Discussion
The present study explores the effect of long-term experimental diabetes on different parameters like hyperglycemia including HbA1, nephropathy and retinopathy, plasma and lipoprotein lipids including sd-LDL and lb-LDL, plasma and liver lipid peroxidation including ex vivo and in vitro oxidation of LDL, sd-LDL and lb-LDL, both in the presence and absence of glucose, erythrocytes MDA release and membrane bound Na⁺, K⁺-ATPase and erythrocytes as well as liver antioxidant enzymes including glutathione. In addition, therapeutic roles of tocotrienols (Tocomin) or Lovastatin in terms of their efficacy to normalize the alterations occurred during 14 weeks of diabetes have been delineated.

Although, previous studies have labeled α-tocopherol (α-T) as the most efficient chain breaking antioxidant, tocotrienols (T₃S) are known to be more potent than tocopherols (Suarna et al., 1993; Kamat and Devasagayan, 1995; Kamat et al., 1997). Tocotrienols have been shown to exhibit greater free radical scavenging properties as cell membrane constituents than tocopherols (Yamaoka and Carrillo, 1990; Serbinova et al., 1991). They quench free radicals in cell membranes and protect them against lipid peroxidation. The higher antioxidant potency of α-T₃ as compared to α-T is attributed to the combined effects of three properties: its higher recycling efficiency from chromanoxyl radical, its more uniform distribution in membrane bilayer, and its stronger disordering of membrane lipids which makes interaction of chromanols with lipid radicals more efficient. Since Tocomin is a mixture of α-T₃, γ-T₃, δ-T₃ and α-T, we have examined efficiency of individual T₃S, α-T and Tocomin as a scavenger of peroxyl radical. By using DPPH, the order of antiradical activity or hydrogen donating ability, expressed in terms of half quenching concentration (IC₅₀) was δ-T₃ > α-T₃ > γ-T₃ > Tocomin > α-T. Our results are in agreement with earlier reports (Qureshi et al., 2000) indicating that in intact membranes, including LDL particles, tocotrienols may have a significantly greater antioxidant effect than α-T and they may provide greater protection against CAD. The possible mechanism for this superior efficacy of tocotrienols compared to α-T has been reported elsewhere (Packer, 1995). The reduction in the free radical quenching efficiency of Tocomin (50 μM) in comparison to δ-T₃, α-T₃, γ-T₃ is due to the
presence of 23% α-T, which has a lowest peroxyl radical scavenging efficiency of 71 μM. These results are consistent with a previous report indicating the presence of higher percentage of α-T in TRF, reduces the antioxidant activity (Qureshi et al., 2000). Previous reports in hyperlipidemic-diabetic hamsters (El-Swefy et al., 2000), atherosclerotic rabbits (Chen et al., 1997; Singh et al., 1997), in vitro as well as in vivo studies in patients with hypercholesterolemia (Aviram et al., 1992) and the results presented in the thesis have demonstrated antioxidative properties of Lovastatin. However, under our experimental conditions used for Tocomin, T3S and α-T, 10 min incubation of 5-100 μM of Lovastatin did not exhibit any DPPH radical scavenging activity. Similarly, Rikitake et al. (2001) have also reported a weak radical scavenging activity for Lovastatin after its incubation with DPPH up to 30 h. These results indicate that the in vivo and in vitro mechanism(s) of antioxidant effects of Lovastatin, described in the present study, may be different than tocotrienols (Tocomin) including α-tocopherol and needs further investigation.

In animal model, STZ is known to induce diabetes along with hyperlipidemia/atherosclerosis, diabetic nephropathy, retinopathy, and neuropathy. Although the exact mechanism of STZ mediated toxicity is not known, one proposed site of action of STZ is at nuclear DNA. During the decomposition of STZ, highly reactive carbonium ions are formed, which cause alkylation of DNA bases (Doux et al., 1986) and also STZ may damage pancreatic β-cell membrane and break the DNA strand which leads to the activation of poly (ADP-ribose) synthetase and NAD depletion, which ultimately leads to cell death (Portha et al., 1989; Okamato, 1981). It has been previously reported that rats administered large dosages of STZ become substantially hyperglycemic as well as hyperlipidemic, but at the same time a marked weight loss, ketosis, and a high rate of mortality is produced (Bar-On, et al., 1976). Our study demonstrated that 12 days after a single injection of 60 mg/kg STZ was associated with an average fasting blood glucose level of 257 mg/dl when compared to a level of 99 mg/dl in normal control rats. However, in diabetic rats there was a decline in body weight from 248 g in N-C to 193 g. After 14 weeks of diabetes induction, average blood glucose levels in diabetic control rats were further increased to 329 mg/dl, whereas, there was no further decrease in body weight (205 g). Treatment of diabetic rats with 6 mg Tocomin or 0.50 mg Lovastatin/rat/day for 14
weeks lowered the elevated blood glucose levels, from 329 mg/dl to near normal level (83 mg/dl). The weight loss and mortality rate of STZ-induced diabetic rats seen in the present study is consistent with earlier findings (Bar-On, et al., 1976). 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 (Kennedy and Baynes, 1984). Our results demonstrate a substantial decrease in hemoglobin and a significant increase in HbA1c levels in diabetic rats. After 14 weeks of Tocomin or Lovastatin treatment, both Hb and HbA1c levels were restored close to normal control values. Nazaimoon and Khalid (2002) have initially reported that feeding of TRF-rich diet (1 g/kg) to STZ-induced diabetic rats for 12 weeks was associated with a reduction of blood glucose level from 556 to 376 mg/dl. Similarly, the TRF-rich diet also mediated a decrease in HbA1c level from 13.1 to 10.0 mg/dl. Although, in principle our results are in agreement with their findings but the hypoglycemic effect of TRF was minimal and both the glucose and HbA1c levels after TRF treatment were still within diabetic range (Nazaimoon and Khalid, 2002). In another report (El-Swefy et al., 2000), where hyperlipidemia was amplified by feeding a cholesterol-saturated fat-rich diet to STZ-induced diabetic Syrian hamsters for 12.5 weeks; 10-week feeding of vitamin E (d-l-α-tocopheryl acetate), probucol or Lovastatin supplemented with the above diet, failed to reduce significantly elevated fasting blood glucose. Our results showing a strong hypoglycemic effect of Lovastatin in diabetic-hyperlipidemic rats is in complete disagreement with the findings in hyperlipidemic-diabetic hamsters (El-Swefy et al., 2000). In addition, efficacy of other statins (simvastatin, pravasatin and atorvastatin) in terms of their beneficial effects on glucose metabolism also remains controversial (Pyorolla et al., 1997; Freeman et al., 2001; HPSCG, 2002; Sever et al., 2003; Sabatine et al., 2004). Among statins, only pravastatin has been shown to exhibit beneficial effects on glucose metabolism especially in the postprandial state in CAD patients with IGT (Sugiyama et al., 2006).

Our results demonstrate that treatment of STZ-induced diabetic rats with Tocomin or Lovastatin, for 14 weeks, mediated a reduction in the elevated fasting blood glucose levels, ranging from 257 to 329 mg/dl, to near normal range. STZ selectively destroys pancreatic insulin secreting β-cells (Doux et al., 1986; Portha et al., 1989; Okamato, 1981) causing diabetes close to type 2 in humans. The elevated
blood glucose levels in the diabetic rats used in this study were in the range of 236 to 329 mg/dl, which resembles type 2 diabetes (150 to about 250 mg/dl) with partially functional pancreas as well as type 1 (above 300 mg/dl) with considerable amount of damaged pancreas. This shows that both Tocomin and Lovastatin may be useful in the treatment of both type 2 and type 1 diabetes, irrespective of whether the pancreas is partially functional or almost totally dysfunctional. In contrast, sulphonylurea drugs act only when there is a functional pancreas (Sharma et al., 1997; Ivora, 1988). Chronic hyperglycemia is a major determinant of the development of secondary complications of diabetes such as nephropathy and retinopathy. At present the most important factor influencing the occurrence of diabetic nephropathy and retinopathy is the duration of disease. In addition, there is a positive correlation between the presence of diabetic nephropathy and retinopathy. Our results demonstrate that 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. Furthermore, an increase in the number of mesangial cells and enlarged Bowman space was also seen. After 14 weeks of Tocomin treatment, kidney from chronic diabetic rats showed a significant regression in glomerulosclerosis and normalization of basement membrane. In addition, histologically, the overall appearance was similar to normal kidney. Similarly, feeding of Lovastatin to diabetic rats was associated with a substantial reduction in basement membrane thickening and glomerulosclerosis. However, unlike Tocomin, Lovastatin therapy induced interstitial inflammation of lymphocytes. After 14 weeks of sustained hyperglycemia and increased oxidative stress, retina from diabetic rats exhibited poliferative retinopathy, characterized by complete retinal detachment. Tocomin or Lovastatin therapy prevented these changes and almost fully restored retinal detachment, similar to retinal membrane from normal control rats. Since Lovastatin is known to have host of side effects, it’s feeding to diabetic rats was associated with a substantial separation of retinal membrane, which resulted in an open space between retina and lens. In conclusion, both Tocomin and Lovastatin mediated a significant decline in blood glucose and HbA1 levels and restored the glycemic state similar to normal control rats. In addition, histologically, all the untoward features of nephropathy and retinopathy were significantly regressed and normalized by the administration of Tocomin or Lovastatin. However, treatment of chronic diabetic rats with Lovastatin
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 (Fig. 3.2-3.10). Our results are consistent with previous reports indicating that improved glycemic control is strongly associated with decreased development or regression of diabetic complications in both type 1 and type 2 diabetes (UKPDS, 1998; Cusick et al., 2002). Furthermore, recently it has been reported that a combined therapy of chronic diabetic rats with a hypoglycemic agent, *Ocimum sanctum Linn* and an antioxidant, α-tocopherol, for 16 weeks completely reversed the retionpathic changes (Eshrat and Mukhopadhyay, 2006). Similar to these findings, our results demonstrate a potent hypoglycemic property of both Tocomin and Lovastatin, which in conjunction with their strong antioxidant activity, as described in the present study, may have contributed to the prevention of diabetic nephropathy and retinopathy. The effectiveness of vitamin E (α-T) supplementation in preventing or reducing diabetic complications has been demonstrated in several studies. D-α-tocopherol was shown to act directly on the diacylglycerol-protein kinase-C pathway (Fig. 1.4), preventing glomerular dysfunctions (Koya et al., 1997) and normalizing the abnormal retinal blood flow (Kunisaki et al., 1998) in diabetic rats. Vitamin E could also exert its protective effects indirectly by improving insulin action (Paolisso et al., 1993b), and as an antioxidant in many pathological disorders associated with oxidative stress (Jialal et al., 1995; Rimm et al., 1993; Watson and Leonard, 1986). In the present investigation we have shown that the treatment of chronic diabetic rats with Tocomin or Lovastatin mediated a decline in blood glucose and HbA₁ levels close to normal values, as well as offered a significant protection against nephropathy and retinopathy. These results imply that there is a significant association between improved glycemic control and Tocomin or Lovastatin. Although a detailed investigation is needed to elucidate the possible mechanism(s) involved, it is intriguing to postulate that both Tocomin and Lovastatin being potent antioxidants may have effectively protected the β-cells from total damage by STZ and/or glucotoxicity. In the presence of residual functional islet cells, the blood glucose and HbA₁ levels of diabetic rats treated with Tocomin or Lovastatin were significantly lower. Our results appear to be in agreement with an earlier study, which showed that by pretreating rats with α-tocopherol before STZ administration, the severity of
pancreatic damage could be significantly reduced (Slonim et al., 1983). Similar effects were also reported for soybean diet, postulated to be due to the presence of soybean trypsin inhibitor that promotes the binding capacity of insulin receptor and arginine, a potent insulinotrophic agent (Lee and Park, 2000). Our data also suggest that the amount of total antioxidants present in the circulation or perhaps in the tissues during the chemical insult was an important factor. In diabetic rats, plasma total antioxidants level was reduced to 37 μmole/dl from a normal value of 50 μmole/dl. Treatment of diabetic rats with Tocomin or Lovastatin significantly increased the total antioxidant levels to a value of 72 and 59 μmole/dl, respectively (Table 9). This increase in plasma total antioxidant levels in Tocomin or Lovastatin treated rats, along with their novel and potent hypoglycemic activity, seems to be adequate for the normalization of glycemic state.

Diabetic mellitus confers an increased propensity to accelerated atherogenesis. Abnormalities in lipid profile are one of the major contributing causes of vascular complications in diabetic patients (Ono et al., 1998; Giugliano et al., 1996). It has been well documented that diabetic patients have increased glycation and increased lipoprotein oxidation and a reduced antioxidant status (Bellomo et al., 1995; Maxwell et al., 1997). Thus, oxidative modification of lipoproteins, particularly LDL, could be one of the mechanisms for an early development of atherosclerosis in diabetes. An enhanced susceptibility to oxidative modification could contribute to the greater atherogeneity of sd-LDL (De Graff et al., 1991; Tribble et al., 1992; Chait et al., 1993). The report of the Diabetes Control and Complications Trial (DCCT, 1996) clearly showed the benefit of antioxidants in the prevention of diabetic microvascular disease. However, to date, the treatment of diabetic macrovascular disease is far from optimal. We have investigated the role of Tocomin and Lovastatin in the prevention of hyperlipidemia, which is known to be associated with a long-term STZ-induced diabetes in rats. As expected, our data showed a striking increase in TG, TC, and non-HLD-C in diabetic-hyperlipidemic rats. Treatment of these rats with Tocomin or Lovastatin mediated a significant reduction in the above lipid parameters, which were close to their respective normal values. It is important to note that Lovastatin, in comparison to Tocomin, was more effective in selectively reducing the atherogenic lipoprotein particles (non-HLDL-C). Similarly, VLDL-C, LDL-C, HDL-C and its
subfractions, HDL\textsubscript{2}-C and HDL\textsubscript{3}-C levels were significantly increased in diabetic rats, which were significantly reduced after treatment with either Tocomin or Lovastatin. Plasma HDL-C and its subfractions, HDL\textsubscript{2}-C and HDL\textsubscript{3}-C levels, were substantially increased after 14 weeks of STZ-induced diabetes. These results are consistent with other reports, where a significant increase in HDL-C level in diabetic rats has been reported (Ebara et al., 1994; Kobayshi et al., 2000). Since these diabetic rats are considered as essentially models of type 1 diabetes (Halliwell, 2002), an increased plasma HDL-C level has been reported in type 1 diabetic patients (Drexel et al., 1992; Guerin et al., 2000). In contrast, type 2 diabetics, as well as hyperlipidemic-diabetic hamsters (El-Swefy et al., 2000) exhibited a decrease in plasma HDL-C. Tocomin treatment to diabetic rats (D-TT) significantly suppressed (41%) the increase in HDL-C levels; however, this decreased HDL-C value was higher than N-C value by 14%. On the other hand, feeding of Lovastatin mediated a decrease of 32% in HDL-C level, which was higher than N-C value by 31%. A similar increase of 29% in HDL-C of Lovastatin treated hyperlipidemic-diabetic hamsters has been reported (El-Swefy et al., 2000). The cholesterol content of large, light, cholesteryl ester-rich HDL\textsubscript{2}, which is considered to be a strong predictor of presence and extent of CAD (Drexel et al., 1992), was significantly and equally reduced (37%) in both Tocomin and Lovastatin treated groups. Small, dense, lipid-poor HDL\textsubscript{3}, which exerts potent protection of atherogenic LDL against oxidative stress, was significantly reduced (45%) in D-TT and to a less extent (31%) in D-LT. Similar to HDL-C, the reduced levels of HDL\textsubscript{3}-C in D-TT and D-LT were still higher by 10% and 21%, respectively, when compared to HDL\textsubscript{3}-C value in N-C, indicating a two-fold higher increase in HDL-C and HDL\textsubscript{3}-C levels in Lovastatin treated diabetic rats. These data are in agreement with published evidence indicating that Lovastatin treatment of hyperlipidemia was associated with a significant increase in antiatherogenic HDL-C concentrations (Helve and Tikkanen, 1988; El-Swefy et al., 2000). The above results indicate that both Tocomin and Lovastatin mediated a significant decline of plasma and lipoprotein lipids in a long-term experiment in diabetic rats. In general, both Tocomin and Lovastatin were equally effective in terms of their lipid lowering efficacy. These results are in agreement with our previous finding in type 2 diabetic-hyperlipidemic patients (Baliarsingh et al., 2005), where after 60 days of TRF treatment, subjects showed a significant decline in serum total lipids, TC and LDL-C
with no significant effect on TG and HDL-C levels. However, in another study, feeding of TRF to type 2 diabetic patients for 60 days caused no significant change in TG, TC, LDL-C and HDL-C levels (Nazaimoon et al., 1996). The results of present study are also consistent with our previous findings and reports from other laboratories indicating strong hypolipidemic effects of TRF or purified tocotrienols (Tocomin) in normolipidemic and hyperlipidemic rats (Beg et al., 1996b; Minhajuddin et al., 1999; Minhajuddin et al., 2005; Sharma and Rukmini, 1986 and 1987; Seetharamaiah and Chandrasekhara, 1989; Watkins et al., 1993); normolipidemic and genetically hyperlipidemic swines and chickens (Qureshi et al., 1991c; Qureshi and Qureshi, 1993; Qureshi et al., 2000); hyperlipidemic rabbits (Teoh et al., 1994); cholesterol/oxidized cholesterol-induced hyperlipidemic/atherosclerotic rabbits (Zainuddin, 2004; Beg and Zainuddin, 2003); normolipidemic and hyperlipidemic humans (Beg et al., 1995; Beg et al., 1996b, Beg et al., 1997; Minhajuddin et al., 1999; Tan et al., 1991; Qureshi et al., 1991a, Qureshi et al., 1997; Qureshi et al., 2001b; Qureshi et al., 2002) and hyperlipidemic hamsters (Raederstorff et al., 2002). Similarly, our results showing lipid lowering effects of Lovastatin are consistent with previous findings indicating strong hypolipidemic effects of Lovasatin and other statins. Statins have been used to reduce plasma cholesterol levels in many animal species (Alberts et al., 1980; Kovanen et al., 1981; Tobert et al., 1982). In another study, feeding of Lovastatin to hyperlipidemic-diabetic hamsters mediated a significant reduction in TG, TC, non-HDL-C and phospholipids, with an increase in HDL-C levels (El-Swefy et al., 2000). In clinical studies, Lovastatin and compactin effectively reduced plasma LDL-C in normal (Tobert et al., 1982) as well as subjects with heterozygous familial hypercholesterolemia (Bilheimer et al., 1983).

LDL particles are heterogeneous with respect to their size, density and lipid composition. Compared with lb-LDL, sd-LDL is thought to be more atherogenic as a result of their better penetration into the arterial wall, lower binding affinity for the LDL-receptor, prolonged plasma half life, and lower resistance to oxidative stress (Chapman et al., 1998; Bjornheded et al., 1996). Several studies have reported a 2- to 3-fold increase in CHD risk among patients with a predominance of sd-LDL particles (Austin et al., 1988; Austin et al., 1990; Austin et al., 1994). Hirano et al. (2004) have shown that not only the prevalence of sd-LDL but also its concentration was
substantially increased in patients with diabetes, CHD or diabetes with CHD. Similarly, Quebec cardiovascular study has confirmed that a predominance of sd-LDL is a strong and independent predictor of CHD in the first seven years of follow-up (St-Pierre et al., 2005). Recently, Koba et al. (2006) have reported that the progression of CHD was closely linked not to the LDL particle size, but to the concentration of sd-LDL. Therefore, sd-LDL has been highlighted as a new powerful and useful marker for the risk of CHD or type 2 diabetes with and without CHD (Austin et al., 1995; Hirano et al., 2004; Koba et al., 2006). The results presented in Table 5 demonstrate that sd-LDL-C and its percent share of total LDL-C, as well as sd-LDL-apoB and its percent share of total LDL-apoB have always increased or decreased in tandem, indicating a very strong correlation between sd-LDL-C and sd-LDL-apoB values. Furthermore, these data suggest that the measurement of cholesterol content may be sufficient to evaluate the sd-LDL mass and that apoB measurement is not essential. It is likely that both the cholesterol and apoB content of each particle is homogeneous in the sd-LDL fraction. These results are fully consistent with earlier reports (Hirano et al., 2003; Hirano et al., 2004), where only sd-LDL-C was measured and lb-LDL-C was estimated as difference between LDL-C and sd-LDL-C. In the present study, mean sd-LDL-C level in normal rats was 32 mg/dl, which was 32 % of total LDL-C. Similarly, sd-LDL-apoB level in N-C rats was 36 mg/dl, which was 27 % of total LDL-apoB. As expected, in diabetic-hyperlipidemic rats, sd-LDL-C was markedly increased (245 %) and the majority (63 %) of LDL-C was recovered in the sd-LDL fraction. Similarly, sd-LDL-apoB level was significantly increased by 154 %, which was 62 % of total LDL-apoB. Our results indicating a substantial increase in the levels of sd-LDL-C, sd-LDL-apoB and their percent share of total LDL-C or LDL-apoB in diabetic-hyperlipidemic rats are similar to the data reported in patients with diabetes, CHD alone, diabetes with CHD, hypertriglyceridemia and combined hyperlipidemia (Hirano et al., 2004). However, in these patients, sd-LDL-apoB levels were not reported.

Treatment of diabetic-hyperlipidemic rats with Tocomin or Lovastatin was associated with a significant reduction in sd-LDL-C levels by 53 % in D-TT and 56 % in D-LT. Whereas, in both the treated groups, sd-LDL-C as a percent of total LDL-C was reduced to a level of 42-43 %, which is close to normal control value of 32 %.
Similar to sd-LDL-C, sd-LDL-apoB content and its percent share of total LDL-apoB was significantly reduced after 14 weeks of Tocomin or Lovastatin treatment. As expected, the mean lb-LDL-C and its apoB content in normolipidemic rats were 65 and 91 mg/dl, respectively, which was 66% of total LDL-C and 69% of total LDL-apoB. These results represent a significant increase in comparison to sd-LDL-C and sd-LDL-apoB values in N-C. Due to substantial increase in sd-LDL-C level in diabetic-hyperlipidemic rats, no increase in lb-LDL-C was seen in comparison to N-C value. In addition, the levels of lb-LDL-C in D-TT and D-LT rats remained unchanged and were similar to values in D-C or N-C. In contrast to sd-LDL-apoB, lb-LDL-apoB content as well as its percent share of total LDL-apoB in diabetic rats was significantly reduced (40-47%), when compared to corresponding lb-LDL-apoB values of normal rats. Treatment of diabetic-hyperlipidemic rats with Tocomin or Lovastatin mediated a significant increase in lb-LDL-apoB levels close to normal value. It is interesting to mention that sd-LDL is known to be generated from large triglyceride-rich VLDL particle, production of which is enhanced by insulin resistance during diabetes, thus resulting in an increased prevalence of sd-LDL (Packard and Shephered, 1997; Tribble et al., 2001; Berneis and Krauss, 2002). Our results are consistent with these findings, showing a significant increase in the level of plasma VLDL-C (76%), TG (76%) and sd-LDL-C (244%) in chronic diabetic rats. Therefore, based on our results, the greater atherogenic potential of sd-LDL in comparison to lb-LDL may explain the higher incidence of CHD in diabetic patients than in isolated hypercholesterolemia (Hirano et al., 1998; Hirano et al., 2004). In addition, because of greater preponderance of sd-LDL, a moderately high LDL-C (between 130 and 160 mg/dl) in a type 2 diabetic patient is equivalent to a much higher LDL-C in terms of CHD risk for a nondiabetic subject (Grundy et al., 1999; Haffner et al., 1998; NCEP Expert Panel, 1993). Therefore, the primary target of therapy in type 2 diabetic patients is lowering LDL-C to a level ≤ 100 mg/dl as suggested by NCEP Expert Panel for patients with preexisting CHD (NCEP Expert Panel, 1993). Similarly, the substantial increase in more atherogenic sd-LDL in our study as well as in diabetic patients with and without CHD requires an immediate attention. However, no therapeutic interventions to reduce the elevated levels of sd-LDL-C in diabetic-hyperlipidemic patients or animals have been reported (Hirano et al., 2004; Hirano et al., 2005; Koba et al., 2006). In the present study, which
represents an initial demonstration, administration of dietary tocotrienols (Tocomin) or Lovastatin to diabetic rats was associated with a concomitant and significant decline in the levels of both LDL-C and more atherogenic sd-LDL-C. In D-TT rats, LDL-C was reduced from 172 to 124 mg/dl, whereas, in D-LT it was reduced to 111 mg/dl. The decline registered in sd-LDL-C from an elevated level of 109 mg/dl in D-C to a value of 52 mg/dl in D-TT and 48 mg/dl in D-LT rats represent a very potent cholesterol lowering therapeutic impacts of Tocomin and Lovastatin. It has been established that LDL-C/HDL-C and HDL-C/TC ratios are good predictors for the presence and severity of CAD (Drexel et al., 1992). In addition to LDL-C/HDL-C and HDL-C/TC ratios, we have also presented the ratios of sd-LDL-C/HDL-C and HDL-C/sd-LDL-C (Table 6), which were increased (80 %) or decreased (44 %) in D-C rats, respectively, in comparison to the corresponding ratios in N-C. Tocomin or Lovastatin treatment of diabetic rats for 14 weeks resulted in a significant improvement in these ratios, indicating normalization of above lipid and lipoprotein lipid parameters. Our results also demonstrate that similar to plasma TG and TC, liver TG and TC levels were significantly increased in diabetic-hyperlipidemic rats. Feeding of Tocomin or Lovastatin to diabetic rats resulted in a significant decline of both TG and TC to nearly normal values. The combined results indicate that increased levels of plasma and hepatic lipids as well plasma lipoprotein lipids in diabetic rats were significantly reduced after treatment with 6.0 mg Tocomin or 0.50 mg Lovastatin/rat/day for 14 weeks.

It has previously been established that diabetic rats deficient in insulin are known to express low levels of hepatic HMG-CoA reductase activity (Ness et al., 1994a; Ness et al., 1994b; Ness and Gertz, 2004) and frequently exhibit elevated serum cholesterol levels (Eshrat and Mukhopadhyay, 2006; Chen and Cheng, 2006). In addition, diabetic rats given replacement doses of insulin restored high levels of hepatic HMG-CoA reductase (Ness and Chambers, 2000; Lakshmanan et al., 1973; Ness et al., 1994a; Ness et al., 1994b). Our results also show a significant decrease (57 %) in liver HMG-CoA reductase activity and a substantial increase in plasma and tissue lipid levels in rats after 14 weeks of STZ-induced diabetes. This decrease in hepatic HMG-CoA reductase activity in diabetic rats may be due to a sustained insulin deficiency/hyperglycemia and hyperlipidemia. Treatment of these diabetic-
hyperlipidemic rats with Tocomin or Lovastatin for 14 weeks was associated with a significant reduction in glucose and lipid levels as well as restoration of hepatic HMG-CoA reductase activity to a level similar to normal control value. This increase in hepatic HMG-CoA reductase activity in D-TT and D-LT rats close to normal levels may be due to an increase in insulin activity along with a concomitant decline/normalization of both glucose and lipid levels. Both tocotrienols (Tocomin) and Lovastatin are known to exert their hypolipidemic effects in hyperlipidemic animals by reducing HMG-CoA reductase activity (Minhajuddin et al., 1999; Beg et al., 2000; Beg et al., 2000b; Minhajuddin et al., 2005; Endo et al., 1979; Tanaka et al., 1982). However, our present results indicate that the mechanism(s) of their cholesterol lowering effects in diabetic-hyperlipidemic rats may be quite different. Support for the existence of such a mechanism is obtained from a recent report (Chen and Cheng, 2006), where rice bran oil containing γ-tocotrienol or γ-oryzanol have been shown to exert their hypolipidemic effects in diabetic rats by increasing fecal neutral sterol and bile acid excretion, via upregulating cholesterol synthesis and catabolism. After 4 weeks of feeding RBO containing γ-tocotrienol or γ-oryzanol to STZ/nicotinamide-induced diabetic rats, there was a 50% increase in hepatic HMG-CoA reductase mRNA, 89% increase in the hepatic LDL-receptor and ~100% increase in the mRNA of 7α-hydroxylase - the rate controlling enzyme in the biosynthetic pathway of bile acids (Chen and Cheng, 2006). It is likely that the mechanism described for the cholesterol lowering actions of RBO containing γ-tocotrienol or γ-oryzanol in diabetic rats, may also be operative for tocotrienols (Tocomin) and Lovastatin in diabetic-hyperlipidemic rats.

Increased lipid peroxidation can be detected in early stages of type 2 diabetes, long before the development of any diabetic complications (Halliwell, 2002). Since most animal studies of diabetes are induced by administration of STZ or alloxan, toxic agents that target β-cells, they are thus essentially considered as models of type 1 diabetes. Nevertheless, such animals show increased lipid peroxidation (Halliwell, 2002), hyperlipidemia and other diabetic complications seen in type 2 diabetes. In addition, hyperglycemia directly causes cellular dysfunctions (Koya and King 1998; Nishikawa et al., 2000), glycation (Brownlee, 1994) and oxidative stress (Wolff, 1993). All these factors aggravate the consequences of the diabetic dyslipidemia by
increasing susceptibility to lipid peroxidation. This leads to accumulation of products of oxidative damage to lipids (Griesmacher et al., 1995), foam cell formation and eventually increased atheroma plaque deposition (Ross, 1990). When the lipid peroxides formed and accumulate to a certain degree, they leak from the organ or tissue in to the blood stream and increase the lipid peroxide level in the blood. Thus, the increased blood lipid peroxide level obviously indicates the occurrence of some membrane damage in cells of some organ or tissue provoked by diabetes. Accordingly, the blood lipid peroxide level also indicates the severity of the disease (Yagi, 1987). Several studies have shown that supplementation of antioxidants, such as vitamin E, prevent membrane lipid peroxidation in vivo, thereby decreasing the extent of diabetic complications (Halliwell, 2002). In our study, the increase in plasma lipid peroxidation products in diabetic-hyperlipidemic rats is consistent with a substantial decline (27%) in plasma total antioxidant levels. Elevated plasma concentrations of conjugated dienes, lipid hydroperoxides and TBARS suggest that lipid peroxidation is significantly enhanced in diabetic-hyperlipidemic rats. Several other workers have also reported elevated lipid peroxidation products in blood samples of type 1 and type 2 diabetic patients (Karasu et al., 1997; Slonim et al., 1983; Laight et al., 1999; Ihara et al., 2000; Tanaka et al., 1999) as well as in STZ-induced diabetic rats (Bukan et al., 2003; Eshrat, 2002). The reduction in the levels of these products in both the treated groups is directly related with a significant increase in the plasma total antioxidant levels. This increase in the levels of plasma total antioxidant was above the normal value by 43 % in D-TT and 17 % in D-LT indicating an increased scavenging capacity against elevated lipid peroxidation process in diabetic rats. As α-tocopherol (vitamin E) is known for its antioxidative property (Gapor et al., 1989; Asmah et al., 1993; Bieri et al., 1983; Aoki et al., 1992; Vannucchi et al., 1999), the decreased levels of MDA and other intermediate products of lipid peroxidation in our study was apparently due to increased consumption of the antioxidants, tocotrienols (Tocomin) or Lovastatin. Our observation that there is a significant inverse association between plasma lipid peroxidation products and total antioxidant levels in treated diabetic rats in D-TT and D-LT, further supports the potent antioxidative role of tocotrienols (Tocomin) and Lovastatin. In another report (Nazaimoon and Khalid, 2002), no increase in serum MDA level was seen in STZ-induced diabetic rats. In addition, feeding of TRF or vitamin C-rich diet to diabetic
rats for 12 weeks failed to influence serum MDA levels (Nazaimoon and Khalid, 2002). This was rather unexpected as we in the present investigation and others have reported significant increase in MDA levels when rats became diabetic (Aoki et al., 1992) or when diabetic rats were on vitamin E (α-tocopherol) deficient diet (Vannucchi et al., 1999). In our opinion, no increase in serum MDA level in diabetic rats, as reported by Nazaimoon and Khalid (2002), was apparently due to the feeding of TRF or vitamin C-rich diet for 4 weeks prior to induction of diabetes. Recently, Zhu et al. (2005) have demonstrated an increase in the plasma MDA level in 12-week STZ-induced diabetic rats. This increase in MDA levels of diabetic rats was less pronounced after simvastatin treatment for 12 weeks. In another report, El-Swefy et al. (2000) have demonstrated a substantial and significant increase in plasma MDA concentration in hyperlipidemic-STZ-induced diabetic hamsters. Hyperlipidemic-diabetic hamsters treated with Lovastatin or α-tocopherol had significantly lower plasma MDA levels. Our results are consistent with two reports (Zhu et al., 2005; El-Swefy et al., 2000), indicating an increase in plasma MDA levels in diabetic rats and hyperlipidemic-diabetic hamsters, and reduction in MDA levels following treatment with simvastatin or Lovastatin. Our results are also in agreement with a previous report, where basal serum MDA levels in type 2 diabetic patients were significantly higher than in normal subjects. Daily intake of TRF for 60 days caused a significant decrease in MDA levels (Nazaimoon et al., 1996).

It is well known that the increase of free radicals in diabetic-hyperlipidemic condition is further aggravated due to the increased lipid peroxidation and the damage of antioxidant defense systems (Wolf, 1993), which may lead to disruption of cellular functions and oxidative damage to membranes. Lipid peroxidation products including MDA reflect the oxidant/antioxidant balance in diabetes. Our results demonstrate that treatment of diabetic-hyperlipidemic rats with two potent antioxidants (Tocomin and Lovastatin) may mediate a reduction in lipid peroxidation products by scavenging cellular free radicals, thus improving overall oxidant/antioxidant balance as well as possibly protecting the oxidative damage to membranes and tissues. Several abnormalities have been identified in the erythrocytes of diabetic patients as reviewed by Jones and Peterson (1981). In particular, erythrocytes of diabetic patients have a reduced life span (Lehrman, 1977; Pescarmona et al., 1982), excessive aggregation
(Schmid-Schonbein and Volger, 1976; Satoh et al., 1984), altered membrane phospholipid asymmetry (Wali et al., 1988), and an increased tendency to adhere to endothelial cells (Wali et al., 1988; Wautier et al., 1981). Erythrocytes of diabetic patients are exposed to continuous oxidative stress, because oxygen radicals are continuously generated by the autooxidation of hemoglobin (Misra and Fridovich, 1972). Oxygen radicals formed over and above the detoxifying capacity of erythrocytes can cause peroxidative breakdown of phospholipid fatty acids and accumulation of MDA (Halliwell and Gutteridge, 1984). In addition, erythrocytes of diabetics are more susceptible to lipid peroxidation when treated with hydrogen peroxide in vitro (Uzel et al., 1987). Our results show a greater susceptibility to hydrogen peroxide-induced lipid peroxidation in erythrocytes from diabetic-hyperlipidemic rats than those from normal rats. A significant increase of 117% in the percent release of MDA from erythrocytes in D-C was markedly decreased to a level similar to normal value following treatment with Tocomin or Lovastatin. Consistent with these results, MDA content of erythrocytes hemolysate was significantly increased (105%) in diabetic-hyperlipidemic rats. Similarly, a highly significant decrease in MDA content was seen in diabetic rats treated with Tocomin or Lovastatin. Our results in diabetic rats are consistent with previous reports indicating that erythrocytes of diabetic patients are more susceptible to lipid peroxidation when treated with hydrogen peroxide in vitro (Matkovics et al., 1982; Uzel et al., 1987). However, in these studies no antioxidant treatment of diabetics was included. The exact mechanism by which elevated blood glucose leads to membrane lipid peroxidation of erythrocytes of diabetic-hyperlipidemic subjects is not known (Jain et al., 1989). However, based on our results it seems plausible that oxygen radicals formed over and above the detoxifying capacity of erythrocytes can cause peroxidative breakdown of phospholipid fatty acids and accumulation of MDA and hence membrane damage. This free radical mediated enhanced peroxidation of membrane fatty acids is significantly blocked in diabetic rats treated with antioxidants, Tocomin or Lovastatin.

As indicated above, alterations in lipid composition can affect the physico-chemical properties of the erythrocytes membrane, including plasma membrane, Na⁺, K⁺-ATPase and Mg²⁺-ATPase activities (Tsakiris and Deliconstantinos, 1984). In
previously published reports (Raccah et al., 1996; Mimura et al., 1994; Llewelyn and Thomas, 1987), a decrease in $\text{Na}^+\text{, K}^+\text{-ATPase}$ activity in various tissues including erythrocytes of diabetic animals as well as humans have been observed. Consistent with these reports, our results indicate a significant reduction of 37 % and 78 % in total, and $\text{Na}^+\text{, K}^+\text{-ATPase}$ activities, respectively, in erythrocyte membranes of diabetic rats. Consistent with hypolipidemic, antioxidant and hypoglycemic properties, Tocomin and Lovastatin significantly prevented the decline in total ATPase activity in D-TT and D-LT and restored to a level similar to normal rats. Since the decline in $\text{Na}^+\text{, K}^+\text{-ATPase}$ activity in D-C was more drastic, treatment with Tocomin or Lovastatin partially restored this activity in comparison to normal value. A decrease in this enzyme activity has been implicated in the pathogenesis of diabetic polyneuropathy via an activation of the polyol pathway (Fig. 1.3) leading to an accumulation of sorbitol and fructose and decrease in myoinositol concentrations in the peripheral nerve (Greene et al., 1987a). It has also been previously observed that this enzyme activity was lower in the erythrocyte membranes of male type 1 diabetic patients with neuropathy than those without it (Raccah et al., 1992). A decreased activity of this enzyme has been linked to the development of atherosclerosis in diabetes (Stojadinovic et al., 1996). Previous report showing a reduced erythrocytes $\text{Na}^+\text{, K}^+\text{-ATPase}$ activity in type 2 diabetics has explained this effect on an alteration in membrane fluidity (Mazzanti et al., 1989), which has a strong influence on important membrane function such as the conformation and thus the activities of membrane associated enzymes. The enhanced oxidative damage of membranes in diabetes may also contribute to the alteration in activities of membrane bound enzymes (Jain and Lim, 2001). Insulin therapy to diabetic patients normalizes lipid composition and other abnormal changes. It stimulates $\text{Na}^+\text{, K}^+\text{-ATPase}$ activity and translocation to plasma membrane via phosphorylation of the $\alpha$-subunits by protein kinase-C (Al-Khalili et al., 2004). Previous studies have shown that a number of dietary components may influence membrane characteristics such as fluidity, stability and susceptibility to membrane oxidative damage (Peck, 1994; Gutteridge and Halliwell, 1996). Dietary modulators of membrane structure and function include several antioxidants such as flavonoids (Gutteridge and Halliwell, 1996), $\beta$-carotene (Gutteridge and Halliwell, 1996; Thurnham, 1994), ascorbic acid (Gutteridge and Halliwell, 1996; Thurnham, 1994) and vitamin E (Gutteridge and Halliwell, 1996;
Morrissey et al., 1994). Based on potent antioxidative and protective properties of Tocomin and Lovastatin described here, the mechanism of action of these antioxidants, in terms of effectively preventing the dysfunction of erythrocytes membrane bound Na\(^+\), K\(^+\)-ATPase activity induced by diabetes, may be similar to other antioxidants including \(\alpha\)-tocopherol described elsewhere (Gutteridge and Halliwell, 1996; Thurnham, 1994; Morrissey et al., 1994).

Similar to plasma, the conjugated dienes, lipid hydroperoxides and TBARS in liver and kidney were significantly increased. Treatment of diabetic rats with Tocomin or Lovastatin for 14 weeks mediated a significant decline in all lipid peroxidation products, except the hepatic conjugated diene formation in D-LT did not respond to Lovastatin treatment. Failure of Lovastatin to reduce the level of hepatic conjugated dienes in diabetic rats and a lesser (9 %) reduction in hepatic hydroperoxides is similar to another report (Duthie, 1991).

An increasing number of studies have pointed out a possible role of LDL peroxidation in the occurrence of atherogenic lesions, which are one of the most frequent complications of diabetes (Deckert et al., 1978; Stout, 1979; Clowell et al., 1981). The involvement of increased oxidative stress in diabetes is supported by several data such as increased concentrations of lipid peroxidation products (conjugated dienes, hydroperoxides and TBARS) and decreased levels of antioxidants in the plasma of patients (Sato et al., 1979; Karpen et al., 1984; Wolf, 1987). In addition, an elevated glucose concentration in plasma could promote LDL oxidation by leading to glycated forms of the particle, which are more sensitive to transition metal-dependent autooxidation (Sakurai et al., 1991). High glucose concentrations have been reported to enhance \textit{in vitro} LDL oxidative modification induced by copper ions (Hunt et al., 1990; Hunt et al., 1994). The observation that elevated glucose concentrations in the culture medium enhances superoxide production and the subsequent ability of endothelial and smooth muscle cells to oxidatively modify LDL, brings a new insight to the pathogenesis of atherosclerotic lesions in diabetes (Mazeri et al., 1995). Otero et al. (2002) have demonstrated that the consumption of vitamin E in normal human LDL subjected to Cu\(^{+2}\)-induced oxidation was delayed by a glucose concentration frequently found in subjects with poorly controlled type 1 diabetes. Since vitamin E is one of the first compounds to be consumed during the LDL
oxidation process (Esterbauer et al., 1991), this finding indicates that glucose delays the early phases of LDL oxidation. However, high glucose concentration accelerated the LDL oxidation once LDL associated vitamin E was consumed. This prooxidant effect of glucose was reflected by increased formation of conjugated dienes, TBARS and a decreased lag phase (Otero et al., 2002). Although lipid oxidation in the vessel wall is thought to occur as result of local deficiency of endogenous antioxidants or an excess of free metal ions, only limited data support these hypotheses (Jialal and Devraj, 1996; Steinberg, 1997). Research has shown that human atherosclerotic plaques contain massive amounts of lipid peroxidation products, despite the presence of large quantities of α-tocopherol and ascorbate (Suarna et al., 1993). Thus, it is unclear whether oxidized lipoproteins originate in the arterial wall or are produced in the circulation and then enter the intimal space. Nevertheless, oxidative modification of lipoproteins would be one of the possible mechanisms for an early development of atherosclerosis in diabetes. This hypothesis has led to great interest in the development and evaluation of antioxidants as potential antiatherosclerotic agents. As discussed earlier (p. no. 157-160), increase in the concentration of more atherogenic sd-LDL has directly been linked to the progression of diabetes with and without CHD or CHD alone. Therefore, we sought to determine the differential ex vivo and in vitro oxidative susceptibility of sd-LDL and lb-LDL as well as LDL isolated from plasma of normal and diabetic-hyperlipidemic rats. Considering the potent hypoglycemic, hypolipidemic and antioxidant properties of Tocomin and Lovastatin shown in the present study, possible protective impacts of these agents on the in vivo oxidative modification of these lipoproteins in diabetic rats were determined. Consistent with ex vivo BDC levels of LDL, sd-LDL and lb-LDL, susceptibility of these particles to copper-induced oxidation, as measured by their lag time, was decreased in D-C. 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 high oxidative susceptibility to oxidation. After treatment with Tocomin or Lovastatin, the lag phase time of sd-LDL was increased from a value of 8.5 min in D-C to an identical value of 12.5 min in D-TT and D-LT. In contrast to sd-LDL, the lag phase of lb-LDL was reduced to 40 min in D-C from a value of 50 min in N-C. However, the lag phase of lb-LDL was fully restored to 50 min in D-TT and 52 min in D-LT. It is worth mentioning that in comparison to N-C, the decline in
lag phase values of LDL and sd-LDL in D-C was significantly higher (~50%) than the decrease in the lag phase of lb-LDL, which was only 20%. On the other hand, the lag phase time of LDL was partially restored in D-TT and D-LT rats; however, the increase in D-LT was more pronounced.

As expected, the *ex vivo* BDC levels of LDL, sd-LDL and lb-LDL isolated from plasma of N-C, D-C, D-TT and D-LT rats were not affected in the presence of glucose. However, with glucose the lag phases of copper-induced conjugated diene formation in LDL, sd-LDL and lb-LDL of N-C group were reduced, when compared to their corresponding lag phase values obtained in the absence of glucose. In D-C rats, which were deficient in antioxidants, and exhibit a high plasma glucose level, addition of glucose in the incubation medium further mediated a prooxidant effect only on sd-LDL lag phase, which was reduced to 6.5 min from a lag phase value of 10.0 min in N-C. No prooxidant effect of glucose was seen on the lag phase values of LDL and lb-LDL of D-C rats. The substantial prooxidant effect of glucose on sd-LDL lag phase of N-C and D-C rats is consistent with the previous reports indicating it's enhanced susceptibility to oxidation (De Graaf et al. 1991; Tribble et al., 1992; Dejager et al., 1993; Tribble et al. 1994; Tribble et al., 1995) and reduced content of antioxidants in sd-LDL, relative to lb-LDL (De Graaf et al. 1991; Tribble et al., 1992; Dejager et al., 1993; Tribble et al. 1994; Tribble et al., 1995). In D-TT and D-LT rats, due to the presence of high plasma levels of antioxidants, tocotrienols (Tocomin)/Lovastatin, prooxidant effect of glucose was not seen and the lag phase values of sd-LDL were increased to a level similar to corresponding values in D-TT and D-LT obtained after the oxidation of sd-LDL in absence of glucose. Our results show that due to sustained oxidative stress in D-C, the *ex vivo* base line diene conjugation (BDC) levels of LDL, sd-LDL and lb-LDL were significantly increased. However, in comparison to *ex vivo* BDC level of lb-LDL, the BDC level of sd-LDL was higher by 3.5 fold, indicating a markedly enhanced susceptibility of sd-LDL to *in vivo* oxidation. Treatment of diabetic rats with Tocomin or Lovastatin significantly reduced the *ex vivo* BDC levels. It is interesting to mention that in both the treated groups, the decline mediated by Tocomin and Lovastatin in BDC levels of sd-LDL was more pronounced. This increase in BDC levels of lipoprotein particles in D-C and
subsequent decrease in treated groups is consistent with the plasma values of conjugated dienes, lipid hydroperoxides and TBARS (Table 9).

Based on the ex vivo results described above, we investigated prooxidant/antioxidant effect of glucose on in vitro Cu^{++}-induced oxidative modification of LDL, sd-LDL and lb-LDL isolated from normal plasma pretreated with no antioxidant, Tocomin, α-tocopherol or Lovastatin. These results (Tables 15-17) show that due to markedly enhanced in vivo oxidation of sd-LDL, it’s ex vivo BDC levels, measured before the initiation of Cu^{++}-induced oxidation, represented a 3-fold increase in comparison to BDC values of lb-LDL. Similar increase in the ex vivo BDC levels of sd-LDL was observed in N-C rats, when compared to BDC levels of lb-LDL (Table 13 and 14). 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, as expected, 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. Under these conditions, the addition of glucose (250 mg/dl) to the media delayed the oxidation of antioxidant-enriched LDL, sd-LDL and lb-LDL, and markedly increased the lag phase values to a level similar to their corresponding lag phases in non antioxidant treated control samples obtained in absence of glucose.

Consistent with these results, an inverse relationship between lag time of LDL oxidation and severity as well as progression of coronary atherosclerosis has been reported (De Rijke et al., 1995; van de Vijver et al., 1998). Since sd-LDL isolated from diabetic rats is markedly more susceptible to ex vivo oxidation, which suggests that diabetic sd-LDL may be more susceptible to further oxidation in the vessel wall. Consistent with previous findings (De Graaf et al. 1991; Tribble et al., 1992; Dejager et al., 1993; Chait et al., 1993; Tribble et al. 1994; Reaven et al., 1994; Tribble et al., 1995; Jansen et al., 1995; Sevanian et al. 1996), our results demonstrate that sd-LDL particles are substantially more susceptible to both in vivo and in vitro oxidation than lb-LDL or LDL. This property has been proposed to contribute to the increased
disease risk associated with the sd-LDL phenotype. Differences in oxidative susceptibility between sd-LDL and lb-LDL have been attributed to differences in their physical-chemical properties. Relative to lb-LDL, sd-LDL has a reduced content of antioxidants (De Graaf et al. 1991; Tribble et al., 1992; Dejager et al., 1993; Tribble et al. 1994; Tribble et al., 1995) and free cholesterol (De Graaf et al. 1991; Tribble et al., 1992; Tribble et al. 1994; Jansen et al., 1995), and are enriched with polyunsaturated fatty acids (De Graaf et al. 1991), and, possibly, hydroperoxides (Sevanian et al., 1996). Antioxidants concentrations and free cholesterol content have been shown to predict differences in the oxidative susceptibility of LDL density subfractions (De Graaf et al. 1991; Tribble et al., 1992; Tribble et al. 1994). The origins of these physical-chemical differences are unclear, but may include differential metabolic processing of buoyant and dense LDL and their metabolic precursors. Kinetic tracer studies indicate that sd-LDL particles are metabolic products of both intermediate density lipoproteins and large, buoyant LDL (Griffin and Packard, 1994; Packard and Shepherd, 1997). Hence sd-LDL particles have a greater residence time in plasma, and undergo more metabolic processing, than do IDL and lb-LDL. Moreover, because sd-LDL particles bind less avidly to the LDL receptor and are degraded less efficiently (Nigon et al., 1991; Campos et al., 1996; Musliner et al., 1987), these subfractions may include some very long lived, extensively lipolysed particles. Tribble et al. (2001) reported that IDL from subjects with sd-LDL phenotype had a greater oxidative susceptibility and lower antioxidant concentrations than corresponding particles from subjects with the lb-LDL phenotype. Therefore, the differences in oxidative behavior of lb-LDL and sd-LDL arise from differences in their metabolic precursors, suggesting that factors contributing to an enhanced oxidation response are amplified in subjects with sd-LDL phenotype. An enhanced oxidative susceptibility of IDL and their LDL end-products could be one important consequence of reduced lipoprotein clearance in subjects with sd-LDL phenotype. This hypothesis is consistent with the fact that in diabetes, due to insulin resistance, generation of triglyceride-rich VLDL is increased which results in increased prevalence and concentration of sd-LDL (Hirano et al., 2004). Our results also show a substantial increase in VLDL (Table 4) and sd-LDL (Table 5) mass in diabetic-hyperlipidemic rats and hence a markedly increased in vivo and in vitro susceptibility to oxidation. Among the other physical-chemical factors that could be
important in determining differences in lipoprotein oxidative susceptibility are fatty acid composition and hydroperoxide content (De Graaf et al. 1991; Tribble et al., 1995; Sevanian et al., 1996; Thomas et al., 1994; Kontush et al., 1994; Frei and Gaziano, 1993). Previous reports have suggested that sd-LDL may be enriched in more oxidizable polyunsaturated fatty acids and preformed hydroperoxides (De Graaf et al. 1991; Sevanian et al., 1996). Tribble et al. (2001) also reported that in subjects with either the lb-LDL or the sd-LDL phenotype, oxidative susceptibility increased, and antioxidant concentrations decreased, from IDL to lb-LDL to sd-LDL. Moreover, they also showed that parinaric acid oxidation lag times were correlated with concentrations of ubiquinol-10 and α-tocopherol among all three lipoprotein subfractions. Our results are consistent with extremely low antioxidant content of sd-LDL in diabetic rats, which apparently was responsible for the prooxidant effect of glucose only on sd-LDL. It has been reported by Otero et al. (2002) that glucose may act either as LDL antioxidant or prooxidant, depending on the LDL vitamin E (α-tocopherol) content. Consistent with these findings, our combined ex vivo and in vitro results demonstrate that glucose may act either as antioxidant or prooxidant during copper-induced oxidative modification of LDL, sd-LDL and lb-LDL, depending on their antioxidant (α-tocopherol, Tocomin or Lovastatin) content.

Based on the findings reported by Otero et al. (2002), a model of LDL oxidation has been proposed, where the free radicals generated in the water phase by copper, will be taken up by the vitamin E attached to the LDL surface and also by the glucose of the media, thus, delaying the vitamin E consumption. As the process continues and the LDL associated vitamin is consumed, the copper mediated oxidation of fatty acids starts generating peroxides, as has been described in other systems containing transitional metal ions, can also oxidize glucose, generating glucose-derived aldehydes (Mlakar et al., 1996). Both the oxidation of glucose-derived aldehydes will generate more free radicals, further accelerating the LDL fatty acids oxidation as described by Hunt et al. (1990). This mechanism is very similar to the one proposed to explain the antioxidant and prooxidant effects of ascorbic acid (Halliwell, 1996b). It is worthwhile to mention the structural similarities between ascorbic acid and glucose and the fact that both of them when subjected to oxidation may generate the same aldehydes (Mlakar et al., 1996). In addition, glucose, like
ascorbic acid, may also reduce the oxidized copper in the presence of lipid peroxides, as described previously (Hunt et al., 1990; Halliwell, 1996a; Niki, 1991; Kadiiska et al., 1992; Thornalley and Stern, 1984). A phenomenon like this could also lead to an acceleration of the LDL oxidation by allowing further generation of free radicals from the copper oxidation (Hunt et al., 1990; Halliwell, 1996a). Therefore, glucose as other compounds like ascorbic acid, dehydroascorbic acid, or flavonoids (Otero et al., 1997b) could act either as LDL antioxidant or prooxidant, depending on the presence of vitamin E in the LDL.

The mechanism for enhanced lipoprotein oxidation in vivo in diabetic rats may be similar to the one described by Maziere et al. (1995), where preculture of endothelial or smooth muscle cells in glucose-enriched medium induced a dose-dependent increase in LDL oxidative modification. This phenomenon was correlated to a marked stimulation of superoxide anion secretion by cells. Regardless of the mechanism(s) involved in the in vivo oxidation of LDL, sd-LDL and lb-LDL, it will accelerate the atherosclerotic process, particularly in diabetes where mass and oxidative susceptibility of sd-LDL is considerably increased. Our results also indicate that LDL, sd-LDL and lb-LDL associated with higher content of antioxidants, Tocomin, Lovastatin or α-tocopherol, will be less susceptible to the prooxidation effects of glucose in vivo and, therefore, less prone to the development of atherosclerosis. In contrast, in diabetics due to low intake of antioxidants, their antioxidant-deficient LDL, sd-LDL and lb-LDL would be more susceptible to the oxidative effects of hyperglycemia. Indeed, some studies in type 2 diabetic patients have shown lower levels of vitamin E, making them more susceptible to the prooxidant effects of hyperglycemia (Otero et al., 1997a; Jain et al., 1991). Several other studies in animal model have shown that some of the complications secondary to diabetes can be prevented with the administration of antioxidants, including vitamin E, despite no improvement in hyperglycemia (Srivastava and Ansari, 1988; Viana et al., 1996; Cameron et al., 1993). Furthermore, based on the present results, there is a possibility of minimizing the effects of glucose on the oxidation of LDL, sd-LDL, and lb-LDL by increasing the antioxidant content of these particles. Finally, since the present study describes a potent hypoglycemic, hypolipidemic and antioxidant impacts of Tocomin and Lovastatin in diabetic-hyperlipidemic rats, intake
of these compounds will automatically prevent the prooxidant effect of glucose on lipoprotein oxidation, especially sd-LDL, by normalizing hyperglycemia as well as hyperlipidemia. To the best of our knowledge, the above results represent an initial demonstration of \textit{ex vivo} and \textit{in vitro} oxidative modification of sd-LDL, lb-LDL as well as LDL. In addition, in response to substantial increase in oxidative stress, evoked in experimental diabetes coupled with hyperlipidemia, as reflected by higher \textit{ex vivo} BDC levels of sd-LDL, and lb-LDL including LDL, as modified \textit{in vivo}, and a decrease in lag phase time of their oxidation was substantially blocked by Tocomin or Lovastatin, also represents an initial demonstration.

Our results indicating strong antioxidant impacts of Tocomin in diabetic-hyperlipidemic rats are in agreement with earlier findings of our laboratory in normolipidemic and hyperlipidemic rats as well as hyperlipidemic/atherosclerotic rabbits (Beg \textit{et al.}, 2000a; Beg \textit{et al.}, 2000b; Beg and Zainuddin, 2003), and other reports indicating an inhibition in the formation of conjugated dienes and TBARS by TRF or individual tocotrienols and tocopherols when fed to rats along with an atherogenic diet. These results also indicate that \(\gamma\)-tocotrienol exerts a significantly more potent impact as compared to \(\alpha\)-tocopherol (Watkins \textit{et al.}, 1993). Support to our results is also obtained from another study where feeding of a mixture of tocotrienols along with an atherogenic diet to rabbits was associated with a significant reduction in the formation of serum lipid peroxides (Teoh \textit{et al.}, 1994). TRF treatment of patients with hyperlipidemia and carotid stenosis caused a significant decrease in TBARS, an \textit{ex vivo} indicator of maximal platelet peroxidation (Tomeo \textit{et al.}, 1995; Theriault \textit{et al.}, 1999\textit{b}). The antioxidant activity of the \(\alpha\)-tocotrienol (T3) homologue has been shown to be more than 3-fold greater than that of \(\alpha\)-tocopherol (T) (Packer, 1995). Using an \textit{in vitro} liposome system, antioxidant activities for several tocotrienols were 4-33-fold higher than that for \(\alpha\)-T. The order of activity was d-P25-T3 > d-P21-T3 > TRF25 > \(\delta\)-T3 > \(\gamma\)-T3 > \(\alpha\)-T3 > \(\alpha\)-T (Qureshi \textit{et al.}, 2000). These results indicate that in intact membranes, including LDL particles, tocotrienols may have a significantly greater antioxidant effect than tocopherols and they may provide greater protection against CAD. D-P25-T3 is the most potent homologue of all natural forms of unsaturated (T3) and saturated (T) vitamin E tested. These findings were further confirmed by determining the antioxidant activities involving coupled
autoxidation of β-carotene and linoleic acid. The antioxidant activities of known α-, γ- and δ-tocotrienols and TRF25 (prepared from stabilized and heated rice bran with 6 % α-T) were 22 %, 27 %, 32 % and 24 % better than α-T, respectively (Qureshi et al., 2000). The possible mechanism for this superior efficacy of tocotrienols compared to tocopherols has been reported elsewhere (Packer, 1995). Comparative protective effects of TRF (75 % tocotrienols and 25 % α-tocopherol) and α-tocopherol on copper-induced oxidation of plasma LDL and indices of lipid peroxidation in human umbilical vein endothelial cells revealed that TRF is a more (2.5-fold) potent antioxidant than α-tocopherol (Mutalib et al., 2003). Differences in the transport and tissue uptake of the saturated (T) and unsaturated (T3) tocols have been reported by other investigators (Kayden and Traber, 1993; Hayes et al., 1993; Pearson and Barnes, 1970). A hepatic binding protein with high specificity for α-T in the liver results in the subsequent enrichment of this tocol in the VLDL moiety and as a consequence, the LDL moiety. The tocotrienols on the other hand are transported non-specifically like other lipid soluble compounds (Kayden and Traber, 1993; Pearson and Barnes, 1970; Suama et al., 1993). Thus, although tocotrienols was found to be more potent in vitro, the poor absorption of these compounds in the intestine is suggested to significantly affect their efficacy in vivo. It has been previously reported that treatment of hypercholesterolemic humans with TRF25 resulted in substantial increases in the levels of LDL-bound antioxidants, especially tocotrienols, which are known to have significantly greater antioxidant activity than tocopherols (Qureshi et al., 1997). Therefore, it appears that tocotrienols as TRF or Tocomin exert their antioxidant effect on plasma LDL oxidation while being attached to LDL particle.

In general, our results indicating a protective effect of Lovastatin in the in vivo and in vitro oxidation of LDL, sd-LDL and lb-LDL is consistent with another report, where Lovastatin, in addition to lowering cholesterol, also prevented the foam cell formation by inhibiting the oxidation of LDL in hyperlipidemic-diabetic hamsters. This is evidenced by data showing that Lovastatin decreased plasma lipid peroxide level, increased the lag time, and reduced the conjugated diene formation, during in vitro LDL oxidation (El-Swefy et al., 2000). The antioxidant activity of Lovastatin has also been reported in atherosclerotic rabbits (Chen et al., 1997; Singh et al., 1997) and in vitro as well as in vivo studies in patients with hypercholesterolemia (Aviram et
al., 1992). However, in this study, Lovastatin did not exhibit any radical scavenging activity. Whereas, Rikitake et al. (2001) have reported a strong radical scavenging activity for fluvastatin and a very weak activity for Lovastatin, simvastatin and pravastatin. In addition, direct antioxidative effect on LDL oxidation in vitro was observed at nontherapeutic concentrations of Lovastatin (Aviram et al., 1992). These results indicate that the mechanism(s) of antioxidant activity of Lovastatin is not clear. However, inhibitory effect of Lovastatin on LDL oxidation has been suggested to be caused mainly by increased removal of aged plasma LDL as a result of upregulation of LDL receptor. Hussein et al. (1997) have demonstrated that fluvastatin exerted its antioxidative effect as a result of binding to LDL. It is likely that Lovastatin may also exert its antioxidative effect after binding to LDL. Alternatively, the open ring of Lovastatin is probably an effective metal chelating agent and thus it is possible that the ester groups present in the Lovastatin molecules chelate the copper ions during in vitro LDL oxidation. However, antioxidative activity of Lovastatin was not evident in terms of inhibiting the formation of oxysterols in the lipoproteins of hyperlipidemic-diabetic hamsters (El-Swefy et al., 2000). Due to above discrepancies further investigation is needed to elucidate the exact mechanism(s) involved in the antioxidative actions of Lovastatin.

A low concentration of HDL-C is a powerful and independent predictor of premature CHD (Miller and Miller, 1975; Gordon et al., 1989). However, type 1 diabetes is associated with an increased incidence of CHD (Laing et al., 1999), despite normal or increased HDL-C concentrations (Dullaart, 1995). Our results also show increased levels of HDL-C in diabetic rats. This paradox may be explained by quantitative changes in HDL that affect its functional properties, such as its antioxidant capacity, related to paraoxonase (PON)/arylesterase activity. PON is a HDL-associated enzyme that protects LDL from oxidative stress by destroying biologically active phospholipids (Mackness et al., 1996). Castellani et al. (1997) have shown that depletion of PON results in the loss of the antioxidant function of HDL, and addition of PON to HDL restores the protective function of HDL. Aviram et al. (1998) also reported that purified PON is a potent inhibitor of LDL and HDL oxidation in vitro. Finally, a PON knockout mice model where absence of serum PON is associated with greater susceptibility of lipoproteins to oxidation and more
extensive atheromas (Shih et al., 1998), an extrapolation of these studies suggest that lower serum PON activity constitutes a risk factor for atherosclerotic disease. The enzyme has been identified as an independent genetic risk factor for vascular disease, particularly in type 2 diabetics (Ruiz et al., 1995; Blatter Garin et al., 1997; Odawara et al., 1997; Pfohl et al., 1999). In patients with type 1 diabetes, Boemi et al. (2001) have reported a lower PON activity and concentration, higher LDL: PON concentration ratio and a reduced capacity to prevent LDL oxidation. Serum PON activity is also reported to be reduced in STZ-induced diabetic rats (Patel et al., 1990). Antioxidant supplements may be expected to influence PON activity by altering oxidative stress. Inactivation of PON by oxidized LDL can be inhibited by antioxidants (Aviram et al., 1999). Although the effects of PON on LDL oxidation appear to be independent of the function of antioxidant vitamins (Watson et al., 1995), vitamin C and E (α-tocopherol) have been shown to inhibit LDL oxidation (Jialal et al., 1990; Harats et al., 1990). Therefore, any reduction in oxidative stress related to vitamin C and E intake may preserve PON activity. Consistent with these findings, our results (Table 18) show a significant decline in plasma arylesterase activity of diabetic-hyperlipidemic rats. In addition, the ratio of LDL-C: arylesterase activity was significantly higher in D-C, indicating a reduced capacity to protect LDL from oxidation. Tocomin treatment significantly blocked the reduction in arylesterase activity and increase in LDL-C: arylesterase activity ratio and restored these levels close to normal values, indicating an increased capacity to protect LDL from oxidation. In D-LT, Lovastatin not only prevented the decline in arylesterase activity but also significantly increased it to a level above N-C value. The mechanism of action of Lovastatin appears to be different than tocotrienols (Tocomin) and may be similar to simvastatin, which has been reported to be involved in the upregulation of PON at the gene level in vitro. In addition, hypercholesterolemic patients with CAD treated with simvastatin showed a significant increase in serum concentrations and activities of PON (Deakin et al., 2003).

It has been reported that xanthine oxidase, a superoxide radical generating enzyme is increased in plasma and liver of diabetic rats (Desco et al., 2002). In diabetic animals, xanthine oxidase is released by the liver into the plasma and is bound to the vascular endothelial cells (Adachi et al., 1993). The role of xanthine
oxidase in the vascular dysfunction that occurs in atherosclerosis was reported by White et al. (1996). Arterial rings from diabetic rabbits produce superoxide in presence of xanthine. The fact that the production of such a reactive molecule as superoxide is increased in the vessel wall of diabetic animals may be relevant in explaining some of the arterial complications of diabetes and underscores the importance of xanthine oxidase in this process. Consistent with published reports, our results show a significant increase in plasma and liver xanthine oxidase activity of diabetic-hyperlipidemic rats. Treatment of diabetic rats with Tocomin or Lovastatin significantly inhibited the elevated levels of plasma and hepatic xanthine oxidase activity. Results described here show that both Tocomin and Lovastatin in addition to their potent antioxidant activity also exhibit a xanthine oxidase inhibitory property, indicating an additional therapeutic benefit in the treatment of diabetes with and without CHD.

Increasing evidence in both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both type 1 and type 2 diabetes. Free radicals are formed disproportionately in diabetes by glucose degradation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to enhanced lipid peroxidation, which may mediate cellular damage in diabetes. The hyperlipidemia associated with diabetes may also lead to increased lipid peroxidation (Chirico et al., 1993; Palombo et al., 1999) perhaps because an increased lipid load allows lipoproteins to reside for longer periods in the circulation and in the vessel walls, giving them a greater exposure to free radicals (Walzem et al., 1995). Hyperketonemia may also promote lipid peroxidation (Jain et al., 1999). Several reports have demonstrated that blood MDA levels, a lipid peroxidation product and a marker of oxidative stress, were significantly elevated in type 1 and type 2 diabetes (Mahboob et al., 2005; Maritim et al., 2003; Sekeroglu et al., 2000; Sundaram et al., 1996; Telei et al., 2000). Higher plasma and erythrocyte MDA levels were observed in diabetics with retinopathy relative to diabetics without retinopathy (Atamer et al., 1998; Uzel, et al., 1987; Jennings, et al., 1991; Gallau et al., 1993; Augustin et al., 1993; Katoh, 1992). In the present study, consistent with above findings, we have
seen a substantial increase in plasma (101 %, Table 9) and erythrocytes (105 %, Table 10) MDA levels of chronic (14-week) diabetic-hyperlipidemic rats. In addition, we have also observed an increase of 117 % in MDA release of intact erythrocytes from diabetic rats. Increased erythrocyte MDA concentrations are known to cause a decrease in the membrane fluidity of the membrane lipid bilayer and increased osmotic stability of cells (Raccah et al., 1996). Consistent with these findings, we have observed a significant decrease in erythrocytes membrane bound Total ATPase and Na⁺, K⁺-ATPase activities (Table 11). It seems likely that oxidation process and MDA accumulation can contribute directly to changes in the properties of diabetic erythrocytes and may cause the development of long-term complications. Therefore, oxidative stress is the imbalance between production and removal of ROS. Increased oxidative stress, which contributes substantially to the pathogenesis of diabetic complications, is the consequences of either enhanced ROS production or attenuated ROS scavenging capacity. Several tissues 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 the balance between ROS production and 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. An impaired ROS scavenging function has been linked to the decreased activity of enzymatic and nonenzymatic scavengers of free radicals. Reports about the status of antioxidants and scavengers as defense mechanism in diabetes are very contradictory. Increase, decrease or no change in antioxidant enzyme activities have been reported (Siddiqui et al., 2005; Bukan et al., 2003). These discrepancies may be partly explained by the variability in the diabetes models used, including the strain and sex of the animals, their age at the induction of diabetes, the severity of the resulting insulin deficiency, and the duration of diabetes (Szaleczky et al., 1999).

Our results demonstrate a significantly lower catalase and SOD activities in erythrocytes hemolysate of chronic diabetic rats with retinopathy, which are consistent with an earlier report where plasma catalase and SOD levels were significantly decreased in type 2 diabetics with retinopathy (Atamer et al., 1998). Other reports also indicate that catalase and SOD activities were reduced in
uncontrolled diabetics, but unchanged in well-controlled diabetics (Cousins, 1985; Faure et al., 1993; Hayakawa and Kuzuya, 1990; Balashova et al., 1993). In contrast, our results are inconsistent with other reports (Wohaieb and Godin, 1987; Matkovics et al., 1982) where no change in erythrocytes catalase activity in diabetic animals was observed. Our results indicating a decline in erythrocytes SOD activity is consistent with the results reported by Loven et al. (1986) in STZ-induced diabetic rats. The decreased activities of catalase and SOD may be a response to increased production of H$_2$O$_2$ and O$_2^-$ by the autooxidation of excess glucose and nonenzymatic glycation of proteins (Argano et al., 1997). Hodgson and Fridovich (1975) and Pigeolet et al. (1990) have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The decreased activity of catalase and SOD could also be due to their decreased protein expression levels in the diabetic condition as reported in liver (Sindhu et al., 2004). As expected, treatment of the diabetic rats with antioxidants, Tocomin or Lovastatin restored the reduced activities of catalase and SOD. The restoration of enzymatic activity of catalase was partial (78-87%), whereas, for SOD it was similar to normal activity levels.

Reduced glutathione is a major intracellular non-protein sulphydryl compound. It has many biological functions, including maintenance of membrane protein and lipoprotein SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function. Glutathione cycle operates in the erythrocytes for the disposal of H$_2$O$_2$ generated in the cell supplementing the function of catalase. GSH and H$_2$O$_2$ are twin substrates for glutathione peroxidase. GSH is formed from its oxidized form, GSSG by the enzyme glutathione reductase, which requires NADPH as a cofactor. Glucose oxidation through the pentose phosphate pathway operates in the erythrocytes precisely to supply this NADPH (Halliwell, 2002). Normally, insulin promotes the operation of this pathway. In diabetes, due to inadequate function of insulin, the efficiency of this pathway is hampered, which results in reduced formation of NADPH (Fig. 1.3). Our observation of decreased levels of intracellular GSH and glutathione reductase in diabetic erythrocytes is corroborated by previous reports (Riemersma et al., 2001; Stojadinovic et al., 1996; Mukherjee et al., 1994; Murakami, 1991; Uzel et al., 1987). This decrease in GSH levels in diabetes may be due to combined effect of inhibited Gred and reduced
supply of NADPH. In our study, activity of Gpx is enhanced in diabetic rat erythrocytes, thus causing a further decline in GSH content. In addition, the increased Gpx activity represents a compensatory mechanism to degrade \( \text{H}_2\text{O}_2 \). This result is consistent with the findings of Matkovics et al. (1982), where erythrocytes Gpx activity was increased in diabetics. Decreased GSH content indicate a decreased scavenging capacity of glutathione-dependent antioxidant defensive system against the elevated lipid peroxidation processes in diabetic-hyperlipidemic rats. It has been suggested that diabetic complications may be the result of a long-term effect of small deficiency in intracellular GSH (Mazzanti et al., 1989). Tocomin or Lovastatin treatment of diabetic-hyperlipidemic rats for 14 weeks, mediated a significant reversal to near normal levels of Gpx, Gred and a partial restoration (51-61 \%) of GSH content, indicating a strong anti-lipid peroxidative effect of these antioxidants.

As discussed above, hyperglycemia is the most important factor in the onset and progress of diabetic complications mainly by producing oxidative stress, which is mainly based on the evidence of increased lipid peroxidation, or by indirect evidence of reduced antioxidant reserve (Palanivel et al., 1998). Increased production of free radicals may lead to disruption of cellular functions and oxidative damage to membranes (Oberley, 1988). It has also been reported that free radicals generate during diabetes deteriorate membrane structure and decrease membrane fluidity. Changes in membrane fluidity have been implicated in disease process and diabetic complications (Hong et al., 2004). Enhanced lipid peroxidation and oxidation of thiol groups have been implicated in the development of liver necrosis (Ellenhorn and Mathew, 1997). The levels of ROS are controlled by antioxidant enzymes, SOD, catalase, Gpx, Gred and nonenzymatic scavengers such as GSH. Earlier reports indicate increased or decreased SOD, catalase, Gpx and Gred activities in various tissues including liver (Ramanathan et al., 1999; Kinalski et al., 2000). Thus, the hepatic antioxidant status seems to have an important role in the etiology of diabetic complications. Antioxidant activity or inhibition of generation of free radicals plays a crucial role in providing protection against hepatic damage. Consistent with the above findings, our results show that chronic diabetes in rats is associated with a significant increase in hepatic lipid peroxidation, as evidenced by elevated levels of conjugated dienes, hydroperoxides and TBARS. Lipid peroxidation has been used as a measure...
of oxidative stress, which was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems. Once this imbalance appears, cellular macromolecules may be damaged by the predominant free radicals. The role of ROS in causing cell injury or death is increasingly recognized. Superoxide and hydroxyl radicals are involved in a large number of degenerative changes often associated with an increase in peroxidation process and linked to low antioxidant concentrations (Romero et al., 1998). Mammalian cells are equipped with both enzymatic and nonenzymatic antioxidant defense mechanisms to minimize the cellular damage resulting from interaction between cellular constituents and ROS (Irshad and Chaudhuri, 2002). The enzymatic antioxidant defense mechanism contains various forms of SODs, catalase and Gpx, as well as the enzymes involved in the recycling of GSSG such as Gred and glucose-6-phosphate dehydrogenase, the major enzyme in pentose phosphate pathway for generation of NADPH (Ho et al., 1998). An unbalanced production of ROS in localized compartments has been reported to play a role in the pathogenesis of diabetic complications, which illustrates the importance of antioxidant defense system in maintaining normal cellular physiology. Results of the present study show decreased activities of hepatic antioxidant enzymes, SOD and catalase, in chronic diabetic rats, which could be due to harmful effects of free radicals on these enzymes. A reduced activity of SOD and catalase may lower its cellular efficacy to detoxify these potentially active oxyradicals, thus leading to an increased level of lipid peroxidation products. Diabetic-hyperlipidemic rats treated with Tocomin or Lovastatin exhibited near normal activities of SOD and catalase enzymes, which indicates an antioxidant and possibly hepatoprotective nature of Tocomin and Lovatatin. Our results are consistent with the findings of Wohaieb and Godin (1987), where in a long-term experiment, a decrease in liver SOD activity of STZ-induced diabetic rats was observed. They proposed that this reduction in SOD activity might be due to the direct damaging effect of free radicals on the enzyme. The activity of catalase in the liver of diabetic animals is generally believed to decrease (Wohaieb and Godin, 1987; Asayama et al., 1989), although there are also reports of it's increase (Dohi et al., 1988). In another recent report, Eshrat and Mukhopadhyay (2006) have demonstrated a significant decline in hepatic SOD, and catalase activities in 16-week STZ-induced diabetic rats.
Treatment of these rats with vitamin E for 16 weeks showed a partial reversal of these antioxidant enzymes.

GSH acts as a reducing agent and a vital substance in detoxification. It provides antioxidant protection in the aqueous phase of cellular systems (Rana et al., 2002); its antioxidant activity is through the thiol group of its cysteine residue. Like ascorbic acid, another important water soluble antioxidant, GSH can directly reduce a number of ROS and is oxidized to GSSG in the process. Liver is viewed as a glutathione-generating site, which supplies the kidney and intestine with other constituents for glutathione resynthesis (Rana et al., 2002). Intrahepatic glutathione is reported to afford protection against liver dysfunction by at least two ways: (i) as a substrate for Gpx, GSH serves to reduce large variety of hydroperoxides before they attack unsaturated lipids or convert already formed lipid hydroperoxides to the corresponding hydroxy compounds. (ii) As a substrate of glutathione-S-transferase, it enables the liver to detoxify foreign compounds or other metabolites and to excrete the products, preferably in to bile. Decline in GSH, Gpx, Gred and GST levels in the liver of diabetic-hyperlipidemic rats, and their subsequent reversal to near normal levels in Tocomin or Lovastatin treated rats demonstrates strong anti-lipid peroxidative effects of these compounds. This decrease in hepatic GSH levels in diabetic rats may be due to the effect of inhibited Gred activity and also apparently due to reduced supply of NADPH. In addition, since GSH also acts as substrate and cosubstrate in essential enzymatic reactions of Gpx and GST, inhibition of their activities may also be due to decreased levels of GSH in diabetic liver. Thus, during oxidative stress, depletion of GSH, which is of clinical importance in tissue injury, mediated a significant impact on the antioxidant poise of liver cells. Treatment of the chronic diabetic rats with Tocomin or Lovastatin for 14 weeks, significantly restored the reduced hepatic activities of SOD, catalase, Gpx, Gred and GST including GSH, indicating an almost total alleviation of such damage by these antioxidants. Our results are consistent with other reports indicating a reduction in levels of hepatic GSH and Gred of STZ-induced diabetic rats (Mukherjee et al., 1994). Others have reported reductions in mitochondrial GPx and Gred activities in liver of diabetic (Sukalski et al., 1993). Wohaieb and Godin (1987) found that in the liver, which normally contains high content of GSH and strong Gpx activity, induction of diabetes
caused a decrease in their levels. Our results are also in agreement with the findings of Eshrat and Mukhopadhyay (2006), where in a long-term experiment a decrease in liver GPx and GST activities of STZ-induced diabetic rats was observed. In addition, treatment of diabetic rats with vitamin E (α-tocopherol) fully restored the enzymatic activities of Gpx and GST.

The combined results demonstrate that strong hypoglycemic, hypolipidemic and antidiabetic impacts of dietary tocotrienols (Tocomin) and Lovastatin coupled with their potent antioxidative property can provide additional benefits in the inhibition of oxidative stress, particularly resistance of highly atherogenic sd-LDL to oxidation and hence in the prevention and treatment of nephropathy, retinopathy, and diabetes linked hyperlipidemia with and without CHD. Although multiple therapeutic benefits of Tocomin and Lovastatin observed in the present study were comparable, but Lovastatin, which is known to exert a host of adverse effects (Haffner et al., 1998; Assmann et al., 1998), induced interstitial inflammation of lymphocytes in kidney and also mediated separation of retinal membrane from the lens, thus creating an open space. In contrast, dietary tocotrienols (Tocomin) are vitamin E and have no toxicity, therefore, did not induce any side effect. In conclusion, based on our combined findings, daily intake of dietary tocotrienols (Tocomin) or Lovastatin may be useful in the prevention and treatment of type 1 and type 2 diabetes, including nephropathy and retinopathy, diabetes linked hyperlipidemia with and without CHD and atherosclerosis. However, in view of the several toxic effects exhibited by Lovastatin including the ones exhibited in the present study, use of dietary tocotrienols (Tocomin) as an effective hypoglycemic, hypolipidemic, antioxidant and antidiabetic agent should be preferred. Furthermore, daily intake of Tocomin as a dietary supplement will be an excellent source of vitamin E. In addition, therapy with tocoomin will be both efficacious and cost effective.