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Original Article

# MORPHOLOGICAL EFFECT OF COMBINATION OF FENOFIBRATE AND SAXAGLIPTIN ON KIDNEY OF DIABETIC RATS

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**ABSTRACT**

**Objective:** Diabetes mellitus associated structural alterations are considered as early adaptive shifts and are associated with progression of nephropathy. Structural changes may already be present once functional markers such as microalbuminuria occur. The correlation between structural and functional consequences coerce for more insightful on structural marker for evaluating any pharmacological agent for its renoprotective potential. Thereby, the present study has been designed to investigate the effect of combination of PPAR agonist (fenofibrate) and DPP-4 inhibitor (saxagliptin) on detailed pathological changes for their direct renoprotective effect in the kidney of diabetic rats.

**Methods:** After 8 weeks of streptozotocin administration the kidneys were excised and sections of 3-5 µm in thickness were made and stained with H&E and PAS using standard histologic procedures to assess mesangial cell expansion; tubular injury and glomerular basement membrane thickening using light microscopy. In addition, kidney/body weight ratio was assessed.

**Results:** The increased kidney/body weight ratio, glomerular basement membrane thickening, tubular injury, mesangial matrix expansion, interstitial inflammation and tubular atrophy were observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Concurrent administration of fenofibrate (30 mg/kg *p.o.,* 7 weeks) and saxagliptin (3 mg/kg *p.o.,* 7 weeks) markedly prevent the interstitial inflammation and tubular atrophy in the diabetic kidney as compared to treatments with lisinopril (1 mg/kg, *p.o.,* 7 weeks) in diabetic rats.

**Conclusion:** The present study provides the evidence for direct nephroprotective effect of combination of fenofibrate and saxagliptin. However, long-term clinical studies demonstrating their rationale in preventing diabetic nephropathy are mandatory.

**Keywords:** Diabetes, DPP-4 inhibitor, Glomerular basement membrane, mesangial expansion, PPAR- α agonist.

## INTRODUCTION

Diabetes mellitus, a metabolic syndrome is known to trigger various complications including retinopathy, neuropathy, nephropathy etc. Diabetic nephropathy is the leading cause of end stage renal disease and becoming a serious social and health problem worldwide. In clinical practice, functional parameters such as albuminuria, serum creatinine and blood urea nitrogen are commonly used marker of diabetic nephropathy. Although numerous experimental effort have been made to develop ground-breaking therapeutic concepts in preventing the development and progression of diabetic nephropathy. But in recent reports most attention has focused on functional markers and has drawn attention from structural markers. Interestingly, structural changes may already be present once micro-albuminuria or other markers occurs. It's worth noting that diabetic nephropathy is characterized by a series of ultra structural changes in all renal compartments and the hallmarks of diabetic nephropathy include glomerular hyper-filtration, thickening of glomerular basement membranes, expansion of extracellular matrix in mesangial areas which ultimately results in functional consequences, including progressive albuminuria, and reduction in glomerular filtration rate [1-3]. Thereby, the correlation between structural and functional consequences coerce for more insightful on structural marker for evaluating any pharmacological agent for its renoprotective potential during diabetes as all the kidney cellular elements, i.e., glomerular endothelia, mesangial cells, podocytes, and tubular epithelia, are targets of hyperglycemic injury. Hyperglycemia, an important risk factor for the development of diabetic nephropathy cause tubulo-interstitial damage. Most detrimental effects of glucose are mediated indirectly through diverse metabolic pathways that include mainly AGEs formation, increased activity of the polyol pathway, activation of protein kinase C (PKC) and increased flux through the hexosamine pathway. It's worth mentioning that activation of these pathways in turn causes dysregulation of a number of effectors molecules i.e transforming growth factor- β (TGF-β), reactive oxygen species (ROS), vascular growth factor (VEGF), nitric oxide (NO), inflammatory cytokines and adipokines which cause cellular damage and dysfunction [4-6]. In addition to hyperglycemia, dyslipidemia has been identified as a significant contributor to the development of diabetic nephropathy. Several studies have demonstrated altered lipid profile is associated with increased mesangial cell synthesis of extracellular matrix components, mesangial cell proliferation, glomerulosclerosis and tubulointerstitial fibrosis [7, 8]. This suggests that hyperlipidemia causes continuous renal injury and diabetes associated hyperlipidemia act synergistically in induction and progression of diabetic nephropathy. Dipeptidyl peptidase-4 (DPP-4) inhibitor is a new class of anti-diabetic drug which exerts its glucose-lowering action by suppressing the degradation of a gut incretin hormone glucagon-like peptide-1 (GLP-1). Treatment with exendin-4 (GLP-1 receptor agonists) was found to show the significant reduction in glomerular hypertrophy, mesangial matrix expansion in db/db mice. Fenofibrate, a peroxisome proliferator-activated receptor α (PPARα) agonist that has been widely used to treat dyslipidemia [9]. In addition to its hypolipidemic activity, fenofibrate has been noted to afford renoprotection by reducing the occurrence of albuminuria, glomerular injury, renal inflammation and tubulointerstitial fibrosis by suppressing nuclear factor- κB (NF-κB) and TGF-β1/Smad3 signaling pathways in experimental diabetic models [10, 11].

We previously reported that combination of fenofibrate and DPP-4 inhibitor saxagliptin provide the direct renoprotective effect in diabetic rats by reducing serum creatinine, blood urea nitrogen, albumin urea, renal oxidative stress, improving lipid profile and histopathological changes [12], but the effect of combination of fenofibrate and saxagliptin on detailed pathological changes is remained to be defined for their direct renoprotective effect in diabetic rats. Thus, this paper is more focused to illustrate the effect of combination of fenofibrate and saxagliptin on structural markers in the kidney of diabetic rats.

## MATERIAL AND METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched young wistar rats weighing about 200-240 g were employed in the present study. Rats were fed on standard chow diet and water *ad libitum*. They were acclimatized in institutional animal house and were exposed to normal cycles of day and night.

### Drugs and chemicals

Streptozotocin was obtained from Sigma-Aldrich Ltd., St. Louis, USA. 1,1,3,3-tetra methoxypropane and carboxymethyl cellulose were purchased from R. K. Enterprises, Meerut, India. Fenofibrate was obtained from Ranbaxy Laboratory Ltd., Gurgaon, India. Saxagliptin was obtained from Bristol-Myers Squibb, Mumbai, India. Lisinopril was obtained from Dr. Reddy's Laboratory Ltd., Hyderabad, India. All other chemicals used in the present study were of analytical grade.

### Induction and assessment of diabetes

The experimental diabetes mellitus was induced in rats by single injection of streptozotocin (STZ) (55 mg/kg *i.p*.,) dissolved in freshly prepared ice cold citrate buffer of pH 4.5. The blood sugar level was monitored once daily for first week after administration of STZ. Then, at the end of the experimental protocol (8 weeks after administration of STZ), the blood samples were collected and serum was separated.

The serum samples were frozen until analysing the biochemical parameters. The serum glucose concentration was estimated by glucose oxidase peroxidase (GOD-POD) method [13] using the commercially available kit (Transasia Bio-Medical Ltd., Solan, India).

### Histopathological study

The early diabetic changes in glomeruli were assessed histologically as previously described [14, 15]. The kidney was excised and immediately immersed in 10% formalin. The sections from kidney were dehydrated in graded concentrations of alcohol, immersed in xylene and then embedded in paraffin.

From the paraffin blocks, sections of 3-5 µm thickness were made and stained with hematoxylin-eosin and periodic acid-Schiff (PAS) using standard histologic procedures to assess the pathological changes occur in kidney using light microscopy.

### Experimental protocol

Eight groups were employed in the present study and each group comprised 6 rats. The fenofibrate and saxagliptin were suspended in 0.5% w/v of carboxy methyl cellulose (CMC). Group I (*Normal Control*), rats were maintained on standard food and water and no treatment was given. Group II (*Diabetic Control*), rats were administered STZ (55 mg/kg, *i.p*., once) dissolved in citrate buffer (pH 4.5). Group III (*Fenofibrate per se)*, the normal rats were administered fenofibrate (30 mg/kg *p.o*.) suspended in 0.5% w/v of CMC for 7 weeks. Group IV (*Saxagliptin per se*), the normal rats were administered saxagliptin (3 mg/kg *p.o*.) suspended in 0.5% w/v of CMC for 7 weeks. Group V (*Fenofibrate Treated*), the diabetic rats, after 1 week of STZ administration, were treated with low dose of fenofibrate (30 mg/kg *p.o*.) for 7 weeks. Group VI (*Saxagliptin Treated*), the diabetic rats, after 1 week of STZ administration, were treated with saxagliptin (3 mg/ kg *p.o*.) for 7 weeks. Group VII (*Fenofibrate + Saxagliptin Treated*), the diabetic rats, after 1 week of STZ administration, were treated with the combination of low dose of fenofibrate (30 mg/ kg, *p.o*.) and saxagliptin (3 mg/kg *p.o*.) for 7 weeks. Group VIII (*Lisinopril Treated*), the diabetic rats after 1 week of STZ administration, were treated with lisinopril (1 mg/kg *p.o*.) for 7 weeks.

### Statistical analysis

All values were expressed as mean ± SD. The data obtained from various groups were statistically analysed using one way ANOVA, followed by Tukey's multiple comparison test. The p value of less than 0.05 was considered to be statistically significant and the p values were of two tailed.

## RESULTS

Administration of fenofibrate at lower dose (30 mg/kg *p.o.,* 7 weeks) or saxagliptin (3 mg/kg *p.o.,* 7 weeks) did not produce any significant *per se* effect on various parameters assessed in normal rats. Administration of streptozotocin (STZ) (55 mg/kg, *i.p.,* once) produced hyperglycemia after 72 h (serum glucose >180mg/dL). After 7 days of STZ administration, the rats showed blood glucose level of greater than 270 mg/dL were selected and were named as diabetic rats. Fenofibrate (30 mg/kg *p.o.,* 7 weeks), saxagliptin (3 mg/kg *p.o.,* 7 weeks) and lisinopril (1 mg/kg, *p.o.,* 7 weeks) were administered to diabetic rats after 7 days of single injection of STZ and their treatments were continued for 7 weeks. All the parameters were assessed at the end of 7 weeks in normal and diabetic rats with or without drug treatments. Less than 12% of mortality rate was observed in diabetic rats with or without drug treatments.

### Effect of pharmacological interventions on serum glucose

The marked increase in serum concentration of glucose was noted in diabetic rats as compared to normal rats. Treatment with fenofibrate (30 mg/kg *p.o.,* 7 weeks) did not affect the serum glucose concentration in diabetic rats. However, treatment with saxagliptin (3 mg/kg *p.o.,* 7 weeks) incompletely reduced the elevated glucose level in diabetic rats. In addition, the effect of saxagliptin in incompletely reduction of elevated serum glucose level in diabetic rats was not altered by its combination with fenofibrate (30 mg/kg *p.o.,* 7 weeks). The lisinopril (1 mg/kg *p.o.,* 7 weeks) treatment slightly lowered the glucose level, but the results were not statistically significant. (Figure 1)

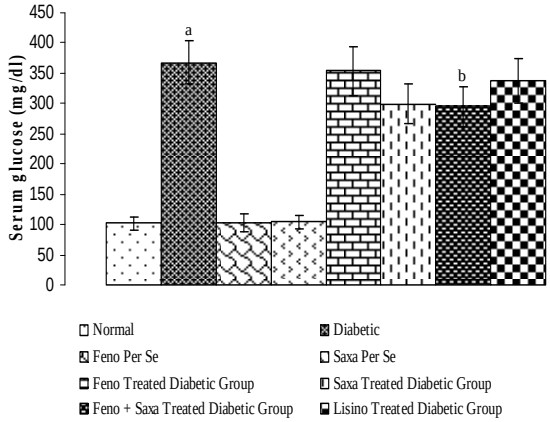


**Fig. 1: Effect of fenofibrate (feno), saxagliptin (saxa) and lisinopril on serum glucose and lipid profile. All values are represented as mean ± SD. a = p< 0.05 versus normal control; b = p< 0.05 versus diabetic control.**

### Effect of pharmacological interventions on bodyweight, kidney weight and kidney/body weight ratio

Diabetic rats after 7 weeks (8 weeks after STZ administration) showed decrease in body weight as compared to normal rats. In addition, the kidney weight of diabetic rats was noted to be increased as compared to normal rats. Treatment with either fenofibrate (30 mg/kg *p.o.,* 7 weeks) or saxagliptin (3 mg/kg *p.o.,* 7 weeks) partially prevented the diabetes-induced decrease in body weight and increase in kidney weight. The concurrent administration of fenofibrate (30 mg/kg *p.o.,* 7 weeks) and saxagliptin (3 mg/kg *p.o.,* 7 weeks) markedly attenuated the diabetes-induced decrease in body weight and increase in kidney weight as compared to treatments with either drug alone or lisinopril (1 mg/kg, *p.o.,* 7 weeks) in diabetic rats (Table 1). Increased kidney/body weight ratio in the diabetic rats as compare to the normal rats was found to be reversed by the concurrent administration of fenofibrate (30 mg/kg *p.o.,* 7 weeks) and saxagliptin (3 mg/kg *p.o.,* 7 weeks) as compared to treatments with either drug alone in diabetic rats (Figure 2).

**Table 1: Effect of fenofibrate (feno), saxagliptin (saxa) and lisinopril on body weight and kidney weight**

| Assessment | Normal Control | Diabetic Control | Feno *per se* | Saxa *per se* | Feno Treated Diabetic Group | Saxa Treated Diabetic Group | Feno + Saxa Treated Diabetic Group | Lisino Treated Diabetic Group |
|---|---|---|---|---|---|---|---|---|
| Body weight (gm) | 231.66 ± 8.16 | 182.5 ± 10.36[a] | 226.66 ± 9.83 | 227.5 ± 9. 35 | 199.16 ± 7.35 | 205.83 ± 9.70 | 217.5 ± 5.24[b] | 207.5 ± 6.89[b] |
| Kidney weight (gm) | 0.922 ± 0.08 | 1.342 ± 0.082[a] | 0.904 ± 0.051 | 0.903 ±0.068 | 1.300 ± 0.051[b] | 1.272 ± 0.059 | 1.128 ± 0.081[b] | 1.246 ± 0.082 |

All values are represented as mean ± SD. a = p< 0.05 versus normal control; b = p< 0.05 versus diabetic control.

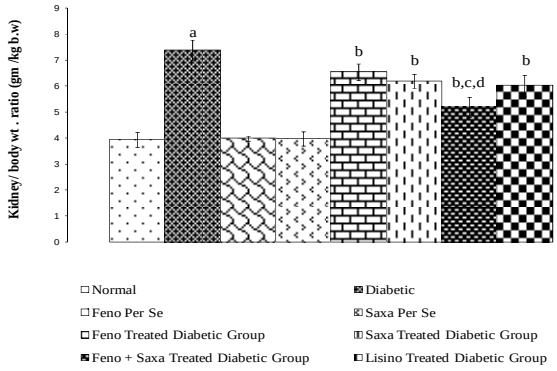


**Fig. 2: Effect of combination of fenofibrate and saxagliptin on kidney/body weight ratio. a = p< 0.05 versus normal control; b = p< 0.05 versus diabetic control; c = p< 0.05 versus fenofibrate treated diabetic group; d = p< 0.05 versus saxagliptin treated diabetic group.**

**Effect of pharmacological interventions on glomerular basement membrane and tubular injury**

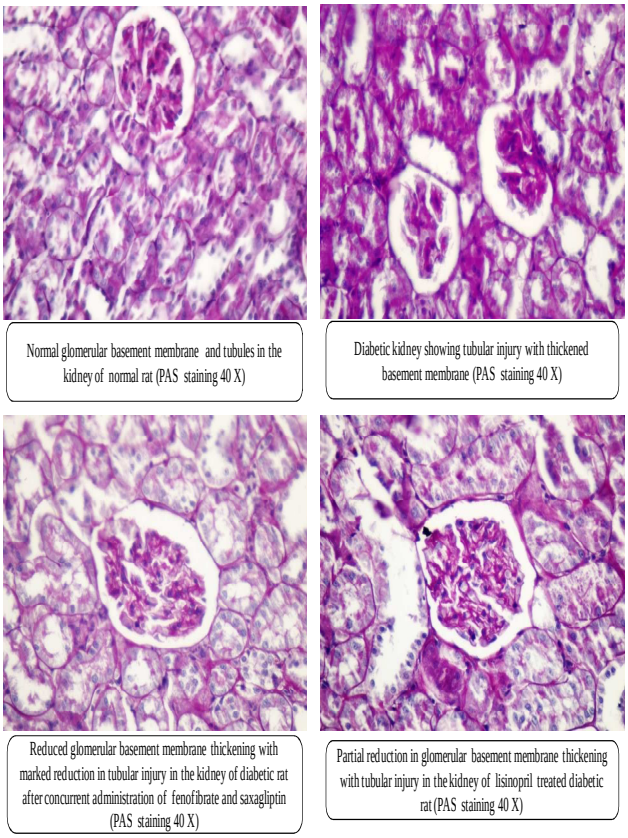


**Fig. 3: Effect of combination of fenofibrate and saxagliptin on glomerular basement membrane and tubular injury.**

The glomerular basement membrane thickening and tubular injury was observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Treatment with concurrent administration of fenofibrate (30 mg/kg *p.o.,* 7 weeks) and saxagliptin (3 mg/kg *p.o.,* 7 weeks) markedly protected the diabetic kidney by halting the glomerular basement membrane thickening and tubular injury as compared to treatments with lisinopril (1 mg/kg, *p.o.,* 7 weeks) in diabetic rats (Figure 3).

**Effect of pharmacological interventions on mesangial matrix expansion**

The mesangial matrix expansion was observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Treatment with concurrent administration of fenofibrate (30 mg/kg *p.o.,* 7 weeks) and saxagliptin (3 mg/kg *p.o.,* 7 weeks) markedly prevent the mesangial matrix expansion in the diabetic kidney as compared to treatments with lisinopril (1 mg/kg, *p.o.,* 7 weeks) in diabetic rats (Figure 4).

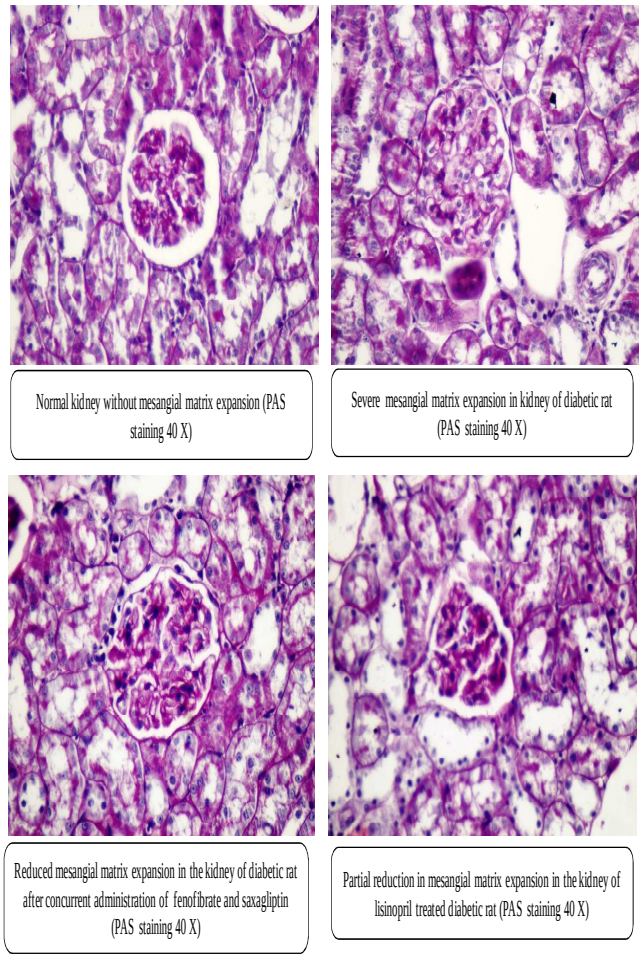


**Fig. 4: Effect of combination of fenofibrate and saxagliptin on mesangial matrix expansion.**

**Effect of pharmacological interventions on interstitial inflammation and tubular atrophy**

The interstitial inflammation and tubular atrophy was observed in the kidney of diabetic rats after 7 weeks as compared to normal rats. Concurrent administration of fenofibrate (30 mg/kg *p.o.,* 7 weeks) and saxagliptin (3 mg/kg *p.o.,* 7 weeks) markedly prevent the interstitial inflammation and tubular atrophy in the diabetic kidney as compared to treatments with lisinopril (1 mg/kg, *p.o.,* 7 weeks) in diabetic rats (Figure 5).

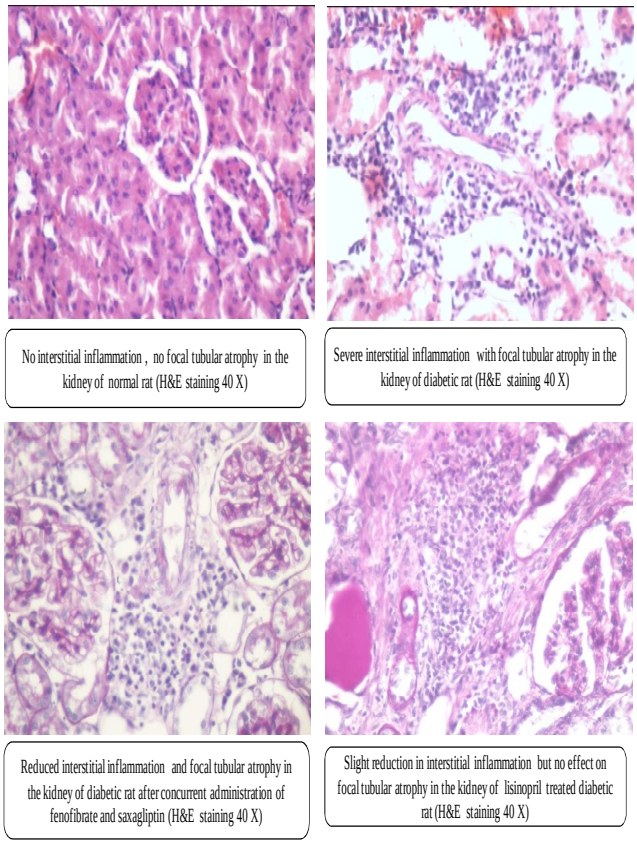


**Fig. 5: Effect of combination of fenofibrate and saxagliptin on interstitial inflammation with tubular atrophy.**

## DISCUSSION

In the present study, we corroborate our previous report that combination of fenofibrate and saxagliptin affords renoprotective potential by preventing the development and progression of diabetic nephropathy. Present data expands our previous reports by illustrating effect of combination of fenofibrate and saxagliptin on detailed morphological study in the kidney of diabetic rats. 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 [16, 17]. Studies analyzing structural-functional relationships have demonstrated that the development of proteinuria correlates with the degree of mesangial expansion [18, 19]. 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 [20]. Fenofibrate is known to possess renoprotective potential by downregulating the renal expression of TGF-β [11] 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 [9], suggesting the possible underlying mechanism involved in mesangial expansion reduction by concurrent administration of fenofibrate and saxagliptin in the present study. In addition, renal lipid accumulation has been reported to play an important role in mesangial matrix accumulation independent to TGF-β over expression [21]. Thereby, it may suggested that hypercholesterolemia causes renal injury in a continuous insult manner, and it supports the fact that lowering cholesterol levels may lead to improved renal injury. 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 [22, 23]. However, in late state nephropathy intrinsic basement membrane components are no longer produced. Instead, massive accumulation of PAS positive material occurs [24]. 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. Although diabetic nephropathy was traditionally considered a primarily glomerular disease, it is also accepted that the rate of deterioration of function correlates best with the degree of renal tubulointerstitial fibrosis [18, 25]. Morphometric studies indicate that glomerular and tubulointerstitial injury is interdependent: thus, in glomerulonephritis, damage of a single nephron may progress from initial glomerular injury to interstitial inflammation, tubular atrophy, and interstitial scarring [26]. Diabetes has been well thought-out as a state of increased oxidative stress, and over the past several years, ROS mediated over expression/activation of PKC, NFκB, MCP-1and TGF-β in renal compartments has been recognized as a central player to induce glomerular and tubular injury during diabetic nephropathy [27-30]. Previously, we have reported that, concurrent administration of fenofibrate and saxagliptin markedly prevented the development of renal oxidative stress in diabetic rats with nephropathy by reducing renal TBARS and concomitantly elevating renal GSH levels [12]. Indeed, this previous data supports the possible underlying mechanism involved in marked reduction in interstitial inflammation and focal tubular atrophy by concurrent administration of fenofibrate and saxagliptin in the present study.

## CONCLUSION

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 improving the altered structural markers of diabetic nephropathy, including renal enlargement; mesangial cell expansion; tubular injury and GBM thickening. The present study provides the evidence for their direct nephroprotective effect. However, long-term clinical studies demonstrating the rationale of combination of fenofibrate and saxagliptin in preventing diabetic nephropathy are mandatory.

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