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NEPHROPROTECTIVE EFFECTS OF CANDESARTAN ON DIABETIC NEPHROPATHY IN RATS

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Abstract

Diabetic nephropathy (DN) stands out as a primary contributor to end-stage kidney damage. The renin-angiotensin system (RAS) plays a pivotal role in the advancement of DN, making angiotensin receptor blockers (ARBs) particularly noteworthy due to their influence on angiotensin II in DN development. This study investigated the impact of the angiotensin receptor blocker candesartan (CAN) on rats with streptozotocin (STZ)-induced DN, characterized by albuminuria, renal hypertrophy, and mild glomerulosclerosis. DN was induced in normotensive Wistar rats through a single injection of STZ (60 mg/kg ip).

STZ administration led to diabetes mellitus (DM) symptoms and DN indicators, such as poor general condition, weight loss, increased kidney weight, elevated serum creatinine levels and BUN, augmented diuresis, and notable albuminuria. These manifestations were prominent at 4 weeks, intensifying further at 8 and 12 weeks post-STZ injection. Commencing candesartan treatment (5 mg/kg BW) at the 4-week mark post-STZ injection significantly alleviated all DN symptoms, reducing serum creatinine values and BUN, albuminuria, and diuresis.

Histopathological examination at 8 and 12 weeks revealed that candesartan effectively mitigated glomerulopathy progression, improved the glomerulosclerotic index, and attenuated renal histological abnormalities induced by STZ. In conclusion, candesartan treatment ameliorates STZ-induced nephropathic changes in DM rats.

Key words: Diabetic Nephropathy, streptozotocin, candesartan, Glomerulosclerosis, Rats.

Introduction

Diabetic nephropathy (DN) poses a significant challenge in nephrology, contributing to 40% of end-stage renal disease (ESRD) cases. DN manifests with persistent albuminuria, escalating arterial blood pressure, a gradual decline in glomerular filtration rate (GFR), and an increased risk of cardiovascular morbidity [1] and mortality [2,3].

Although the exact mechanisms of DN remain somewhat elusive, recent evidence highlights the crucial role of the renin-angiotensin system (RAS) in its progression [4-6].

Angiotensin II, the primary vasoactive hormone of the renin-angiotensin-aldosterone system (RAAS), is implicated in mediating proteinuria through various mechanisms, including hyperfiltration, alterations in the glomerular basement membrane composition, and reduced nephrin expression on podocytes [7].

Physiological effects of angiotensin II, such as vasoconstriction, aldosterone stimulation, regulation of salt and water homeostasis, and cell growth stimulation, predominantly occur through the angiotensin type 1 (AT1) receptor [8].

Systemic and glomerular hypertension, partly resulting from RAAS activation, are recognized as key factors driving the progression of chronic kidney disease (CKD), regardless of the initial insult [9-11].

Recent insights suggest that renal inflammation, characterized by macrophage infiltration, also contributes to renal disease progression, with angiotensin II acting as a proinflammatory cytokine, playing a crucial role in the advancement of diabetic microvascular and tubulointerstitial conditions [12-15].

Candesartan (CAN), a potent and selective, long-acting angiotensin II type 1 receptor blocker (ARB), tightly binds to and dissociates slowly from the AT1 receptor. Its adaptable dosage regimen

suggests it as an effective and well-tolerated alternative for treating a diverse range of hypertensive patients [16-18]. The streptozotocin (STZ)-induced DN in rats serves as an excellent model for evaluating ARB treatment, specifically candesartan. This model exhibits progressive development of severe glomerular sclerosis and tubulointerstitial fibrosis in STZ-induced diabetes mellitus (DM) rats, along with a high similarity in intrarenal enzyme distribution to that in humans. The primary focus remains on examining the effects of candesartan treatment in rats with STZ-induced DM, characterized by albuminuria, renal hypertrophy, and mild glomerulosclerosis.

Materials and Methods

Animal model

A total of 75 normotensive Wistar rats, both male and female, aged 9 to 11 weeks and weighing between 160-300g, were utilized for this study. All rats were housed in a controlled environment, maintaining a temperature of 20±2ºC, with a 12-hour light/dark cycle. They had continuous access to fresh water and a standard laboratory diet.

Diabetes mellitus (DM) was induced through a single intraperitoneal (ip) injection of streptozotocin (STZ) obtained from Sigma-Aldrich, Chemie GmbH, Germany, at a dosage of 60 mg/kg dissolved in 0.1 M citrate buffer (pH 4.5). The control group received an ip injection of citrate buffer alone, serving as control nondiabetic rats.

Diabetes was confirmed 72 hours post-STZ application by measuring blood glucose levels using an Accu-Chek blood glucose monitor from Roche Diagnostic, Germany. Blood samples for glucose determination were collected through tail bleeding. Rats with blood glucose levels $\geq$ 11 mmol/L under fasting conditions (morning blood samples) were included in the study. To induce diabetic nephropathy (DN), animals were maintained in a diabetic state without any treatment for the subsequent 4 weeks.

The diabetic rats (n=50) were randomly allocated to two experimental groups: the STZ group (n=25), left untreated for the next 8 weeks to evaluate symptoms and signs of diabetic nephropathy, and the STZ+CAN group (n=25), treated with candesartan (CAN) at a dose of 5 mg/kg/day via gavage from week 4 to week 12. The control group (nondiabetic rats, n=25) received saline in the same volume and at the same intervals as the groups receiving the tested drugs.

Biochemical parameters

The blood glucose levels were assessed at different time points throughout the study. Specifically, measurements were taken before the commencement of the investigation, 72 hours after the administration of streptozotocin (STZ), and subsequently at 4, 8, and 12 weeks from the start of the study. These periodic assessments provided a comprehensive understanding of the changes in blood glucose levels over the course of the experiment.

Renal functional tests

Throughout the study, the renal function of the examined animals was evaluated through the determination of serum creatinine and blood urea nitrogen (BUN) using an autoanalyzer (Cobas Integra 400 Plus; Roche Diagnostics, Germany). Additionally, the 24-hour urine volume was measured. Urine albumin in 24-hour urine samples was determined using the same autoanalyzer.

These assessments were conducted at multiple time points: before the initiation of the study (Day 0), and at 4, 8, and 12 weeks from the beginning of the study. To determine serum creatinine and BUN levels, blood samples were obtained by venepuncture from the orbital sinus of rats under light ether anesthesia. Serum separation was performed with 400 µl of blood, and 200 µl were used for analysis. Metabolic cages were employed to collect 24-hour urine samples for the quantification of urinary albumin levels.

Body weight

The body weight of the examined animals was measured weekly throughout the entire study. Additionally, at the conclusion of the 12-week study period, the body weight/kidney weight ratio was calculated as part of the assessment.

Histopatological examination and renal histology

At 8 and 12 weeks following streptozotocin (STZ) administration, euthanasia of the animals was carried out by intraperitoneal injection of pentobarbital (50 mg/kg; Boehringer Ingelheim). Subsequently, a midline incision was made to open the abdomen, and both kidneys were removed. The kidneys were immediately bisected, fixed in 10% buffered formaldehyde, and then embedded in paraffin. At least 6 sections were cut at 4-6 μm thickness and subjected to staining with haematoxylin and eosin, periodic acid-Schiff (PAS), silver methenamine Jones, and trichrome Masson.

To assess the severity of glomerular sclerosis, 20 randomly selected non-overlapping fields per kidney were examined at a magnification of x 200 using a Nikon light microscope. Glomerular sclerosis was characterized by glomerular basement membrane thickening, mesangial hypertrophy, and capillary occlusion.

The degree of glomerular damage was evaluated through a semi-quantitative scoring method: grade 0 for normal glomeruli, grade 1 for sclerotic area up to 25% (minimal sclerosis), grade 2 for sclerotic area 25 to 50% (moderate sclerosis), grade 3 for sclerotic area 50 to 75% (moderate-severe sclerosis), and grade 4 for sclerotic area 75 to 100% (severe sclerosis). The glomerulosclerotic index (GSI) was calculated using the formula: $GSI = (1 \times n1) + (2 \times n2) + (3 \times n3) + (4 \times n4)/n0 + n1 + n2 + n3 + n4$, where n_x represents the number of glomeruli in each grade of glomerular sclerosis [19]. This analysis was conducted with the observer masked to the treatment groups in a double-blinded manner to prevent bias.

The changes identified through light microscopy were further validated by transmission electron microscopy (TEM). Kidney tissue samples, measuring 1-2mm², underwent a deparaffinization and rehydration procedure before being postfixed in 1% OsO4 for 1 hour. The samples were then processed for embedding in Durcupan resin. Semi-thin sections were stained with Toluidine blue, while ultrathin sections obtained from an ultramicrotome (PT-PC PowerTome Ultramicrotomes-RMC Products) were contrasted in an autostainer (QG-3100 Automated TEM Stainer-RMC Products) for ultrathin sections using Uranyl acetate and Lead citrate. Subsequently, samples were analyzed on a transmission electron microscope (JEOL JEM 1400, JAPAN) equipped with a digital camera (Veleta TEM Camera, Olympus, Germany) and controlled by iTEM software v.5.2.

Statistical analyses

The statistical analysis of the data involved expressing all results as mean ± SD. To compare more than two groups, Kruskal-Wallis variance analysis was utilized, followed by the Mann-Whitney U-test to determine which groups exhibited significant differences. A p-value less than 0.05 was considered statistically significant. This rigorous statistical approach ensured reliable interpretation of the experimental outcomes.

Results

The streptozotocin (STZ) rats experienced an overall deterioration in condition, characterized by notable body-weight loss and increased urine volume at 4, 8, and 12 weeks after drug administration compared to the control (nondiabetic) rats. Moreover, both kidney weight and the body/kidney weight ratio of the STZ rats exhibited a significant increase by the end of the 12th week in comparison to the control group (Table 1).

Conversely, diabetic rats treated with candesartan from week 4 to week 12 demonstrated less pronounced body weight loss and a mitigated increase in the kidney/body weight ratio. Although there was a significant difference in the kidney/body weight ratio compared to the control group (3.20 ± 0.29 vs. 4.31 ± 0.48; $p < 0.05$) at the study's conclusion, this ratio was significantly lower than that in the STZ group (4.31 ± 0.48 vs. 5.41 ± 0.69; $p < 0.05$) (Table 1).

Table 1. Effects of candesartan on body weight/renal weight (mg/g) ratio in rats with STZ induced DN

Body weight/renal weight mg/g (12 week)			
	Control	**STZ**	**CAN**
X	3.20	5.41[a]	4.31[a,b]
SD	0.29	0.69	0.48
Min	2.81	4.40	3.73
Max	3.68	6.49	5.50

[a]<0.05 vs Control

[b]<0.05 vs STZ

Renal function

Serum concentrations of creatinine, blood urea nitrogen (BUN), and urinary excretion of albumins served as fundamental parameters for assessing renal function. In the STZ group, the serum concentrations of creatinine were doubled after 8 weeks compared to baseline values ($p < 0.05$). By the end of the 12-week study, there was an additional increase in serum creatinine values. In contrast, the group of animals receiving candesartan treatment exhibited only a mild increase in serum creatinine concentrations after 8 and 12 weeks (Figure 1).

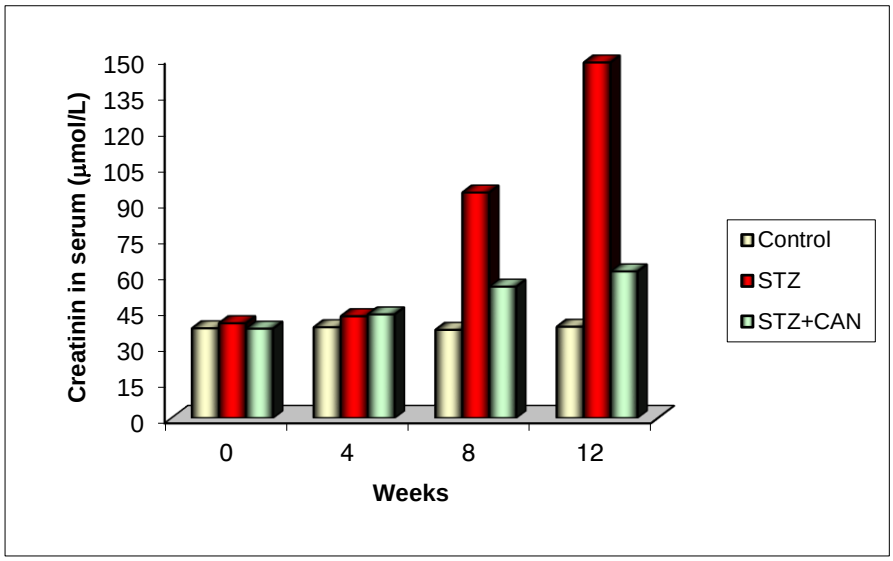

Figure 1. Effects of candesartan on serum creatinine levels

A comparative statistical analysis of serum creatinine levels between the STZ and STZ+CAN groups revealed a significant difference after week 8, reaching a peak disparity at week 12. At this point, the serum concentrations in the STZ and STZ+CAN groups were 148.32 ± 67.91 and 61.18 ± 19.18 µmol/L, respectively ($p < 0.05$) (Table 2).

Table 2. Effects of candesartan treatment on serum creatinine levels in rats with STZ induced DN

	Serum creatinine (μmol/L)			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	37.44	37.89	36.81	38.11
SD	3.18	3.78	3.53	3.37
Min	30.10	31.01	30.3	31.0
Max	41.9	46.05	44.2	43.5
STZ				
X	39.58	42.49	94.09 [a]	148.32 [a]
SD	5.43	7.37	41.63	67.91
Min	30.5	35.25	35.7	57.3
Max	49.6	58.14	158.3	253.8
STZ+CAN				
x	37.31	43.27	54.82[a,b]	61.18[a,b]
SD	4.27	9.12	18.95	19.18
Min	32.15	32.51	28.9	32.3
Max	45.3	61.22	95.9	94.7

[a]<0.05 vs Control
[b]<0.05 vs STZ

A notable increase in blood urea nitrogen (BUN) values was observed four weeks after the administration of streptozotocin (STZ). Subsequently, the serum concentrations of BUN in the STZ group showed further increases at 8 and 12 weeks. Treatment with candesartan consistently led to a reduction in BUN levels, although the values remained significantly higher compared to the control group ($p < 0.05$) (Figure 2).

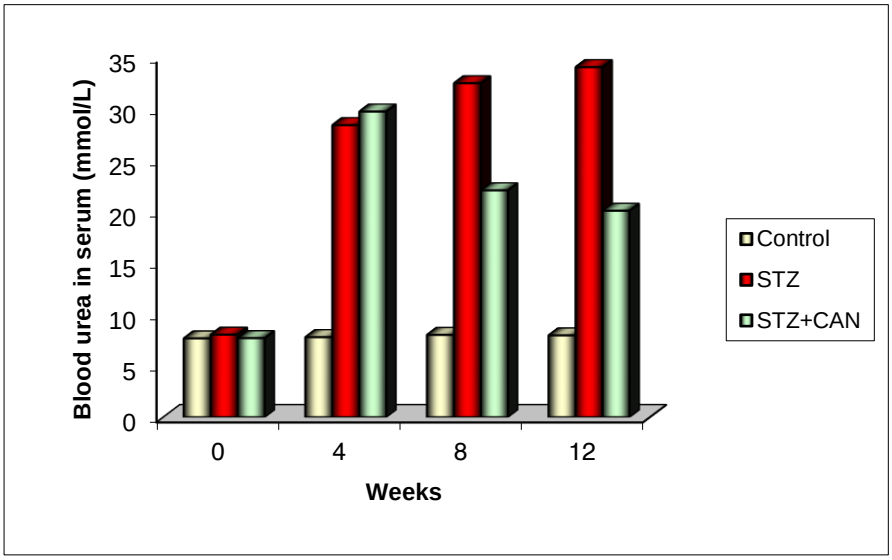

Figure 2. Effects of candesartan on BUN levels

The comparison of blood urea nitrogen (BUN) levels after 8 and 12 weeks between the two experimental groups (STZ and STZ+CAN) demonstrated a significant positive effect of candesartan on the reduction of BUN levels ($p < 0.05$) (Table 3).

Table 3. Effects of candesartan treatment on BUN levels in rats with STZ induced DN

	BUN (mmol/L)			
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	7.67	7.79	8.01	7.96
SD	1.90	1.75	1.45	1.85
Min	4.30	5.30	5.40	5.20
Max	11.50	10.30	10.60	11.60
STZ				
X	8.03	28.41 [a]	32.48 [a]	34.05 [a]
SD	1.79	12.48	16.45	20.63
Min	4.50	8.70	11.95	12.90
Max	11.20	53.50	77.60	85.40
STZ+CAN				
x	7.69	29.73[a]	22.07[a,b]	20.08[a,b]
SD	2.05	10.92	9.53	8.30
Min	4.90	11.50	9.15	8.45
Max	12.20	43.70	44.16	39.60

[a]<0.05 vs Control
[b]<0.05 vs STZ

A significant increase in urine albumin values was observed four weeks after the administration of streptozotocin (STZ). These values in the STZ group further increased after 8 weeks and peaked significantly after 12 weeks (3.316 ± 0.822 mg/24 h). Treatment with candesartan in the STZ+CAN group resulted in a significant decrease in urine albumin values after 8 weeks and even more pronounced reduction after 12 weeks since the beginning of the study (1.236 ± 0.505 mg/24 h) (Figure 3).

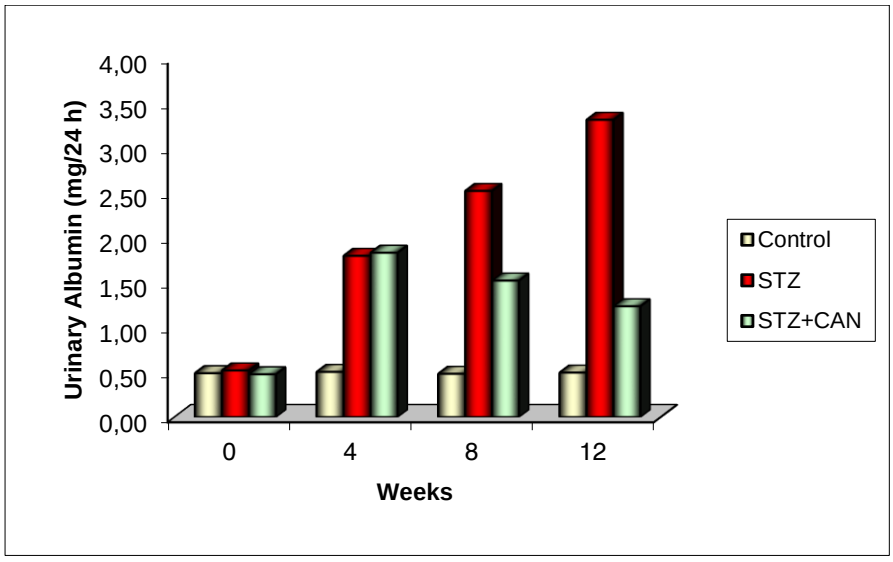

Figure 3. Excretion of candesartan on urinary albumin levels

The treatment with candesartan resulted in a significant ($p < 0.05$) decrease in urine albumin values in the STZ+CAN group compared to the STZ group after 8 weeks, with a peak difference observed at week 12 (Table 4).

Table 4. Effects of candesartan treatment on urinary albumin excretion in rats with STZ induced DN

Urine 27lbumin (mg/24h)				
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	0.490	0.504	0.483	0.496
SD	0.118	0.120	0.109	0.116
Min	0.335	0.345	0.340	0.318
Max	0.714	0.756	0.652	0.707
STZ				
X	0.520	1.799 [a]	2.523 [a]	3.316 [a]
SD	0.158	0.775	0.824	0.822
Min	0.295	0.765	0.953	1.696
Max	0.836	3.454	3.765	4.364
STZ+CAN				
x	0.478	1.834[a]	1.523[a,b]	1.236[a,b]
SD	0.127	0.796	0.556	0.505
Min	0.327	0.664	0.735	0.651
Max	0.687	3.53	2.512	2.324

[a]<0.05 vs Control
[b]<0.05 vs STZ

Histopathological research

The macroscopic analysis of renal parenchyma in the control group revealed preserved corticomedullary construction, patent calyces, and renal pelvis. Microscopic evaluation of the renal cortex in nondiabetic rats (control group) showed a normal appearance in most glomeruli after 8 and 12 weeks, with a normal glomerular basement membrane and no increased intra- or extracapillary cellular effusions or other exudative changes. Tubular excretory ducts within the tubulointerstitium exhibited normal morphology, while the interstitium showed slight alterations (Figure 4A).

Four weeks after administering streptozotocin (STZ), most changes in renal tissue were found in the glomerular compartment, with minor changes in the tubular compartment of the corticomedullary region affecting both proximal and distal tubules. Macroscopic analysis of the kidneys in the STZ group revealed a paler cortical region with pronounced discoloration of the medulla and lower kidney weight. Calyces and renal pelvis retained normal morphology.

Renal tissue examination of the STZ group under light microscopy, 8 weeks after STZ administration, showed a moderate degree of glomerulopathy characterized by basement membrane thickening, expansion of the mesangial matrix, arteriolar hyalinosis, and insudative protein deposits obstructing some capillaries (Figure 4B). Similar and even more severe glomerular damage was found in the same animal group 12 weeks after STZ administration (Figure 4C and D).

Histopathological examination of renal samples 8 and 12 weeks after STZ administration revealed signs of interstitial expansion with interstitial fibrosis and dilatation of the tubules with atrophy of the epithelium in the corticomedullary region. Some renal samples showed tubular epithelial vacuolization with the presence of tubular lumen dilatation and glycogen deposits. Blood vessels in the

renal parenchyma exhibited medial hypertrophy with a low level of intimal thickening.

Ultrastructure analysis identified uneven fusion of the podocytes as well as widening of the mesangial matrix with glomerular basement membrane thickening due to the deposition of basal membranaceous sclerotic material.

The macroscopic appearance of kidney samples from diabetic rats treated with candesartan (STZ + CAN) did not show significant deviations compared to the morphology of samples from the STZ group, except for a macroscopically visible reduction in the size of the kidneys. In the STZ + CAN group, a light level of diabetic glomerulopathy was detected, in contrast to the STZ group. Basement membrane thickening, glomerulosclerosis, and changes in the tubulointerstitium were present in both experimental groups but significantly less pronounced in the STZ + CAN group compared to the STZ group (Figure 4E and F). A reduction in the thickness of the arterial wall in affected vessels was also observed.

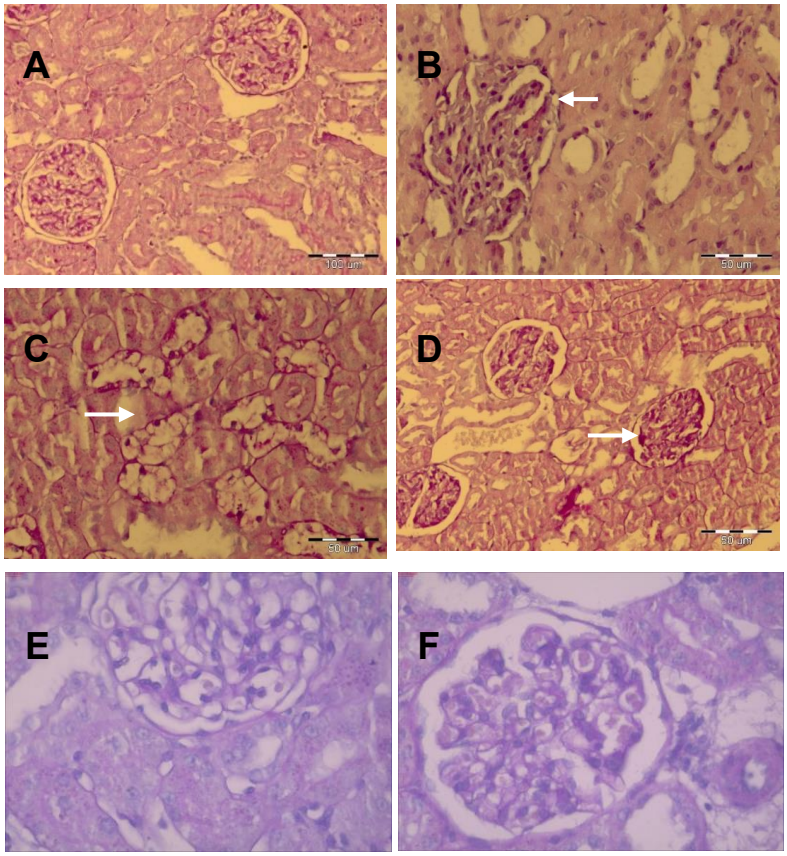

Figure 4. Histopathologic features of kidney from nondiabetic, from STZ induced DN rats and STZ induced DN rats treated with perindopril. Sections were stained with PAS reagent. Magnification x 100 and x 200. (A) Untreated nondiabetic rats at 12 wk. (B) Untreated diabetic rat at 8 wk. (C) Untreated diabetic rat at 12 wk. (D) Untreated diabetic rat at 12 wk. with damaged glomeruli, thickened GBM, altered tubular epithelium with clear cytoplasm due to intracellular glycogen accumulation and areas of partial tubular dilatation. (E) Diabetic rat treated with candesartan at 8 wk. (F) Diabetic rat treated with candesartan at 12 wk.

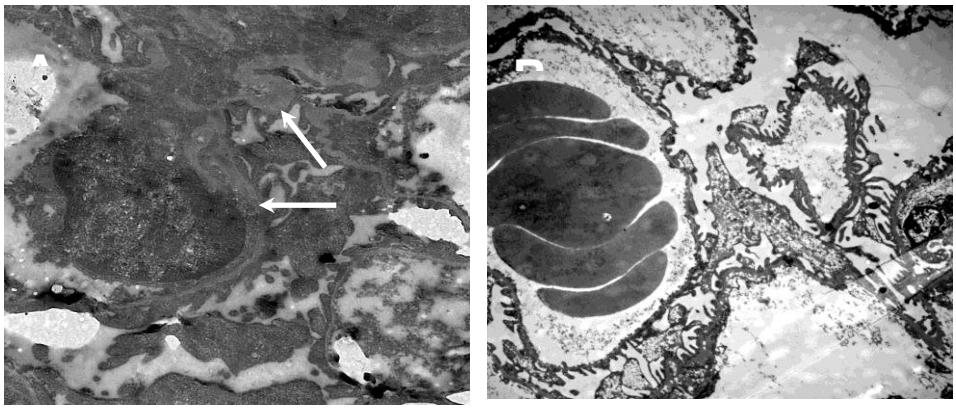

Figure 5. An electron micrographs from STZ induced DN rats and STZ induced DN rats treated with candesartan. (A) Untreated diabetic rat at 12 wk., uneven fusion of the podocytes as well as widening of the mesangial matrix with sclerotic GBM thickening (magnification x 20000). (B) Diabetic rat treated with candesartan at 12 wk., showing mild thickening of the GBM and regenerated podocyte (magnification x 15000).

Glomerulosclerotic Index

The glomerulosclerotic index exhibited a significant increase in the STZ group after 8 weeks (1.636 ± 0.674), further intensifying at the end of the study (2.077 ± 0.862). In contrast, the STZ+CAN group displayed a lower increase in the glomerulosclerotic index (Figure 4).

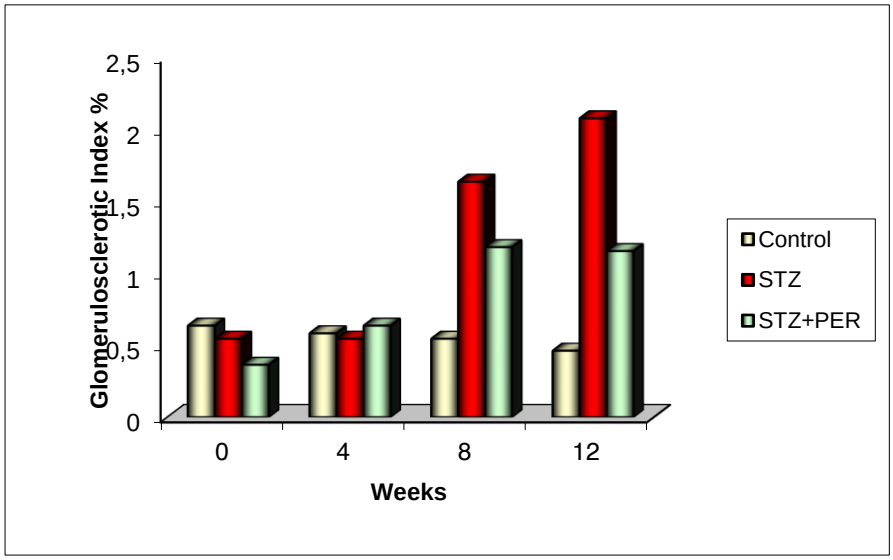

Figure 6. Glomerulosclerotic index (GSI) of STZ and STZ+CAN groups

The comparative statistical analysis of the glomerulosclerotic index (GSI) in the STZ and STZ+CAN groups showed a significant difference after week 8, with the peak difference occurring at week 12 (1.182 ± 0.405 and 1.154 ± 0.555, respectively) ($p < 0.05$) (Table 5).

Table 5. Glomerulosclerotic index in kidneys from nondiabetic, STZ induced DN rats and STZ induced DN rats treated with candesartan

Glomerulosclerotic Index %				
	0-Day	**4-Weeks**	**8-Weeks**	**12-Weeks**
Control				
X	0.636	0.583	0.545	0.462
SD	0.505	0.515	0.522	0.519
Min	0	0	0	0
Max	1	1	1	1
STZ				
X	0.545	0.545	1.636 [a]	2.077 [a]
SD	0.522	0.522	0.674	0.862
Min	0	0	1	1
Max	1	1	3	3
STZ+CAN				
X	0.364	0.636	1.182	1.154
SD	0.505	0.505	0.405	0.555
Min	0	0	1	0
Max	1	1	2	2

[a]<0.05 vs Control
[b]<0.05 vs STZ

Discussion

Diabetic nephropathy (DN) is a severe complication of diabetes mellitus (DM) and a crucial prognostic factor for diabetic patients. Currently, there is no specific treatment for DN, and prevention through early detection and treatment of microalbuminuria is considered the most effective approach. In this study, the efficacy of the angiotensin receptor blocker (ARB) candesartan was evaluated for treating the symptoms and signs of diabetic nephropathy using an experimental model for the induction of DM and subsequently induced diabetic nephropathy.

The administration of streptozotocin (STZ) induced diabetes with distinct symptoms and signs of DN, including poor general condition, body weight loss, increased kidney weight, elevated levels of blood urea nitrogen (BUN) and serum creatinine, augmented diuresis, and significant albuminuria in the experimental animals. These manifestations were prominent at 4 weeks, intensifying further at 8 and 12 weeks after STZ injection.

Commencing candesartan treatment at the 4-week mark post-STZ injection significantly alleviated all DN symptoms. The treatment resulted in a significant improvement in BUN and serum creatinine values, as well as a reduction in albuminuria and diuresis. The study concludes that ARBs, such as candesartan, although not fully, largely alleviate the functional renal disorder resulting from experimentally induced DN with STZ. This research suggests that ARBs may hold promise as a therapeutic option for diabetic nephropathy, potentially mitigating its symptoms and improving renal function.

The results obtained regarding albuminuria underscore the significance of this parameter in detecting diabetic nephropathy (DN) induced by streptozotocin (STZ) and highlight the therapeutic effects of candesartan. Microalbuminuria is recognized as an independent predictor of the later progression of nephropathy and is associated with an increased risk of cardiovascular morbidity and mortality [20].

The primary cause of albuminuria during DN is the increased permeability of the basal

membrane, attributed to a reduction in the number of proteoglycans that are integral to the basal membrane. Microalbuminuria may occur intermittently in the early stages and become persistent in the later stages. As the condition advances, glomerular sclerosis can lead to additional deterioration of renal function, distinct proteinuria, and chronic renal insufficiency.

According to the glomerular hyperfiltration theory, the elevation of blood osmotic pressure due to hyperglycemia and the increase in circulating blood volume stimulate the hypersecretion of atrial natriuretic peptide (ANP), leading to the dilation of glomerular afferent arterioles. Additionally, hyperglycemia induces the production of angiotensin II, which not only causes arteriolar constriction but more prominently affects glomerular efferent arterioles. This results in increased intraglomerular pressure, contributing to the development and progression of diabetic nephropathy [21, 22].

There are different theories and explanations of the beneficial effects of the ARBs on DN. In DM rats glomerular angiotensin II levels are increased due to increase in angiotensinogen [23]. ACE inhibitors do not fully block production of angiotensin II from angiotensinogen because of up regulation of some non-ACE pathways (chymase enzyme) in kidneys [24]. Angiotensin II is known to produce deleterious effects on kidney by affecting the blood pressure and renal hemodynamics, production of growth promoting and profibrotic factors, renal tubular and glomerular hypertrophy and oxidative stress in kidney [25]. Therefore, blockade of angiotensin II is very important factor for halting the progress of DN.

The histopathological analysis of renal samples, conducted 8 weeks after the administration of streptozotocin (STZ), unambiguously confirmed the development and progression of diabetic nephropathy (DN) in the experimental animals. Light microscopy examination of renal samples from diabetic rats revealed the presence of glomerulopathy characterized by basement membrane thickening, expansion of the mesangial matrix, arteriolar hyalinosis, and insudative protein deposits obstructing some capillaries. Similar but more severe histopathological renal changes were observed within the same experimental group 12 weeks after administering STZ. The deterioration of the glomerulosclerotic index after 8 weeks and an even more pronounced decline at the end of the study confirmed the progression of glomerulopathy. These findings align with the conclusions of Nevin E et al. (2004) [26], who stated that glomerulopathy represents the most significant structural change in DN, manifested by thickening of the glomerular basement membrane and mesangial expansion, resulting in a progressive decrease in the glomerular filtration surface.

Furthermore, the histopathological examination of the kidneys revealed signs of interstitial fibrosis and tubular dilatation, similar to the study by Huang et al. (2001) [27], reporting glomerular hypertrophy, sporadic interstitial fibrosis, and tubular atrophy without overly distinct glomerular sclerosis in diabetic rats.

Indeed, the histopathological examinations support the conclusion that candesartan has renoprotective effects. The examination of renal samples at 8 and 12 weeks after the beginning of the study clearly indicates that candesartan significantly mitigates the progression of glomerulopathy, improves the glomerulosclerotic index, and alleviates histological abnormalities in the tubular compartment, interstitium, and blood vessels of kidneys induced with streptozotocin (STZ).

Concluson

In summary, the results obtained from this study lead to the conclusion that candesartan, while not providing complete resolution, significantly ameliorates the functional renal disorder. Moreover, it demonstrates a substantial reduction in the progression of glomerulopathy and histological abnormalities in the tubular compartment, interstitium, and blood vessels induced by streptozotocin (STZ).

References

1. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T. Coronary heart disease in young Type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk-factors. Diabetologia 1987; 30:144-148.
2. Borch-Johnsen K, Kreiner S. Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus. Br Med J 1987; 294:1651-1654.

3. Parving H-H, Osterby R, Ritz E. Diabetic nephropathy. In: Brenner BM, Levine S, editors. The Kidney. Philadelphia: WB Saunders, 2000; 1731-1773.
4. Rincon-Choles H, Kasinath BS, Gorin Y, Abboud HE. Angiotensin II and growth factors in the pathogenesis of diabetic nephropathy. Kidney Int 2002; 82 (Supp l): S8-S11.
5. Berl T. Angiotensin-converting enzyme inhibitors versus AT1 receptor antagonist in cardiovascular and renal protection: The case for AT1 receptor antagonist. J Am Soc Nephrol 2004; 15(Supp l): S71-S76.
6. Deferrari G, Ravera M, Deferrari L, Vettoretti S, Ratto E, Parodi D. Renal and cardiovascular protection in type 2 diabetes mellitus: Angiotensin II receptor blockers. J Am Soc Nephrol 2002; 13(Supp l3): S224-S229.
7. Lasaridis AN, Sarefidis PA. Diabetic nephropathy and antihy-pertensive treatment: what are the lessons from clinical trials? Am J Hypertens. 2003; 16:689-97.
8. Xi XP, Graf K, Goetze S, Fleck E, Hsueh WA, Law RE. Central role of the MAPK pathway in ang II-mediated DNA synthesis and migration in rat vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 19: 73–82, 1999.
9. Jackson B, Franze L, Whitty M. Systemic and renovascular hypertension. Tohoku J Exp Med 1992; 166: 155 –164.
10. Dworkin LD, Feiner HD, Parker M, Tolbert E. Effects of nifedipine and enalapril on glomerular structure and function in uninephrectomized SHR. Kidney Int 1991; 39: 1112 –1117.
11. Weir MR. Hypertensive nephropathy: Is a more physiologic approach to blood pressure control an important concern for the preservation of renal function? Am J Med 1992; 93: 27S –37S.
12. Eddy AA. Molecular basis of renal fibrosis. Pediatr Nephrol 2000; 15: 290 –301.
13. Erwig LP, Kluth DC, Rees AJ. Macrophages in renal inflammation. Curr Opin Nephrol Hypertens 2001; 10: 341 –347.
14. Ridker PM, Hennekens CH, Buring JE, Rifai N: C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342: 836 –843.
15. Schulz R, Heusch G. Angiotensin II type 1 receptors in cerebral ischaemia-reperfusion. Initiation of inflammation. J Hypertens 2006; Suppl 24: S123 –S129.
16. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. RENAAL Study Investigators: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001; 345: 861-869.
17. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, et al. Collaborative Study Group: Renoprotective effect of the angiotensinreceptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 2001; 345: 851-860.
18. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P, et al. The effect of irbesatan on the developmnet of diabetic nephropathy in patients with type 2 diabetes. N Engl J Med 2001; 345: 870-878.
19. Saito T, Sumithran E, Glasgow EF, Atkins RC. The enhancement of aminonucleoside nephrosis by the co-administration of protamine. Kidney Int 1987; 32: 691–699.
20. Kilaru P, Bakris GL. Microalbuminuria and progressive renal disease. J Hum Hypertens 1994; 8: 809–817.
21. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus.Kidney Int. 1981; 19(3): 410-5.
22. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis oprogressiveglomerular sclerosis in aging, renal ablation, and intrinsic renal disease.N Engl J Med. 1982; 307(11): 652-9
23. Anderson S, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest. 1986; 77(6):1993-2000.
24. Huang XR, Chen WY, Truong LD, Lan HY. Chymase is upregulated in diabetic nephropathy: Implications for an alternative pathway of angiotensin II mediated diabetic renal and vascular disease. J Am Soc Nephrol. 2003; 14:1738-47.

25. Cooper ME. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia. 2001; 44:1957-72.
26. Nevin E. Selma Y. Melek O, Matem T et al. Effects of ACE inhibition on the expression of type IV collagen and laminin in renal glomeruli in experimental diabetes. Acta Histochemica 2004; 106 (4): 279-287.
27. Huang W, Yves G, Nadine B, Patrick B, Didier H, Pierre M et al. Genetically increased angiotensin I converting enzyme level and renal complications in the diabetic mouse. Proc Natl Acad Sci USA, 2001; 98(23):13330-34.