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# Role of lupeol and its ester on cyclophosphamide-induced hyperlipidaemic cardiomyopathy in rats

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## Abstract

Cyclophosphamide, an alkylating agent widely used in cancer chemotherapy, causes fatal cardiotoxicity. In this study, lupeol, a pentacyclic triterpene isolated from *Crataeva nurvala* stem bark, and its ester, lupeol linoleate, were investigated for their possible hypocholesterolaemic effects against cyclophosphamide-induced lipidaemic instabilities. Male albino Wistar rats were categorized into 6 groups. Group I served as control. Rats in groups II, V and VI were injected intraperitoneally with a single dose of cyclophosphamide (200 mg $kg^{-1}$) dissolved in saline. Cyclophosphamide-treated groups V and VI respectively received lupeol and lupeol linoleate (50 mg $kg^{-1}$), dissolved in olive oil, for 10 days by oral gavage. Groups III and IV served as drug controls and were administered lupeol and lupeol linoleate, respectively. Cyclophosphamide administration induced abnormal changes in serum lipoproteins and lipid fractions in both serum and cardiac tissue. The activity of lipid metabolizing enzymes was distorted significantly in the cyclophosphamide-treated rats. The cyclophosphamide-treated rats also showed extensive intermuscular haemorrhage in histology. Lupeol and its ester reversed the above alterations induced by cyclophosphamide. This study encapsulates the early lipaemic abnormalities in the heart tissue of cyclophosphamide-treated rats. Treatment with lupeol linoleate was more effective than lupeol in rendering protection to the cardiac tissue challenged by cyclophosphamide.

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## Introduction

Cyclophosphamide is an anti-tumour drug commonly used for the chemotherapy of human cancer. Cyclophosphamide needs to be bioactivated by cytochrome P450 enzymes to produce its anti-cancer, cytotoxic and teratogenic effects. Cytochrome P450-mediated oxidation of cyclophosphamide yields 4-hydroxycyclophosphamide, which subsequently undergoes spontaneous degradation into phosphoramide mustard and acrolein. These metabolites are potential sources of free radicals, which may be involved in the development of cardiotoxicity after cyclophosphamide administration (Lee et al 1996). The peroxidation of unsaturated membrane lipids by free radicals in biomembranes and tissues causes the leakage of these lipids into circulation and consequently leads to hyperlipidaemia. Cyclophosphamide administration induces lipid peroxidation, hyperlipidaemia, hypertriglyceridaemia and a defect in vascular lipoprotein lipase (Muralikrishnan et al 2001). Defects in cholesterol metabolism are the major cause of cardiovascular disease. Therefore, lowering of blood cholesterol by regulating its metabolism is a prerequisite for the reduction of cardiovascular disease risk. The direct relationship between lipid peroxidation and subsequent alteration in lipid metabolism has already been well established (Watkins et al 1993).

Lupeol, a pentacyclic triterpene isolated from *Crataeva nurvala* Buch Ham (Capparidaceae), protects biological membranes from lipid peroxidation and is also associated with many interesting biological activities (Patocka 2003). It possesses hypotensive activity (Saleem et al 2003). There is evidence that triterpenes reduce plasma cholesterol by blocking the absorption of cholesterol from the gut and thus have a beneficial effect on health by reducing the risk of developing atherosclerosis and coronary heart disease (Sanders et al 2000). Linoleic acid, an omega-6 fatty acid, is an important component of animal and plant cell membranes. Within the body, linoleic

acid can be converted to other polyunsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. It is a nutraceutical that has a cardioprotective effect. It lowers cholesterol, triglyceride levels and blood pressure, thereby reducing the risk of heart disease (Nicolosi et al 1997).

A number of human studies have shown that triterpenes esterified with fatty acids are effective in lowering blood cholesterol (Weststrate & Meijer 1998). In our study, lupeol was further esterified with linoleic acid to form lupeol linoleate as reported by Geetha & Varalakshmi (1998). Lupeol and its ester, lupeol linoleate, effectively scavenge free radicals and reduce oxidative stress indices by enhancing the antioxidant capacity of the cell (Sunitha et al 2001). No side effects have been reported, thus far, with the administration of lupeol and lupeol linoleate. However, the effect of lupeol and its ester in cyclophosphamide-induced lipaemic abnormalities has not been addressed to date. Hence, the main objective of this study is to understand and implicate the pharmacological efficacy of lupeol and lupeol linoleate on cyclophosphamide-induced hyperlipidaemic cardiomyopathy in albino rats.

## Materials and Methods

### Drugs and chemicals

Cyclophosphamide (Endoxan) was purchased from German Remedies Ltd (Goa, India). Lupeol was isolated from *Crataeva nurvala* stem bark, as reported earlier by Baskar et al (1996). The isolated lupeol was further esterified to lupeol linoleate by adding equimolar amounts of pyridine and linoleoyl chloride as reported earlier. All other chemicals used were of analytical grade.

### Experimental design

Male albino Wistar rats, $140 \pm 10$ g, were used for the study and were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The rats were acclimatized to a 12-h light–dark cycle and fed with commercially available standard pelleted feed (Gold Mohur; Hindustan Lever Ltd, Bombay) and had free access to water. Experimental rats were used after obtaining permission and handled according to the University and institutional legislation as regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Rats were divided into six groups of six. Group I served as the vehicle-treated control. Group II rats were injected intraperitoneally with a single dose of cyclophosphamide ($200\,mg\,kg^{-1}$) dissolved in saline on the first day of the experimental period. Group III rats received lupeol ($50\,mg\,kg^{-1}$), dissolved in olive oil, for 10 days by oral gavage. Group IV rats received lupeol linoleate ($50\,mg\,kg^{-1}$), dissolved in olive oil, for 10 days by oral gavage. Group V rats were administered cyclophosphamide as in group II, immediately followed by supplementation with lupeol for 10 consecutive days. Group VI rats were administered cyclophosphamide as in group II, immediately followed by supplementation with lupeol linoleate for 10 consecutive days.

At the end of the experimental period (10 days), all rats were anaesthetized and decapitated. Heart tissues were immediately excised and blood was collected for analysis of biochemical parameters. The heart tissues were homogenized in 0.1 M Tris HCl buffer (pH 7.4) and samples of this homogenate were used for the assays. A section of the heart was kept aside for histological processing.

### Lipid profile

Lipids were extracted from the cardiac tissue according to the method of Folch et al (1957) using chloroform–methanol (2:1 v/v). Cholesterol was estimated by the method of Parekh & Jung (1970) using ferric acetate–uranyl acetate as the chromogenic reagent. The free cholesterol was precipitated as its digitonide according to the method of Sperry & Webb (1950) and its cholesterol content was evaluated. The esterified cholesterol was arrived at from the difference between the total and free cholesterol analysed. Phospholipids were determined by the method of Rouser et al (1970). Serum triglycerol and free fatty acid were quantitated colorimetrically (Rice 1970; Horn & Menahan 1981).

### Lipoprotein fractions

Serum lipoproteins were fractionated by a dual precipitation method/technique of Wilson & Spiger (1973) and the cholesterol content of each fraction was estimated as mentioned earlier. Values were expressed as mg per dL of serum.

### Lipid metabolizing enzymes

The lipid metabolizing enzyme, cholesterol ester hydrolase (CEH), was estimated by the method of Kothari et al (1970) with slight modification of Kritchevsky & Kothari (1973). The free cholesterol liberated from cholesterol oleate was precipitated. The precipitate was processed and then dissolved in 3.0 mL of uranyl acetate reagent and the cholesterol content was estimated as described earlier. Cholesterol ester synthase (CES) was estimated by the method of Kothari et al (1973). The cholesterol digitonide, which was precipitated after centrifugation, was washed twice with acetone–ether mixture and finally with ether. The cholesterol content was estimated as mentioned earlier. The activity of lipoprotein lipase (LPL) was estimated in the cardiac tissue according to the procedure of Schmidt (1974). Lecithin cholesterol acyl transferase (LCAT) was assayed in the plasma by the method of Legrand et al (1979) with the modifications of Hilts et al (1983). Protein content was determined by the procedure of Lowry et al (1951).

### Histopathologic studies

Immediately after rats were sacrificed, heart tissue was fixed in 10% formalin. The washed tissue was dehydrated

in descending grades of isopropanol and finally cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut (5 $\mu$m thick), and stained with haematoxylin and eosin. The sections were then viewed under light microscope for histopathological changes.

### Statistical analysis

The results are expressed as mean ± standard deviation (s.d.) for six rats in each group. Differences between groups were assessed by analysis of variance using the SPSS software package for Windows. Post-hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test; significance at $P$ values < 0.001, < 0.01 and < 0.05 have been given respective symbols in the tables.

## Results

### Body and heart weights

Cyclophosphamide administration decreased ($P < 0.001$) the weight gain and increased ($P < 0.05$) the heart weight in group II rats. At the end of the experimental period, cyclophosphamide-treated rats recorded an average weight loss of 8%. With the supplementation of lupeol and lupeol linoleate, the body and heart weights remained unaltered compared with their basal values (Table 1).

### Serum lipid profile

The lipid levels were found to be significantly increased ($P < 0.001$) in cyclophosphamide-treated rats when compared with group I (Table 2). Supplementation with lupeol and lupeol linoleate to groups V and VI reduced the levels of the serum lipids nearly to control values.

### Tissue lipid profile

Table 3 highlights the effects of cyclophosphamide, lupeol and its ester on lipid fractions of the heart. Cyclophosphamide-treated rats showed a significant increase in the content of esterified cholesterol ($P < 0.001$) and free cholesterol ($P < 0.05$) whereas there was a significant decline in the levels of phospholipids ($P < 0.001$) and free fatty acids ($P < 0.05$). Supplementation with lupeol and lupeol linoleate brought about a significant increase in the content of both phospholipids ($P < 0.001$) and free fatty acids ($P < 0.05$) and a significant decline in the free cholesterol ($P < 0.05$) and esterified cholesterol ($P < 0.001$) content of the heart, relative to the treatment with cyclophosphamide alone.

### Lipoprotein fractions

Table 4 shows the serum lipoprotein-cholesterol profile of high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels of all groups. The

**Table 1** Effect of cyclophosphamide, lupeol and lupeol linoleate on body and heart weight of rats

| Parameter | Group I (control) | Group II (cyclo-phosphamide, CP) | Group III (lupeol) | Group IV (lupeol linoleate) | Group V (CP + lupeol) | Group VI (CP + lupeol linoleate) |
|---|---|---|---|---|---|---|
| Initial body weight (g) | 141.5 ± 5.99 | 143.16 ± 3.31 | 139.50 ± 7.45 | 141.6 ± 6.38 | 144.16 ± 6.01 | 141.8 ± 6.52 |
| Final body weight (g) | 154.16 ± 6.4 | 144.70 ± 3.32 | 152.33 ± 6.80 | 154.36 ± 6.26 | 154.57 ± 6.14 | 152.5 ± 6.12 |
| Change in body weight (g) | 12.66 ± 1.36 | 1.53 ± 0.12$^{a@}$ | 12.83 ± 1.60 | 12.70 ± 1.75 | 10.4 ± 2.55$^{a*b@}$ | 10.7 ± 1.21$^{a*b@}$ |
| Heart weight (g/kg body weight) | 0.50 ± 0.04 | 0.56 ± 0.03$^{a*}$ | 0.50 ± 0.03 | 0.51 ± 0.05 | 0.51 ± 0.04$^{b*}$ | 0.50 ± 0.05$^{b*}$ |

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]group I and groups II, III, IV, V, VI; [b]group II and groups V, VI. The symbols * and @ represent statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

**Table 2** Effect of cyclophosphamide, lupeol and lupeol linoleate on serum lipids in rats

| Parameter (mg/dL serum) | Group I (control) | Group II (cyclo-phosphamide, CP) | Group III (lupeol) | Group IV (lupeol linoleate) | Group V (CP + lupeol) | Group VI (CP + lupeol linoleate) |
|---|---|---|---|---|---|---|
| Free cholesterol | 36.38 ± 3.47 | 60.15 ± 7.21$^{a@}$ | 35.67 ± 3.48 | 34.67 ± 2.62 | 42.07 ± 4.00$^{a*b@}$ | 39.83 ± 3.34$^{b@}$ |
| Esterified cholesterol | 48.43 ± 3.58 | 77.42 ± 7.50$^{a@}$ | 48.7 ± 3.60 | 47.65 ± 2.95 | 56.98 ± 7.73$^{a\#b@}$ | 52.75 ± 4.09$^{b@}$ |
| Phospholipids | 105.57 ± 12.45 | 150.68 ± 19.58$^{a@}$ | 106.47 ± 12.35 | 105.01 ± 13.44 | 117.77 ± 12.48$^{a\#b@}$ | 109.35 ± 13.55$^{b@}$ |
| Free fatty acids | 13.87 ± 1.74 | 32.43 ± 4.20$^{a@}$ | 14.17 ± 1.67 | 13.27 ± 1.39 | 15.15 ± 1.19$^{b@}$ | 14.91 ± 1.95$^{b@}$ |
| Triglycerides | 73.47 ± 4.54 | 120.15 ± 16.10$^{a@}$ | 73.42 ± 5.04 | 72.07 ± 6.31 | 81.33 ± 5.70$^{b@}$ | 79.67 ± 4.99$^{b@}$ |

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]group I and groups II, III, IV, V, VI; [b]group II and groups V, VI. The symbols *, # and @ represent statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

**Table 3** Effect of cyclophosphamide, lupeol and lupeol linoleate on cardiac lipid status in rats

| Parameter (mg/g wet tissue) | Group I (control) | Group II (cyclo-phosphamide, CP) | Group III (lupeol) | Group IV (lupeol linoleate) | Group V (CP + lupeol) | Group VI (CP + lupeol linoleate) |
|---|---|---|---|---|---|---|
| Free cholesterol | 3.78 ± 0.41 | 4.47 ± 0.41[a*] | 3.77 ± 0.45 | 3.76 ± 0.41 | 3.81 ± 0.41[b*] | 3.80 ± 0.42[b*] |
| Esterified cholesterol | 1.57 ± 0.14 | 2.73 ± 0.33[a@] | 1.56 ± 0.09 | 1.51 ± 0.15 | 1.53 ± 0.13[b@] | 1.52 ± 0.16[b@] |
| Phospholipids | 14.47 ± 1.43 | 10.67 ± 0.68[a@] | 14.53 ± 0.81 | 14.55 ± 0.95 | 13.68 ± 0.82[b@] | 14.3 ± 0.48[b@] |
| Free fatty acids | 3.07 ± 0.32 | 2.58 ± 0.28[a*] | 3.11 ± 0.36 | 3.12 ± 0.31 | 3.02 ± 0.23[b*] | 3.05 ± 0.34[b*] |
| Triglycerides | 4.42 ± 0.33 | 7.37 ± 0.53[a@] | 4.34 ± 0.45 | 4.33 ± 0.53 | 4.76 ± 0.64[b@] | 4.65 ± 0.62[b@] |

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]group I and groups II, III, IV, V, VI; [b]group II and groups V, VI. The symbols *, # and @ represent statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

**Table 4** Effect of cyclophosphamide, lupeol and lupeol linoleate on serum lipoprotein fractions in rats

| Parameter (mg/dL serum) | Group I (control) | Group II (cyclo-phosphamide, CP) | Group III (lupeol) | Group IV (lupeol linoleate) | Group V (CP + lupeol) | Group VI (CP + lupeol linoleate) |
|---|---|---|---|---|---|---|
| LDL-cholesterol | 12.25 ± 0.93 | 29.90 ± 3.97[a@] | 12.33 ± 1.17 | 11.96 ± 1.13 | 16.55 ± 2.30[a#b@] | 14.17 ± 2.08[b@] |
| HDL-cholesterol | 42.65 ± 4.60 | 28.98 ± 3.18[a@] | 42.67 ± 5.12 | 43.38 ± 4.77 | 38.25 ± 3.83[a#b@] | 41.18 ± 5.06[b@] |
| VLDL-cholesterol | 30.67 ± 3.25 | 47.85 ± 5.45[a#] | 30.83 ± 3.32 | 30.43 ± 3.65 | 34.55 ± 4.28[b@] | 32.35 ± 3.26[b@] |

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]group I and groups II, III, IV, V, VI; [b]group II and groups V, VI. The symbols *, # and @ represent statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

level of HDL was significantly ($P < 0.001$) decreased in group II rats, when compared with group I rats. The levels of VLDL ($P < 0.01$) and LDL ($P < 0.001$) were significantly increased in cyclophosphamide-treated rats when compared with control. However, significantly ($P < 0.001$; $P < 0.05$) decreased levels of VLDL and LDL and increased levels of HDL ($P < 0.001$) were observed in group V and VI rats; when compared with group II rats. Hence, it can be proposed that lupeol and its ester reduces lipid-mediated abnormalities in cyclophosphamide-treated rats.

### Lipid metabolizing enzymes

Table 5 represents the activity of lipid-metabolizing enzymes. Compared with controls (Group I), the activity of CES was significantly ($P < 0.001$) increased and the activity of CEH ($P < 0.01$) and LPL ($P < 0.001$) decreased in cardiac tissues of cyclophosphamide-treated rats (Group II). The activity of LCAT showed a significant ($P < 0.001$) decline in the plasma of cyclophosphamide-treated rats. Lupeol and its ester restored the activity of cholesterol-metabolizing enzymes to near normal.

**Table 5** Effect of cyclophosphamide, lupeol and lupeol linoleate on the activities of lipid metabolizing enzymes in rats

| Parameter | Group I (control) | Group II (cyclo-phosphamide, CP) | Group III (lupeol) | Group IV (lupeol linoleate) | Group V (CP + lupeol) | Group VI (CP + lupeol linoleate) |
|---|---|---|---|---|---|---|
| Heart | | | | | | |
| CES | 10.82 ± 1.32 | 19.18 ± 1.96[a@] | 10.80 ± 1.06 | 10.79 ± 1.51 | 11.08 ± 0.82[b@] | 10.93 ± 0.99[b@] |
| CEH | 16.17 ± 1.53 | 13.27 ± 1.30[a#] | 16.45 ± 1.80 | 16.48 ± 1.59 | 15.55 ± 1.39[b*] | 15.88 ± 1.56[b#] |
| LPL | 13.45 ± 1.32 | 9.55 ± 1.16[a@] | 13.51 ± 1.86 | 13.66 ± 1.63 | 12.66 ± 1.37[b#] | 13.06 ± 1.44[b@] |
| Plasma | | | | | | |
| LCAT | 7.07 ± 0.79 | 5.35 ± 0.68[a@] | 7.31 ± 0.61 | 7.36 ± 0.59 | 6.28 ± 0.58[b*] | 6.63 ± 0.39[b#] |

V Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]group I and groups II, III, IV, V, VI; [b]group II and groups V, VI. The symbols *, # and @ represent statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Units of enzyme activity: CES and CEH, nmol of cholesterol esterified/mg protein/hour at 37°C; LPL, $\mu$mol of free fatty acids liberated/mg protein/hour at 37°C; and LCAT, $\mu$mol of cholesterol liberated/mg protein/hour at 37°C.

### Histopathology

Groups I, III and IV rats presented normal cardiac histology when stained with haemotoxylin & eosin (H & E, × 200; Figures 1A, C and D, respectively). Figure 1B reveals cardiac muscle fibre destruction and hypertrophy in cyclophosphamide-treated rats. The photomicrograph shows cardiac muscle with karyorrhexis, pyknotic nuclei and extensive intermuscular haemorrhage. Lupeol treatment after cyclophosphamide administration reverted the cardiac histology to near normal, wherein the changes are less marked (Figure 1E). Figure 1F depicts a more or less normal cellular architecture of the heart muscle cells in rats supplemented with lupeol linoleate after cyclophosphamide administration. The linoleate ester of lupeol was found to be more effective than lupeol in mitigating the above alterations overall.

## Discussion

High-dose cyclophosphamide was introduced as an integral part of numerous preparative regimens for haematopoietic

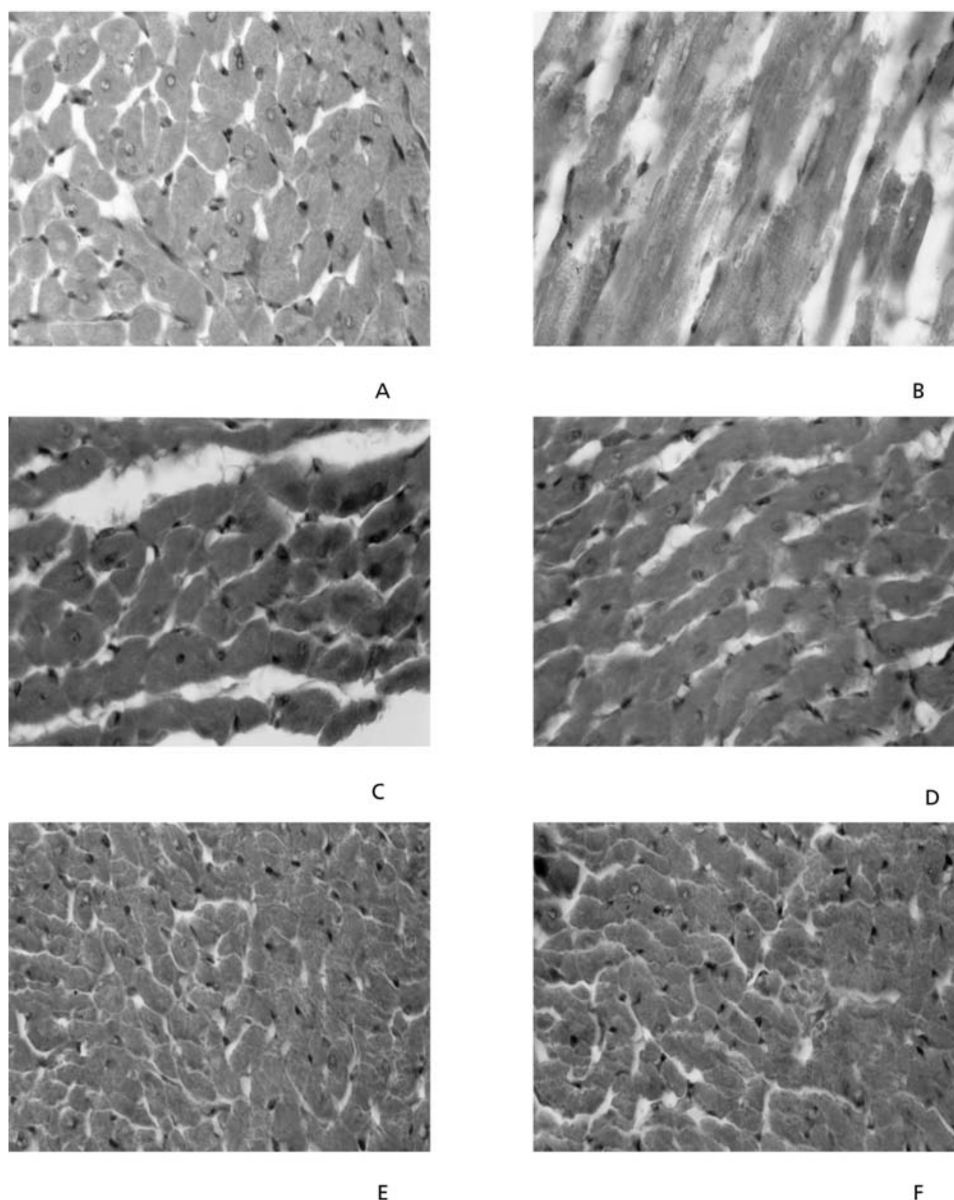


**Figure 1** Histopathology of heart tissue in the six experimental groups of rats (H&E, ×200). A. Control group shows normal architecture. B. Cyclophosphamide-induced group II shows abnormal cardiac muscle with karyorrhexis, pyknotic nuclei and extensive intermuscular haemorrhage. C. Lupeol drug control group III exhibits normal architecture. D. Lupeol linoleate drug control group IV exhibits normal architecture. E. Lupeol treatment in cyclophosphamide-induced group V brought about a significant recovery in the cardiac fibres. F. Lupeol linoleate treatment in cyclophosphamide-induced group VI brought about a significant recovery in the cardiac fibres.

stem-cell transplantation in the early 1970s and its potential to cause myocardial damage was soon recognized. When the differences in body surface area of rats and man are taken into account, cyclophosphamide administered at a dose of $200\,mg\,kg^{-1}$ to rats corresponds to the doses typically administered to man for cancer chemotherapy and immunosuppression (Wheeler et al 1962). In a previous study we demonstrated that high-dose administration of cyclophosphamide increased the serum cardiotoxicity indices, such as creatinine phosphokinase and lactate dehydrogenase activity. This increase in cardiac enzymes could be due to an increase in their release following cyclophosphamide-induced lipid peroxidation of cardiac membranes. By scavenging free radicals and bolstering the cardiac antioxidant defence system, lupeol and its ester, lupeol linoleate, were effective in alleviating the cardiac oxidative injury imposed by cyclophosphamide (Sudharsan et al 2005). Further, the linoleate ester of lupeol was found to be more potent than lupeol.

In a number of experiments conducted, cyclophosphamide-treated rats were observed daily for changes in body weight and the heart weight was documented after sacrifice. Cyclophosphamide-treated rats showed marked decrease in body weight and increase in heart weight in relation to control rats. We consider that the severe interstitial oedema present in the heart might have contributed to the increased cardiac weight in cyclophosphamide-treated rats. Supplementation with lupeol and its ester restored the above alterations induced by cyclophosphamide.

The liver indirectly contributes to the control of the serum and cardiac lipid profiles. It plays an important role in the synthesis and metabolism of plasma lipoproteins. It has active enzyme systems for synthesizing and oxidizing fatty acids and for synthesizing triacylglycerols and phospholipids. It facilitates the digestion and absorption of lipids by the production of bile. We observed hypercholesterolaemia in cyclophosphamide-treated rats, which may be due to decreased utilization of cholesterol. In the serum and cardiac tissue we observed significant increase in free cholesterol and esterified cholesterol fraction in cyclophosphamide-treated rats. McClure & Stupans (1992) previously reported that after 7 days following a single dose of cyclophosphamide ($200\,mg\,kg^{-1}$) there was a decrease in cytochrome P450 activity in male rats, which may in turn depress cholesterol 7$\alpha$-hydroxylase activity, the key enzyme in the conversion of cholesterol to bile acids.

Phospholipids are essential structural components of animal cell membrane and cytoskeleton. Muralikrishnan et al (2001) observed an increase in the content of phospholipids in the serum of cyclophosphamide-treated rats. Our results are consistent with that report. The decrease in tissue phospholipids may perhaps be due to increased activity of phospholipase, which eventually releases large amount of fatty acids into the serum of cyclophosphamide-treated rats. We also observed a decline in the free-fatty-acid content of the heart. The moderate increase in the rate of serum triglycerol synthesis by the liver contributes to the occurrence of hypertriglyceridaemia in cyclophosphamide-treated rats (Lespine et al 1993). The increased levels of free cholesterol and triglycerides in cyclophosphamide-treated rats may be explained by a reduction in the activity of fat-splitting enzymes, such as LCAT and LPL. LCAT is responsible for the esterification of free cholesterol in plasma and indirectly controls the level of free cholesterol in various cells and tissues. This enzyme is secreted by hepatocytes and released into plasma. The esterification of cholesterol on the surface lipoproteins by LCAT leads to the remodelling of the lipoprotein HDL and results in the formation of large HDL particles that are known to offer protection against coronary artery disease. We observed a deficiency in the levels of LCAT in the plasma of cyclophosphamide-treated rats. Deficiency of LCAT has been demonstrated to cause hyperlipidaemia (Furbee et al 2002). LPL, an extra cellular enzyme that is most active within the capillaries of adipose tissue and cardiac muscle, is the clearing factor for triglycerides in plasma and cleaves serum triglycerol into free fatty acid and glycerol. Our observation is consistent with the previous reports on cyclophosphamide (Lespine et al 1997), where a defect in the secretion of heart LPL may account for its poor expression in the vascular compartment, leading to reduced lipolysis. An increase in the activity of CES, with significant decrease in the activity of CEH, was observed in cyclophosphamide-treated rats. Cholesterol esterification in the tissue is reported to be mediated through CES (Proudlock & Day 1972). The activity of CEH may be reduced due to excessive increase in CES.

In this study, lupeol and its ester, lupeol linoleate, reduced serum and tissue cholesterol levels significantly to near normal in cyclophosphamide-treated rats. They proved to be extremely useful as they countered the lipaemic disturbances and oxidative injury, thereby protecting the cardiac tissue against hypercholesterolaemia. The inhibitory activity of lupeol can be attributed to its ability to directly scavenge free radicals and thus prevent their attack on the membrane by increasing its negative surface charge (Sunitha et al 2001). Moreover, the naturally occurring pentacyclic triterpenes have been reported to possess hypoglycaemic, antihyperlipidaemic and antioxidant activity (Patocka 2003). Romijn et al (1998) demonstrated that a linoleic-acid-rich diet may cause increased metabolism of serum cholesterol by LCAT in rats. This is due to changes in the chemical composition of endogenous lipoprotein substrates. Moreover, the efficacy of linoleic acid against lipid peroxidation has also been studied (Kikugawa 2001). Lupeol and its ester had a better effect on the antioxidant status of the liver, which may protect cytochrome P450 against alterations (Sunitha et al 2001). In this study, lupeol and lupeol linoleate restored the lipid-metabolizing enzyme activity in cyclophosphamide-treated rats significantly to near normal. It has been shown that triterpenes inhibit the uptake of endogenously produced cholesterol from the intestine. The mechanism is based on the fact that cholesterol must enter mixed micelles, containing bile salt and phospholipids, before it can be absorbed into the enterocytes. Triterpenes reduce circulating cholesterol by displacing and preventing its absorption (Sanders et al 2000).

The supplementation with lupeol and its ester showed favourable modulation of lipoproteins, while the un-supplemented cyclophosphamide-treated rats had a high risk of heart disease on account of decreased HDL and increased LDL concentrations. The alterations in lipoprotein transport and metabolism play an important role in the context of changes in plasma lipids. The liver is the major source of VLDL and HDL. LDL is removed from plasma by liver and extra-hepatic tissues. The observed increase in LDL cholesterol in cyclophosphamide-treated rats may be due to the suppression of LDL receptor activity. The increased synthesis of apo-B decreases the catabolism of LDL. This leads to an increase in LDL level, which ultimately raises the total cholesterol concentration in the plasma of cyclophosphamide-treated rats, as it is the major transporter of cholesterol. The reduction in HDL cholesterol in cyclophosphamide-treated rats is further worsened by an increase in LDL cholesterol. The increased levels of triglycerides observed in Group II rats may be due to the increased synthesis of VLDL cholesterol. The increase in lipids caused by a metabolic disorder due to cyclophosphamide seems to imply a remodelling of the VLDL core, which is enriched by triacylglycerol and cholesteryl esters (Muralikrishnan et al 2001). Lupeol has been shown to have LDL-protective activity during LDL oxidation studies performed in-vitro (Andrikopoulos et al 2002). A possible mechanism by which linoleic acid lowers the triglycerol level in plasma may be via an inhibition of triglycerol synthesis in the liver, thereby reducing the triglycerol content in circulating VLDL (Benner et al 1990).

On histological examination, the cardiac sections from cyclophosphamide-treated rats showed karyo-pyknosis of myocyte nuclei and extensive intermuscular haemorrhage. These findings corroborate with earlier reports wherein multiple areas of haemorrhage were identified after cyclophosphamide administration (Buja et al 1976). Lupeol (and its ester)-treated rats exhibited significant recovery from cyclophosphamide-induced cytotoxic damage to cardiac fibres, presenting a normal cardiac architecture. The study showed that lupeol and its ester exerted a protective effect in the heart, consequent to cyclophosphamide administration.

## Conclusion

The major proposal for its lipid-lowering potential and efficacy of lupeol and its ester, lupeol linoleate, seems to be its ability to intercept free radicals and protect cellular macromolecules (Patocka, 2003). The ability of lupeol, and its ester with its antilipaemic activity, in ameliorating cyclophosphamide-induced hyperlipidaemic cardiomyopathy in rats has thus been highlighted. Cancer chemotherapy is often disturbed by adverse drug reactions. These results suggest that cyclophosphamide treatment is better when carried out in conjunction with lupeol and more advantageous when administered with lupeol linoleate, on the basis of biochemical assessment confirmed by histopathological examination. Further studies should be focused on exploring the beneficial effects of these drugs and the mechanisms underlying their actions.

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