SUMMARY

Hypercholesterolemia is the dominant risk factor for atherosclerosis. It is a common clinical disorder that may begin early in life in humans and is characterized as a systemic disease which can restrict blood flow in numerous tissues resulting in ischemia. The underlying mechanisms responsible for these abnormalities may derive from high lipid levels and activation of oxidative, inflammatory and nitrosative mechanisms. Treating hypercholesterolemia is therefore a major challenge to biomedical research. Hence, a drug with hypolipemic, antioxidative and anti-inflammatory potential may be an appropriate choice to alleviate the abnormalities associated with hypercholesterolemia. The present work was aimed to evaluate the therapeutic potency of pentacyclic triterpene, lupeol and its ester derivative lupeol linoleate, a known antioxidative and anti-inflammatory drug. in reducing the hypercholesterolemia induced abnormalities. The salient findings of the study are briefly highlighted here under.

- Cellular lipid homeostasis is an important concern for preventing atherosclerosis. The levels of total cholesterol, free cholesterol, triglycerides, free fatty acids and phospholipids were increased in the serum and tissues (heart, liver and kidney) of HCD fed rats. Further, a sharp increase in LDL and VLDL level accompanied with a marked decline in HDL level were observed. But treatment with lupeol and lupeol linoleate reduced the above changes/alterations significantly in HCD fed rats, which highlights their hypolipemic effect.

- Increased lipid profile in turn troubled the lipid metabolism through the alterations in fecal cholesterol and bile acid excretion and the activities of lipid metabolizing enzymes in the plasma and tissues of HCD fed
rats. HCD fed rats showed a decrease in the activities of LPL, CEH and LCAT and an increase in the activity of CES in plasma and tissues, when compared with the control animals. Treatment with triterpenes favorably influenced the activities of these enzymes to an appreciable extent, and they also increased the levels of fecal cholesterol and bile acid excretion compared to HCD fed rats. Reduced absorption of cholesterol together with enhanced degradation in response to triterpenes administration may significantly contribute to the decrease in cholesterol content in blood and tissues.

- ACAT is another key enzyme involved in the esterification and absorption of cholesterol, secretion of LDL cholesterol and cholesterol accumulation in the arterial wall. HCD fed rats showed a significant increase in the mRNA expression of ACAT in liver. Triterpenes supplementation significantly decreases the mRNA expression of ACAT, suggesting their protective effect in hypercholesterolemic conditions.

- Increase in the biochemical variables like urea, uric acid and creatinine with concomitant decrease in albumin levels were observed in the serum of HCD fed rats. Triterpenes played a protective role in subduing the above biochemical abnormalities.

- Maintenance of cellular integrity is crucial for a tissue function. The activities of marker enzymes like LDH, ALP, AST and ALT were significantly increased in tissues and plasma of HCD fed rats when compared with control rats. The results obtained in this study indicate that the treatment with lupeol and lupeol linoleate normalized the activities of these enzymes within control level, emphasizing the cytoprotective action of triterpenes in preventing the tissue damage.
The illustrative evidence for the significant lesions that are characteristic of atherogenesis has been provided as photomicrographs of heart, liver, kidney and aortic sections. The protective role of triterpenes is well substantiated by the reversal of morphological changes to normal architecture in the treated group. In addition, the hypocholesterolemic effect of triterpenes was confirmed by transmission electron microscopic studies, wherein triterpenes decreased the accumulation of lipid droplets in the cardiac tissue of HCD fed rats.

Oxidative stress is one of the causative factors that link hypercholesterolemia with the pathogenesis of atherosclerosis. Hypercholesterolemia significantly increased the intracellular accumulation of ROS in heart, liver and kidney tissues. Triterpenes treatment considerably reduced the ROS level through their antioxidant property.

Oxidative stress results from an imbalance between the production of free radicals and the effectiveness of the antioxidant defense systems. A decline in the activities of enzymatic and levels of non-enzymatic antioxidants with associated increased LPO was observed in the tissues of hypercholesterolemic rats compared to control rats. Triterpenes treatment significantly reversed the increase in lipid peroxide levels and the altered antioxidant status, which shows that triterpenes alleviate the oxidative stress generated during hypercholesterolemia.

ROS are known to cause peroxidation of lipid, protein and DNA, which could be involved in hypercholesterolemia-induced vasculopathies. Increased susceptibility of plasma lipoproteins and cellular macromolecules to oxidation was noted in hypercholesterolemic group when compared with the control group. In addition, HCD fed rats
showed peroxidative damage in membrane ATPases. Treatment with triterpenes was effective in countering the macromolecular oxidative modifications.

- The erythrocyte biochemistry was also altered in hypercholesterolemic condition. Membrane derangement in the form of cholesterol accumulation, oxidative indices and alteration in erythrocyte membrane were observed in erythrocyte of HCD fed rats. These abnormalities were counteracted by triterpenes treatment, which implies the anti-hemolytic effect of triterpenes.

- Dietary cholesterol intake and hypercholesterolemia may increase the production of atherogenic inflammatory cytokines, which are known to exert systemic effects in atherogenesis. The levels of TNF-α, IL-1β and IL-6 were significantly increased in HCD fed animals. Further, the nuclear translocation of NF-κB, a redox sensitive factor increased in hypercholesterolemic group. Treatment with triterpenes substantially decreased the proinflammatory cytokines level and NF-κB expression.

- The proinflammatory cytokines in turn increase the production of acute phase proteins. Hypercholesterolemia is characterized by a sharp increase in acute phase proteins like CRP and fibrinogen. Additionally, lysosomal instability, characterised by the release and increase in the activities of its constituent hydrolases, was observed in the HCD fed groups. The anti-inflammatory effect of lupeol and its ester derivative were signified by their lowering of the acute phase inflammatory markers and lysosomal enzymes.

- The nitrosative stress associated with hypercholesterolemia and the possible role of triterpenes have been highlighted in this study.
Increased NO levels with concomitant increase in iNOS mRNA and protein expression were noted in the pathologic models. Triterpenes treatment substantially prevented the above nitrosative abnormalities.

- 25-OHC is the oxidized product of cholesterol, and the cytotoxic effects of this compound in liver cells were studied under *in vitro* condition. 25-OHC-induced ROS and mitochondrial dysfunction preceding apoptotic cell death in Chang liver cells. Decline in the expression of antiapoptotic Bel-2 coupled with the cytosolic release of cytochrome c from the mitochondria were noted in 25-OHC treated cells.

- In the cytosol, cytochrome c release triggered downstream cleavage of substrates such as α-fodrin, a membrane protein. These apoptotic changes along with DNA fragmentation were observed after 25-OHC exposure in Chang liver cells. Triterpenes were found to be beneficial in preventing the apoptotic changes induced by 25-OHC.

The findings of the present study indicate that hypercholesterolemia induced significant lipemic-oxidative and inflammatory abnormalities with a concomitant increase in nitrosative stress in rats and also that the oxidized product of cholesterol was cytotoxic in liver cells. The study further highlights the protective role of lupeol and lupeol linoleate in combating the lipid associated abnormalities.