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ORIGINAL ARTICLE

Anti-diabetic and renoprotective effects of aliskiren in streptozotocin-induced diabetic nephropathy in female rats

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Abstract Since chronic kidney disease due to diabetic nephropathy (DN) is becoming an ever larger health burden worldwide, more effective therapies are desperately needed. In the present study, the anti-diabetic and renoprotective effects of aliskiren have been evaluated in streptozotocin (STZ)-induced DN in rats. DN was induced by a single intraperitoneal injection of STZ (65 mg/kg). Three weeks after STZ, rats were divided into four groups; normal, diabetic, diabetic treated with gliclazide (10 mg/kg/day) for 1 month, and diabetic treated with aliskiren (50 mg/kg/day) for 1 month. At the end of the experiment, mean arterial blood pressure and heart rate were recorded. Rats were then euthanized and serum was separated for determination of glucose, insulin, kidney function tests, superoxide dismutase activity (SOD), adiponectin, and tumor necrosis factor-alpha (TNF-α). One kidney was used for estimation of malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide (NO) contents. Other kidney was used for histopathological study and immunohistochemical measurement of caspase-3 and transforming growth factor beta (TGF-β). In addition, islets of Langerhans were isolated from normal rats by collagenase digestion technique for in vitro study. Aliskiren normalized STZ-induced hyperglycemia, increased insulin level both in vivo *and* in vitro, normalized kidney function tests and blood pressure, and alleviated STZ-induced kidney histopathological changes. This could be related to the ability of aliskiren toward preserving hemodynamic changes and alleviating oxidative stress and inflammatory and apoptotic markers induced by STZ in rats. However, aliskiren was more effective than gliclazide in relieving STZ-induced DN. These findings support the beneficial effect of aliskiren treatment in DN which could be attributed to its anti-diabetic, renoprotective, antioxidant, anti-inflammatory, and anti-apoptotic effects. Moreover, clinical studies are required to establish the effectiveness of aliskiren treatment in patients suffering from hypertension and diabetes.

Keywords Renin-angiotensin system · Diabetic nephropathy · Oxidative stress · Gliclazide · Aliskiren

Abbreviations

ACEIs	Angiotensin converted enzyme inhibitors
ARB	Angiotensin II receptor blocker
BUN	Blood urea nitrogen
DM	Diabetes mellitus
DN	Diabetic nephropathy
GSH	Reduced glutathione
HR	Heart rate
MDA	Malondialdehyde
MAP	Mean arterial pressure
NO	Nitric oxide
PRA	Plasma renin activity
RAAS	Renin-angiotensin aldosterone system
ROS	Reactive oxygen species
STZ	Streptozotocin
SOD	Superoxide dismutase activity

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TGF-β Transforming growth factor-beta
TNF-α Tumor necrosis factor-alpha

Introduction

Diabetic nephropathy (DN) is one of the most serious complications of diabetes and the most common cause of end-stage renal failure in the world. Since the 1950s, kidney disease has been recognized as a common complication of diabetes mellitus (DM). Fifty percent of patients with DM of more than 20 years' duration were having this complication. About 3 % of diagnosed patients with type 2 DM have overt DN (De Boer et al. 2011).

DN was defined clinically by microalbuminuria, that is, a urinary albumin excretion of more than 300 mg in a 24-h collection or macroalbuminuria and abnormal renal function (Shlipak 2011). Coca et al. (2012) cited that intensive glycemic control is important for nephropathy prognosis. Intensive treatment of hyperglycemia would prevent DN, including development of microalbuminuria, and could slow progress of chronic kidney disease (Coca et al. 2012). The cause of DN is multifocal and may include altered glucose metabolism, ischemia, and superoxide-induced free radical formation and increased oxidative stress (Fowler 2008).

Interventions that inhibit the activity of the renin-angiotensin aldosterone system (RAAS) like angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) have become a standard and essential therapy in the management of DN (Lewis et al. 1993). However, most studies to date show kidney disease progression in many patients despite treatment with ACEI or ARB (Morgan et al. 2004; Forclaz et al. 2003; Doulton 2006). A potential explanation for this limitation in success is the increase in renin secretion and synthesis (Azizi et al. 2004; Kramer et al. 1998), as a result of negative feedback from the suppression of angiotensin II synthesis or activity by these agents. Besides, they have little effect on basal glucose and insulin levels, in lean animals (Akbar et al. 2012; Michel et al. 2016).

Renin is the initial and rate-limiting substance in the RAS. The direct renin inhibitor (DRI) may locally have some advantages in terms of specificity by blocking angiotensin I generation. Therefore, there is currently renewed interest in DRIs. Aliskiren, the first renin inhibitor to reach the market, lowers elevated blood pressure efficiently by decreasing plasma and/or local renin activity (Jensen et al. 2008). Recently, increasing evidence shows that aliskiren has an anti-proteinuric effect in patients with diabetes and also exerts renoprotective, cardioprotective, anti-atherosclerotic, and antioxidant effects in animal models independent of its blood pressure lowering activity (Komers 2013; Pilz et al. 2005; Lu et al. 2008; De Mello 2015; Kamal 2013).

In a recent study, aliskiren was found to be equally effective to ACEI and ARB in slowing the progression of DN in db/db mice. However, the use of combination therapy with aliskiren and ACEI/ARB was not recommended (Zhou et al. 2015).

A very recent study showed that aliskiren has a potential anti-fibrotic effect in bleomycin-induced pulmonary fibrosis in rats (Abuelezz et al. 2016). Another study showed that aliskiren protects liver tissues of rats during paracetamol-induced toxicity by preventing oxidative stress and cytokine changes (Karcioglu et al. 2016).

So in the present study, we have therefore designed experiments to investigate the different mechanisms of action underlying the therapeutic action of aliskiren in renal damage induced by streptozotocin (STZ) type 1 diabetes using rats as the working model and focusing on its anti-diabetic effect by both in vivo and in vitro studies, besides comparing its effects with a reference anti-diabetic drug as gliclazide.

Materials and methods

Animals

Female Wistar rats weighing 180–200 g were obtained from the National Scientific Research Center (Giza, Egypt). Rats were housed under controlled temperature (25 ± 2 °C) and constant light cycle (12 h light/dark) and allowed free access to a standard rodent chow diet and water ad libitum. The investigation complies with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (permit number PT 1180).

Drugs and chemicals

STZ, gliclazide, and study chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Aliskiren was provided as 150 mg tablets from Novartis Company. For in vivo experiments, gliclazide was suspended in 2 % Tween 80 and administered in a dose of 10 mg/kg/day I.P. for 1 month (Pushparaj et al. 2007). For in vitro experiments, 1 mmol/L was prepared by dissolving 0.16 g gliclazide in 10 mL saline using a sonicator. Aliskiren tablets were crushed, dissolved in normal saline, and filtered. Aliskiren was administered in a dose of 50 mg/kg/d I.P. for 1 month (De Mello 2015) or used as 100 μmol/L for in vitro experiments.

Induction of experimental diabetic nephropathy

Rats were fasted for 18 h; diabetes was induced by intraperitoneal injection of 65 mg/kg STZ (Pushparaj et al. 2007). STZ was freshly prepared in 0.1 M citrate buffer just before injection. After injection of STZ, rats were fed on 5 % glucose

solution for 24 h to prevent hypoglycemia during the hyperinsulinemic phase caused by β cell lyses. Hyperglycemia was tested by measuring glucose using blood from the tail and OneTouch Glucometer (LifeScan, Inc., Milpitas, CA, USA). Only rats with glucose levels more than 250 mg/dL were selected and considered as diabetic animals and left for 3 weeks for induction of DN (Barzegar-Fallah et al. 2015).

Islet isolation

Islets of Langerhans were isolated from normal rats by collagenase digestion technique according to the method mentioned before (Lacy and Kostianovsky 1967). It was isolated from non-fasting normal rats since fasting would diminish the responsiveness of the islets to stimulation of insulin secretion in vitro. Rats were pretreated with pilocarpine nitrate (20 mg/kg) I.P. 2–3 h before islet isolation. Pilocarpine depletes zymogens from the exocrine pancreatic tissues and thus minimizes the destruction of islet membranes which could occur during collagenase digestion of the tissue.

In vivo experiments

Three weeks after STZ injection, rats were divided into four groups. The first is the normal control group, two to four groups, composed of STZ-diabetic rats. The second group served as a diabetic control group and treated with normal saline. The third group was treated with gliclazide (10 mg/kg I.P.). The fourth group was treated with aliskiren (50 mg/kg I.P.). Drug treatments were done for 1 month. At the end of the experiment, animals were euthanized 1 h after the last drug dose.

In vitro experiments

Isolated β cells were divided into four groups: normal control, 20 μL gliclazide (40 μmol/L), 20 μL aliskiren (100 μmol/L), and a combination of 20 μL gliclazide and 20 μL aliskiren. Drugs were incubated with isolated islets for 1 h, then 0.5 mL of the supernatant was separated and kept frozen for measurement of insulin concentration. The number of experiments was prespecified as six per group before the start of the experimental series.

Biochemical analysis

Serum glucose level

Fasting serum glucose level was determined by colorimetry at 546 nm at the end of the experiment just after animals were euthanized according to the method of Trinder (1969) using a commercial reagent kit and was expressed as milligrams per deciliter.

Insulin immunoassay

Fasting rats' serum insulin level and insulin concentration in supernatant of isolated islets were determined using a commercial ELISA Kit (Li Ka Shing Faculty of Medicine, University of Hong Kong, AIS). Insulin was expressed as micro international units per milliliter serum and micro international units per hour per islet. Serum samples were collected after animals were euthanized and kept frozen at −20 till insulin measurements.

Kidney function tests

Blood urea nitrogen (BUN), serum creatinine, and albumin were determined according to the methods described before (Urea 1984; Murray 1984). BUN and creatinine were expressed as milligrams per deciliter, and serum albumin was expressed as grams per deciliter.

Oxidative stress biomarkers

Kidney GSH content was determined at 405 nm by a spectrophotometer using Ellman's reagent according to the method described by Beutler et al. (1963) and was expressed as milligrams per gram of kidney (Beutler et al. 1963).

Kidney MDA was determined at 534 nm using a spectrophotometer according to the method of Satoh (1978) using a commercial reagent kit (Satoh 1978).

Serum SOD activity was determined by colorimetry at 450 nm according to the pyrogallol autoxidation method of Marklund and Marklund (1974) and was expressed as units per milliliter (Marklund and Marklund 1974).

Serum nitric oxide

Nitric oxide (NO) was determined by colorimetry at 450 nm using Griess reagent after reduction of nitrate to nitrite by vanadium trichloride and expressed in serum as micromoles per microliter (Miranda et al. 2001).

Kidney tumor necrosis factor-alpha

Tumor necrosis factor-alpha (TNF-α) level was assessed using rat TNF-α ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions, and the results are expressed as nanograms per milligram of protein (Petrovas et al. 1999).

Serum adiponectin

Adiponectin was measured in serum using a commercial ELISA kit and expressed as micrograms per milliliter (Mehany et al. 2013).

Measurement of hemodynamic parameters

Blood pressure and heart rate were assessed by CODA™, a computerized non-invasive blood pressure system (Kent Scientific, Torrington, CT, USA). It measures tail blood pressure by means of volume pressure.

Rats were held in a restrainer on a preheated platform with the tail exposed. Both an occlusion cuff and a volume-pressure recording cuff were placed close to the tail base. The digital value for the systolic and diastolic blood pressures and heart rate were recorded. Readings were taken for 20 cycles from each rat, and the highest and lowest odd values were excluded. Measurements were taken in the same peaceful environment at the same time of the day to minimize stress according to Kurtz et al. (2005). Heart rate was expressed as beats per minute (bpm), and mean arterial blood pressure was expressed as millimeters of mercury.

Histopathology

Kidney sections were stained with periodic acid-Schiff's reagent to assess glomerulosclerosis and tubulointerstitial fibrosis (Banchroft et al. 1996). Caspase-3 and transforming growth factor-beta (TGF-β) were determined by immunohistochemistry as described before (El Nahas 1992), using anti-caspase-3 antibody (ab52181), unconjugated rabbit polyclonal caspase-3 and anti-TGF-β2 antibodies (ab66045), and unconjugated rabbit polyclonal TGF-β2 antibody. The person scoring the histology slides had been blinded regarding group allocation.

Statistical analysis

All data obtained were presented as mean ± SD. Results were analyzed using one-way analysis of variance test (one-way ANOVA) followed by Tukey-Kramer multiple comparison test, using SPSS software, version 16. For all the statistical tests, the level of significance was fixed at $p < 0.05$ (Sendecor and Cochran 1980).

Results

Serum glucose and insulin

STZ (65 mg/kg) resulted in hyperglycemia accompanied by significant decrease in serum insulin concentration. Treatment with gliclazide (10 mg/kg) for 4 weeks after induction of diabetes resulted in a significant decrease in serum glucose to 36.7 % and a significant increase in serum insulin concentration to 256 % as compared to the diabetic control group. However, aliskiren treatment significantly decreases serum glucose to 30.8 % and increase serum insulin to 640 % as compared to diabetic control rats (Table 1).

Kidney function tests

STZ resulted in a significant increase in BUN and serum creatinine and a significant decrease in serum albumin. Gliclazide treatment resulted in a significant decrease in BUN to 79.2 % and serum creatinine to 71.3 %, but did not change serum albumin as compared to diabetic control rats. Aliskiren treatment resulted in a significant decrease in BUN to 74.2 % and serum creatinine to 38.3 % and a significant increase in serum albumin to 125.7 % as compared to diabetic control rats. However, aliskiren was more effective than gliclazide in relieving kidney damage (Table 2).

Oxidative stress biomarkers

Induction of DN by STZ resulted in a state of oxidative stress as shown by a significant decrease in kidney GSH, a significant increase in kidney MDA, a significant decrease in serum SOD, and a significant increase in kidney NO. Treatment with gliclazide resulted in a significant increase in kidney GSH to 143.2 % and serum SOD to 127.5 % and a significant decrease in kidney MDA to 78.8 % and kidney NO to 73.5 % as compared to diabetic control rats. Treatment with aliskiren resulted in a significant increase in kidney GSH to 142.2 % and serum SOD to 193 % and a significant decrease in kidney MDA to 73.8 % and kidney NO to 73.2 % as compared to diabetic control rats (Table 3).

MAP and HR

STZ resulted in hypertension and tachycardia. Treatment with gliclazide resulted in a significant decrease in mean arterial pressure (MAP) to 90.7 % and heart rate (HR) to 90 % as compared to the diabetic control group. Treatment with aliskiren resulted in a significant decrease in MAP to 79.4 % and HR to 86.2 % as compared to the diabetic control group.

Table 1 Effect of one month treatment with gliclazide and aliskiren on serum glucose and insulin in STZ-induced diabetic nephropathy in female rats

Parameters Treatments	Serum glucose (mg/dL)	Serum insulin (μIU/mL)
Normal control	120 ± 1.4	61.17 ± 1.9
Diabetic control	400 ± 5.4[a]	23.5 ± 1[a]
Gliclazide (10 mg/kg)	146.7 ± 12[a,b]	60.33 ± 1.8[b]
Aliskiren (50 mg/kg)	123.3 ± 4.3[b,c]	150.33 ± 2[a,b,c]

The values are the means ± S.D. from eight animals in each group

[a] $p < 0.05$ vs. normal group

[b] $p < 0.05$ vs. diabetic group

[c] $p < 0.05$ vs. gliclazide-treated group

Table 2 Effect of one month treatment with gliclazide and aliskiren on kidney function tests in STZ-induced diabetic nephropathy in female rats

Parameters Treatments	BUN (mg/dL)	Serum creatinine (mg/dL)	Serum albumin (g/dL)
Normal control	25.9 ± 0.32	0.59 ± 0.08	4.7 ± 0.26
Diabetic control	33.7 ± 0.62[a]	0.94 ± 0.14[a]	3.5 ± 0.08[a]
Gliclazide (10 mg/kg)	26.7 ± 0.84[b]	0.67 ± 0.16[b]	3.3 ± 0.19[a]
Aliskiren (50 mg/kg)	25 ± 0.68[b,c]	0.36 ± 0.01[a,b,c]	4.4 ± 0.44[b,c]

The values are the means ± S.D. from eight animals in each group

[a] $p < 0.05$ vs. normal group

[b] $p < 0.05$ vs. diabetic group

[c] $p < 0.05$ vs. gliclazide-treated group

However, aliskiren was more effective than gliclazide (Table 4).

Adiponectin

STZ resulted in a significant decrease in serum adiponectin. Both gliclazide and aliskiren resulted in a significant increase in serum adiponectin to 129 and 138 % as compared to the diabetic control group (Table 4).

TNF-α

STZ resulted in a significant increase in kidney TNF-α (2.27 ± 0.06 vs. 1.62 ± 0.04 ng/mL). Gliclazide and aliskiren resulted in a significant decrease in kidney TNF-α to 75.8 and 48.5 % as compared to the diabetic control group. However, aliskiren was more effective than gliclazide (Table 4).

In vitro study

The mean value of insulin secreted from isolated islets incubated in 3 mM glucose was 37.5 ± 1.2 (μIU/h/islet). Incubation of islets for 1 h with 20 μL gliclazide (1 mmol/L) resulted in a significant increase in insulin secretion to 253 % compared to normal control. Incubation of islets for 1 h with 20 μL aliskiren (100 nM) leads to a significant increase in insulin secretion to 206 % as compared to normal control. Aliskiren combination with gliclazide resulted in a significant increase in insulin secretion to 345 % as compared to normal control (Fig. 1).

Histopathological findings

DN resulted in degenerative changes and coagulative necrosis (nephrosis) detected in the lining epithelium of these individual tubules at the cortex (Fig. 2b) vs. normal control (Fig. 2a). Gliclazide and aliskiren improved the histopathological alteration in the tubules of both cortex and corticomedullary parts induced by STZ (Fig. 2c, d).

Induction of DN by STZ resulted in a severe elevation in caspase-3 immunoreactivity (Fig. 3b) vs. normal control (Fig. 3a). Gliclazide resulted in a mild elevation in caspase-3 immunoreactivity (Fig. 3c). Aliskiren resulted in a faint expression of caspase-3 immunoreactivity (Fig. 3d).

STZ (65 mg/kg) resulted in a significant elevation in TGF-β immunoreactivity (Fig. 4b) as compared to the normal control group (Fig. 4a). This elevation was suppressed by concomitant administration of gliclazide (Fig. 4c) or aliskiren (Fig. 4d).

Discussion

Diabetic nephropathy is one of the major microvascular complications of both type 1 and 2 DM. It is considered the major cause of end-stage renal disease worldwide which causes premature death in diabetic patients. It is found that between 20 and 40 % of all diabetic patients are prone to developing renal failure (Sheela et al. 2013), so more effective therapies are needed.

In the present study, nephropathy was resulted from type 1 diabetes which was induced in adult female albino rats by a single I.P. injection of STZ, at 65 mg/kg. Nephropathy was noted in rats 4 weeks after administering STZ as assessed in terms of a significant increase in BUN, serum creatinine, and albuminuria. Besides, hypertension, tachycardia, and disturbance in oxidant/antioxidant balance were observed by the

Table 3 Effect of one month treatment with gliclazide and aliskiren on oxidative stress biomarkers in STZ-induced diabetic nephropathy in female rats

Parameters Treatments	Kidney GSH (mg/g kidney)	Kidney MDA (nmol/g kidney)	Serum SOD (U/mL)	Kidney NO (μmol/μL)
Normal control	28.3 ± 1.9	26.7 ± 0.87	87.13 ± 2.3	25.8 ± 1.2
Diabetic control	19.9 ± 1.2[a]	38.6 ± 1.6[a]	50.33 ± 2.1 [a]	38.5 ± 1.9[a]
Gliclazide (10 mg/kg)	28.5 ± 1.5[b]	30.4 ± 1.5[a,b]	64.17 ± 1.4[a,b]	28.3 ± 1.5[a,b]
Aliskiren (50 mg/kg)	28.3 ± 1.7[b]	28.5 ± 1.3[a,b,c]	97.15 ± 1.8[a,b,c]	28.2 ± 1.4[a,b]

The values are the means ± S.D. from eight animals in each group

[a] $p < 0.05$ vs. normal group

[b] $p < 0.05$ vs. diabetic group

[c] $p < 0.05$ vs. gliclazide-treated group

Table 4 Effect of one month treatment with gliclazide and aliskiren on MAP, HR, serum adiponectin and kidney TNF-α in STZ-induced diabetic nephropathy in female rats

Parameters Treatments	MAP (mmHg)	Heart rate (bpm)	Serum adiponectin (μg/mL)	Kidney TNF-α (ng/mL)
Normal control	141.7 ± 1.57	345 ± 7.1	3 ± 0.17	1.62 ± 0.1
Diabetic control	163.5 ± 4.3[a]	400 ± 1.9[a]	2.27 ± 0.15[a]	2.27 ± 0.1[a]
Gliclazide (10 mg/kg)	148.3 ± 2.2[b]	360 ± 1.5[a,b]	2.93 ± 0.25[b]	1.72 ± 0.14[b]
Aliskiren (50 mg/kg)	129.9 ± 2.3[a,b,c]	345 ± 2.6[b,c]	3.13 ± 0.21[b]	1.1 ± 0.23[a,b,c]

The values are the means ± S.D. from eight animals in each group

[a] $p < 0.05$ vs. normal group

[b] $p < 0.05$ vs. diabetic group

[c] $p < 0.05$ vs. gliclazide-treated group

increase in kidney content of MDA and NO. Reduction of kidney GSH and serum SOD, increased inflammatory mediators as TNF-α and TGF-β, and increased caspase-3 also were observed. In addition, degenerative changes and coagulative necrosis (nephrosis) were detected in the lining epithelium of some individual tubules at the cortex.

These results may be due to hemodynamic alterations caused by STZ and hyperglycemia, as well as alterations in the glomerular basement membrane composition, reactive oxygen species (ROS), glycation of proteins, and podocyte biology as explained by Pilmore (2010).

Various factors may be involved in the pathogenesis of DN. It is believed that hyperglycemia induces a defect in the mitochondrial electron transport chain, resulting in increased production of ROS and increased oxidative stress. This is a common mediator of the pathophysiological effects of hyperglycemia and subsequent DN (Ziyadeh 2004). The increased oxidative stress activates glycation and formation of advanced glycation end products, cytokines, and growth factors. An increased influx of glucose through the hexosamine pathway leads to increased formation of the observed TGF-ß. TGF-ß plays an important part in the development of glomerulosclerosis and tubulointerstitial fibrosis by stimulating extracellular matrix protein collagen types I, III, and IV and fibronectin (Kiran et al. 2012).

Administration of gliclazide (10 mg/kg, I. P) normalized hyperglycemia and increased serum insulin concentration as compared to diabetic control rats. These effects are emphasized by the in vitro measurements of insulin secretion from isolated beta cells of normal rats. Gliclazide also increased serum adiponectin level.

The hypoglycemic effect of gliclazide is through pancreatic action by stimulating endogenous insulin secretion in response to physiologic secretagogues (Kumar et al. 2015), besides extrapancreatic action through increasing the glucose utilization in muscles and adipose tissues (Al-Salami et al. 2008). These effects are related to its ability to restore insulin-stimulated glucose transporter 4 translocation in peripheral tissues (Shimoyama et al. 2006). In addition, gliclazide decreased BUN and serum creatinine as compared to diabetic control rats. These actions may be a cause of the observed normalization of BP and HR. The improved BP and HR with gliclazide have been observed in a previous study of Pagano et al. (1998) who reported that treatment with gliclazide enhances ACh-induced relaxation in isolated aortic segments of alloxan-induced diabetic rabbits. In addition, the improvement in endothelial function has been reported in STZ-induced diabetic rats receiving gliclazide (Vallejo et al. 2000).

Gliclazide resulted in a significant increase in renal GSH and serum SOD and a decrease in renal MDA and NO. This

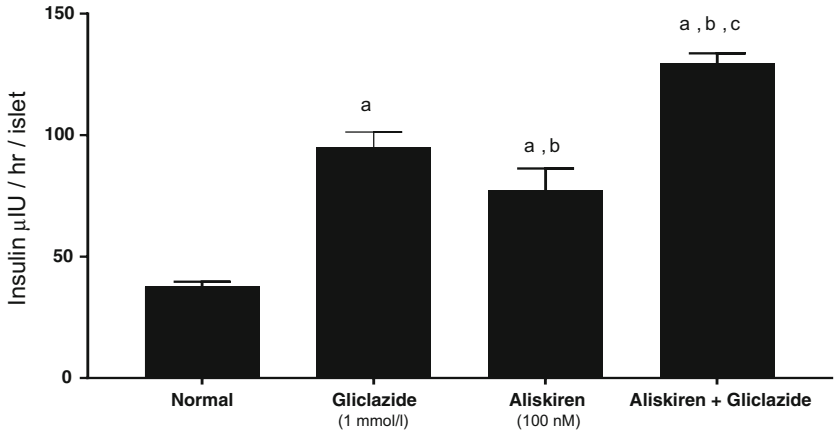

Fig. 1 Effect of gliclazide and aliskiren each in 20 μL on insulin secretion from isolated pancreatic islets of normal female rats. The values are the means ± SD from eight animals in each group. Statistical analysis was done using one-way ANOVA followed by Tukey's post hoc test. [a] $p < 0.05$ vs. normal group, [b] $p < 0.05$ vs. diabetic group, [c] $p < 0.05$ vs. gliclazide-treated group

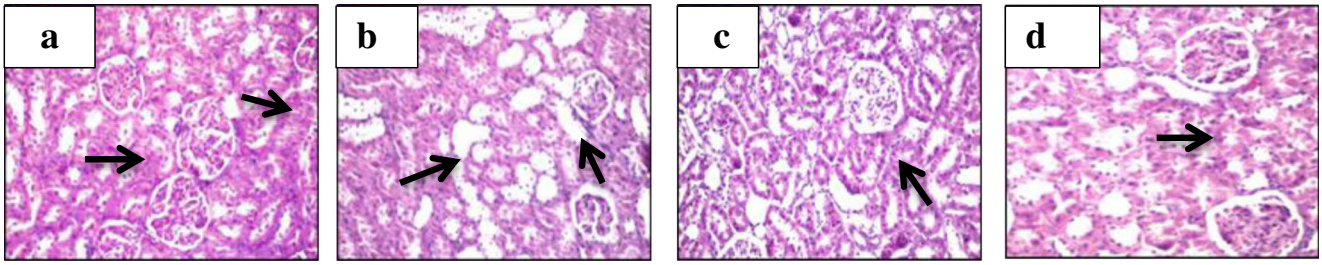

Fig. 2 Photomicrographs of kidney sections from rats treated with gliclazide and aliskiren for 1 month in STZ-induced nephrotoxicity (H&E-stained)(×200). **a** Kidney of rat in the normal control group: showing normal histological structures of the glomeruli and tubules of the cortex. **b** Kidney of rat in the diabetic control group: showing degeneration and coagulative necrosis in lining epithelial cells of some individual tubules at the cortex. **c** Kidney of rat in the gliclazide-treated group: showing normal histological structure. **d** Kidney of rat in the aliskiren-treated group: showing normal histopathological structure with less degeneration and necrosis in lining epithelial cells in some tubules of the cortex

finding also was consistent with the idea that gliclazide improves diabetic endothelial dysfunction by an antioxidant mechanism (Sena et al. 2009; Desfaits et al. 1997). Another study has also reported that gliclazide has antioxidant properties in vitro, perhaps reversing the endothelial dysfunction caused by glycosylated oxyhemoglobin in human mesenteric microvessels (O'brien and Luo 1997). Gliclazide resulted in a significant decrease in kidney TNF-α, TGF-β, and caspase-3 as compared to diabetic control rats. STZ-induced histopathological and ultrastructural changes were reversed by treatment with gliclazide; these support its renoprotective effects, and these results are compatible with earlier studies (Vallejo et al. 2000; Desfaits et al. 1997).

Treatment with aliskiren (50 mg/kg, I. P) for 1 month after induction of DN by STZ resulted in normalized hyperglycemia and increased serum insulin concentration as compared to diabetic control rats. This effect was supported by the in vitro study where aliskiren stimulated insulin secretion from beta cells of normal rats; however, aliskiren was more effective than gliclazide. In addition, aliskiren synergized gliclazide-induced insulin secretion in this in vitro study. Furthermore, aliskiren resulted in a significant increase in serum adiponectin as compared to diabetic control rats, which was associated with enhanced insulin sensitivity.

The observed glucose lowering effect of aliskiren in the present study is novel and may be due to its ability to stimulate insulin secretion or decreased insulin resistance by decreasing serum adiponectin or increase insulin sensitivity by its antioxidant and anti-inflammatory effects.

The anti-diabetic effect of aliskiren is proven in the study of Gandhi et al. (2013) who found that diabetic rats experienced approximately an 81 % decrease in plasma/serum pancreatic insulin content. However, aliskiren treatment significantly reduced blood glucose and increased total body weight as compared to diabetic rats. They explained the improved insulin sensitivity effect of aliskiren by the improved liver and muscle glucotransporter expression levels (Gandhi et al. 2013). This effect was explained by the study which demonstrated that renin inhibition attenuates insulin resistance and improves systemic insulin sensitivity in transgenic Ren2 rats that overexpress renin (Habibi et al. 2008). Thus, a possible link between renin activation and insulin resistance was suggested.

Another study of Kang et al. (2010) found an improvement in insulin resistance and lipid abnormality, as well as direct anti-fibrotic effect in target organs in db/db mice by aliskiren treatment. They explained this effect by the significant decrease in plasma levels of the homeostasis model assessment index, lipid abnormalities, and insulin sensitivity confirmed by insulin tolerance test with aliskiren treatment (Kang et al. 2010).

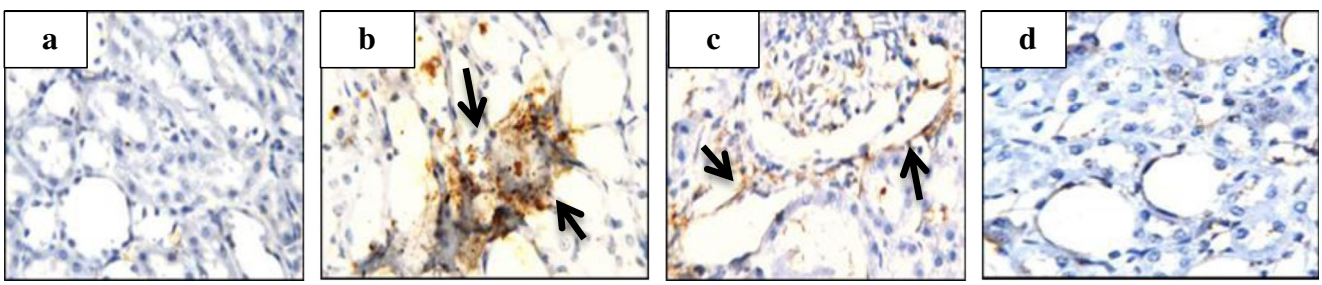

Fig. 3 Effect of 1-month treatment with gliclazide and aliskiren on caspase-3 immunohistochemical staining of rats' kidney tissues in STZ-induced diabetic nephropathy in female rats (×200). **a** Kidney of rat in the normal control group showing negative immunoreaction using caspase-3. **b** Kidney of rat in the diabetic control group showing positive immunoreaction using caspase-3. **c** Kidney of rat in the gliclazide-treated group showing positive immunoreaction using caspase-3. **d** Kidney of rat in the aliskiren-treated group showing positive immunoreaction using caspase-3

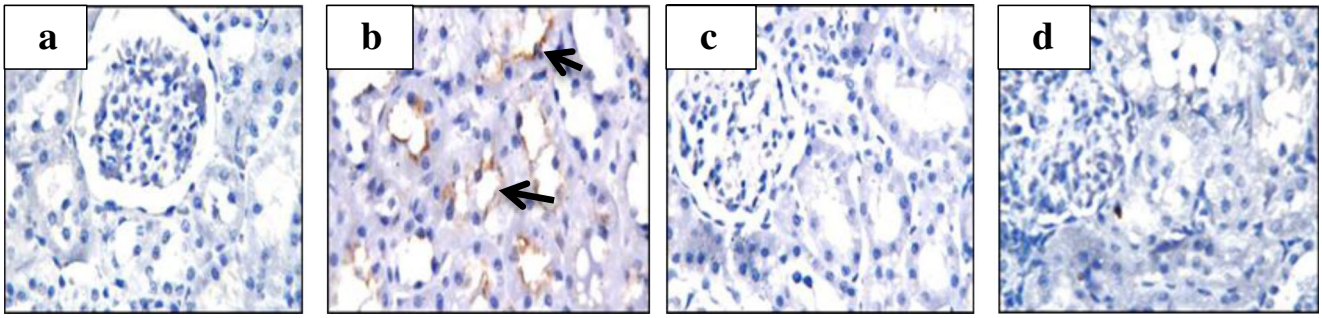

Fig. 4 Effect of 1-month treatment with gliclazide and aliskiren, on transforming growth factor beta (TGF-β) immunohistochemical staining of rats' kidney tissues in STZ-induced diabetic nephropathy in female rats. (×200). **a** Kidney of rat in the normal control group showing negative immunoreaction using TGF-β. **b** Kidney of rat in the diabetic control group showing positive immunoreaction using TGF-β. **c** Kidney of rat in the gliclazide-treated group showing negative immunoreaction using TGF-β. **d** Kidney of rat in the aliskiren-treated group showing negative immunoreaction using TGF-β

Renoprotective effects of aliskiren were manifested by its ability to normalize BUN, serum creatinine, and serum albumin as compared to diabetic control rats, in addition to normalized BP and HR. Aliskiren also showed antioxidant effects which were manifested by a significant decrease in renal MDA and NO and an increase in GSH and SOD. In this study, the anti-inflammatory effect for aliskiren was manifested by a significant decrease in kidney TNF-α and TGF-β as compared to diabetic control rats, and an anti-apoptotic effect was seen by reduction of caspase-3. STZ-induced histopathological and ultrastructural changes were reversed by treatment with aliskiren.

The renoprotective effect of aliskiren was suggested before by Hamed et al. (2013) who found that aliskiren (150 mg/day) treatment in hypertensive patients significantly reduced the urinary albumin excretion rate after 6 and 9 months of treatment. The mechanism of these actions is inhibition of the rate-limiting step in the RAS (conversion of angiotensinogen to angiotensin I via renin) by aliskiren leading to a potent renoprotective effect by blocking angiotensin II (Schernthaner 2008; Rashikh et al. 2013). This also leads to preservation of podocyte architecture, mitochondrial function, and epithelial integrity (Huby et al. 2009).

Similar results were obtained by Dong et al. (2010), who found that aliskiren protected against DN and enhanced the protective effects of valsartan against DN by decreasing albuminuria and glomerular mesangial matrix expansion in db/db mice. This is associated with increased glomerular TGF-β and type IV collagen expressions and macrophage infiltration, besides decreased glomerular nephrin expression in db/db mice. They explained that the protective effects of aliskiren attributed to the attenuation of $p22^{phox}$-related nicotinamide adenine dinucleotide phosphate oxidase-induced superoxide (Dong et al. 2010). Aliskiren showed also protective effects against tacrolimus-induced nephrotoxicity in rats through an antioxidant mechanism (Naif et al. 2014). STZ-induced histopathological and ultrastructural changes were reversed by treatment with aliskiren. Aliskiren attenuated glumerosclerosis and tubulointerstitial fibrosis, which are considered as another important predictors of renal dysfunction (Kelly et al. 2007).

Likewise, decreased blood pressure after aliskiren treatment also offers renoprotective activity. This action is simply explained via inhibition of renin and RAAS by aliskiren (Feldman et al. 2008). Esch et al. (2010) also reported that aliskiren improves coronary endothelial function and decreases cardiac hypertrophy in spontaneous hypertensive rats; this was confirmed in the present study by normalization of HR in diabetic rats (Esch van et al. 2010). Another possible explanation is the antioxidant properties of aliskiren as seen in this study and reported before (Lee et al. 2013). Moreover, aliskiren reduced nitric oxide which was suggested to increase in the renal expression of the p47phox component of NAD(P)H oxidase and eNOS. These decrease the indices of systemic and renal oxidative/ nitrosative stress leading to renoprotection (Sonta et al. 2005).

The observed anti-inflammatory activity in the present study confirms that obtained before (Gandhi et al. 2013), where aliskiren significantly reduced TGF-β1, which is further supported by a significant reduction in glomerulosclerosis. The anti-inflammatory effect may be due to inhibition of the binding of renin and prorenin to the prorenin receptor. This stimulates inflammatory mediators as TGF-β, fibronectin, and collagen through the angiotensin-independent extracellular signal-regulated kinase 1 and 2 pathways (Abassi et al. 2009). Furthermore, the observed anti-apoptotic effect of aliskiren is related to its antioxidant activity. This is because oxidative stress disturbs the proapoptotic-anti-apoptotic balance and activated mitochondria-dependent apoptosis via caspase-3 (Pal et al. 2014).

Therefore, aliskiren (rennin inhibitor) has a protective effect on kidney against DN like other ACEIs and ARBs through inhibition of RAS and oxidative stress and enhancement of anti-apoptosis activity. Besides, it has a different potent anti-diabetic effect as shown by the ability to decrease serum glucose, stimulate insulin secretion from isolated β cells, and increase insulin sensitivity. However,

aliskiren was more effective than gliclazide in treating DN as observed in the present study by its more potent antioxidant, anti-inflammatory, anti-apoptotic, and renoprotective effects.

Limitations of the present study include the use of aliskiren in tablet form and absence of metabolic parameters.

Conclusion

This study provides an additional evidence of the beneficial effects of aliskiren treatment in STZ-induced DN in rats. The curative effect of aliskiren could be attributed to its anti-diabetic, renoprotective, antioxidant, anti-inflammatory, and anti-apoptotic effects. Moreover, clinical studies are required to establish the effectiveness of aliskiren treatment in patients suffering from hypertension and diabetes.

Contribution statement HA and AM conceived and designed the study and conducted the systematic review. AM conducted the statistical analysis and drafted the paper. AM and LA contributed to interpreting the findings and revised the paper. NH, AS, and HA are involved in the critical revision of the paper. All authors edited and approved the final version of the manuscript to be published. AM had full access to data in the study and had final responsibility for the decision to submit for publication.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

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