Summary
Results presented in the thesis demonstrate that rats after 14 weeks of administration of a single dose (60 mg/Kg) of STZ became substantially hyperglycemic as well as hyperlipidemic. In addition, long-term diabetes was associated with fully developed nephropathy and retinopathy. Daily treatment of these diabetic-hyperlipidemic rats with 6 mg Tocomin (tocotrienols) or 0.50 mg Lovastatin for 14 weeks significantly lowered the elevated blood glucose levels to near normal values. Since the extent of hemoglobin glycation is currently used as a cumulative index of glycemia over the previous few weeks in the clinical management of diabetes, our results demonstrate a significant increase in HbA1 levels in diabetic rats, which were restored close to normal control values after 14 weeks of Tocomin or Lovastatin treatment. After 14 weeks of STZ-induced diabetes, histology of kidney resulted in the progression of diffused nodular glomerulosclerosis along with thickening of basement membrane in capillaries. An increase in the number of mesangial cells and enlarged Bowman space was also seen. Similarly, after 14 weeks of sustained hyperglycemia and increased oxidative stress, retina from diabetic rats exhibited proliferative retinopathy, characterized by complete retinal detachment. Consistent with the Tocomin and Lovastatin mediated decline in blood glucose and HbA1 levels, and normalization of glycemic state, histologically, all the untoward features of nephropathy and retinopathy were regressed and normalized in Tocomin or Lovastatin treated diabetic rats. However, treatment of chronic diabetic rats with Lovastatin, which is known to have host of side effects, induced interstitial inflammation of kidney lymphocytes as well as separation of retinal membrane from the lens, resulting in an open space. In contrast, dietary tocotrienols (Tocomin), are vitamin E and have no toxicity, therefore, did not exhibit any side effect. These results represent an initial demonstration of strong hypoglycemic and antidiabetic action of Tocomin and Lovastatin in rats induced with chronic diabetes coupled with hyperlipidemia.

Since long-term STZ-induced diabetes in rats is associated with a substantial hyperlipidemia, feeding of Tocomin or Lovastatin significantly prevented the increase in plasma TG, TC, atherogenic non-HDL-C, VLD-C, LDL-C, HDL-C and its subfractions, HDL2-C and HDL3-C levels. The cholesterol content of HDL2, which is considered to be a strong predictor of presence and extent of CAD, was significantly and equally reduced
in both Tocomin and Lovastatin treated diabetic rats. However, Lovastatin, in comparison to Tocomin, was more effective in selectively reducing the atherogenic non-HDL-C, whereas, levels of plasma antiatherogenic HDL-C and HDL₃-C in Lovastatin treated diabetic rats were significantly higher than Tocomin treated group.

Consistent with published reports that relative to lb-LDL, the concentration of more atherogenic sd-LDL was substantially increased in patients with diabetes, CHD alone or diabetes with CHD, sd-LDL-C and sd-LDL-apoB levels of diabetic-hyperlipidemic rats were increased by 245 % and 154 %, respectively, in comparison to corresponding values in N-C. Thus, > 60 % of total LDL-C and LDL-apo B were recognized in sd-LDL fraction, isolated from LDL of diabetic rats. Treatment of diabetic rats with Tocomin or Lovastatin significantly reduced both the cholesterol and apoB content of sd-LDL, as well as their percent share of total LDL, close to normal control values. In addition, sd-LDL-C/HDL-C ratio was increased (80 %), HDL-C/sd-LDL-C ratio was reduced (44 %) in diabetic-hyperlipidemic rats. Treatment with Tocomin or Lovastatin during diabetes resulted in significant improvement in these ratios, indicating normalization of sd-LDL levels and strong antiatherogenic property of Tocomin and Lovastatin. The therapeutic intervention of dietary tocotrienols (Tocomin) and Lovastatin in diabetic-hyperlipidemic rats, indicating a significant decline in the levels of sd-LDL-C, represents an initial demonstration.

Both tocotrienols (Tocomin) and Lovastatin are known to exert their hypolipidemic effects in hyperlipidemic animals by reducing hepatic HMG-CoA reductase activity. However, results in the present study show a decrease of 57 % in liver HMG-CoA reductase activity along with a substantial increase in plasma and tissue lipid levels after 14 weeks of chronic diabetes. This decrease in hepatic HMG-CoA reductase activity in diabetic animals may be due to a sustained insulin deficiency/hyperglycemia and hyperlipidemia. Treatment of these rats with Tocomin or Lovastatin for 14 weeks was associated with a significant reduction in glucose and lipid levels as well as restoration of HMG-CoA reductase activity to near normal levels. This increase in enzyme activity in treated diabetic rats may be due to an increase in insulin activity as a result of normalization of both glucose and lipid levels. Recently, it has been shown that RBO containing γ-T3 or γ-oryzanol exert their hypolipidemic effects in diabetic rats by
increasing fecal neutral sterol and bile acid excretion, via up regulating cholesterol synthesis and catabolism. In the present study involving diabetic-hyperlipidemic rats, dietary tocotrienols and Lovastatin may exert their hypolipidemic effects in a similar fashion.

In response to oxidative stress, evoked in experimental diabetes/hyperlipidemia, as reflected by increased formation of plasma and liver lipid peroxidation products, conjugated dienes, lipid hydroperoxides and TBARS, and increased release of MDA from intact erythrocytes subjected to hydrogen peroxide-induced lipid peroxidation was substantially and significantly blocked by Tocomin and Lovastatin. In addition, the enhanced oxidative damage of membranes during long-term diabetes is also associated with a decrease in membrane bound Na⁺, K⁺-ATPase activity, which is implicated in the pathogenesis of several diabetic complications. Consistent with these reports, our results show a significant reduction in total and Na⁺, K⁺-ATPase activities in erythrocyte membranes of diabetic rats. Consistent with hypoglycemic, hypolipidemic and antioxidant properties, Tocomin or Lovastatin feeding during diabetes prevented the decline in total and Na⁺, K⁺-ATPase activities and restored to a level close to normal values, apparently by blocking and protecting the ROS-mediated membrane damage. Similarly, due to enhanced oxidative stress and increased consumption of antioxidants in chronic diabetic rats, total antioxidant concentrations in plasma was significantly reduced, which was substantially increased to a level higher than normal control value in both the treated groups.

Our results show that due to substantial oxidative stress in diabetic-hyperlipidemic rats, the ex vivo base line diene conjugation (BDC) levels of sd-LDL and lb-LDL including LDL were increased. However, in comparison to lb-LDL BDC level, sd-LDL BDC value was higher by more than 3-fold indicating a markedly enhanced susceptibility of sd-LDL to in vivo oxidation. Similarly, treatment of diabetic rats with Tocomin or Lovastatin reduced the ex vivo BDC levels of sd-LDL, lb-LDL and LDL, with a maximum effect on sd-LDL. Consistent with ex vivo BDC levels of LDL, sd-LDL, and lb-LDL, susceptibility of these particles to Cu²⁺-induced oxidation, as measured by their lag time, was decreased in diabetic rats. It is important to mention that in comparison to a lag phase value of 98 min and 50 min for LDL and lb-LDL, respectively,
in N-C, the lag phase of sd-LDL was only 17.0 min, indicating a substantially increased 
in vitro oxidative susceptibility to oxidation. In treated groups, both Tocomin and Lovastatin increased the resistance of sd-LDL to oxidative modification, as shown by an increase in lag time from a value of 8.5 min in D-C to 12.5 min. In contrast to sd-LDL, the lag time of lb-LDL was reduced from 50 min in N-C to 40 min in D-C, which was fully restored to 50 min in Tocomin or Lovastatin treated groups. Consistent with known property of glucose that it may act either as LDL antioxidant or prooxidant depending on the vitamin E/antioxidant content of LDL, presence of glucose during Cu²⁺-induced oxidation of LDL, sd-LDL and lb-LDL from normal rats, further reduced their lag phases. However, in diabetic rats, which were deficient in antioxidants, and had a high plasma glucose level, addition of glucose in the incubation medium further mediated a prooxidant effect on sd-LDL lag phase with no effect on lb-LDL and LDL. The substantial prooxidant effect of glucose on lag phase of sd-LDL from normal and diabetic rats is consistent with its enhanced susceptibility to oxidation and reduced content of antioxidants, relative to lb-LDL. In Tocomin or Lovastatin treated rats due to the presence of high plasma concentrations of antioxidants, tocotrienols (Tocomin)/Lovastatin, prooxidant effect of glucose was blocked and lag phase value of sd-LDL was increased.

Based on the ex vivo results, prooxidant/antioxidant effect of glucose was investigated on Cu²⁺-induced oxidative modification of LDL, sd-LDL and lb-LDL, isolated from normal rat plasma pretreated with no antioxidant, Tocomin, α-tocopherol or Lovastatin. Similar to ex vivo studies, BDC level of untreated sd-LDL represented a 3-fold increase in comparison to BDC value of lb-LDL. As expected, glucose mediated a maximum prooxidant effect by decreasing the lag phase of sd-LDL, which was isolated from untreated control plasma and apparently had a least amount of associated antioxidants. When Tocomin, α-tocopherol or Lovastatin enriched LDL, sd-LDL and lb-LDL were subjected to oxidation with copper, a marked increase in the lag phase of LDL was observed. However, apparently due to low retention of antioxidants in sd-LDL and lb-LDL particles, after their fractionation from LDL, the increase in lag phase values of sd-LDL and lb-LDL was less pronounced. Addition of glucose to the media delayed the oxidation of antioxidant-enriched LDL, sd-LDL and lb-LDL, and markedly increased the
lag phase values similar to their corresponding values in untreated control samples obtained in the absence of glucose. These results represent an initial demonstration of ex vivo and in vitro oxidative modification of sd-LDL and lb-LDL, isolated from LDL. In addition, in response to substantial increase in oxidative stress, evoked in experimental diabetes coupled with hyperlipidemia as reflected by higher ex vivo BDC levels of sd-LDL and lb-LDL including LDL, as modified in vivo, and a decrease in lag phase time of their oxidation, was substantially and effectively blocked by Tocomin or Lovastatin, also represents an initial investigation.

Xanthine oxidase is known to be an important biological source of superoxide radical generating enzyme. In diabetic animals, xanthine oxidase is released by the vascular endothelial cells. Serum xanthine oxidase activity is known to be increased in various pathological disorders, such as carcinogenesis, hepatitis, inflammatory diseases, aging, hyperlipidemia and diabetes and that free radicals generated in the enzymatic processes are involved in the oxidative damage. Thus, it is possible that the inhibition of this enzymatic pathway by compounds, such as tocotrienols (Tocomin) and Lovastatin, that have both antiradical and xanthine oxidase inhibitory properties may have additional therapeutic importance in the treatment of diabetes with hyperlipidemia and other pathological disorders. Consistent with this hypothesis, our results show a significant increase in plasma and liver xanthine oxidase activity of diabetic-hyperlipidemic rats. Feeding of Tocomin or Lovastatin to diabetic rats was associated with a significant inhibition of the elevated levels of plasma and hepatic xanthine oxidase activity. These results show that both Tocomin and Lovastatin, in addition to their potent antioxidant activity, also exhibit xanthine oxidase inhibitory property, indicating an additional therapeutic benefit in the treatment of diabetes with and without CHD.

It is well known that diabetes may enhance oxidative stress not only through the increased production of ROS but also through weakening the antioxidant defense system. In this context the antioxidant role of serum HDL-complexed paraoxonase (PON)/arylesterase enzyme in the protection of LDL as well as HDL from oxidative modification is important. In diabetics, a lower PON/arylesterase activity and concentration, higher LDL: PON concentration ratio and a reduced capacity to prevent LDL oxidation have been reported. In addition, antioxidant supplementation may be
expected to influence PON/arylesterase activity by altering oxidative stress. Consistent with these reports, our results show a significant decrease in plasma arylesterase activity and increase in the LDL-C: arylesterase activity ratio in diabetic-hyperlipidemic rats, indicating a reduced capacity to protect LDL from oxidation. Both Tocomin and Lovastatin treatment significantly blocked the reduction in arylesterase activity and increase in LDL-C: arylesterase activity ratio, indicating an increased capacity to protect LDL from oxidation. However, Lovastatin not only prevented the decline in arylesterase activity but significantly increased its level above normal control value. Similarly, in D-LT, LDL-C: arylesterase activity ratio was reduced to a level lower than N-C value.

As indicated above, increased oxidative stress, which contributes substantially to the pathogenesis of long-term diabetic complications, is the consequences of either enhanced ROS production or attenuated ROS scavenging capacity. Several tissues, including erythrocytes and liver, have an effective mechanism to neutralize and prevent the free radical induced damage, which is accomplished by a set of endogenous enzymes such as catalase, SOD, Gpx and Gred, as well as nonenzymatic antioxidant, GSH. As the balance between ROS production and combined antioxidant defenses is lost, the resultant oxidative stress through a series of events deregulates the cellular functions leading to various pathological conditions. An antioxidant compound might contribute partial or total alleviation of such damage. Our results demonstrate that feeding of 6 mg Tocomin or 0.50 mg Lovastatin/rat/day for 14 weeks were found to be therapeutically quiet effective in restoring/normalizing the altered enzymatic and nonenzymatic antioxidant defense system in erythrocytes and liver.

Based on the above combined results, dietary tocotrienols (Tocomin) or Lovastatin can be used in the prevention and treatment of both type 1 and type 2 diabetes, including long-term complications, such as nephropathy and retinopathy, diabetes associated hyperlipidemia with and without CHD and atherosclerosis. However, considering the host of side effects exhibited by Lovastatin, use of dietary Tocomin as a multitherapeutic agent should be preferred. In addition, daily use of Tocomin as a dietary supplement will be highly cost effective as well as a good source of vitamin E.