Numerous efforts have been made to identify the major culprit involved in the pathogenesis of this disease and these findings argue that numerous pathways such as hemodynamic pathway involving the renin-angiotensin-aldosterone and urotensin systems, profibrotic and inflammatory cytokines including TGF-β and TNF-α, various kinases such as PKC and Janus kinase pathway and most importantly oxidative stress mediator like NADPH oxidase are activated during the course of this disease. But, the clinical strategies based on these pathways for the management of diabetic nephropathy remains unsatisfactory, as the numbers of diabetic patients with nephropathy are increasing year-by-year. The present study has been aimed to explore the possible therapeutic strategy to prevent diabetic nephropathy. Our study showed the renoprotective effects of combination of fenofibrate and saxagliptin in diabetes-induced experimental nephropathy. Creatinine, a non-protein waste product is freely filtered by the kidney and as the serum creatinine level depends on the glomerular filtration rate (GFR), thereby is considered to be an index of renal dysfunction (Finco and Duncan, 1976; Perrone et al., 1992; Reder and Hartmann, 1994). Another waste product of protein metabolism namely urea is cleared from the bloodstream by the kidney and it has been considered as an important bio-marker for dysfunction of the kidney (Finco and Duncan, 1976; Lyman, 1986). Increased cortical interstitial fibrous tissue is correlated with increased mesangial enlargement. Since mesangial expansion was related to albuminuria, GFR, and hypertension, it follows that the index of interstitial fibrosis predicted these clinical manifestations of diabetic nephropathy (Remuzzi et al., 1992; Wolf and Ziyadeh, 2007). Altogether, elevated levels of serum creatinine, blood urea nitrogen, and proteinuria, and pathological changes in glomeruli have been recognized as an index of experimental diabetic nephropathy. In
the present study, the serum creatinine, blood urea nitrogen, and proteinuria were noted to be increased in diabetic rats in 7 weeks as compared to normal rats. In addition, the diabetic rats showed marked renal pathological changes after 7 weeks. These results clearly signify the development of diabetes-induced nephropathy. Treatment with either fenofibrate or saxagliptin prevented the diabetes-induced nephropathy to a degree by decreasing serum creatinine, blood urea nitrogen and proteinuria and the same effect was observed on renal pathological changes. On the other hand, concurrent administration of fenofibrate and saxagliptin noticeably prevented the development of diabetes-induced nephropathy as compared to treatment with either drug alone.

Diabetic kidneys specifically expressed several genes normally found in adipocytes, including adipose differentiation-related protein (ADRP, or adipophilin in humans), suggesting a switch of kidney phenotype in favour of lipid accumulation in diabetes (Varghese et al., 2006). In addition, abnormal high concentration of serum lipids is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production and this altered lipid profile during the diabetic state has been noted to be associated with the increased expression of TGF-β1, fibronectin, collagen-IV, MAPKs and NF-κB which ultimately accounts for glomerulosclerosis and tubulointerstitial fibrosis (Figarola et al., 2008). Therefore, Lipid-mediated injury plays an important role in the pathogenesis of many renal diseases, including diabetic nephropathy. This contention is supported by the results obtained in the present study that marked increases in serum cholesterol and triglycerides and consequent decrease in serum HDL levels were noted in diabetic rats with nephropathy. Decreased
expression of PPAR-α has been noted to be associated with the progression of diabetic nephropathy through an increase in circulating lipids, induction of inflammation and extracellular matrix formation in diabetic mice (Park et al., 2006). Fenofibrate, one of the most widely used PPAR-α, along with its well established actions on lipids and multiple mechanisms have been postulated regarding the direct renoprotection by decreasing the renal COX-2 expression and reducing the nitrosative stress in the kidney of diabetic rats with early stages of nephropathy (Chen and Quilley, 2008). TGF-β plays an important role in mediating the hypertrophic and fibrotic/sclerotic manifestation diabetic nephropathy. Numerous in-vitro as well as in-vivo investigations have shown that renal TGF-β production is increased during the development of diabetic kidney disease by increasing the expression of CTGF, VEGF, collagen I, collagen IV, and fibronectin that results in disassembly and hypertrophy of mesangial cells (Border and Noble, 1998; Hoffman et al., 1998; Goldfarb and Ziyadeh, 2001; Jeong et al., 2004), thereby implicated in the pathogenesis of diabetic nephropathy. In addition, TGF-β1 upregulates the expression of p22phox, p47phox, p67phox, and gp91phox in rat mesangial cells and p22phox mRNA in tubular epithelial cells suggesting that TGF-β1-induced ROS may be NADPH oxidase dependent (Lee et al., 2003). It is worth-mentioning that activation of PPAR-α by fenofibrate has been reported to produce renoprotective effect by downregulating the renal expression of TGF-β (Li et al., 2010). These studies explicate the possible mechanisms involved in fenofibrate-mediated renoprotective effects noted in the present study.
Hyperglycemia is an important contributor for the cardiovascular diseases risk. The animal studies revealed that hyperglycemia produces glycation and peroxidation of proteins which cause damage to the vascular walls (Aronson and Rayfield, 2002). Uncontrolled hyperglycemia plays an important role in the induction and progression of nephropathy by upregulating the renal expression of Ang-II, PKC, TGF-β and other intermediary growth factors (Kanwar et al., 2011; Arora et al., 2013). Moreover, high glucose-induced ROS generation through an activation of NADPH oxidase play a crucial role in induction and progression of diabetic nephropathy (Arora et al., 2013). GLP-1 receptor agonists (incretin mimetics) and inhibitors of DPP-4 activity (incretin enhancers) have been found to be promising in lowering the glucose level in the experimental as well as clinical studies. Interestingly, GLP-1 expression has been found in the kidney of rats and the rGLP-1 found to inhibit the Na\(^+\) reabsorption in the proximal tubule and increases glomerular filtration rate in kidneys (Dunphy et al., 1998; Moreno et al., 2002). In addition, GLP-1 has been noted to improve endothelial dysfunction in type 2 diabetic patients with coronary heart disease (Nystrom et al., 2004). Moreover, treatment with exendin-4 (GLP-1 receptor agonists) was found to show the significant reduction in glomerular hypertrophy, mesangial matrix expansion, TGF-β1 expression, and type IV collagen accumulation and associated glomerular lipid accumulation in db/db mice (Park et al., 2007). These studies support the direct renoprotective effect of saxgliptine observed in the present study.

The major structural markers of diabetic nephropathy, namely renal enlargement; mesangial cell expansion; tubular injury and glomerular basement membrane (GBM) thickening were noted in kidney of untreated diabetic rats. Renal enlargement is one of the key features occurring during initial changes of diabetes.
Marked increase in the kidney/body weight ratio was observed in diabetic rats as compare to normal rats, treatment with concurrent administration of fenofibrate and saxagliptin markedly preserve the alteration in kidney/body weight ratio. In addition, mesangial expansion is considered as an initial morphological change during diabetic nephropathy which may be due to mesangial cell proliferation and excessive production of mesangial matrix components and a mild increase in mesangial cellularity (Floege et al., 1992; Floege et al., 1993). Studies analysing structural-functional relationships have demonstrated that the development of proteinuria correlates with the degree of mesangial expansion (Mauer et al., 1984; White and Bilous, 2000). In the present study, treatment with combination of fenofibrate and saxagliptin showed a marked reduction in mesangial expansion in kidney of diabetic rats. From various experimental studies it has been noted that TGF-β plays an important role in mediating the hypertrophic and fibrotic/sclerotic manifestations of diabetic nephropathy by affecting extracellular matrix (ECM) metabolism that leads to excessive production of ECM, resulting in glomerular fibrosis, and ultimately loss of renal function (Nishibayashi et al., 2010). Fenofibrate is known to possess renoprotective potential by downregulating the renal expression of TGF-β (Li et al., 2010) and exendin-4 (GLP-1 receptor agonists) reported to have significant effect on mesangial matrix expansion by reducing the TGF-β1 expression in db/db mice (Park et al., 2007), suggesting the possible underlying mechanism involved in mesangial expansion reduction by concurrent administration of fenofibrate and saxagliptin in the present study. The GBM plays a crucial role in both structural support and functional operation of the glomerulus and it forms the boundary between blood and urine. The GBM is built of a meshwork of fused basal lamina mainly composed of laminin and collagen IV. An increased synthesis of type IV collagen may lead to increased
permeability of the GBM and permanently unbalanced synthesis of BM components finally results in destruction of the capillary lumen (Tervaert et al., 2010; McCarthy and Wassenhove-McCarthy, 2012). However, in late state nephropathy intrinsic basement membrane components are no longer produced. Instead, massive accumulation of PAS positive material occurs (Schleicher et al., 1988). In present study, Light microscopy findings showed slight increase in the solid areas of the tuft, most frequently observed as PAS positive material in diabetic rats and concurrent treatment with fenofibrate and saxagliptin markedly reduce the PAS positive material in kidney of diabetic.

Accumulating research suggests that oxidative stress is a significant contributor to the pathogenesis of diabetic nephropathy. Lipid peroxidation is initiated by free radicals attack of membrane lipids, generating large amounts of reactive products, which have been implicated in pathogenesis of diabetic complications including diabetic nephropathy nephropathy. Thereby, increase in renal TBARS and decrease in GSH levels are considered to be an index of development of oxidative stress (Kakkar et al., 1997). In the present study, diabetes has been noted to increase the renal TBARS and decrease the GSH levels in the kidney. Thus, it may be suggested that diabetes-induced development of oxidative stress may develop nephropathy by damaging renal architecture. In the present study, the administration of either fenofibrate or saxagliptin partially reduced the development of oxidative stress in diabetic rats with nephropathy by reducing renal TBARS and concomitantly elevating renal GSH levels. However, their combination markedly prevented the development of renal oxidative stress in diabetic rats with nephropathy. Thus, it may be suggested that the direct renoprotective potential of combination of fenofibrate and
saxagliptin in preventing diabetic nephropathy may be due to their anti-oxidant properties on diabetic kidney. Taken together, the overall observed beneficial effect of combination of fenofibrate and saxagliptin in preventing the development of diabetic nephropathy may be attributed to their direct renoprotective action, improving altered lipid profile and reversing renal oxidative stress. The renoprotective effect of lisinopril has been well reported in basic and clinical studies (Amann et al., 2003; Benigni et al., 2003). Therefore, lisinopril has been employed as a standard drug in the present study. The renoprotective effect of combination of fenofibrate and saxagliptin observed in present study was slightly better than lisinopril treatment in ameliorating diabetes-induced nephropathy.

On the basis of above discussion, it may be concluded that the concurrent administration of fenofibrate and saxagliptin may have prevented the development of diabetes-induced nephropathy by reducing the altered lipid profile, decreasing the renal oxidative stress, and providing the direct nephroprotective action. In addition, their combination strategy may provide synergistic renoprotective effect against diabetic nephropathy. Therefore, long-term clinical studies demonstrating the rationale of combination of fenofibrate and saxagliptin in preventing diabetic nephropathy are mandatory.