Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in albino rats

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Preventive role of lipistat against doxorubicin induced myocardial toxicity in rats has been reported. Cardiotoxicity was produced by doxorubicin administration (15 mg/kg for 2 weeks). Lipistat (350 mg/kg, orally) was administered as pretreatment for 2 weeks and then for 2 weeks alternated with doxorubicin. The general observations, mortality, histopathology, biomarker enzymes like lactate dehydrogenase (LDH) and creatine phosphokinase (CPK), serum lipid profiles like total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL), antioxidant enzymes such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were monitored after 3 weeks of last dose. Pretreatment with the lipistat significantly protected myocardium from the toxic effects of doxorubicin by reducing the elevated level of biomarker enzymes like LDH and CPK to the normal and serum lipids such as total cholesterol, triglyceride and LDL back to normal. Lipistat increases the decreased level of GSH, SOD and CAT and decreases the increased level of malondialdehyde in cardiac tissue. The repeated administration of doxorubicin causes cardiomyopathy associated with an antioxidant deficit and increased level of lipid profiles by interfering with fatty acid metabolism. The results support the lipid lowering and antioxidant properties of lipistat, which indicate the cardioprotective property against doxorubicin induced cardiotoxicity.

Keywords: Antioxidant, Cardiotoxicity, Doxorubicin, Lipistat

Doxorubicin an anthracycline is well-established and highly efficacious drug in the fight against many kinds of cancers like solid tumors, leukemia, soft tissue sarcoma, breast cancer, small cell carcinoma of the lung and oesophageal carcinomas.1,2 But, its clinical usefulness is still restricted due to its specific toxicities to cardiac tissue.3 Congestive heart failure, cardiomyopathy and electrocardiographic changes were demonstrated after cumulative doxorubicin administration.4 The mechanisms proposed for cardiotoxic effects of doxorubicin include free radical induced myocardial injury, lipid peroxidation,5 mitochondria damage,6 decreased activity of Na+ K+ ATPase,7 vasoactive amine release,8 impairment in myocardial adrenergic signaling/regulation, increase in serum total cholesterol, triglyceride and low density lipoproteins.9 Generation of reactive oxygen species like superoxide anion and hydrogen peroxide by doxorubicin leads to causing impairment of cell functioning and cytolysis.10 Due to the presence of less developed antioxidant defense mechanisms, heart is particularly vulnerable to injury by anthracycline-induced reactive oxygen species. Liberation of free radicals is central to the mechanism of doxorubicin-induced damage to the myocardium.11 It also causes the elevation of serum enzymes like lactate dehydrogenase (LDH) and creatine phosphokinase (CPK).12 Endogenous antioxidant deficits have been suggested to play a major role in doxorubicin induced cardiomyopathy and heart failure.13

Polyherbal formulations/antioxidant compounds have shown protective effects in doxorubicin induced cardiotoxicity without reducing their therapeutic efficacy. Moreover, there is a growing interest in the usage of natural antioxidants as a protective strategy against the cardiovascular related problems in experiments such as ischemia reperfusion14 and doxorubicin induced cardiotoxicity.15 Probucol a lipid lowering agent and potent antioxidant, provides complete protection against doxorubicin induced cardiomyopathy, without interfering with the antitumor properties of this antibiotic.16 Lipistat capsule contains 500 mg of Terminalia arjuna, Inula racemosa and Commiphora mukul in equal...
proportions (Dabur India). Lipistat (1 capsule twice daily) promotes hypolipidemia and improves optimum cardiac function. *Terminalia arjuna* bark has remarkable cardioprotective and heart muscle strengthening properties\(^{17}\). It possesses cardiac stimulant properties with variable effect on heart, associated with increase in threshold for myocardial ischemia and angina\(^{18}\). The bark powder of *Arjuna* exerts hypocholesterolaemic and antioxidant effect\(^{19}\). It is thought that saponin glycosides may be responsible for the inotropic effect of *T. arjuna*, while the flavonoids and oligomeric proanthocyanidins provide free radical antioxidant activity\(^{20}\) and vascular strengthening. *Inula racemosa* is another ingredient with potent beneficial effects on cardiovascular system. It has antianginal and beta blocking activity\(^{21}\). *Commiphora mukul* has hyperlipidemic\(^{22}\), thrombosis and fibrinolytic activity\(^{23,24}\). Efforts have been made to use antioxidants, iron chelators, beta blockers and hypolipidemic agents to prevent doxorubicin induced cardiotoxicity.

The present study has been undertaken to investigate the effect of lipistat against doxorubicin induced myocardial toxicity in rats.

**Materials and Methods**

**Animals** — Albino rats of either sex weighing 150-200 g were procured from animal house of K.L.E.S’s College of Pharmacy, Hubli, were used for the study after the clearance from Institutional Animal Ethical Committee. Animals were acclimatized for one week to laboratory conditions before starting the experiment; they had free access to water and standard rat feed but 12 hr prior to an experiment, the rats were deprived of food but not water.

**Materials** — Doxorubicin was a generous gift from Doxorubicin, Daman, India. Lipistat and other chemicals used were of analytical grade and procured locally. Analyzing kits were obtained from ERBA Diagnostics, Daman, India.

**Dosage fixation** — Lipisat (350 mg/kg) showed protection against isoproterenol induced myocardial necrosis\(^{25}\). The same dose was used in the present study.

**Experimental design** — After one week of acclimatization, the animals were randomly divided into 4 groups of 6 animals in each. Group 1 served as normal control and received normal saline 5 ml/kg body weight (ip). Group 2 animals were treated with doxorubicin (2.5 mg/kg body weight ip) in 6 equal injections alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight. Group 3 animals received lipistat (350 mg/kg body weight po) for 2 weeks and then alternatively with vehicle for next 2 weeks. Group 4 animals received lipistat (350 mg/kg body weight po, for two weeks) as a pretreatment followed by doxorubicin administration as in group 2.

**Enzyme assays** — After 36 hr of the last treatment, orbital blood samples were obtained under light ether anesthesia using heparinized microcapillaries for the estimation of cardiac biomarkers CPK\(^{26}\) and LDH\(^{27}\). Both control and treated animals were observed for as long as 3 weeks after the last injection for the general appearance, behaviour and mortality. At the end of 3 weeks post treatment period, animals were sacrificed under ether anesthesia and a midline abdominal incision was performed and heart tissue was quickly dissected out and washed in ice cold saline, dried on filter paper and weighed immediately. A portion of each heart was taken from all the groups and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of glutathione (GSH)\(^{28}\), superoxide dismutase (SOD)\(^{29}\), catalase (CAT)\(^{30}\) and malondialdehyde (MDA)\(^{31}\). Orbital blood samples were collected before sacrificing the animals and used for estimation of cholesterol\(^{32}\), triglycerides\(^{33}\), low density lipoprotein (LDL)\(^{34}\) and high density lipoprotein (HDL)\(^{35}\). The remaining portion of the heart tissue was used for histopathological studies.

**Statistical analysis** — The results were expressed as the mean ± SE. The results obtained were analyzed using one-way ANOVA followed by Dunnnett’s multiple comparison tests. Data were computed for statistical analysis by using Graph Pad Prism Software.

**Results**

Chronic administration of doxorubicin induced cardiac toxicity and effect of lipistat was established by significant increase in cardiac biomarker enzymes and endogenous antioxidants and heart tissue histopathology.

**General observations** — The general appearance of all groups of animals was recorded throughout the study. In doxorubicin treated group, the animal fur became scruffy and developed a pink tinge. These rats also had red exudates around the eyes and nose, soft watery feces and enlargement of abdomen. These observations were significantly decreased in lipistat treated group.
Heart weight, body weight and ratio of heart weight to body weight — Effect of doxorubicin on heart weight, body weight, liver weight, ratio of heart weight to body weight and liver weight to body weight is shown in Table 1. The food and water intake in doxorubicin treated group was significantly decreased as compared to group 1. The food and water intake in the group 4 was significantly increased as compared to group 2. The heart weight, body weight, liver weight, ratio of heart weight to body weight and liver weight to body weight in group 2 treated rats were significantly increased compared with normal rats. The heart weight, body weight, liver weight, ratio of heart weight to body weight and liver weight to body weight in group 4 was significantly decreased compared with group 2.

Serum lipid levels — Animals treated with doxorubicin produced significant increase in the levels of cholesterol, triglycerides and LDL compared to group 1 and there was very slight difference in HDL levels compared to group 1 (Table 2). Group 4 produced significant decrease in the level of cholesterol, triglycerides and LDL but significant increase in the level of HDL as compared to group 2.

Serum enzyme biomarkers — Animals treated with doxorubicin produced significant increase in the levels of CPK and LDH compared to group 1 (Table 3). Group 4 produced significant decrease in the level of CPK and LDH as compared to group 2.

Antioxidant status — Effect of doxorubicin on tissue lipid peroxidation, antioxidant and antioxidant enzymes is shown in Table 4. The malondialdehyde level was increased; GSH, SOD and CAT level were significantly decreased in doxorubicin treated group as compared to normal animals. Group 4 produced significant decrease in the level of MDA and increase in the status of antioxidant and antioxidant enzymes.

Histopathological observation — The histology of the heart tissue from control and lipistat treated animals showed normal morphological appearances, whereas in group 2 disruption of loss of myofibrils and vacuolization of the cytoplasm were observed. The histology of heart tissues from group 4 showed less loss of myofibrils and vacuolization of the cytoplasm.
Discussion

The study entails the cardioprotective effect of lipistat against doxorubicin-induced cardiotoxicity. Lipistat, an Indian herbal formulation possesses cardioprotective, cardiotonic and lipid lowering properties. The present study is aimed to explore the cardioprotective effects of oral administration of lipistat against doxorubicin-induced cardiotoxicity in rats.

The existing experimental evidence suggests that doxorubicin oxidative stress is due to the generation of free radicals in the heart tissue. The generated reactive oxygen species such as superoxide radicals and hydroxyl radicals are potential to cause damage to various intracellular components. Heart tissue is particularly susceptible to free-radical injury, because it contains low levels of free-radical detoxifying enzymes/molecules like SOD, GSH and CAT. Further, doxorubicin also has high affinity for the phospholipid component of mitochondrial membrane in cardiac myocyte, leading to accumulation of doxorubicin in the heart tissue. The doxorubicin induced mitochondrial injury is critical to the heart because it would presumably have extreme adverse effects on the contractile functioning of the cardiac myocytes by alterations in the energy metabolism. Hence a protocol that would initiate doxorubicin induced oxidative stress followed by lipistat intervention was used to explore the extent of control of progressive tissue damage.

Pretreatment of lipistat was able to reduce the doxorubicin-induced cardiotoxic manifestations in multiple ways. Increase in the level of plasma triglycerides, total cholesterol and low density lipoproteins in the doxorubicin treated group indicate doxorubicin may be interfering with metabolism or biosynthesis of lipids. Pretreatment with lipistat showed reduction in blood lipid profile levels with concomitantly increase in HDL cholesterol was observed. Decrease in the blood lipid profiles and increase in HDL cholesterol in lipistat treated group may be due to the presence of C. mukul. Lipid lowering effect of lipistat is due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of LDL cholesterol and increase in the uptake of LDL from blood by liver. Heart tissue injury induced by doxorubicin in rats was indicated by elevated level of the marker enzymes such as serum LDH and CPK. The increase of LDH level in serum and extracellular fluid suggests an increased leakage of this enzyme from mitochondria as a result of toxicity induced by treatment with doxorubicin. This index has been recently used in other studies to test for cardiotoxicity.

Lipistat was found to inhibit the doxorubicin-induced CPK and LDH release in the serum of rats. It is widely reported that doxorubicin-induced free-radical generation triggers membrane peroxidation and disruption of cardiac myocytes, which can lead to increased release of CPK in the serum. Lipistat pretreatment led to inhibition of CPK and LDH release which resulted in either complete reversal or considerable recovery of the serum enzyme activities. The present results are in good agreement with those of Murat et.al. Another effect of doxorubicin induced cardiotoxicity is characterized by decreased body weight and increase in the heart weight. The results of the present study confirmed the earlier findings that doxorubicin administration caused decrease in the body weight and increase in heart weight.

Cardioprotective activity of lipistat was further supported by increased myocardial antioxidant

| Table 4—Effect of lipistat on malondialdehyde, glutathione, catalase and superoxide dismutase in doxorubicin induced cardiotoxicity in rats |
|---------------------------------|----------------|---------------|----------------|----------------|
| Treatment                       | Malondialdehyde (n mol MDA/g of wet tissue) | Glutathione (n mol/g of wet tissue) | Catalase (units mg of protein) | Superoxide dismutase (units/mg of protein) |
| Normal                          | 17.467 ± 0.6086 | 2.867 ± 0.1606 | 60.568 ± 2.124 | 36.710 ± 0.8072 |
| Doxorubicin                     | 48.173 ± 0.7414a | 1.100 ± 0.1758a | 40.643 ± 2.029a | 23.907 ± 1.31a |
| Lipistat                        | 17.840 ± 0.8242b | 2.432 ± 0.1840b | 61.425 ± 1.084b | 37.677 ± 0.821b |
| Lipistat + doxorubicin          | 36.855 ± 1.948b | 1.697 ± 0.1106b | 53.30 ± 1.084b | 29.312 ± 1.075b |

P values; <0.01; compared with a normal, b doxorubicin.
ns = not significant
† - µmole of H₂O₂ consumed / min.
∥ - one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NTB reduction in one min.
enzymatic activity and decreased extent of lipid peroxidation. Lipid peroxidation is known to cause cellular damage and is primarily responsible for reactive oxygen species induced organ damage. Increased level of MDA and decreased levels of GSH, SOD and CAT were observed in heart tissue in doxorubicin treated animals. Pretreatment with lipistat efficiently counteracted the doxorubicin induced cardiac tissue damage by significant decrease in MDA and increase in GSH, SOD and CAT levels. The observed increase in CAT activity in doxorubicin treated animals supports the above hypothesis that this increase is possibly required to overcome excessive oxidative stress.

Histopathological report suggest lipistat pretreated group attenuates the doxorubicin induced loss of myofibrils, vacuolization of the cytoplasm and swelling of mitochondria. The histopathological changes observed in the doxorubicin treated rats were similar to those previously reported. Flavonoids and oligomeric proanthocyanidin present in T. arjuna and guggulsterones in C. mukul may be responsible for reducing oxidative damage. Hence, the antioxidant activity of lipistat may be attributable to T. arjuna or C. mukul. The other possible protective effect mediated through the adrenergic blocking property of I. racemosa, one of the important constituent of lipistat, may be contributing to protective action against doxorubicin induced cardiotoxicity. β-Blockers have been reported to be useful in the treatment of doxorubicin induced cardiomyopathy.

In conclusion, the present results suggest that lipistat prevented the doxorubicin induced myocardial toxicity by boosting the endogenous antioxidant activity. The cardioprotective property of lipistat could be due to lipid lowering and antioxidant properties. Further studies are needed to elucidate the exact mechanisms of action of lipistat and its clinical application.

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