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The combined strategy with PPARα agonism and AT_1 receptor antagonism is not superior relative to their individual treatment approach in preventing the induction of nephropathy in the diabetic rat

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ABSTRACT

We have previously shown that the low-dose combination of fenofibrate and rosiglitazone might halt the progression of diabetes-induced nephropathy in rats. The present study investigated the combined effect of fenofibrate (PPARα agonist) and telmisartan (AT_1 receptor antagonist) in diabetes-induced onset of nephropathy in rats. The single administration of streptozotocin (STZ, 55 mg/kg *i.p.*) produced diabetes mellitus, which subsequently produced nephropathy in 8 weeks by markedly elevating serum creatinine, blood urea nitrogen and microproteinuria. In addition, histopathological studies revealed the development of renal structural abnormalities such as mesangial expansion, glomerular and tubular damage. Moreover, diabetes-induced nephropathy was accompanied with high renal oxidative stress and lipid alteration. Treatment with fenofibrate (80 mg/kg/day, *p.o.*, 4 weeks) and telmisartan (10 mg/kg/day, *p.o.*, 4 weeks) either alone or in combination did not affect the elevated glucose levels in diabetic rats. Albeit treatment with fenofibrate normalizes the altered lipid profile in diabetic rats, telmisartan treatment has no effect on it. Treatment with fenofibrate and telmisartan either alone or in combination markedly prevented diabetes-induced onset of nephropathy and renal oxidative stress. Their combination was as good as to their individual treatment, but not superior in attenuating the diabetes-induced nephropathy and renal oxidative stress. It may be concluded that diabetes-induced oxidative stress and lipid alteration, besides hyperglycemia, could play a key role in the induction of nephropathy. Fenofibrate and telmisartan individual treatment was equipotent in preventing the onset of diabetes-induced experimental nephropathy, while their combination did not afford additional benefits in preventing the disease induction of the diabetic kidney.

1. Introduction

The prevalence of diabetes mellitus is continuously increasing worldwide, and it has been estimated to reach more than 360 million peoples with diabetes mellitus by 2030 [1]. The uncontrolled and chronic diabetes mellitus often results in numerous complications in which nephropathy is considered the most challenged one. About 20–30% patients with type I and type II diabetes mellitus develop signs of nephropathy [2]. Diabetic nephropathy is associated with renal structural alterations such as glomerular basement membrane thickening, mesangial cell expansion, glomerulosclerosis, interstitial fibrosis, podocyte loss and tubular atrophy [3–7]. These alterations during diabetic nephropathy could result in proteinuria, decrease in glomerular filtration rate, and increase in serum creatinine and urea nitrogen levels [4,8–10].

Renin-angiotensin-aldosterone system (RAAS) overactivation plays a pivotal role in the induction and progression of diabetic nephropathy [11,12]. Angiotensin-II AT_1 receptor blockers are frequently employed for the management of diabetic nephropathy [13,14]. However, the mortality rate pertaining to diabetic nephropathy remains high [15], and this may due to incomplete knowledge on the exact cause of diabetic nephropathy. Accumulating evidence suggests that elevation of circulating lipids could contribute to the renal disease pathogenesis [16,17]. The diabetes mellitus-associated lipid alteration and dyslipidemia contributes significantly to the development of diabetic nephropathy [15,18,19]. Peroxisome proliferator-activated receptor alpha (PPARα) plays an important role in lipid regulation and metabolism [20]. Accelerated diabetic nephropathy was noted in mouse lacking PPARα [21], suggesting the modulatory role of PPARα in diabetic nephropathy. In fact, fenofibrate, an activator of PPARα, has a renoprotective effect against experimental and clinical diabetic

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nephropathy [22–25]. These studies certainly suggest that dyslipidemia could play a significant role during the induction of diabetic nephropathy.

We have recently demonstrated that the pre-treatment with fenofibrate and rosiglitazone (PPARγ agonist) could have ability to attenuate the development of diabetic nephropathy in rats [26]. However, it must be realized that rosiglitazone combination might need an attention since its clinical use is associated with cardiovascular adverse events, including myocardial infarction [27]. Telmisartan is a potent AT_1 receptor blocker that has additional potential to activate PPARγ partially without producing cardiovascular adverse events that are associated with PPARγ full agonists like rosiglitazone [28,29]. Therefore, the present study has been designed to investigate the combined effect of fenofibrate and telmisartan in comparison to their individual effects in treating diabetic nephropathy in rats.

2. Materials and methods

The experimental protocol used in the present study was approved by Institutional Animal Ethics Committee (IAEC) in accordance to the guidelines given by 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA). Wistar albino rats of either sex weighing about 170–250 g were employed in the present study. The rats were acclimatized in the Institutional animal house and maintained on rat chow (Ashirwad Industries, Mohali, India) and tap water. Rats were given *ad libitum* access to food and water. They were exposed to normal day and night cycles.

2.1. Induction of experimental diabetes mellitus

Experimental diabetes mellitus was induced in rats by single administration of streptozotocin (STZ) (55 mg/kg, *i.p.*) dissolved in freshly prepared citrate buffer (pH 4.5). The blood sugar level was monitored once after 72 h and serum glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method using the commercially available kit (Transasia Bio-medicals Ltd., Solan, India). The rats showing blood glucose level of greater than 200 mg/dL were selected and named as diabetic rats. At the end of the experimental protocol, the serum glucose level was again estimated.

2.2. Assessment of diabetic nephropathy

The development of diabetic nephropathy, 8 weeks after the administration of STZ, was assessed in rats by estimating serum creatinine, blood urea nitrogen and protein in urine using commercially available kits.

2.2.1. Estimation of serum creatinine

The serum creatinine concentration was estimated by alkaline picrate method using the commercially available kit (Angstrom Biotech Pvt. Ltd., Vadodara, India). Briefly, 100 μL serum sample and 100 μL standard creatinine solution (2 mg/dL) were taken separately in glass tubes, which were named as test (T) and standard (S), respectively. The working reagent (1000 μL) containing alkaline picrate solution was added in both tubes, mixed and the reaction temperature was kept at 30 °C. The absorbance of test and standard at 20 s (T_1, S_1) and again at 80 s (T_2, S_2) was noted against blank spectrophotometrically. The formation of a colored complex as a result of a reaction between creatinine present in serum sample and alkaline picrate present in working reagent was measured at 510 nm.

The serum creatinine concentration was calculated using the following formula:

$$\text{Serum creatinine concentration } \left(\frac{\text{mg}}{\text{dL}}\right) = \frac{(T_2 - T_1)}{(S_2 - S_1)} \times 2$$

2.2.2. Estimation of blood urea nitrogen

The blood urea nitrogen was estimated by modified Berthelot method using the commercially available kit (Reckon Diagnostics Pvt. Ltd., Vadodara, India). Briefly, 10 μL distilled water, 10 μL standard solution (40 mg/dL) and 10 μL serum sample were taken separately in blank, standard (S) and test (T) glass tubes, respectively. The working enzyme reagent (1000 μL) (containing urease and a mixture of salicylate, hypochlorite and nitroprusside) was added in all glass tubes with thorough mixing. All glass tubes were incubated at 37 °C for 5 min. Then, the working color reagent (1000 μL) (containing alkaline buffer) was added to all glass tubes, and they were again incubated at 37 °C for 5 min. The absorbance of standard and test was noted against blank spectrophotometrically. The principle involved in this estimation follows. Urease breaks down urea into ammonia and carbon dioxide. In alkaline medium, ammonia reacts with hypochlorite and salicylate to form a colored compound, dicarboxyindophenol. This reaction is catalysed by sodium nitroprusside. The intensity of the color produced was measured spectrophotometrically at 578 nm.

Blood urea concentration was calculated using the following formula:

$$\text{Blood urea concentration } \left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 40$$

Blood urea nitrogen (BUN) concentration (mg/dL) = 0.467 × Blood urea concentration (mg/dL)

2.2.3. Estimation of protein in urine

The microproteinuria was assessed by pyrogallol red method using the commercially available kit (Crest Biosystems, Goa, India). The reagent (containing pyrogallol dye) (1000 μL) was added to 10 μL distilled water, 10 μL standard protein and 10 μL urine sample to prepare blank, standard (S) and test (S), respectively. All test tubes were mixed and incubated at 37 °C for 5 min. The absorbance of test and standard samples were noted against blank at 600 nm spectophotometrically within 30 min. When the pyrogallol red-molybedate complex binds to basic amino groups of protein molecules, there is a shift in reagent absorbance and forms a blue colored complex. The intensity of color formed is directly proportional to protein concentration present in the sample.

The urinary protein was calculated using the following formula:

$$\text{Microproteins } \left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 100$$

$$\text{Total microprotein excreted } \left(\frac{\text{mg}}{24\,\text{h}}\right) = \text{Urinary protein concentration } \left(\frac{\text{mg}}{\text{dL}}\right) \times 10 \times \text{total volume of urine (l) excreted for 24 h}$$

2.2.4. Histopathological study

The early diabetic changes in glomeruli and tubules were assessed histologically with the help of Mangalam Pathological Laboratory, Haryana, India. The kidney was excised and immediately immersed in 10% formalin solution. The kidney was dehydrated in graded concentrations of alcohol, immersed in xylene and then

embedded in paraffin. From the paraffin blocks, sections of 5 μm in thickness were made and stained with hematoxylin and eosin to assess the pathological changes occurred in glomeruli and tubules using light microscopy (Motic Digital Microscope BA310 (Motic, USA) at 40×.

2.3. Assessment of lipid profile

The lipid profile was assessed by estimating total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol. The serum total cholesterol concentration was estimated by cholesterol oxidase peroxidase (CHOD/POD) method using the commercially available kit (Transasia Bio-Medicals Ltd., Solan, India). The HDL cholesterol was estimated by Precipitation method using phosphotungstate magnesium acetate reagent employing the commercially available kit (Agappe Diagnostics Ltd., Kerala, India).

2.4. Assessment of renal oxidative stress

The development of oxidative stress in the rat kidney was assessed by estimating renal thiobarbituric acid reactive substances (TBARS) [30] and reduced form of glutathione (GSH) [31,32].

2.4.1. Preparation of renal homogenate

The kidney was excised and washed with ice cold isotonic saline and weighed. The kidney weight to body weight ratio was calculated. The kidney was then minced, and a homogenate (10% w/v) was prepared in chilled 1.15% KCl. The homogenate was used for the estimation of renal TBARS and GSH.

2.4.2. Estimation of renal thiobarbituric acid reactive substances

The renal thiobarbituric acid reactive substances, an index of lipid peroxidation, were estimated according to the method described earlier [30]. The reaction mixture was prepared by mixing 0.2 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid (TBA). The reaction mixture was made up to 4.0 mL with distilled water, and then incubated at 95 °C for 60 min. After cooling in tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of *n*-butanol and pyridine (15:1 v/v) were added to reaction mixture and shaken vigorously using vortex shaker. The test tubes were centrifuged at 4000 rpm for 10-min (REMI Cooling Centrifuge, India). The absorbance of developed pink colour was measured spectrophotometrically at 532 nm. The standard curve using 1,1,3,3-tertramethoxyopropane (1–10 nM) was plotted to calculate the concentration of TBARS, and the results were expressed as nM/g wet weight of renal tissue.

2.4.3. Estimation of reduced glutathione

The renal GSH level was estimated using the methods described by Ellman [31] and Boyne and Ellman [32]. The renal homogenate of the rat was mixed with 10% w/v trichloroacetic acid in 1:1 ratio and centrifuged at 4 °C for 10 min at 5000 rpm. The supernatant (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 mL of distilled water. Then, 0.25 mL of 0.001 M freshly prepared DTNB [5,5′-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added to the reaction mixture, and then incubated for 10-min. The absorbance of the yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using the reduced form of glutathione (0.1–1 μM), and the results were expressed as μM/g wet weight of renal tissue.

2.5. Experimental protocol

Six groups were employed in the present study and each group comprised six rats. Fenofibrate and telmisartan were suspended in 0.5% w/v of carboxy methyl cellulose. *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), and allowed for 8 weeks to develop diabetic nephropathy. *Group III*, *fenofibrate + telmisartan per se*: the normal rats were administered fenofibrate (80 mg/kg/day, *p.o.*) and telmisartan (10 mg/kg/day, *p.o.*) for 4 weeks. *Group IV*, *fenofibrate treated*: the diabetic rats after 4 weeks of STZ administration were treated with fenofibrate (80 mg/kg/day, *p.o.*) for 4 weeks. *Group V*, *telmisartan treated*: the diabetic rats after 4 weeks of STZ administration were treated with telmisartan (10 mg/kg/day, *p.o.*) for 4 weeks. *Group VI*, *fenofibrate + telmisartan treated*: the diabetic rats after 4 weeks of STZ administration were treated with fenofibrate (80 mg/kg/day, *p.o.*) and telmisartan (10 mg/kg/day, *p.o.*) combination for 4 weeks.

2.6. Statistical analysis

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

2.7. Drugs and chemicals

Streptozotocin and 1,1,3,3-tetra methoxypropane were obtained from Sigma-Aldrich Ltd., St. Louis, USA. Reduced glutathione was obtained from SD Fine, Mumbai, India. Fenofibrate and telmisartan were obtained as gift samples from Glenmark Generics Ltd., Mumbai, India. Carboxy methyl cellulose and trichloroacetic acid were obtained from RANKEM, New Delhi, India. Thiobarbituric acid and DTNB were obtained from Otto-Chemika-Biochemika, Mumbai, India. All other chemicals used in the present study were of analytical grade.

3. Results

Administration of fenofibrate (80 mg/kg/day *p.o.*, 4 weeks) and telmisartan (10 mg/kg/day *p.o.*, 4 weeks) did not produce any significant *per se* effect on various parameters performed in normal rats. Rats showed serum glucose level of more than 200 mg/dL after 72 h of STZ (55 mg/kg, *i.p.*, once) administration were named as diabetic rats and included in the present study. Fenofibrate (80 mg/kg/day *p.o.*, 4 weeks) and/or telmisartan (10 mg/kg/day *p.o.*, 4 weeks) treatments were started after 4 weeks of STZ administration and their treatments were continued for 4 weeks. All the parameters were assessed at the end of 8 weeks of experimental protocol.

3.1. Effect of pharmacological interventions on serum glucose

As compared to normal rats, the serum glucose level was noted to be markedly elevated in diabetic rats. Treatment with either fenofibrate or telmisartan did not affect the elevated serum glucose level in diabetic rats. Treatment with their combination slightly lowered the glucose level in diabetic rats; however, the results were not statistically significant (Table 1).

3.2. Effect of pharmacological interventions on lipid profile

An increase in serum total cholesterol and consequent decrease in HDL cholesterol were noted in diabetic rats as compared to

Table 1
Effect of fenofibrate, telmisartan and their combination on serum glucose and lipid profile and KW/BW.

Assessments	Normal control	Diabetic control	Fenofibrate + telmisartan *per se*	Fenofibrate treated	Telmisartan treated	Fenofibrate + telmisartan treated
Glucose (mg/dL)	90.61 ± 8.43	289.56 ± 29.54[a]	98.57 ± 7.99	277.90 ± 26.29	271.26 ± 27.05	264.18 ± 28.84
TC (mg/dL)	52.74 ± 5.78	100.97 ± 7.18[a]	54.31 ± 5.43	72.74 ± 11.29[b]	88.03 ± 5.23	62.40 ± 8.65[b]
HDL-C (mg/dL)	48.23 ± 4.83	31.76 ± 6.22[a]	48.43 ± 4.90	39.21 ± 3.91	32.14 ± 5.10	41.36 ± 8.46
KW/BW (mg/g)	5.0 ± 0.46	7.43 ± 0.45[a]	5.16 ± 0.59	5.28 ± 0.79[b]	5.05 ± 0.71[b]	5.03 ± 0.71[b]

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; KW/BW, kidney weight to body weight ratio. All values were represented as mean ± S.D.
[a] $p < 0.05$ *versus* normal control.
[b] $p < 0.05$ *versus* diabetic control.

normal rats. Treatment with fenofibrate significantly attenuated diabetes-induced increase in serum total cholesterol where as telmisartan treatment did not produce any significant effect in the elevated total cholesterol in diabetic rats. Like wise, telmisartan addition did not affect the cholesterol-lowering action of fenofibrate in diabetic rats. On the other hand, treatment with fenofibrate and telmisartan either alone or in combination did not affect the reduction in HDL cholesterol in diabetic rats (Table 1).

3.3. Effect of pharmacological interventions on the kidney weight to body weight ratio

A significant increase in the kidney weight to body weight ratio (mg/g) was noted in diabetic rats (7.43 ± 0.45) as compared to normal rats (5.0 ± 0.46). Treatment with either fenofibrate (5.28 ± 0.79) or telmisartan (5.05 ± 0.71) or their combination (5.03 ± 0.71) significantly reduced diabetes-induced increase in the kidney weight to body weight ratio. However, their combination did not produce any significant additive effect as compared to their individual treatment effect in reducing the increase in kidney weight to body weight ratio in diabetic rats (Table 1).

3.4. Effect of pharmacological interventions on serum creatinine and blood urea nitrogen

The serum creatinine and blood urea nitrogen levels were noted to be markedly increased in diabetic rats as compared to normal rats. Treatment with fenofibrate significantly reduced the elevated levels of serum creatinine and blood urea nitrogen in diabetic rats. Telmisartan treatment significantly reduced diabetes-induced increase in serum creatinine and blood urea nitrogen levels. Like wise, their combination significantly reduced diabetes-induced increase in serum creatinine and blood urea nitrogen levels. However, their combination did not produce any additive effect as compared to their individual treatment effect in reducing the elevated levels of serum creatinine and blood urea nitrogen in diabetic rats. It should be noted that their combination produced a significant reduction in the elevated level of blood urea nitrogen as compared to fenofibrate treatment but not to telmisartan treatment in diabetic rats (Figs. 1 and 2).

3.5. Effect of pharmacological interventions on microproteinuria

An increase in the occurrence of microproteinuria was noted in diabetic rats as compared to normal rats. Treatment with either fenofibrate or telmisartan or their combination significantly reduced diabetes-induced increase in the occurrence of microproteinuria. However, their combination did not produce any significant additive effect as compared to their individual treatment effect in reducing the occurrence of microproteinuria in diabetic rats (Fig. 3).

3.6. Effect of pharmacological interventions on renal TBARS and GSH

Diabetic rats, after 8 weeks of STZ administration showed a marked increase in renal TBARS as compared to normal rats. Moreover, the renal concentration of GSH was noted to be decreased in diabetic rats as compared to normal rats. Treatment with either fenofibrate or telmisartan or their combination significantly

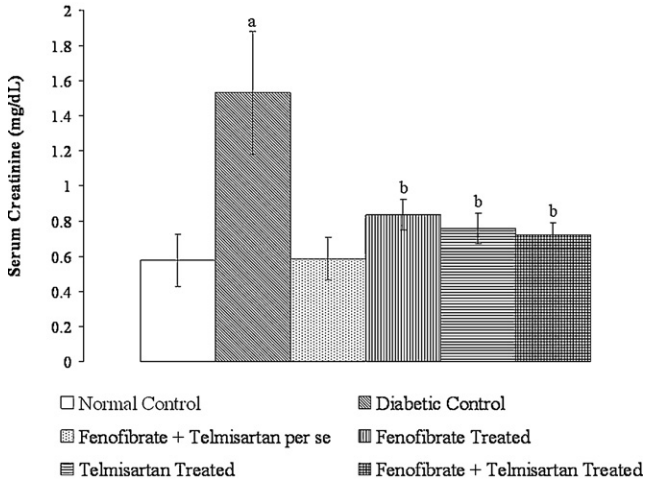

Fig. 1. Effect of fenofibrate, telmisartan and their combination on serum creatinine (mg/dL). All values were represented as mean ± S.D. $a = p < 0.05$ *versus* normal control; $b = p < 0.05$ *versus* diabetic control.

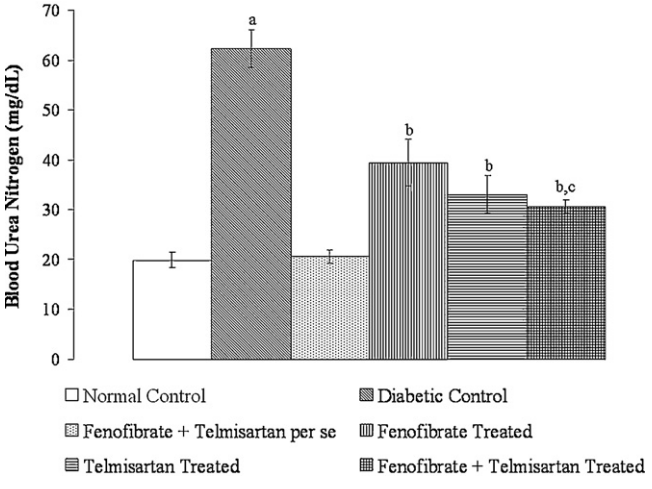

Fig. 2. Effect of fenofibrate, telmisartan and their combination on blood urea nitrogen (mg/dL). All values were represented as mean ± S.D. $a = p < 0.05$ *versus* normal control; $b = p < 0.05$ *versus* diabetic control; $c = p < 0.05$ *versus* fenofibrate treated diabetic group.

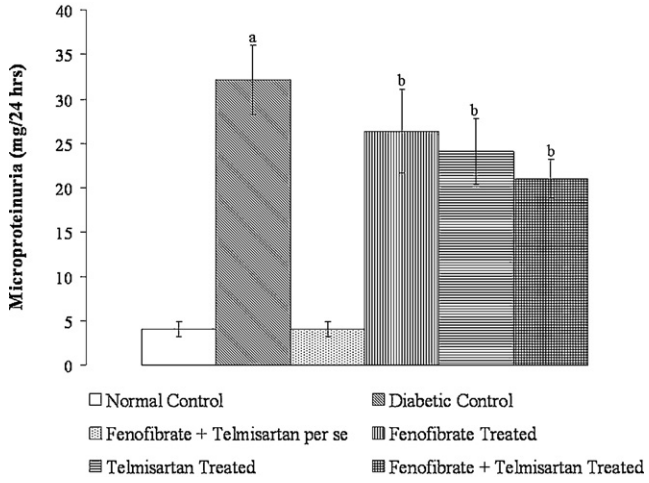

Fig. 3. Effect of fenofibrate, telmisartan and their combination on microproteinuria (mg/24 h). All values were represented as mean ± S.D. $a = p < 0.05$ *versus* normal control; $b = p < 0.05$ *versus* diabetic control.

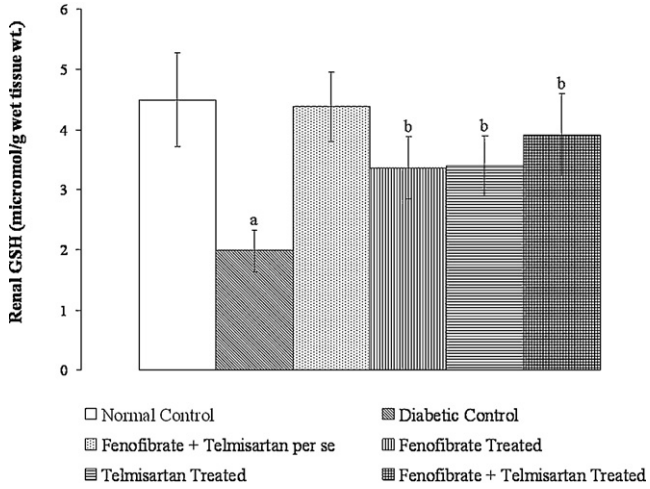

Fig. 5. Effect of fenofibrate, telmisartan and their combination on renal GSH (μM/g wet tissue wt.). All values were represented as mean ± S.D. $a = p < 0.05$ *versus* normal control; $b = p < 0.05$ *versus* diabetic control.

attenuated diabetes-induced increase in renal TBARS and decrease in renal GSH. Though their combination markedly attenuated diabetes-induced increase in renal TBARS and decrease in renal GSH, the results were not statistically significant as compared to their individual treatment effects (Figs. 4 and 5).

3.7. *Effect of pharmacological interventions on renal histopathology*

The diabetic rats were noted to develop pathological changes in the glomerulus such as glomerular capsular wall distortion, mesangial cell expansion, microvascular condensation and tubular damage as compared to normal rats. Treatment with either fenofibrate or telmisartan prevented the diabetes-induced pathological changes in glomerulus and tubules. Moreover, their combined effect was slightly better than their individual treatment effect in preventing diabetes-induced pathological changes in glomerulus and tubules (Figs. 6 and 7).

4. Discussion

The present study compared the renoprotective effect of fenofibrate and telmisartan combination with their individual treatment

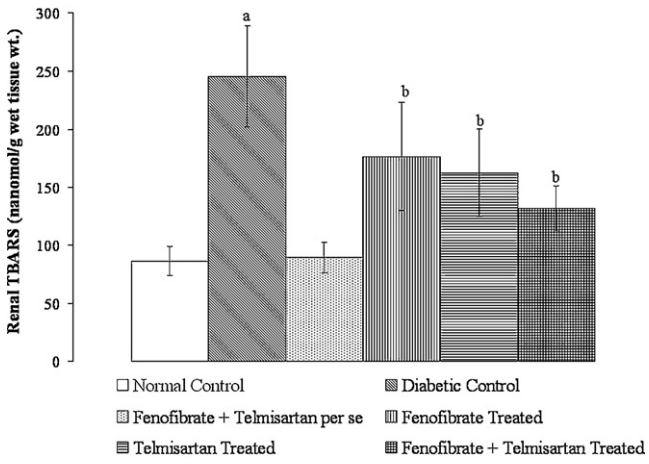

Fig. 4. Effect of fenofibrate, telmisartan and their combination on renal TBARS (nM/g wet tissue wt.). All values were represented as mean ± S.D. $a = p < 0.05$ *versus* normal control; $b = p < 0.05$ *versus* diabetic control.

effect in diabetic rats with the onset of nephropathy. We found out that treatment with either fenofibrate or telmisartan was equipotent in preventing the onset of diabetes mellitus-induced experimental nephropathy where as their combination afforded considerably no additional renoprotective effect in preventing the onset of nephropathy in the diabetic rat.

The elevation of serum creatinine and blood urea nitrogen is considered an index of renal dysfunction [33–35]. The incidence of proteinuria might correlate with the progression of glomerulosclerosis and tubular alterations [36]. In the present study, a marked increase in serum creatinine and blood urea nitrogen, and apparent microproteinuria were noted in diabetic rats (after 8 weeks of STZ administration) as compared to normal rats. It indicates the development of renal dysfunction in diabetic rats. Moreover, diabetic rats developed renal pathological changes such as glomerular capsular wall distortion, mesangial cell expansion, microvascular condensation and tubular damage. These results certainly suggest the development of diabetic nephropathy.

In the present study, pharmacological treatment with fenofibrate and telmisartan either alone or in combination (from 4 to 8 weeks after STZ administration) markedly prevented the elevated levels of serum creatinine and blood urea nitrogen and reduced the occurrence of microproteinuria in diabetic rats, suggesting the anti-nephropathic potential of these agents in diabetic rats. Moreover, treatment with either fenofibrate or telmisartan markedly prevented the pathological alterations in glomerulus and tubules of diabetic rats. However, their combination did not produce any additive effect as compared to their individual treatment effect in preventing afore-mentioned renal structural and functional abnormalities associated with rats afflicted to diabetic nephropathy. It should be noted that treatment with either fenofibrate or telmisartan or their combination did not affect the elevated serum glucose level in diabetic rats. It indicates that the protective effect of fenofibrate or telmisartan in preventing the development of diabetic nephropathy is pertaining to their direct renoprotective action independent to glucose concentration in diabetic rats.

Hyperlipidemia is an independent risk factor for the pathogenesis of diabetic nephropathy [37–40]. The elevation of circulating lipids might contribute to renal disease pathogenesis [16,17]. The diabetes mellitus-associated dyslipidemia could contribute to the development of diabetic nephropathy [15,18,19,41]. In the present study, serum total cholesterol was noted to be markedly increased while a marked decrease in HDL level was noted in diabetic rats after 8 weeks of STZ administration. These results

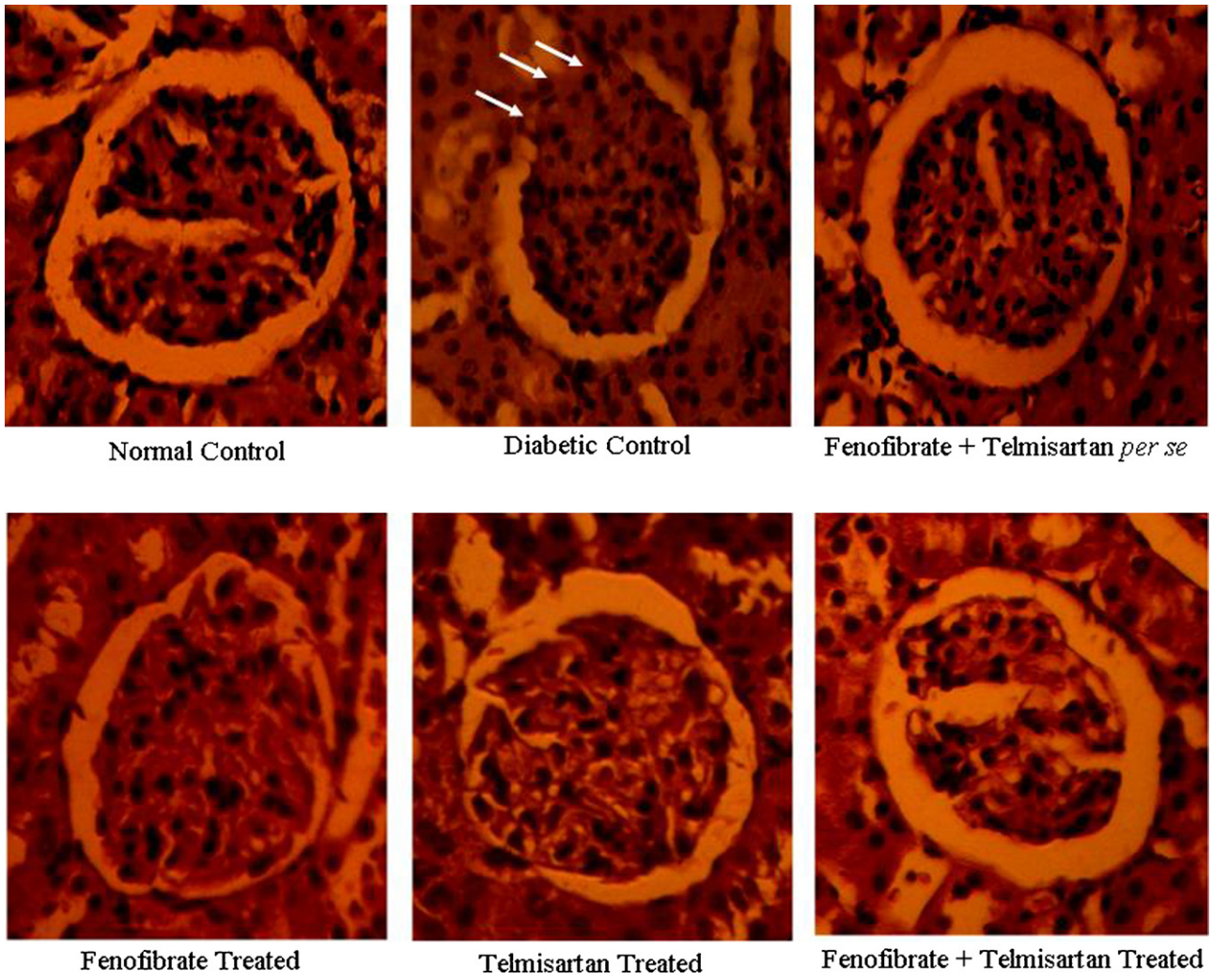

Fig. 6. Effect of fenofibrate, telmisartan and their combination on pathological changes in the glomerulus of the kidney. The kidney of the diabetic rat developed pathological changes in the glomerulus such as glomerular capsular wall distortion (indicated in arrow), mesangial cell expansion and microvascular condensation as compared to the kidney of the normal rat. The administration of fenofibrate or telmisartan and their combination markedly reduced these pathological changes of the glomerulus.

further substantiate that diabetes mellitus-associated lipid alteration could contribute to the renal disease pathogenesis. Treatment with fenofibrate significantly attenuated diabetes mellitus-induced increase in serum total cholesterol where as telmisartan treatment did not have any significant effect in the elevated total cholesterol in diabetic rats. Thus it may be suggested that the renoprotective effect of fenofibrate against the onset of nephropathy in diabetic rats might be associated with its action in reducing circulating lipids while the renoprotective effect of telmisartan noted in the present study may be of lipid-independent.

Experimental and clinical studies have suggested that fenofibrate has a renoprotective potential against diabetic nephropathy [22–26]. Diabetes Atherosclerosis Intervention Study (DAIS) trial suggested that improvement in lipid profile with fenofibrate in patients with type 2 diabetes mellitus was associated with reduced progression from normal albumin excretion to microalbuminuria [42]. Moreover, the fenofibrate intervention and event lowering in diabetes (FIELD) study suggested that fenofibrate could delay albuminuria and glomerular filtration rate impairment in type 2 diabetes mellitus patients [43]. Numerous clinical studies demonstrated the renoprotective potential of telmisartan in diabetic patients. Rysavá et al. [44] demonstrated that telmisartan reduced the incidence of proteinuria in diabetic and non-diabetic, hypertensive, proteinuric patients with chronic kidney disease. The groundbreaking 'Diabetics Exposed to Telmisartan And enalaprIL (DETAIL)' trial in patients with hypertension and early diabetic nephropathy suggested that telmisartan was not inferior to enalapril in reducing the decline in glomerular filtration rate [45]. The Incipient to Overt: Angiotensin II Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION) study reported that normotensive patients with diabetes mellitus treated with telmisartan showed a reduction in the transition rate from microalbuminuria to overt nephropathy, and more number of patients in the telmisartan group was reverted to normoalbuminuria [46], suggesting that telmisartan has ability to prevent the progression of microalbuminuria, and to induce remission in albuminuria. Bakris et al. [47] reported that telmisartan was superior to losartan in reducing proteinuria in patients with diabetic nephropathy though a similar reduction in blood pressure was noted, suggesting a direct renoprotective action of telmisartan. A comparison of telMisartan *versus* losArtan in hypertensive type 2 DiabEtic patients with Overt nephropathy (AMADEO) study reported that telmisartan-based regimen showed a greater anti-proteinuric effect than a losartan-based regimen at similarly achieved blood pressure-lowering effects [48]. These studies certainly support the results obtained in the present study that both fenofibrate and telmisartan have abilities to prevent the pathogenesis of diabetic nephropathy. The present study for the first

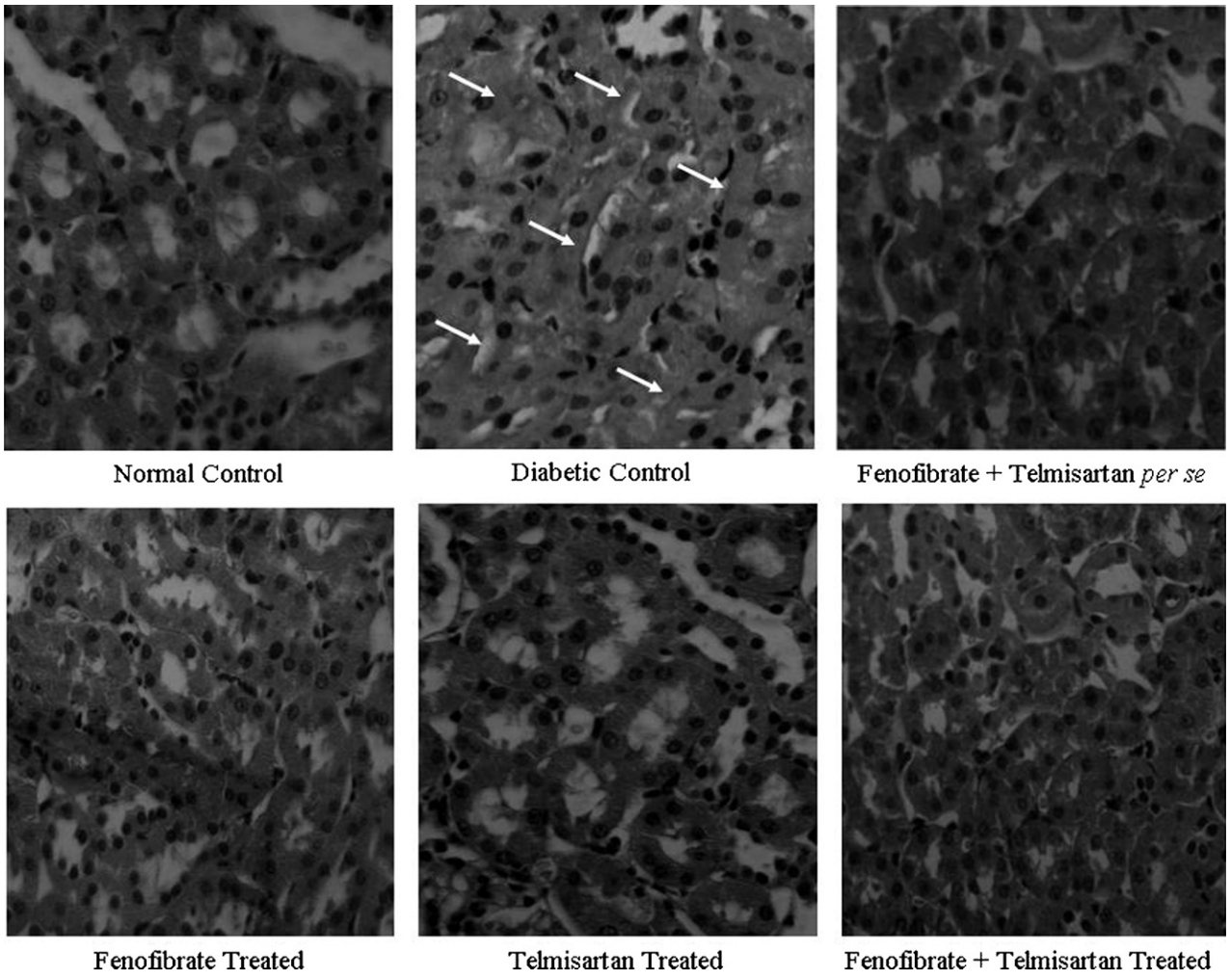

Fig. 7. Effect of fenofibrate, telmisartan and their combination on pathological changes in tubules of the kidney. The kidney of the diabetic rat developed pathological changes in the tubules such as tubular damage (indicated in arrow) as compared to normal rats. The administration of fenofibrate or telmisartan and their combination markedly reduced the pathological changes of tubules (greyscale representation).

time demonstrated that fenofibrate or telmisartan treatment was equipotent in preventing the onset of diabetes mellitus-induced experimental nephropathy where as their combination did not afford additional benefits in protecting the disease induction of the diabetic kidney.

The diabetes-associated hyperglycemia induces oxidative stress to damage the structure and function of the diabetic kidney [35,49]. The increase in TBARS and decrease in GSH are considered an index of the development of oxidative stress [26,50,51]. In the present study, diabetic rats after 8 weeks of STZ administration showed a marked increase in renal TBARS and a marked decrease in renal GSH. These results strongly suggest that diabetes mellitus-induced renal oxidative stress could have contributed significantly to renal structural and functional abnormalities noted in the present study. Treatment with fenofibrate and telmisartan either alone or in combination significantly attenuated diabetes-induced oxidative stress by reducing renal TBARS and consequently increasing renal GSH. However, their combination did not produce any additive effect in reducing the renal oxidative stress in diabetic rats.

On the basis of above discussion, it may be concluded that the diabetes mellitus-induced renal oxidative stress and lipid alteration, besides hyperglycemia, could have played a pivotal role in the induction of nephropathy in diabetic rats. The combination of fenofibrate and telmisartan was comparable to their individual treatment, but not superior, in preventing the diabetes mellitus-induced onset of nephropathy.

Conflict of interest

No conflict of interest has been declared.

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