In the present investigation, the extraction of the powder of the dried leaves of *Lagerstroemia speciosa* L. (*L. speciosa*) was carried out using 50% methanol. The yield of (50% methanolic extract of *L. speciosa* leaves) MEL was found to be 5.13%. Preliminary pharmacological studies with MEL showed that it possesses dose dependent antidiabetic activity. MEL was further fractionated to petroleum ether soluble fraction (PFML) and petroleum ether insoluble fraction. Petroleum ether insoluble fraction was fractionated to solvent ether soluble and solvent ether insoluble fractions (Tannin fraction). MEL and its fractions were checked for their antidiabetic potential and their role in diabetic complications except solvent ether soluble fraction due to very low yield (0.01%). The yield of PFML was 3.33% and that of tannin fraction of MEL (TFML) was 10%.

Phytochemical evaluation was done for presence of various phytoconstituents. Preliminary chemical tests indicated presence of saponins, carbohydrates, steroids, triterpenoids, tannins, phenolics, coumarins, quinone glycosides.

Standardization of the extracts and fractions of the herbal drugs can be performed by estimation of marker compounds that may be chemical markers and biomarkers using modern analytical techniques like HPTLC. HPTLC analysis has emerged as one of the tools for the qualitative and quantitative assessment of the herbal drugs. In the present study, PFML was subjected to HPTLC analysis for the estimation of corosolic acid, a marker compound present in *L. speciosa* (Katta *et al.*, 2006). On HPTLC analysis, corosolic acid found in PFML was 0.25%.

Intravenous injection of streptozotocin (STZ) produces fragmentation of DNA of pancreatic β cells which stimulates poly ADP-ribose and depletes NAD ultimately leading to destruction of β cells. This is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia (Rodrigues *et al.*, 1986). Diabetes is a metabolic disorder characterized by hyperglycemia resulting from low levels of insulin or insulin resistance. Hyperglycemic state is reported to cause a series of vascular changes. Short term elevation of blood glucose level affects the sorbitol metabolism causing generation of diacylglycerol with subsequent stimulation of protein kinase C, which in turn, alters the cellular uptake of myoinositol. Elevation of blood glucose for a longer time causes non enzymatic glycosylation of vital body proteins which leads to thickening of capillary basement membrane along with atherosclerosis (Haller *et al.*, 1991). In the present study, the blood glucose levels were found to be significantly higher in STZ induced type 1 diabetic Sprague-Dawley (SD) rats as compared to non diabetic control rats. Treatment with MEL (100, 300, 500 mg/kg, p.o.) reduced the fasting serum glucose levels but did not affect the serum insulin levels in diabetic rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group. The possible underlying mechanism could be, increased peripheral glucose utilization or stimulation of secretion of insulin at pancreatic level in response to increased glucose which is further supported by Kar *et al.* (1999). Corosolic acid isolated from methanolic extract of *L. speciosa* stimulates glucose uptake (Murakami *et al.*, 1993). Administration of STZ destroys β-cells of the islets of Langerhans in pancreas (Elsner *et al.*, 2000). Destruction of β-cells in the pancreas causes marked decrease in serum insulin levels (Gilman *et al.*, 2001). In present investigation STZ-induced type 1 diabetic rats showed significant decrease in serum insulin level when compared with non diabetic control rats. Treatment with MEL (100, 300, 500 mg/kg, p.o.) did not increase insulin levels significantly in STZ-induced type 1 diabetic rats. These results indicate that MEL did not induce any regeneration of pancreatic beta cells. Thus, the possibility of MEL inducing insulin secretion to elicit the antihyperglycemic activity can be ruled out. Treatment with MEL (100, 300, 500
mg/kg, p.o.) and insulin (5 IU/kg, i.p.) also reduced fed serum glucose level in diabetic rats when compared with diabetic control rats. Delaying or inhibiting glucose absorption at intestinal level or decrease in the endogenous glucose production in the liver could be the underlying mechanism for resultant decrease in fed serum glucose levels. During OGTT when further glucose load was given STZ-induced type 1 diabetic rat there was further rise in glucose levels observed at 0, 30, 60 and 120 min when compared with non diabetic control rats. Treatment with MEL 500 mg/kg p.o., significantly reduced AUC glucose while, 100 and 300 mg/kg, p.o. dose failed to produce this effect in STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced type 1 diabetic group. No significant change was found in AUC insulin at any dose of MEL. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced type 1 diabetic group. These results support the hypothesis of delaying or inhibiting glucose absorption at intestinal level by MEL.

So far there are no published reports of effects of L. speciosa on hexokinase or phosphoenolpyruvate carboxykinase (PEPCK), the enzymes affecting hepatic glucose metabolism. Hexokinase is a key enzyme in the glycolytic pathway. It converts glucose into glucose 6 phosphate in the glycolysis process. Treatment with STZ reduces the activity of liver hexokinase enzyme in diabetic control animals when compared with that of non diabetic control animals (Vats et al., 2004). Insulin deficiency in chemical models of diabetes (STZ and alloxan induced diabetes) causes suppression of hexokinase activities (Grover et al., 2000; Raju et al., 2001; Rathi et al., 2002). Administration of insulin in these animals caused normalization of enzymatic activities (Weber et al., 1966). All these imply that the measurement of hexokinase may be indicative of glucose utilization. In the present study, liver hexokinase activity was found to be decreased in diabetic control rats when compared with the non diabetic rats. Treatment with MEL (500 mg/kg, p.o.) increased the activity of liver hexokinase enzyme in the STZ induced type 1 diabetic rats when compared with non diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced type 1 diabetic rats when compared with diabetic control rats. This indicates that the MEL is effectively increases the utilization of glucose. Thus, increased utilization of glucose in MEL treated STZ induced type 1 diabetic rats could be one of the possible mechanisms of action of their antihyperglycemic activity.

PEPCK is a key enzyme involved in gluconeogenesis in liver (Barthel and Schmoll, 2003). Studies in diabetic animals showed that augmented gluconeogenesis is a major factor in the increased plasma glucose in fasting and post-prandial stage (Hanson and Reshef, 1997). Insulin directly inhibits the gluconeogenesis. Since only a negligible quantity of insulin is secreted in the STZ induced diabetic animals, the suppression of gluconeogenesis by insulin becomes minimal. This could be the reason for higher levels of gluconeogenic enzyme PEPCK in the STZ diabetic animals. The level of mRNA of PEPCK in liver from the STZ induced diabetic rats was 1.7 times that of liver of non diabetic rats (Liu et al., 2000; Cheng et al., 2001). In the present study, the activity of gluconeogenic enzyme PEPCK was found to be increased in the STZ induced type 1 diabetic rats when compared with that of non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) reduced PEPCK activity when compared with the diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced type 1 diabetic group. This indicates that the gluconeogenesis was suppressed by treatment with MEL. Gluconeogenesis significantly contributes to the endogenous hepatic glucose production. The suppressed gluconeogenesis could contribute to the antihyperglycemic activity of MEL at 500 mg/kg, p.o. Increase in the activity of liver hexokinase by MEL that was observed in the present study may also contribute to its anti-hyperglycemic activity.
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In the present study, treatment with MEL (500 mg/kg, p.o.) prevented the loss in body weight in STZ-induced type 1 diabetic rats. This could be due to emaciation of skeletal muscle, dehydration and catabolism of fats and proteins (Oakley, 1968; Hofteizer and Carpenter, 1973; Sevak and Goyal, 1996; Umarani and Goyal, 2002). The other cardinal signs of STZ diabetes viz., polyphagia and polydypsia were observed in the present study. The results are consistent with those reported earlier (Vadlamudi et al., 1982; Tahiliani et al., 1983). STZ induced type 1 diabetic rats showed significant increase in food and water intake when compared with non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) in STZ-induced diabetic rats reduced the elevated food and water intake of diabetic rats.

Diabetic conditions are associated with the elevated lipid levels along with the hyperglycemia (Betteridge, 1994; Brown and Goldstein, 1994). Levels of plasma triglycerides and cholesterol in individuals with various types of diabetes are higher than that of normal subjects (Chase and Glasgow, 1976). In the present study, serum cholesterol levels were found to be significantly higher in STZ-induced type 1 diabetic rats as compared to non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) in STZ-induced type 1 diabetic rats reduced the serum cholesterol levels when compared with diabetic control rats. Earlier studies have shown that in STZ induced diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia (Rodrigues et al., 1986). Insulin deficiency may be responsible for dyslipidemia, since insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles. In the present investigation, LDL levels were found to be significantly higher in STZ-induced type 1 diabetic rats as compared to non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) in STZ-induced type 1 diabetic rats reduced the LDL levels when compared with diabetic control rats.

The most commonly elevated plasma level in diabetes mellitus is that of triglycerides (Denton and Randle, 1967). Insulin deficiency is also associated with hypertriglyceridemia (Rodrigues et al., 1986). The mechanisms responsible for the development of hypertriglyceridemia in uncontrolled diabetes in human beings and possibly in insulin deficient STZ-diabetic rats are metabolic abnormalities that occur sequentially. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyzes triglycerides. Insulin deficiency results in failure to activate the enzymes, thereby causing hypertriglyceridemia (Chase and Glasgow, 1976). In the present investigation, triglyceride levels were found to be significantly higher in STZ-induced type 1 diabetic rats as compared to non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) in STZ-induced type 1 diabetic rats reduced the triglycerides levels when compared with that of STZ-induced diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) significantly reduced VLDL levels when compared with diabetic control rats. The positive relation between plasma triglycerides concentration and coronary events has been reported (Carlson and Botinger, 1972). Data from the Framingham study suggest that high plasma triglyceride is one of the risk factors for coronary heart disease in women (Kannel, 1987). In men also this is true particularly when hyperlipidaemia is associated with low levels of HDL-cholesterol concentration (Castelli, 1986). In the present study, STZ-induced type 1 diabetic rats showed...
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a significant decrease in HDL levels when compared with non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) in STZ-induced type 1 diabetic rats produced significant increase in HDL levels when compared with diabetic control rats.

Our results indicate that the induction of diabetes in rats with STZ elevated the circulating lipid levels. Serum cholesterol level and various fractions of lipoproteins such as LDL, VLDL and triglycerides were found to be increased, while HDL were found to be decreased when compared with non diabetic control rats which might lead to cardiovascular complications. Treatment with insulin brought down the levels of increased lipoproteins and total cholesterol. Administrations of MEL (500 mg/kg, p.o.) also decreased the serum cholesterol level and various fractions of lipoproteins such as LDL, VLDL and triglycerides and increased HDL levels in STZ-induced diabetic rats when compared with diabetic control and will lower the cardiovascular complications. The effect on lipid levels by MEL was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

STZ-diabetic rats have been shown to exhibit elevated plasma SGOT and SGPT levels without morphological changes in liver (Domingo et al., 1991; Cam et al., 1993; Dai et al., 1993). In the present study, significant elevation in SGOT and SGPT levels were observed in STZ induced type 1 diabetic rats as compared to non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) and insulin (5 IU/kg, i.p.) reduced these elevated SGOT and SGPT levels in STZ induced type 1 diabetic rats when compared with diabetic control rats thereby suggesting their beneficial effects on altered liver functions in conditions of diabetes.

Rise in serum creatinine, urea and blood urea nitrogen levels has been reported in patients with diabetes (Mulec et al., 1990). In the present investigation, we found significant elevation in serum creatinine and urea levels in STZ induced type 1 diabetic rat when compared with non diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) and insulin (5 IU/kg, i.p.) prevented rise in serum creatinine and urea levels in STZ induced type 1 diabetic rats when compared with diabetic control rats. Hence, it could be beneficial as it provides some protection against diabetic nephropathy.

In the present investigation, HFD control rats showed significant increase in serum glucose levels as compared to non diabetic control rats. HFD/STZ-induced type 2 diabetic rats showed significant increase in fasting serum glucose level as compared to HFD control rats. In HFD/STZ-induced type 2 diabetic rats following treatment for 3 weeks, MEL (100mg/kg, p.o.) failed to produce any significant change in fasting serum glucose level when compared with HFD/STZ-induced type 2 diabetic control rats. Treatment of HFD/STZ-induced type 2 diabetic rats with MEL (300, 500 mg/kg, p.o.) produced significant decrease in fasting serum glucose level when compared with HFD/STZ-induced diabetic control group after 3 weeks. This glucose lowering effect of MEL (300, 500 mg/kg, p.o.) was comparable to the effect produced by glipizide (10 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.).

In HFD/STZ-induced type 2 diabetic rats following treatment of 8 weeks, MEL (100mg/kg, p.o.) did not produce any significant change in fed serum glucose while significant decrease in fasting serum glucose level was found when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced type 2 diabetic rats with MEL (300, 500 mg/kg, p.o.) produced significant decrease in fed and fasting serum glucose levels as compared to HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Pioglitazone, like other thiazolidinedione based drugs might have reduced the serum glucose level by sensitizing the insulin action in the target tissues mainly through diminishing lipolysis in adipose tissues and subsequent reduction of glucose production in
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Liver and enhancement of insulin-mediated glucose disposal in skeletal muscle (Srinivasan et al., 2004).

In contrast to pioglitazone, the glucose-lowering effect of glipizide was however, associated with significant increase in plasma insulin level, thus suggesting its mechanism to take place through the stimulation of pancreatic beta cell secretion (Masiello et al., 1998). In HFD/STZ-induced type 2 diabetic rats following treatment for 3 weeks, glipizide (10 mg/kg, p.o.) was found to be effective in reducing fasting serum glucose level which was associated with significant increase in serum insulin level, thus suggesting its mechanism of action to take place through the stimulation of pancreatic beta cell secretion. In HFD/STZ-induced type 2 diabetic rats following treatment of 8 weeks, glipizide (10 mg/kg, p.o.) was found to be ineffective to alter fed and fasting glucose and insulin level. With the long term use, there is progressive decrease in the effectiveness of the sulfonylurea resulting from a gradual reduction in and exhaustion of insulin producing capacity of pancreatic beta cells. Though we did not study the extent of beta cell destruction in this model, the normal basal insulin and no elevation in serum insulin levels following the 8 week of administration of insulinotropic agent (glipizide) apparently revealed the presence of insufficient amount of functional beta cell population that was not further sensitive to the effect of insulinotropic agent resembling human type 2 diabetes which is in agreement with the earlier report on the neonatal-STZ diabetic rat where glipizide failed to alter significantly the circulating glucose and insulin levels in vivo. Further their beta cells are completely unresponsive to the insulin stimulatory effect of glipizide without significantly altering plasma total cholesterol levels in vitro (Weir et al., 1981; Shafrir, 1992; Schaffer et al., 1993). This property is distinctly different from that observed in diabetic rats where diabetes was produced by the combination of STZ and nicotinamide treatment (Masiello et al., 1998). The possible mechanism for decreased fasting serum glucose level by MEL might be, increased peripheral glucose utilization or stimulation of secretion of insulin at pancreatic level in response to increased glucose levels (Kar et al., 1999). As glipizide has failed to alter glucose and insulin level in this model, the possibility of MEL inducing insulin secretion to elicit the antihyperglycemic activity can be ruled out. Methanolic and hot water extracts of the leaves of plant L. speciosa have been shown to stimulate glucose uptake in 3T3-L1 cells (Liu et al., 2001). Corosolic acid isolated from methanolic extract of L. speciosa stimulates glucose uptake (Murakami et al., 1993).

Treatment with MEL (300, 500 mg/kg, p.o.) in the present study reduced fed serum glucose levels in HFD/STZ-induced type 2 diabetic rats when compared with HFD/STZ-induced type 2 diabetic control rats. Delaying or inhibiting glucose absorption at intestinal level or decrease in the endogenous glucose production in liver could be the underlying mechanism for resultant decrease in fed serum glucose level. In OGTT, HFD/STZ-induced type 2 diabetic rats showed significant increase in glucose levels when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic group with MEL (100, 300 and 500 mg/kg, p.o.) as well as pioglitazone (20 mg/kg, p.o.) produced significant decrease in serum glucose level when compared with HFD/STZ-induced diabetic control rats while glipizide (10 mg/kg, p.o.) did not produce any significant change in OGTT. The AUC$_{glucose}$ was found to be significantly higher in HFD/STZ-induced type 2 diabetic rats as compared to HFD control rats. Treatment of HFD/STZ-induced type 2 diabetic rats with MEL (100 mg/kg, p.o.) failed to lower while (300 and 500 mg/kg, p.o.) showed significantly lower AUC$_{glucose}$ as compared to HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced type 2 diabetic rats with glipizide (10 mg/kg, p.o.) and pioglitazone (20mg/kg, p.o.) group did not produce any change in AUC$_{glucose}$ when compared with HFD/STZ-induced diabetic control rats. This indicates that MEL might act through decrease
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Hexokinase is a key enzyme in the glycolytic pathway. It converts glucose into glucose 6 phosphate in the glycolysis process. As described earlier, insulin deficiency in chemical models of diabetes (STZ and alloxan induced diabetes) causes suppression of hexokinase activities (Grover et al., 2000; Raju et al., 2001; Rathi et al., 2002). In the present study, liver hexokinase activity was found to be decreased in HFD/STZ-induced type 2 diabetic control rats when compared with the HFD control rats. Administration of the MEL (500 mg/kg, p.o.) increased the activity of liver hexokinase enzyme in the HFD/STZ-induced type 2 diabetic animals when compared with HFD/STZ-induced diabetic control rats. This further indicates that the MEL can increase the utilization of glucose. The increased utilization of glucose upon treatment with MEL in HFD/STZ-induced type 2 diabetic rats could be one of the possible mechanisms for antihyperglycemic activity of MEL.

As already mentioned, PEPCK is a key enzyme involved in gluconeogenesis in liver (Barthel and Schemoll, 2003). Insulin directly inhibits the gluconeogenesis. Since there is a significant insulin resistance in the HFD/STZ-induced type 2 diabetic rats, the suppression of gluconeogenesis by insulin becomes too inadequate to control the gluconeogenesis. This could be the reason for the high levels of PEPCK in high-fat STZ diabetic rats. In the present study, the activity of gluconeogenic enzyme PEPCK was found to be increased in HFD/STZ-induced type 2 diabetic rats when compared with that of HFD control rats. Groups treated with MEL showed significant decrease in PEPCK activity when compared with HFD/STZ-induced type 2 diabetic control rats. This indicates that gluconeogenesis was suppressed. Since, gluconeogenesis significantly contributes to the endogenous hepatic glucose production, suppression of gluconeogenesis can contribute to the antihyperglycemic action of MEL. As MEL also increased the glucose utilization by increasing the enzyme hexokinase, both mechanisms can contribute towards antihyperglycemic action by MEL.

Control rats receiving HFD showed significant increase in serum insulin levels as compared to non diabetic control rats. HFD/STZ-induced type 2 diabetic rats showed significantly lower fed and fasting serum insulin levels when compared with HFD control rats. Following 3 weeks treatment with MEL (100, 300, 500 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.), HFD/STZ-induced type 2 diabetic rats did not produce significant change in fasting serum insulin levels when compared with HFD/STZ-induced diabetic control group while glipizide (10mg/kg, p.o.) produced significant increase in serum insulin levels. After 8 weeks, HFD showed significant increase in fed and fasting serum insulin levels when compared with non diabetic control rats. HFD/STZ-induced type 2 diabetic rats showed significantly higher fed and fasting serum insulin levels as compared to HFD control rats. Following 8 weeks treatment with MEL 300, 500 mg/kg p.o., significantly reduced fed and fasting serum insulin levels while, 100 mg/kg, p.o. dose failed to produce this effect in HFD/STZ-induced type 2 diabetic rats when compared with HFD/STZ-induced diabetic control rats. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Non diabetic rats treated with MEL (500mg/kg, p.o.) did not produce any significant change in insulin levels when compared with non diabetic control rats. HFD rats showed significant increase in AUC\textsubscript{insulin} when compared with non diabetic control rats. Treatment of HFD/STZ-induced type 2 diabetic rats with MEL (300, 500 mg/kg, p.o.) produced significant decrease in AUC\textsubscript{insulin} when compared with HFD/STZ-induced diabetic control group. The above results indicate that the glucose lowering effects of MEL was like pioglitazone and not similar to glipizide as MEL did not reduce glucose level by release of insulin from beta cells of pancreas but, reduced insulin resistance. This was further confirmed by KITT. Serum insulin sensitivity can be estimated using insulin tolerance test
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This test measures insulin sensitivity using KITT as an index of insulin mediated glucose metabolism. ITT, which represents the response of exogenously administered insulin on blood glucose, has been used to estimate insulin sensitivity (Alford et al., 1971). In our study, HFD control rats showed significantly lower insulin sensitivity and higher T1/2 as compared to rats fed on normal pallet diet. HFD/STZ-induced type 2 diabetic rats showed significant decrease in insulin sensitivity and increase in T1/2 when compared with HFD control rats. Treatment with pioglitazone (20mg/kg, p.o.) in HFD/STZ-induced type 2 diabetic rats produced significant decrease in serum insulin and T1/2 when compared with HFD/STZ-induced diabetic control group. Glipizide (10mg/kg, p.o.) was not found to be effective on serum insulin and T1/2. Administration of MEL (500mg/kg, p.o.), in HFD/STZ-induced type 2 diabetic rats significantly increased insulin sensitivity by decreasing serum insulin level and increased glucose disposal rate by decreasing the T1/2 when compared with HFD/STZ-induced diabetic control group.

Levels of plasma triglycerides and cholesterol in individuals with various types of diabetes are higher than that of normal subjects (Chase and Glasgow, 1976). However, the levels of lipids depend on the severity of diabetes, its therapeutic control and the composition of diet. It has been known that dietary ingredients affecting glucose metabolism may also influence the lipid metabolism (Jenkins and Josse, 1995; Jaysooriya et al., 2000). Lipid profile, which is altered in the serum of diabetic patients (Betteridge, 1994), appears to be significant factor in the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol levels.

In the present study, a part from the HFD control rats, insulin-resistant STZ animals also showed abnormalities in lipid metabolism as evidenced from increased plasma triglycerides and plasma total cholesterol levels, as in case of human type 2 diabetic patients. This might contribute to various cardiovascular complications. Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD in the diabetic condition (Shafrir, 2003) and (Colea et al., 1991). The hypertriglyceridermia observed by us in the HFD rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of triglyceride-enriched hepatic VLDL and decreased triglyceride uptake in peripheral tissues (Srinivasan et al., 2004). The positive relation between plasma triglycerides concentration and coronary events has been reported (Carlson and Botinger, 1972).

Data from the Framingham study have suggested that high plasma triglyceride is one of the risk factors for coronary heart disease in women (Kannel, 1987) and in men, particularly when hyperlipidaemia is associated with low levels of HDL-cholesterol concentration (Castelli, 1986). In the present study, there was significant increase in serum cholesterol, triglyceride, VLDL, LDL levels and atherogenic index while decrease in HDL levels in HFD/STZ-induced diabetic control rats when compared with HFD control group. Treatment with MEL (500 mg/kg, p.o.) in HFD/STZ-induced diabetic rats produced significant decrease in serum cholesterol, triglyceride, VLDL, LDL levels and atherogenic index while increase in HDL levels when compared with HFD/STZ-induced diabetic control rats. This showed further decrease the risk of cardiovascular complications associated with diabetes. Treatment with pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic rats produced significant decrease in cholesterol, triglyceride, VLDL and LDL levels while increase in HDL levels when compared with HFD/STZ-induced diabetic control rats. This may further decrease the risk of cardiovascular complications associated with diabetes while glipizide (10 mg/kg, p.o.) was found to be ineffective on lipid levels. Further, the attenuating effect of pioglitazone on hyperlipidemia might result either from the inhibition of triglyceride
synthesis in liver or from increased triglyceride clearance in the periphery by stimulating the enzyme lipoprotein lipase (LPL) and/or inhibition of dietary cholesterol absorption from the intestine (Colca et al., 1991; Srinivasan et al., 2004). These actions are known to be mediated largely by interaction with nuclear peroxisome proliferated activated (PPAR-γ) receptors in target tissues (Spiegelman, 1998).

Lactate dehydrogenase (LDH) and creatine kinase (CK) are cardiac enzymes. HFD/STZ-induced diabetic rats showed significant increase in serum LDH and CK levels when compared with HFD control rats. MEL (500 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) exhibited significant reduction in the serum LDH and CK levels in the HFD/STZ-induced diabetic rats when compared with HFD/STZ-induced diabetic control rats. In severely hypertrophied myocardium, capillary density is reduced, the diffusion for oxygen, nutrients and metabolites are increased and the ratio of energy-production sites to energy-consumption sites is decreased. The metabolic state of severely hypertrophied myocardium is anaerobic, as indicated by the shift of lactate dehydrogenase and creatine kinase marker enzymes (Zhu et al., 1996). Hence, MEL (500 mg/kg, p.o.) might be beneficial in providing protection against failing and infarcted myocardium with severe hypertrophied heart HFD/STZ-induced type 2 diabetic rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not produce any significant change in serum LDH and CK levels as compared to HFD/STZ-induced diabetic control rats.

Collagen in the normal adult heart serves several important functions, which include providing a supportive structural lattice for cardiomyocytes and coronary vessels and connecting individual myocytes and myofibrillar bundles to integrate individual cardiac contractions. However, a disproportionate increase in collagen accretion or collagen resorption from normal levels can cause defect in the function and supporting structural lattice of the heart. It has been widely known that diabetes is associated with alteration in extracellular matrix (ECM) turnover and regulation (Williamson and Kilo, 1976). Pathologic remodeling characterized by ECM deposition might contribute to cardiovascular complications that are the leading cause of morbidity and mortality in diabetic patients (Kannel and McGee, 1979). Alteration in diastolic filling of the left ventricle (LV) associated with reciprocal changes in the LV collagen gene and accumulation of cardiac collagen in diabetic rats (Kitayama et al., 1994; Mizushige et al., 2000) suggest that increased interstitial cardiac collagen might cause cardiac fibrosis and result in greater LV stiffness and decreased LV wall compliance, thus leading to diastolic dysfunction and eventually heart failure in diabetes (Akdemir et al., 2001; van Heerebeek et al., 2009). In present study, we observed significant increase in cardiac collagen deposition in diabetic rats and MEL (500 mg/kg, p.o.) significantly reduced elevated left ventricular collagen level in the HFD/STZ-induced diabetic rats when compared with HFD/STZ-induced diabetic control rats. So probably the decrease in collagen level may help in reducing progression of LV fibrosis, LV stiffness, LV diastolic dysfunction, eventually causing improvement in the failing heart.

Experimental studies with animal models of diabetes have demonstrated impaired heart contractility and relaxation (Riva et al., 1998). In the present study, heart of HFD/STZ-induced diabetic rats has been found to produce an increase in the force of contraction and decrease in heart rate when compared with heart of HFD control rats which was significantly prevented by MEL (500 mg/kg, p.o.). MEL showed improved contractility, heart rate, relaxation of heart and thus ultimately reduction in the force of contraction.

The increased sodium retention is suggested to play an important role in development of hypertension in diabetic patients (Feldt-Rasmussen et al., 1987). The Blood pressure of the HFD control rats was found to be significantly higher as compared to non diabetic control rats. HFD/STZ-induced type 2 diabetic rats showed significant increase in
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Blood pressure when compared with HFD/STZ-induced diabetic control rats. Treatment of MEL (500 mg/kg, p.o.) to HFD/STZ-induced type 2 diabetic rats produced significant decrease in blood pressure when compared with HFD/STZ-induced diabetic control group. This effect was comparable to that of pioglitazone (20 mg/kg, p.o.) and not to that of glipizide (10 mg/kg, p.o.) on HFD/STZ-induced diabetic group.

The development of left ventricular hypertrophy (LVH) constitutes a considerable risk for the morbidity and mortality in hypertension (Kannel and Gorden, 1969; Kannel et al., 1974; Messerli et al., 1983; Carr et al., 1985; Frohlich, 1987). Thickening of the ventricular wall leads to a fall in left ventricular compliance (Smith et al., 1987). Moreover, coronary reserve becomes impaired with the progression of LVH (Wangler et al., 1982; Polese et al., 1991). Also LVH commonly leads to ventricular ectopy. Ventricular ectopy could well explain the increased prevalence of sudden death observed in patient with LVH. Long standing LVH leads to impairment of contractile function (Malik et al., 1974). In the absence of hypertension and atherosclerosis, diabetic patients demonstrate left ventricular dysfunction with lower cardiac output, lower stroke volumes, and prolonged pre-ejection period and shortened left ventricular ejection time (Zarich and Nesto, 1989). But some reports also say that LVH does not usually occur in diabetes unless hypertension is present. Metabolic alterations and microvascular diseases are likely to contribute to these changes. However, in the presence of diabetes, damage to the myocardium from hypertension appears to be accelerated. In the present study, The wet heart weight to body weight ratio, as an index of cardiac hypertrophy and LV weight to heart weight ratio, as an index of LV hypertrophy was found to be increased in HFD/STZ-induced type 2 diabetic rat’s hearts when compared with HFD control rat’s hearts. MEL (500 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) treatment significantly reduced the wet heart weight to body weight ratio, an index of cardiac hypertrophy and LV weight to heart weight ratio as an index of LV hypertrophy in HFD/STZ-induced type 2 diabetic rats when compared with HFD/STZ-induced diabetic control rats while glipizide did not produce any significant change. Thus, MEL appears to delay the progression of the congestive heart failure in diabetic condition possibly by preventing development of hypertrophy and cardiomyopathy.

In conclusion, our data suggest beneficial effects of MEL (500 mg/kg, p.o.) on cardiovascular complications in diabetic animals. This may be due to reduction in cholesterol, triglyceride, LDL, VLDL, atherogenic index, LDH, CK, blood pressure and collagen level in left ventricular muscle, increase in HDL levels, heart rate and prevention of increase in force of contraction in diabetic hearts and decrease in LV hypertrophy and cardiac hypertrophy.

Induction of diabetes with STZ is associated with characteristic loss of body weight which is due to increased muscle wasting due to unavailability of carbohydrate for utilization as energy source (Swanston-Flatt et al., 1990). In the present study, there was a gradual increase in body weight in non diabetic controls, while the diabetic controls continued to lose weight. Prince et al. (1998) and Yadav et al. (2002) have also reported similar findings. Treatment with MEL (500 mg/kg, p.o.) decreased the reduction in the body weight in HFD/STZ-induced diabetic rats when compared with HFD/STZ-induced diabetic control rats. The treatment altered the body weight of diabetic animals towards normalcy. This may be due to the protective effect of MEL in controlling muscle wasting i.e. reversal of gluconeogenesis, which is also evident by decreased levels of the enzyme PEPCK in the rats of MEL-treated groups. The standard reference treatment with pioglitazone (20 mg/kg, p.o.) also decreased the weight loss of diabetic animals and altered the weight towards normalcy during the course of treatment. Treatment with MEL (500 mg/kg, p.o.) and pioglitazone (20
Discussion

mg/kg, p.o.) reduced food intake and water intake in HFD/STZ-induced type 2 diabetic rats when compared with HFD/STZ-induced diabetic control rats.

It has been reported that alteration in sodium homeostasis leads to increased sodium retention in diabetes (DeChatel et al., 1977; O'Hare et al., 1986). In diabetes with ketoacidosis, hyperkalemia in the face of potassium depletion is attributable to reduced renal function, acidosis, and release of potassium from cells due to glycolysis and lack of insulin (Urbarri et al., 1990). In our study, HFD control rats exhibited significant increase in serum sodium and potassium levels while significant decrease in urine sodium and potassium levels when compared with non diabetic control rats. HFD/STZ-induced diabetes produced significant increase in serum sodium and potassium levels and significant decrease in urine sodium and potassium when compared with HFD control rats. Administration of MEL (500 mg/kg, p.o.) to HFD/STZ-induced type 2 diabetic rats produced significant lowering of serum sodium and potassium levels while rise in urine sodium and potassium levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Glipizide (10 mg/kg, p.o.) was not found to be effective on serum and urine levels of sodium and potassium in HFD/STZ-induced diabetic rats.

The onset of microalbuminuria is highly significant since its presence predicts the development of overt renal disease in both type 1 and type 2 diabetes (Viberti et al., 1982; Mogensen, 1984). Furthermore, microalbuminuria is associated with an increased risk of cardiovascular and microvascular complications as well as increase in mortality, especially in type 2 diabetes. Renal histology at this stage reveals typical glomerulosclerosis. Once microalbuminuria is established, proteinuria increases until overt nephropathy develops (Dinneen and Gerstein, 1997). Increased urinary excretions of protein and/or albumin or both are early clinical markers for diabetic renal disease and an increased risk for cardiovascular disease (Simon et al., 2004). Stage III is when diabetic nephropathy is first noticeable. As albumin levels increase in the urine, levels in the blood are lowered, resulting in noticeable edema (Evans and Forsyth, 2004). In addition, levels of creatinine and blood urea nitrogen (BUN) increase (Evans and Forsyth, 2002). The accumulation of these waste products in the blood is called azotemia. Early detection at this stage is vital to preserve kidney function and to delay or prevent ESRD (Foote, 1995). According to the National Institute of Diabetes and Digestive and Kidney Diseases, individuals with type 2 diabetes may remain in this stage for several years (Evans and Forsyth, 2002). In the present study, HFD control rats exhibited significant increase in serum urea and creatinine, while significant decrease in serum albumin and total protein levels when compared with non diabetic control rats. HFD control rats exhibited significant decrease in urine creatinine and creatinine clearance while increase in urine albumin and total protein levels when compared with non diabetic control rats. HFD/STZ-induced diabetic rats showed significant increase in serum urea and creatinine levels while decrease in serum albumin and total protein levels when compared with HFD control rats. HFD/STZ-induced type 2 diabetic rats produced significant decrease in urine creatinine and creatinine clearance while increase in urine albumin and total protein levels when compared with HFD control rats. Administration of MEL (500 mg/kg, p.o.) to HFD/STZ diabetic rats produced significant decrease in urea and creatinine levels while increase in serum albumin and total protein levels when compared with HFD/STZ-induced diabetic control group. Administration of MEL (500 mg/kg, p.o.) to HFD/STZ-induced type 2 diabetic rats produced significant increase in urine creatinine and creatinine clearance while decrease in urine albumin and total protein levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to that of pioglitazone (20 mg/kg, p.o.). Glipizide (10 mg/kg, p.o.) had no effect on serum urea, creatinine, albumin, total protein,
Discussion

Progressive increase in kidney weight and glomerular volume in moderately hyperglycemic STZ diabetic rats during a period of six months of diabetes has been reported (Rasch, 1979). In the present study, HFD control rats exhibited significant increase in kidney to body weight ratio when compared with non-diabetic control rats. HFD/STZ-induced diabetes produced significant increase in kidney to body weight ratio when compared with HFD control rats. Administration of MEL (500 mg/kg, p.o.) to HFD/STZ-induced diabetic rats produced significant decrease in kidney to body weight ratio when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.). There was no effect of Glipizide (10 mg/kg, p.o.) on kidney weight to body weight ratio in HFD/STZ-induced diabetic rats.

In severely hyperglycemic rats without insulin treatment, the glomerular volume is reported to be continuously increased during the first eight months of diabetes (Hirose et al., 1982). Intraglomerular hypertension is consequent upon hyperfiltration that leads to mesangial expansion and basement membrane thickening. These features can be the hallmarks of diabetic glomerulosclerosis. Diabetic nephropathy in humans presents several structural changes that are characterized by early hypertrophy of both glomerular and tubulointerstitial elements, thickening of the glomerular basement membrane, progressive accumulation of extracellular matrix components in the glomerular mesangium, and less well recognized lesions such as tubulointerstitial fibrosis and renal arteriosclerosis (Mauer et al., 1984; Osterby et al., 1988; Ziyadeh et al., 1989; Steffs et al., 1992). The histopathology of kidney of HFD/STZ-induced diabetic rats showed thickening of glomeruli and focal sclerosis while HFD/STZ-induced diabetic rats treated with PFM (300 mg/kg, p.o.) showed normal structure—both outer cortex and inner medulla while Cortex show glomerulus, DCT and PCT with interstitium and associated blood vessels same as in case of non diabetic control. Our data suggest beneficial effects of MEL (500 mg/kg, p.o.) on diabetic renal complications in diabetic animals.

In nutshell study with MEL in type 1 and type 2 diabetic rats is beneficial in treatment of diabetes and associated complications. Further fractionation of MEL was done to have a more active fraction.

In present investigation of type 1 study, treatment with MEL 100 mg/kg, p.o., 300 mg/kg, p.o., 500 mg/kg, p.o. and insulin (5 IU/kg, i.p.) produced 45.87, 53.30, 60.92 and 60.0% decrease in fasting glucose level; 46.01, 58.95, 62.84 and 65.15 % decrease in fed serum glucose level, 29.50, 32.78, 36.59 and 48.80% decrease in AUC\textsubscript{glucose} respectively.

Treatment with PFM 100 mg/kg, p.o., 300 mg/kg, p.o., 500 mg/kg, p.o. and insulin (5 IU/kg, i.p.) produced 53.30, 59.16, 61.39 and 60.0 % decrease in fasting glucose level; 61.72, 64.16, 66.35 and 65.15 % decrease in fed serum glucose level, 38.71, 42.40, 44.76 and 48.80% decrease in AUC\textsubscript{glucose} respectively.

Treatment with TFM 100 mg/kg, p.o., 300 mg/kg, p.o., 500 mg/kg, p.o. and insulin (5 IU/kg, i.p.) produced 2.41, 10.58, 58.03 and 60.0% decrease in fasting glucose level; 2.68, 19.28, 43.27 and 65.15 % decrease in fed serum glucose level, 24.70, 27.84, 34.61 and 48.80% decrease in AUC\textsubscript{glucose} respectively.

In our model of type 2 study, treatment with MEL 100 mg/kg, p.o., 300 mg/kg, p.o. and 500 mg/kg, p.o. produced 21.8, 32.93 and 44.04% decrease in fasting glucose level; 25.55, 36.78, and 50.5% decrease in fed serum glucose level, while 9.35, 14.97 and 31.81% decrease in fasting serum insulin levels, 13.1, 18.9 and 37.69% decrease in fed serum insulin levels, 17.28, 31.57 and 40.81% decrease in AUC\textsubscript{glucose} and 8.53, 13.67, 29.10% decrease in AUC\textsubscript{insulin} levels respectively. As MEL 500 mg/kg, p.o. was found to be most effective in
producing antihyperglycemic effect it was used further to establish its role in diabetic complications. Treatment with pioglitazone (20mg/kg, p.o.) produced 41.77% decrease in fasting glucose level, 52.10% decrease in fed serum glucose level, while 27.54% decrease in fasting serum insulin levels, 23.05% decrease in fed serum insulin levels, 27.06% decrease in AUC_glucose and 25.18% decrease in AUC_insulin levels. Treatment with glipizide (10mg/kg, p.o.) produced 15.61% decrease in fasting glucose level, 16.21% decrease in fed serum glucose level, while 7.75% decrease in fasting serum insulin levels, 8.98% decrease in fed serum insulin levels, 7.91% decrease in AUC_glucose and 7.06% decrease in AUC_insulin levels respectively.

Treatment with PFML 100 mg/kg, p.o., 300 mg/kg, p.o. and 500mg/kg, p.o. produced 35.6, 51.01 and 52.37% decrease in fasting glucose level 40.34, 54.16, and 55.46% decrease in fed serum glucose level, while 16.84, 30.74 and 32.89% decrease in fasting serum insulin levels, 18.17, 36.72, 38.29% decrease in fed serum insulin levels, 36.19, 49.08 and 50.62% decrease in AUC_glucose and 15.39, 28.12, 30.08% increase in AUC_insulin levels respectively. As PFML 300 mg/kg, p.o. was found to be most effective in producing antihyperglycemic effect, it was used further to establish its role in diabetic complications.

Treatment with TFML 100 mg/kg, p.o., 300 mg/kg, p.o. and 500mg/kg, p.o. produced 0.11, 19.35 and 30.08% decrease in fasting glucose level, 13.53, 18.63, and 36.84% decrease in fed serum glucose level, while 6.42, 8.29 and 14.97% decrease in fasting serum insulin levels, 10.55, 12.89, 20.12% decrease in fed serum insulin levels, -6.02, 0.38 and 0.15% decrease in AUC_glucose and 5.84, 7.55, 13.67% increase in AUC_insulin levels respectively. As TFML 500 mg/kg, p.o. was found to be most effective in producing antihyperglycemic effect, it was used further to establish its role in diabetic complications.

When we compared effectiveness of PFML with that of MEL and TFML on cardinal signs, serum glucose, serum insulin, AUC_glucose, AUC_insulin, liver hexokinase and PEPCK, OGTT, KITT and T1/2, 300 mg/kg, p.o. PFML was found to produce comparable effects as compared to MEL 500 mg/kg, p.o. and TFML 500 mg/kg, p.o. At the same time when we compared effectiveness of PFML with MEL and TFML on serum cholesterol, triglycerides, LDL, VLDL, HDL, CK, LDH, collagen, atherogenic index, FOC, HR, blood pressure, LVHI and CHI, PFML was found to produce comparable effect in lower dose i.e. 300mg/kg as compared to MEL 500 mg/kg, p.o. and TFML 500 mg/kg, p.o. When we compared effectiveness of PFML with MEL and TFML on serum GPT, GOT, urea, serum and urine creatinine, albumin, total protein, sodium, potassium, kidney weight to body weight ratio, creatinine clearance, PFML was found to produce effect in lower dose i.e. 300mg/kg as compared to MEL 500 mg/kg, p.o. and TFML 500 mg/kg, p.o.

When we compared effectiveness of PFML with MEL and TFML in diabetes and diabetic complications PFML was found to produce comparable effect in lower dose 300mg/kg with MEL 500 mg/kg, p.o. and TFML 500 mg/kg, p.o.

All these results indicate that PFML, MEL and TFML extracts does not induce any stimulation of pancreatic β cells as their effects were comparable to pioglitazone and not to glipizide. Thus the possibility of their inducing insulin secretion to elicit antihyperglycemic activity can be ruled out. They might be acting through increasing insulin sensitivity. Other possible mechanisms for the antihyperglycemic activity of these extracts might be through delaying or inhibiting glucose absorption at intestinal level. The mechanisms may also involve inhibition of liver PEPCK and/or stimulation of liver hexokinase leading to lowering of blood glucose levels. Petroleum ether fraction of MEL (PFML) was found to be more beneficial not only in diabetes but also in diabetic complications like diabetic cardiomyopathy and diabetic nephropathy and diabetes related liver disorders as compared to 50% methanolic extract of Lagerstroemia speciosa leaves (MEL) and solvent ether insoluble
fraction or tannin fraction of MEL (TFML). Corosolic acid found to be present in the PFML may be responsible for this beneficial effect in diabetes mellitus and associated complications.