Comparative Study between the Effect of Pentoxifylline versus Diltiazem versus Rosuvastatin on the Development and Progression of Nephropathy in Streptozotocin Induced Diabetic Rats

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Abstract. Background. Diabetic nephropathy is the major cause of end stage renal disease especially in developing countries. Reducing proteinuria is the mainstay of therapy in order to delay the progression of chronic kidney disease. Current therapeutic regimens provide only partial renoprotection, and a substantial number of patients who have proteinuria progress to end stage renal disease. Aim. The aim of this study was to evaluate the effects of pentoxifylline and diltiazem versus rosuvastatin on development and progression of nephropathy in streptozotocin induced diabetic rats and possible mechanisms of action. Materials and Methods. Eighty adult albino rats were randomly divided into eight groups: the first and second groups are normal control and diabetic control. Streptozotocin-induced type 1 diabetes mellitus (DM) model was set up in adult male albino rats by single intraperitoneal injection of streptozotocin (65mg/kg, I.P). The third, the fifth, and the seventh groups are non-diabetic groups of rats receiving pentoxifylline (40mg/kg/day, orally), diltiazem (10 mg/kg/day, i.p.), or rosuvastatin (10mg/kg/day, orally), respectively, for eight weeks. The fourth, the sixth, and the eighth groups are diabetic rats receiving pentoxifylline (40 mg/kg per day, orally), diltiazem (10mg/kg per day, i.p.), or rosuvastatin (10mg/kg/day, orally), respectively, for eight weeks. The glomerular filtration rate, serum urea, creatinine, urine albumin, urine volume changes, Na⁺ excretion level, K⁺ excretion level, renal blood flow, renal histopathology, and measurement of antioxidant enzymes activity (GSH, superoxide dismutase) of the different groups were tested compared to control group. Results. At the end of the eight weeks, use of pentoxifylline and rosuvastatin induced significant reduction of serum creatinine, urea, and urine albumin. Also, glomerular filtration rate, renal blood flow, and antioxidant enzymes were significantly improved. There were no significant differences between the two drugs, while diltiazem induced insignificant improvement in the previous parameters. Conclusion. The use of pentoxifylline and rosuvastatin is associated with a reduced risk of diabetic nephropathy by improving oxidative stress. Furthermore, diltiazem insignificantly ameliorates diabetic nephropathy.

Keywords: pentoxifylline, diltiazem, rosuvastatin, diabetic nephropathy, oxidative stress.
1. Introduction

Diabetic nephropathy is one of the major complications of type 1 and type 2 diabetes, and it is currently the leading cause of end-stage renal disease. Hyperglycemia is the driving force for the development of diabetic nephropathy. It is well known that hyperglycemia increases the production of free radicals resulting in oxidative stress [34]. Many reports have demonstrated that increased oxidative stress in diabetes plays an important role in the progression of diabetic complications, including nephropathy [26]. Oxidative stress is determined by the relationship between reactive oxygen species and the antioxidant defense system including antioxidant enzymes. Studies have shown that primary antioxidants or genetic manipulation of antioxidant defenses can ameliorate this oxidative stress and, consequentially, reduce the severity of diabetic complications in animal models [26].

Pentoxifylline, a non-selective phosphodiesterase inhibitor, exerts potent inhibitory effects against cell proliferation, inflammation, and extracellular matrix accumulation, all of which play important roles in chronic kidney progression [21]. Recent evidence shows that use of pentoxifylline reduces both proteinuria and microalbuminuria in subjects with diabetes.

Diltiazem is a non-dihydropyridine calcium channel blocker, which has beneficial effect on both reduction of blood pressure and proteinuria. Hypertension and high levels of proteinuria are independent risk factors for accelerated progression of renal failure. There is an increasing evidence that strict control of both blood pressure (BP) and proteinuria is beneficial in slowing the rate of progression of chronic renal disease in diabetic as well as non-diabetic nephropathy. Previous studies suggest that CCB do not worsen the progression of renal disease, but may rather provide benefit when systemic BP is tightly normalized [7].

Statins (3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors) are synthetic lipid-lowering agents that have antioxidant effects and became the preferred lipid lowering agents in diabetic patients [18]. Statins were reported to have a renoprotective action by reducing albuminuria and maintaining stable glomerular filtration rates in both experimental and clinical DN [11]. Some of these benefits may be due to lipid-lowering action of the drug, which is mainly due to the fact that lipid levels are strongly associated with the development and progression of diabetic nephropathy [11]. Other renoprotective mechanisms of statins, which are independent of the cholesterol lowering effect, are not fully understood [16].

The current therapeutic regimen for diabetic nephropathy is still far from being with a potential beneficial effect. New drugs need to be added. Thus, the aim of this study was to investigate and compare the effect of pentoxifylline and diltiazem versus rosuvastatin on development and progression of nephropathy in streptozotocin induced diabetic rats and possible mechanisms of action.

2. Materials and Methods

2.1. Chemicals and drugs

1. Streptozotocin (STZ) powder creamy white was purchased from Sigma Chemicals Co, USA.
2. Carboxy-methyl cellulose (powder) was purchased from El Nasr Pharmaceutical Chemicals Co.

3. Pentoxifylline, white powder, was purchased from Pfizer, USA.

4. Diltiazem, white powder, was purchased from Pfizer, USA.

5. Rosuvastatin, white powder, was purchased from AstraZeneca, Egypt.

2.2. Animals

Eighty adult male albino rats weighting 120–150gm were used. They were brought from Experimental Animal Breeding Farm, Helwan, Cairo, Egypt. All animals were housed in controlled laboratory conditions at 20–25°C in a 12h light/dark cycle and had free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They have acclimatized for one week and were caged (10/cage) in fully ventilated room (at room temperature) in Pharmacology Department, Benha Faculty of Medicine. All experimental protocols were approved by the ethics committee of Benha University.

2.3. Study design

After acclimatization for 1 week, rats were randomly divided into eight experimental groups, with 10 rats each, and treated for 8 weeks as follows:

1. Control Group, normal control rats.

2. Control Diabetic Group, rats injected with single dose of streptozotocin (65mg/kg/day i.p) in 0.1 mmol/l sodium citrate, pH 4.5 [5].

3. Pentoxifylline (40 mg/kg/day, orally) treated non-diabetic group [13].

4. Pentoxifylline (40mg/kg/day, orally) treated diabetic group [13].

5. Diltiazem (10mg/kg/day, i.p.) treated non-diabetic group [5].

6. Diltiazem (10mg/kg/day, i.p.) treated diabetic group [5].

7. Rosuvastatin (10mg/kg/day, orally) treated non-diabetic group [14].

8. Rosuvastatin (10mg/kg/day, orally) treated diabetic group [14].

2.4. Methods

In groups 2, 4, 6, and 8, diabetes was induced by single intraperitoneal injection of streptozotocin 65mg/kg (STZ powder from Sigma Chemicals Co., USA, with creamy white color that was dissolved in cold 0.1 mole citrated buffer at pH 4.5). After 48 hours, diabetes was confirmed in rats by measuring non-fasting blood glucose. A level of >10 mmol/l (using OneTouch glucometer strips) was considered as development of diabetes. Afterwards, groups 3 and 4 were administered pentoxifylline 40 mg/kg daily by intraperitoneal injection. Groups 5 and 6 were
administered diltiazem 10mg/kg (Pfizer, USA) daily through the same route. Groups 7 and 8 were administered rosuvastatin 10mg/kg, while groups 1 and 2 were administered vehicle. In all groups, blood samples were obtained from the tail vein after 12h fasting to measure the fasting blood glucose level. At the end of every week, during the whole course of study, animals were accommodated in special cage (metabolic cage) for urine collection. At the end of the 8th week, rats were anesthetized with pentobarbital and then were fixed on operating table; the abdominal cavity was opened and the kidneys were exposed. Right renal artery was located and freed. The flow probe was placed on top of the right renal artery for the measurement of renal blood flow by the flowmeter. After measuring RBF, arterial blood samples were collected in heparinized capillary tubes. Samples were incubated at 37°C until blood clotted, then centrifuged for separation of serum, and stored at -20°C for biochemical analysis of serum level of Na⁺, k⁺, urea, and creatinine. After blood collection, kidneys were removed. The left kidney was stored in -20°C for measuring antioxidant enzymes and the right kidney stored in formalin for detecting histopathological changes.

Doppler flowmeter (Hadeco ES 1000 SPM, Japan) was used for the measurement of renal blood flow. Spectrophotometer (CECIL,CE 1020) was used for the measurement of serum urea and creatinine and urine concentration of creatinine using Urea Liquicolor (Human Gmbh-65205, Giesbaden, Germany). Flame photometer (JENWAY, United Kingdom) was used for the measurement of urine and serum Na⁺, K⁺ levels. Cobas c 111 analyzer (Roche Diagnostic’s representative, Germany) was used for measuring urinary albumin using Albumin kit (ALBT2, Tina-quant, Germany). Microplate Reader (Platos R 496) was used for measuring glutathione (GSH). Fluoroskan Ascent (2.6 Thermo Scientific) was used for measuring total antioxidant capacity (TAC) and super oxide dismutase (SOD).

2.4.1. Measurement of glomerular filtration rate

Serum and urinary creatinine concentration were determined using the colorimetric method on spectrophotometer. Clearance is estimated as the ratio of urine to plasma concentration of creatinine multiplied by urine flow rate.

\[
\text{Cr CL} = \frac{[U_{\text{Cr}} \times V]}{S_{\text{Cr}}}
\]

\[
\text{GFR} = \frac{[U_{\text{Cr}} \times V]}{S_{\text{Cr}}}
\]

2.4.2. Measurement of antioxidant enzymes activity

All homogenates were prepared from kidney cortex. The kidney cortex tissue was weighed and used to prepare 20% homogenates with a Potter-Elvehjem Teflon-glass homogenizer. Crude homogenate was centrifuged at 3000rpm in 15 minutes. Estimation of GSH: GSH activity was determined by the procedure of Carlberg and Mannervik 1985. The assay solution contained 10% BSA (bovine serum albumin), 50 mM potassium phosphate buffer (pH 7.6), 2 mM NADPH, and 20 mM oxidized glutathione. Absorbance at 340 nm was recorded at a temperature of 250°C. The activity was calculated using the molar coefficient for NADPH of 6.22 μmol–1 x cm–1 and expressed in U/g of tissue. Levels of superoxide dismutase in the cell free supernatant was measured by the method of Kono et al. (1978). Briefly, 1.3 mL of solution A (0.1 mM doi:10.11131/2018/101367
EDTA containing 50 mM Na2CO3, pH 10.5), 0.5 mL of solution B (90 mM NBT-nitro blue tetrazolium dye), 0.1 mL of solution C (0.6% TritonX-100 in solution A), and 0.1 mL of solution D (20 mM hydroxylamine hydrochloride, pH 6.0) were mixed, and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 mL of the supernatant was added to the test cuvette as well as the reference cuvette, which does not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) required, inhibiting the reduction rate by 50% in one minute. Estimation of total antioxidant capacity: the total antioxidant capacity (TAC) was measured using an antioxidant assay kit (Bio Vision). TAC was measured using the ferryl myoglobin/ABTS spectrophotometric assay by generating the chromogenic ABTS + radical cation from the interaction between ABTS, metmyoglobin, and hydrogen peroxide as previously described. Trolox was used as an antioxidant standard to calculate Trolox equivalent antioxidant capacity; absorbance readings were taken at 520 nm.


Results are presented as mean ± standard error (mean ± SEM); statistical analysis was performed using one-way analysis of variance (ANOVA) to detect significant differences between the group means. Tukey Kramer post-test was used to determine the level of significance. Probability (P) values of < 0.05 were considered as statistically significant.

4. Results

In the present study, a single dose of streptozotocin (65 mg/kg, i.p.) significantly increased (p < 0.05) fasting blood glucose level (FBG) in 24-hour urinary albumin excretion, plasma urea, creatinine, and sodium and potassium levels in all diabetic groups compared with normal control group (Figures 1, 2; Table 1).

Treatment with pentoxifylline at a dose of 40 mg/kg produced marked improvement in diabetic nephropathy manifested by the significant decreases (p < 0.05) in 24-hour urinary albumin excretion, serum levels of urea, creatinine, k+ levels, and Na+ levels compared with diabetic non-treated rats (Figure 2; Table 1). On the other hand, administration of pentoxifylline to non-diabetic rats produced insignificant changes in fasting blood sugar and 24-hour urinary albumin excretion. Also, serum levels of urea, creatinine, and sodium and potassium levels showed no changes compared with normal control group (Figures 1, 2; Table 1).

Treatment with rosuvastatin at a dose of 10 mg/kg produced marked improvement in diabetic nephropathy manifested by significant decreases (p < 0.05) in 24-hour urinary albumin excretion, serum levels of urea, creatinine, k+ levels, and Na+ levels compared with diabetic non-treated rats (Figure 2; Table 1). On the other hand, administration of rosuvastatin to non-diabetic rats produced insignificant changes in fasting blood sugar and 24-hour urinary albumin excretion. Also, serum levels of urea, creatinine, and sodium and potassium levels showed no changes compared with normal control group (Figures 1, 2; Table 1).

Treatment with diltiazem at a dose of 10 mg/kg in diabetic and non-diabetic rats produced insignificant decrease (p > 0.05) in fasting blood glucose, 24-hour urinary albumin excretion,
serum urea, creatinine, and potassium and sodium levels compared with diabetic non-treated and normal control groups, respectively (Figures 1, 2; Table 1).

Figure 1: Effect of treatment with pentoxifylline (40 mg/kg), diltiazem (10mg/kg), and rosuvastatin (10mg/kg) for 8 weeks on fasting blood glucose level in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM. a: Significant difference versus control group at p < 0.05.

Figure 2: Effect of treatment with pentoxifylline (40 mg/kg), diltiazem (10mg/kg), and rosuvastatin (10mg/kg) for 8 weeks on urinary albumin excretion in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM. a: Significant difference versus control group at p < 0.05. b: significant difference versus diabetic control group at p < 0.05.

At eight weeks after induction of diabetic nephropathy, estimation of glomerular filtration rate (GFR) and renal blood flow show that STZ-injection resulted in significant decrease (p < 0.05) in both parameters compared to control group (Figures 3, 4), while there was a significant decrease (p < 0.05) in urine volume compared to control group (Figure 5).

Treatment of diabetic rats with pentoxifylline at a dose of 40mg/kg produced marked improvement in glomerular filtration rate and renal blood flow (p < 0.05) compared with diabetic non-treated group, while urine volume was increased with pentoxifylline administration compared with diabetic non-treated group (Figure 5). On the other hand, pentoxifylline produced insignificant changes in GFR, renal blood flow, and urine volume in non-diabetic group compared with normal control group (Figures 4, 5, and 6).

At the same time, treatment of diabetic rats with diltiazem at a dose of 10mg/kg produced insignificant increase (p > 0.05) in glomerular filtration rate and renal blood flow (p > 0.05) compared with diabetic non-treated group (Figures 3, 4). Also, diltiazem administration produced insignificant increase (p > 0.05) in urine volume compared with diabetic non-treated group (Figure 5).
Table 1: Effects of pentoxifylline (40 mg/kg), diltiazem (10mg/kg), and rosuvastatin (10mg/kg) on serum levels of urea and creatinine, sodium excretion, and K⁺ excretion in STZ induced diabetic nephropathy in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na⁺ excretion (mmol/24h)</th>
<th>K⁺ excretion (mmol/24h)</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.2 ± 5.31</td>
<td>60.5 ± 4.71</td>
<td>18.33 ± 1.1</td>
<td>1 ± 0.07</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>23.41 ± 2.3ᵃ</td>
<td>24.3 ± 2.11ᵃ</td>
<td>120.22 ± 6.7ᵃ</td>
<td>2.8 ± 0.1ᵃ</td>
</tr>
<tr>
<td>PTX non-diab.</td>
<td>69.1 ± 4.2</td>
<td>55.9 ± 3.33</td>
<td>22.13 ± 1.3</td>
<td>0.9 ± 0.03</td>
</tr>
<tr>
<td>PTX diab.</td>
<td>57.8 ± 3.22ᵇ</td>
<td>45.6 ± 2.82ᵇ</td>
<td>45.77 ± 4.3ᵇ</td>
<td>1.2 ± 0.5ᵇ</td>
</tr>
<tr>
<td>Dilt non-diab.</td>
<td>68.3 ± 3.9</td>
<td>57.7 ± 3.11</td>
<td>31.74 ± 2.1</td>
<td>0.92 ± 0.02</td>
</tr>
<tr>
<td>Dilt diab.</td>
<td>29.33 ± 3.9</td>
<td>27.2 ± 2.7</td>
<td>113.3 ± 4.9</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Rosu. non-diab.</td>
<td>75.1 ± 6.1</td>
<td>61.3 ± 1.1</td>
<td>17.2 ± 1.3</td>
<td>1.1 ± 0.03</td>
</tr>
<tr>
<td>Rosu. diab.</td>
<td>61.4 ± 4.1ᶜ</td>
<td>55.5 ± 2.7ᶜ</td>
<td>40.4 ± 5.6ᶜ</td>
<td>1.3 ± 0.01ᶜ</td>
</tr>
</tbody>
</table>

a: Significant difference from control group.
b: Significant difference of PTX diabetic group from diabetic non-treated group.
c: Significant difference of rosuvastatin treated diabetic group from diabetic non-treated group.

Furthermore, treatment of diabetic rats with rosuvastatin at a dose of 10mg/kg produced marked improvement in glomerular filtration rate and renal blood flow (p < 0.05) compared with diabetic non-treated group, while urine volume was increased with rosuvastatin administration compared with diabetic non-treated group (Figure 5). On the other hand, rosuvastatin produced insignificant changes in GFR, renal blood flow, and urine volume in non-diabetic group compared with normal control group (Figures 4, 5, and 6).

As regarding glutathione (GSH), superoxide dismutase (SOD) activity, and total capacity antioxidants (TCA), estimation of GSH, SOD, and TCA levels shows that STZ administration resulted in significant decrease (p < 0.05) in these parameters compared to control group (Figures 6, 7, and 8).

Treatment of diabetic rats with pentoxifylline at a dose of 40mg/kg produced marked improvement in GSH, SOD, and TCA (P < 0.05) compared with diabetic non-treated group (Figures 6, 7, and 8), while pentoxifylline treatment at dose of 40mg/kg produced no change in GSH, SOD, and TCA levels compared with normal control (Figures 6, 7, and 8).
Figure 4: Effect of pentoxifylline, diltiazem, and rosuvastatin treatment on renal blood flow in diabetic and non-diabetic rats. Effect of treatment with pentoxifylline (40 mg/kg), diltiazem (10mg/kg), and rosuvastatin (10mg/kg) for 8 weeks on renal blood flow in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM. a: Significant difference versus control group at p < 0.05. b: Significant difference versus diabetic control group at p < 0.05.

Figure 5: Effect of treatment with pentoxifylline (40mg/kg), diltiazem (10mg/kg), and rosuvastatin (10mg/kg) for 8 weeks on urine volume (ml) in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM.

Treatment of diabetic rats with rosuvastatin at a dose of 10mg/kg produced marked improvement in GSH, SOD, and TCA (P < 0.05) compared with diabetic non-treated group (Figures 6, 7, and 8), while rosuvastatin treatment at a dose of 10mg/kg produced no change in GSH, SOD, and TCA levels compared with normal control (Figures 6, 7, and 8), while treatment of diabetic rats with diltiazem at a dose of 10mg/kg produced moderate improvement in GSH, SOD, and TCA (p < 0.05) compared with diabetic non-treated group (Figures 6, 7, and 8).

Figure 6: Effect of pentoxifylline, diltiazem, and rosuvastatin treatment on GSH (mmol/mg protein) in diabetic and non-diabetic rats. Effect of treatment with pentoxifylline (40 mg/kg) and diltiazem (10mg/kg) for 8 weeks on GSH in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM. a: Significant difference versus control group at p < 0.05. b: Significant difference versus diabetic control group at p < 0.05.
Figure 7: Effect of pentoxifylline, diltiazem, and rosvastatin treatment on SOD (mmol/mg protein) in diabetic and non-diabetic rats. Effect of treatment with pentoxifylline (40 mg/kg, orally), diltiazem (10mg/kg, i.p.), and rosvastatin (10mg/kg, orally) for 8 weeks on SOD in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM. a: Significant difference versus control group at p < 0.05. b: Significant difference versus diabetic control group at p < 0.05.

Figure 8: Effect of pentoxifylline, diltiazem, and rosvastatin treatment on TCA (mmol/mg protein) in diabetic and non-diabetic rats. Effect of treatment with pentoxifylline (40 mg/kg, orally), diltiazem (10mg/kg, i.p.), and rosvastatin (10mg/kg, orally) for 8 weeks on TCA in experimentally STZ induced diabetic nephropathy. Data are presented as mean ± SEM. a: Significant difference versus control group at p < 0.05. b: Significant difference versus diabetic control group at p < 0.05.

At the same time, the histopathological findings also supported these biochemical observations and indicated the presence of diabetic nephropathy in STZ injected rats (Figure 9), revealing diffuse diabetic glomerular sclerotic lesion, which is characterized by diffuse thickening of glomerular capillary wall and generalized increase in mesangial matrix in all mesangial regions of all glomeruli compared with normal control group (Figures 9, 10, 11, 12, 13, 14, and 15).

5. Discussion

Diabetic nephropathy (DN) is the major cause of end stage renal disease (ESRD) in developing countries. End stage renal disease requires dialysis and is becoming a staggering challenge to public health care systems due to the prohibitive costs of renal replacement therapy that could become unaffordable even for developed countries. Advanced diabetic nephropathy is also the leading cause of glomerulosclerosis and end stage renal disease worldwide. Between 20% and 40% of all diabetic patients are prone to developing renal failure [36].

Hyperglycemia is a crucial factor in the development of diabetic nephropathy because of its effects on glomerular and mesangial cells, but alone, it is not causative [37]. Dyslipidemia is an
Figure 9: Light microscopic finding of renal section of normal and diabetic rats. A: Normal control rat showing normal glomerulus (original magnification X400). B: Diabetic rat showing a glomerulus with mild increase in the mesangial matrix and cellularity (original magnification X600). C: An overall appearance of the normal control rat kidney (original magnification X100). D: An overall appearance of the diabetic rat kidney with mild tubular dilatation areas indicated by the stars and foamy cells of the tubules indicated by the arrow (original magnification X100). E: Normal control rat section showing slightly shrinkage of Bowman’s capsule and glomerulus with normal tubular appearance, (original magnification X600). F: Diabetics rat showing shrinkage of Bowman’s capsule and glomerulus (ischemic) (original magnification X600).

Figure 10: Light microscopic finding of renal section of pentoxifylline treated non-diabetic rats. A: A glomerulus with mild increase in mesangial matrix (original magnification X600). B: Normal appearance of the section with mild tubular dilatation (original magnification X100).

important risk factor for the development and progression of diabetic kidney diseases as it leads to microvascular damage and progression of albuminuria [38].
Experimental and clinical studies over the last two decades have suggested that albuminuria may not only be a marker of renal injury but also be an independent risk factor for the progression of kidney damage in diabetes. Amelioration of albuminuria is now considered one of the targets in the prevention and retardation of diabetic nephropathy [39].

The present study was designed to explore the beneficial prophylactic effects of pentoxifylline, diltiazem, and rosuvastatin on the progression of diabetic nephropathy in rats models of streptozotocin induced diabetes (Type I D.M).

The obtained data in the current work revealed that induction of diabetes (Type I D.M) by single intraperitoneal injection (i.p.) of STZ 65 mg/Kg of experimental rats resulted in increases in non-fasting blood glucose levels > 300 mg/100 ml 72 h after injection. These data are in line with those of previous studies [13, 40, 41]. These experimentally induced diabetic untreated rats developed diabetic nephropathy in eight weeks and showed significant increase in 24 hr. urinary albumin excretion, serum urea, creatinine, and K level, while GFR, renal blood flow, and antioxidants levels significantly decreased. This is in agreement with Kurundkar et al. (2010), Edremitlioglu et al. (2011), and Mianzhi et al. (2012).

Administration of pentoxifylline for 8 weeks to diabetic rats ameliorated proteinuria, serum urea, and creatinine. Also, they improved glomerular filtration rate and renal blood flow. These findings are in agreement with those of Han et al. (2010), who reported that pentoxifylline significantly ameliorated proteinuria after eight weeks of administration in STZ induced diabetic nephropathy. Similarly, long term administration of PTX in rats was reported to diminish the elevation in urinary protein excretion, plasma creatinine, and urea levels and reduce the extent of glomerular and tubule-interstitial lesions [6, 42]. In addition, Badri et al. (2011) demonstrated
Figure 12: Light microscopic finding of renal section of diltiazem treated non-diabetic rats. A: A glomerulus with normal mesangial cellularity and matrix and normal tubules (original magnification X600). B: Normal appearance of the section with no tubular dilatation (original magnification X100). C: Higher magnification of the tubules showing increased sized protein droplets, indicated by arrows (original magnification X600).

Figure 13: Light microscopic finding of renal section of diltiazem treated diabetic rats. A: A glomerulus with normal mesangial cellularity and matrix and normal tubules. Arrows indicate the protein droplets (original magnification X600). B: General appearance of the kidney section with increased tubular dilatation indicated by the stars and foamy cells showing darker pink stain areas indicated by the arrows (original magnification X100).

that short term use of PTX produced a significant reduction of proteinuria in subjects with
Figure 14: Light microscopic finding of renal section of rosuvastatin treated non-diabetic rats. A: A glomerulus with normal mesangial cellularity and matrix (original magnification X600). B: General appearance of the section with increased tubular dilatation (original magnification X100). C: Inflammatory infiltrate indicated by the stars and tubular atrophy indicated by the arrows (original magnification X400).

Figure 15: Light microscopic finding of renal section of rosuvastatin treated diabetic rats. A: a glomerulus with normal mesangial cellularity and matrix; foamy vacuolated tubules are seen indicated by the arrows (original magnification X600). B: A section showing increased tubular dilatation indicated by the stars and foamy cells indicated by the arrows (original magnification X400).

diabetic and also non-diabetic kidney diseases. These results are supported by the finding of several previous studies that pretreatment with pentoxifylline before liver ischemic reperfusion ameliorates renal oxidative damage by preservation of cellular GSH concentration and a reduction in MDA levels [31].

Our results demonstrated that rosuvastatin significantly ameliorated diabetic nephropathy as it improved albuminuria, serum urea, and creatinine levels in diabetic rats. Also, rosuvastatin restored normal GFR and improved renal blood flow compared with diabetic non-treated
group. In agreement with our results, Rondi et al. (2014) observed that rosuvastatin significantly decreased serum urea nitrogen and creatinine levels in streptozotocin-induced type 2 diabetic rats undergoing treatment with metformin and glimepiride. Furthermore, it has been reported that rosuvastatin has an antioxidant activity and improves enzymatic antioxidant parameters like superoxide dismutase, catalase, and glutathione peroxidase [33]. Consistent with our reports, Mooradian and Hass (2007) demonstrated that rosuvastatin ameliorates glomerular permeability changes in streptozotocin-induced diabetic rats. And this effect is due to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, because mevalonate treatment reversed the favorable effects of rosuvastatin. Also, Giunti et al. (2010) reported that rosuvastatin prevented diabetes associated renal extracellular matrix accumulation. Rosuvastatin reduced accumulation of advanced glycation end products, NADPH oxidase 4, and nitrotyrosine. In accordance with this work, the results of Hussein and Mahfouz (2016) indicate that rosuvastatin with resveratrol had renoprotective effects through improving glycemic control and attenuating oxidative stress damage in renal tissues of diabetic rats.

On the other hand, our results demonstrated that diltiazem was unable to improve albuminuria or the increased serum urea and creatinine level. Murray (1991) reported that diltiazem had beneficial effects in hypertensive patients with diabetic nephropathy. In line with our work, Praxis (2000) found that diltiazem alone produced moderate reduction of proteinuria, but the combination of ACE with diltiazem produced a significant reduction of proteinuria as well as showing an improvement in kidney function in patients with diabetic nephropathy. Another study by Perez-Maraver et al. (2005) demonstrated that the addition of diltiazem to ACE inhibitors has beneficial effects in type 2 microalbuminuric patients at high risk for progression to diabetic nephropathy. Other previous studies have suggested that calcium channel blockers may improve renal function in patients with essential hypertensive renal disease or diabetic renal disease. These effects may be due to the reduction in renal vascular resistance and increase of renal plasma flow and glomerular filtration rate [28]. Similarly, Anjaneyulu and Chopra (2005) revealed that treatment with diltiazem in diabetic rats significantly reduced creatinine and urea and improved albuminuria, as compared with control rats. The discrepancy between our results and other data may relate to the doses used.

In the present study, besides the increased level of serum urea and creatinine, plasma levels of antioxidants (GSH, SOD, and TCA) were significantly decreased after induction of diabetic nephropathy.

Increased reactive oxygen species (ROS) leads to lipid peroxidation, which increases oxidative injury and causes damage to cellular protein and nucleic acid [34]. Many mechanisms are involved in the production of ROS including glucose autoxidation, non-enzyme protein glycation, and generation of advanced glycation end products activation of protein kinase C and NADPH oxidase. It has demonstrated that glomeruli are especially sensitive to oxidative injury that contributes to the progression of diabetic nephropathy [26].

It is well known that the extent of renal tissue damage in diabetic nephropathy correlates with the levels of inflammatory mediators and oxidative damage [9, 32]. Similarly, the present study confirms and extends the previous findings by Aghadavod et al. (2016) who demonstrated that the STZ induced diabetic nephropathy is accompanied by remarkable increased plasma levels of free radicals together with inflammatory mediators. Davila-Esqueda and Martinez-Morales (2004) found that the increased serum lipoperoxidase levels and decreased levels of
total antioxidant activity were demonstrated after 2 days of STZ injection in rats. Moreover, An et al. (2015) reported that increased serum levels cystatin C, malondialdehyde, and decreased serum and kidney levels of superoxide dismutase were detected 8 weeks after the induction of diabetic nephropathy with STZ in rats versus normal control.

The present study focuses on the effect of pentoxifylline, diltiazem, and rosuvastatin on experimentally diabetic nephropathy and possible antioxidant effects. Our data indicate that they exhibit an antioxidant effect as evidenced by the significant increase in serum GSH, SOD, and TCA. This is confirmed by the increase renal blood flow and histopathological manifestation improvement compared to diabetic control. These results are in agreement with those of Krysztopik et al. (1996) who reported that pentoxifylline improves renal blood flow during bacteremia due to pre- and postglomerular vasodilation. These responses may be a consequence of the increased intracellular cAMP and release of vasodilator prostanoids. Also, Krysztopik et al. (2000) found that pentoxifylline has a protective effect on the renal microcirculation during sepsis by altering renal arachidonic acid metabolism. Also, Li et al. (2005) found that rosuvastatin has a favorable effect on diabetic neuropathy via restoration and preservation of the microcirculation of the sciatic nerve. This effect may be mediated through nitric oxide synthase and phosphatidylinositol 3-kinase signaling pathways.

The histopathological manifestations in this work confirmed other results. In line with our results, Gomez-Garcia et al. (2007) found that rosuvastatin and metformin decrease inflammation and oxidative stress in patients with hypertension and dyslipidemia. Also, Abe et al. (2011) found that rosuvastatin administration reduced albuminuria, oxidative stress, and serum cystatin C levels, and inflammation and improved lipid profiles, regardless of the presence or absence of DM and the degree of the estimated GFR.

In addition, diabetic nephropathy was also confirmed in our study by histopathological changes observed microscopically during the same duration in the present study. Histopathological assessment revealed that induction of diabetic nephropathy revealed diffuse diabetic glomerular sclerotic lesion, which is characterized by diffuse thickening of glomerular capillary wall and a generalized increase in the mesangial matrix in all mesangial regions of all glomeruli. This is in agreement with the previous reports by Davila-Esqueda et al. (2005). Administration of pentoxifylline for 8 weeks showed mild thickening of glomerular capillary wall and mild changes in mesangial matrix. These finding are supported by previous studies, where administration of pentoxifylline to diabetic rats significantly decreased sodium retention by enhancing urinary sodium excretion and reduced renal hypertrophy [8]. Also, the data of the present work showed that diltiazem moderately improved histopathological changes in diabetic rats. This is in line with the results of Gaber et al. (1994) who reported that calcium channel antagonist attenuates morphologic progression of diabetic renal disease. These results supported by Park et al. (2017) who used rosuvastatin and cilostazol alone or in combination in high fat diet-induced nephropathy. They improved histopathological changes, including glomerular mesangial expansion, tubular vacuolation, apoptosis, and lipid accumulation. In addition, Kim et al. (2016) reported that rosuvastatin attenuated the glomerular endothelial proliferation and inhibited interstitial fibrosis by suppressing the increases in transforming growth factor-B-1 and plasminogen activator inhibitor-1 in high fat diet/streptozotocin-induced nephropathy in mice.
6. Conclusion

From the previous observations, we suggest that the use of pentoxifylline and rosuvastatin is associated with a reduced risk of diabetic nephropathy by improving inflammatory status and oxidative stress. Furthermore, diltiazem alone does not ameliorate diabetic nephropathy.

Competing Interests

The authors declare no competing interests.

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