. CP causes 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. The cellular response to CP therapy includes apoptosis and/or necrosis. The present study explores the mechanisms underlying CP-induced abnormalities in the heart, wherein cellular and molecular changes were probed through in vivo and in vitro studies. Further, the possible merits of LA, well known for its role as an ideal antioxidant was evaluated in CP induced cardiotoxicity. The salient findings are briefly highlighted here under.

- Maintenance of myocardial cellular integrity is crucial for cardiac function. Increase in the cardiac markers such as cTnI and activities of enzymes such as CPK, LDH, AST and ALT in serum indicate membrane damage which could in turn suggest possible necrotic modulations in the heart after CP administration. The activities of ATPases were also decreased in the above conditions. LA was effective in restoring the cardiac markers to near normalcy.
CP tilted the prooxidant/antioxidant balance towards the harmful oxidative side in the heart which was evident from enhanced lipid peroxidation, oxidative DNA damage marker such as 8-OHdG and protein carbonyl content. Simultaneously the levels of enzymic and non enzymic antioxidants were depleted in heart tissue. The antioxidant properties of LA came forth in countering the oxidative stress mediated cardiac changes.

ROS interfere with NO function by converting it to harmful ONOO' inducing nitrosative stress. In CP treated animals the oxidative products of NO were increased in plasma along with an increased expression of iNOS mRNA in heart tissue. It may be speculated that the delayed effects of CP may be attributed to changes brought about by it at the level of transcription or translation. LA substantially reverted these nitrosative abnormalities to near normalcy.

Considerable attention has been focused on lysosomal alterations and glycoprotein metabolism of connective tissue constituents that might accompany ischemic or hypoxic myocellular damage. The activities of lysosomal enzymes were increased in both heart and serum of CP treated rats. An increase in tissue hydroxyproline content was noted, which is a marker for fibrosis. Fibrosis is in turn a hallmark of CP induced metabolic derangements, which was prevented by LA supplementation.

Cardiac myocytes are the cells with the highest volume density of mitochondria in the body due to the extraordinary demand for
continuous synthesis of ATP by oxidative metabolism. Decrease in the activities of TCA cycle enzymes such as SDH, MDH, ICDH and electron transport chain complexes in heart tissue were notable after CP administration. These functional changes correlated well with transmission electron microscopic findings of damaged mitochondrial cristae. LA improved mitochondrial function thereby correcting the energy demand deficit in myocytes.

- Histopathological studies revealed extensive haemorrhage and pycnotic nuclei in the heart tissue after CP administration, while LA treated group showed substantial recovery.

- Lipotoxic cardiomyopathy can result from alterations in the lipid metabolism. CP resulted in secondary hyperlipidemia characterized by elevated levels of lipids such as cholesterol and triglycerides. The levels of serum LDL and VLDL cholesterol increased along with a decrease in HDL cholesterol and activities of fat splitting plasma LCAT and cardiac LPL after CP administration. LA, known to possess hypolipemic properties could restore the normal lipidemic status.

- CP is a prodrug that has to be activated by the liver mixed function oxidase system. Hence 4-HC, which yields 4-hydroxycyclophosphamide, the activated derivative of CP was used for in vitro studies. 4-HC-induced ROS and mitochondrial dysfunction preceding apoptotic cell death in H9c2 cardiac cells. A decline in the expression of antiapoptotic Bcl-2 was coupled with the cytosolic release of cytochrome c from the mitochondria in 4-HC cells.
In the cytosol, cytochrome c release triggered the activation of caspase-3, a critical step, that degrades specific intracellular substrates such as DNA or α-fodrin. These apoptotic changes along with DNA fragmentation and expression of α-fodrin products were observed after 4-HC exposure in H9c2 myocytes. LA was found to be beneficial in preventing the apoptotic changes induced by 4-HC.

Cardiac myofilaments are susceptible to ROS, apoptotic or necrotic changes, which leads to progression of heart failure. A significant decline in the calcium sensitivity of cardiac myofilaments was evident in CP administered rats. The pCa50 indicative of the responsiveness of myofibrillar Mg2+-ATPase to calcium was significantly reduced with a rightward shift. The Hill coefficient or slope was significantly reduced in Group II indicating the drop in cooperativity.

Myofibrillar myosin ATPase activities were used to assess whether inherent myosin-ATPase activity function was affected by CP administration. A decline in Ca2+-ATPase and K+-EDTA-ATPase activities was noted in heart tissue of CP treated rats. LA normalized these abnormalities and also improved the calcium sensitivity of myofilaments.

The expression of fetal gene, β-MHC mRNA also a hypertrophy marker was increased in heart ventricles, after CP administration, denoting a shift in the isomyosin expression from the V1→V3 or high to low ATPase form. LA prevented the increase in the expression of
β-MHC mRNA probably by improving energy status or by virtue of its cytoprotective properties.

Transmission electron microscopic findings showed the loss of myofilaments in the heart of CP treated rats. The beneficial effect of LA was confirmed by the significant recovery in the myofibres of CP exposed rats.