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Empagliflozin alleviates diabetic nephropathy in albino rats through its antioxidative and anti-inflammatory action

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ABSTRACT

Background: Diabetes mellitus (DM) is a widespread medical disorder that has serious medical complications, including nephropathy. Empagliflozin is an antidiabetic drug that belongs to selective SGLT2 inhibitors that improve diabetic kidney functions and enhance glycemic management.

Aim of the study: This study aimed to assess the kidney structural changes caused by D.M. and the possible ameliorative role of Empagliflozin.

Materials and methods: Thirty adult male albino rats were divided into three equal groups: The control group, the diabetic group, and the diabetic group treated with Empagliflozin. Groups 2 and 3 received intraperitoneal injections of Streptozocin (STZ) at a dose of 55 mg/kg to induce diabetes; all rats were left for 60 days. Blood analyses were done for kidney functions, glycated hemoglobin, serum inflammatory and fibrotic markers, and kidney tissue specimens were taken to assay oxidative stress markers and structural changes.

Results: Significant increase in the serum level of urea and creatinine, BUN, serum inflammatory markers (TNF-α, IL-1β, and IL-6), and renal tissue level of MDA, with a marked decrease in renal tissue levels of SOD and GSH levels in diabetic rats compared to control rats. Also, various histopathological alterations were detected in the kidneys of diabetic rats in the form of significant damage to the glomeruli with enlarged urinary spaces, glomerular inflammatory cell infiltration, distorted renal tubules, and reduced glomerular size. Treatment with Empagliflozin alleviates those nephropathic changes through antioxidative, anti-inflammatory, and antifibrotic effects.

Conclusion: Empagliflozin could ameliorate diabetic nephropathy in albino rats through its antioxidative and anti-inflammatory pathways.

Introduction

Diabetes mellitus (DM) is a widespread medical disorder that has been more prevalent over the past few decades, making it a severe public health concern of the twenty-first century despite its potentially catastrophic nature. (1). Macrovascular disorders including peripheral artery disease, stroke, and coronary heart disease, as well as microvascular disorders like diabetic kidney disease, retinopathy, and peripheral neuropathy, have historically been linked to complications in people with diabetes mellitus (2). However, the four most devastating consequences of diabetes mellitus (DM) and the vascular issues it causes are nephropathy, retinopathy, neuropathy, and cardiovascular disease. (3).

Diabetic kidney disease (DKD) is the primary cause of kidney failure globally, affecting 25% to 40% of persons with diabetes mellitus (DM)(4) . Specific renal morphological and functional changes, such as glomerular hyperfiltration and hypertrophy, thickening of the glomerular basement membrane, expansion of the mesangial extracellular matrix, and fibrosis, linked to significant proteinuria and a decrease in glomerular filtration rate, are characteristics of diabetic kidney disease (DN) (5). Several variables, including oxidative stress, hyperglycemia, hyperlipidemia, and inflammatory cytokines, accelerate renal damage in DN. (6).

Despite abundant research on human and animal models of experimental DN, successful treatment is still lacking. Thus, finding medications that stop DN from progressing is a top priority in biomedical research. (7).

Because it protects the kidney, sodium/glucose cotransporter 2 (SGLT2) inhibitors are now the treatment for type II diabetics (8). Empagliflozin belongs to a group of potent and selective SGLT2 inhibitors that prevent the kidneys from absorbing glucose, cause glucosuria, and enhance glycemic management (9).

In the present study, we aim to investigate the structural kidney changes caused by D.M. and the possible ameliorative role of Empagliflozin.

Material and methods

Drugs and chemicals

Streptozocin (STZ) was provided by Sigma-Aldrich Inc., St. Louis, MO, USA. Empagliflozin (Jardiance 10 Mg tablet) was purchased from Boehringer Ingelheim Pharma GmbH & Co.KG, Germany.

The U.K. company Abcam supplied the malondialdehyde (MDA), Glutathione (GSH), and superoxide dismutase (SOD) kits. ELISA-based kits for inflammatory markers (TNF-α, TGF-β1, and IL-6) were also acquired from Abcam, UK.

Experimental animals

This study employed 30 male albino rats weighing an average of 110–125gm when they were 10–12 weeks old. The rats were fed a steady diet and unlimited access to fresh water. Before the trial started, every rat was given a week to become used to its new environment. Our faculty of medicine's institutional review board authorized the experimental protocols, and we followed the National Institute of Health's guide for the care and use of laboratory animals.

Induction of diabetes

Fresh streptozotocin was made in a sodium citrate buffer with a concentration of 0.05 M (pH 4.5). The single intraperitoneal injection of STZ (55 mg/kg b.w.) caused diabetes in all animals except the control group. Blood samples were drawn from the tail vein, and the patient's glucose level was determined using a glucometer (gluco doctor, China).

When the fasting blood glucose levels of the STZ-induced rats exceeded 200 mg/dL, they were considered diabetic. (10).

Experimental design

Thirty adult male albino rats (weighing 110g-135g) were acquired from our faculty's animal house. For one week during their acclimatization phase, rats were housed in steel mesh cages with four rats per cage. They were fed commercial standard chow and given free tap water. Rats were provided with a well-balanced food during the experiment.

The animals were randomly divided into three equal groups (n=10/group). The first group served as control and received 2 ml saline solution per rat daily by oral gavage. The second group (Diabetic group) received a single intraperitoneal injection of STZ (55 mg/kg b.w.) and daily oral saline for two months; the third group (Empagliflozin group) received a single intraperitoneal injection of STZ (55 mg/kg b.w.) and a daily oral dose of Empagliflozin (10 mg/kg/day) for two months. (11).

Sampling

Blood samples

The medial canthus of the eye's veins was punctured to obtain blood samples, which were then allowed to clot before being centrifuged for 15 minutes at 3,000 rpm. Before being used to measure urea and creatinine concentrations, sera were divided into dry, sterile tubes using an automated pipette and kept in a freezer at -20 °C.

Tissue samples

After blood collection, rats were sacrificed by decapitation, according to Animal Ethics Committees, and the abdomen was opened, then kidneys were collected.

For histopathological changes

The kidney tissue samples were cut to a thickness of about 1 cm and put into the cassettes after being sliced in 10% buffered formalin. They were then processed for staining with either hematoxylin and eosin stain or Masson trichrome stain.

For biochemical analysis

One gram of kidney tissues was roughly cut and minced before homogenizing with a glass homogenizer in nine volumes of ice-cold potassium phosphate buffer (pH 7.4) to create 10% homogenates. The centrifuge was then run for fifteen minutes at 6000 RPM at four °C. Renal tissue levels of malondialdehyde (MDA) and glutathione (GSH) contents were measured in the supernatant, as well as measuring Superoxide dismutase (SOD) activity.

Analysis

Biochemical analysis

According to the manufacturer's instructions, Elisa kits measured glycated hemoglobin, Serum urea, and creatinine. Also, serum levels of TNF-α, IL-6, and TGF-β1 were measured.

Statistical analysis

Using SPSS (software version 20), the results were represented as mean ± SE. Duncan's test was performed after one-way ANOVA data analysis. A significance level of $p < 0.05$ was considered for the values.

Results

Streptozocin treatment showed a significant increase in glycated hemoglobin, serum levels of creatinine, BUN, and uric acid compared to the control group, while administration of Empagliflozin to diabetic rats resulted in a significant decrease in those parameters compared to diabetic rats (table 1).

Table 1. Assay of empagliflozin effects on renal function parameter

Parameters	Control	D.M	D.M+ Empag.
Glycosylated HbA1c (%)	4.43± 0.08	7.81 ± 0.17[a]	5.79 ± 0.25[b]
Serum creatinine (mg/dl)	0.51 ± 0.04	1.94 ± 0.07[a]	0.81±0.52[b]
BUN (mg/dl)	26.37 ± 2.28	60.16 ± 4.24[a]	31.71 ± 2.39[b]
Serum uric acid (mg/dl)	1.84±0.27	4.15±0.27[a]	1.97±0.34[b]

D.M: diabetes mellitus; Empag.: Empagliflozin: BUN: blood urea nitrogen.
Data are shown as mean ± SD and (n=10).
[a]Significantly different from the control group at p <0.05
[b]Significantly different from the Diabetic group at p <0.05

Tissue levels of inflammatory and fibrotic markers

STZ treatment showed a significant increase in inflammatory markers (TNF-α, IL-6) and the fibrotic marker TGF-β1 compared to the control group. At the same time, administration of Empagliflozin to diabetic rats resulted in a significant decrease in those markers compared to diabetic rats (table 2).

Table. 2. Assay of Empagliflozin effects on serum levels of inflammatory markers

Parameters	Control	D.M	D.M + Empag.
TNF-α (Pg/ml)	41.35 ± 2.41	122.28 ± 8.65[a]	54.14 ± 3.6[b]
IL-6 (Pg/ml)	61.81 ± 0.64	117.39 ± 3.39[a]	81.63 ± 1.7[b]
TGF-β1 (ng/ml)	6.15 ± 0.53	20.68 ± 1.23[a]	8.27 ± 0.47[b]

TNF-α: Tumor necrosis factor-alpha; IL-6: interleukin-6; TGF-β1: transforming growth factor -beta 1.
Data are shown as mean ± SD and (n=10).
[a]Significantly different from the control group at p <0.05
[b]Significantly different from the Diabetic group at p <0.05

Tissue levels of inflammatory and fibrotic markers

STZ treatment showed a significant increase in tissue level of MDA & area percentage of collagen deposition and a substantial decrease in tissue level of SOD & GSH compared to the control group. At the same time, administration of Empagliflozin to diabetic rats resulted in a significant increase in tissue level of MDA & area percentage of collagen deposition and a significant increase in tissue level of SOD & GSH compared to diabetic rats (table 3 & figure 2).

Table 3. Assay of empagliflozin effects on lipid peroxidation, antioxidants, and fibrotic markers tissue levels.

Parameters	Control	D.M	D.M + Empag.
MDA (nmol/mg protein)	0.22 ± 0.14	0.71 ± 0.65[a]	0.32 ± 0.25[b]
SOD (U/mg protein)	7.12 ± 0.24	3.21 ± 0.43[a]	6.26 ± 0.67[b]
GSH (µmol/g protein)	1.39 ± 0.17	0.51 ± 0.38[a]	1.09 ± 0.28[b]
Collagen $(\mu m)^2$	15.17 ± 0.93	46.57 ± 2.64[a]	20.19 ± 1.27[b]

MDA: malondialdehyde; GSH: glutathione; SOD: Superoxide dismutase
Data are shown as mean ± SD and (n=10).
[a]Significantly different from the control group at p <0.05
[b]Significantly different from the Diabetic group at p <0.05

Histopathological results

Structural changes

The kidneys of diabetic rats were characterized by damage to the glomeruli with enlarged urinary spaces, glomerular inflammatory cell infiltration, distorted renal tubules, and reduced glomerular size. Treatment of diabetic rats with Empagliflozin ameliorated the previous structural changes (Figure 1).

Fibrotic changes

The kidney of diabetic rats was characterized by increased deposition of collagen fibers in the renal tissue compared to the control group. Treatment of diabetic rats with Empagliflozin showed decreased deposition of collagen in renal tissue compared to the diabetic group (Figure 2).

Discussion

In the present study, we aimed to investigate the kidney structural changes caused by D.M. and the possible ameliorative role of Empagliflozin. The single intraperitoneal injection of STZ (55 mg/kg b.w.) caused diabetes in all animals in this study except the control group. In agreement with our results, several studies developed diabetes as a result of a single intraperitoneal injection of STZ at different doses (55, 65, 70 mg/kg b.w.) (6, 10, 12).

In the current study, STZ treatment significantly increased glycated hemoglobin. Our results are consistent with earlier studies that reported increased glycated hemoglobin in rats exposed to STZ-induced diabetes. (5, 13). The induction of diabetes by Streptozocin, because it is a highly selective pancreatic islet β-cell cytotoxic drug,

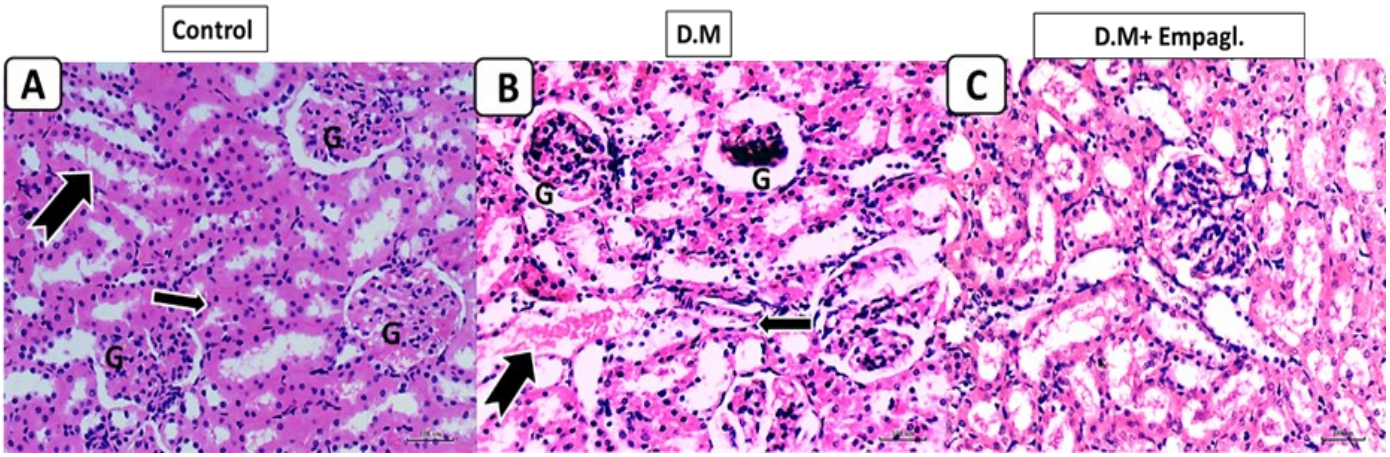

Figure 1. photomicrographs of the kidney of the study groups. (A): control group revealed normal glomeruli (G), proximal (thin arrow), and distal tubules (thick arrow. (B): the kidney of diabetic rats showed shrunk glomerulus, with cellular inflammatory infiltration and widened glomerulus space (G), distorted proximal (thick arrow), and distal tubules (thin arrow). (C): the kidneys of treated rats with Empagliflozin ameliorated the previous structural changes (HX.&E. stain. X 400).

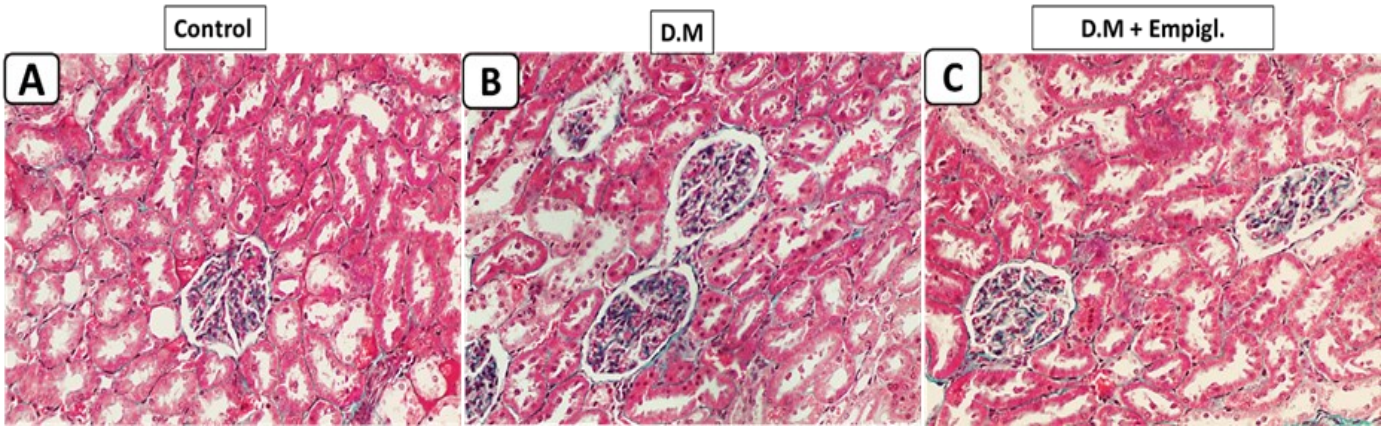

Figure 2. Photomicrographs of the kidney of the control group (A) showed minimal deposition of collagen fibers, while the kidney of diabetic rats (B) showed marked deposition of collagen fibers in the renal tissue. The kidney of the treated group with Empagliflozin (C) showed less deposition of collagen in renal tissue compared to the diabetic group.

commonly given at a single high dose to induce total β-cell necrosis and diabetes in less than 48 hours (14). In this study, STZ treatment resulted in a significant increase in kidney functions, including increased serum levels of creatinine, BUN, and uric acid compared to the control group. Consistent with our findings, several studies revealed that D.M. caused a significant increase in blood urea nitrogen, serum urea, and serum creatinine, indicating severe impairment of renal function due to impaired glomerular filtration membrane barrier function. (5, 15).

Renal pathological alteration is the "gold standard" for the diagnosis of diabetic nephropathy. (15). The impairment of kidney functions in this study is correlated to the alteration in kidney structure as manifested by apparent damage to the glomeruli with enlarged urinary spaces, glomerular inflammatory cell infiltration, distorted renal tubules, and reduced glomerular size. In agreement with our study, D.M. was found to cause severe breakdown of the general structures of the glomeruli, proximal and distal tubules of kidneys of rats. (16, 17).

The diabetic nephropathy induced in this study is primarily caused by oxidative stress, as manifested by increased oxidative enzyme MDA which is exacerbated by high levels of reactive oxygen species (ROS) and compromised antioxidant mechanisms (GSH & SOD). Similar results regarding the oxidative stress markers were recorded in previous studies showed that diabetic rats had much higher renal MDA levels than control animals, along with significantly lower levels of antioxidant enzymes, GSH, and SOD. (18, 19).

This could be explained by the overproduction of reactive oxygen species (ROS) when blood glucose levels are high, and this leads to oxidative damage to the kidneys, which is a significant contributor to the development of diabetic nephropathy (DN) (20).

On the other hand, increased expression of TNF-α, interleukins, and TGF-β1 has been identified as a significant contributor to the development of diabetic nephropathy (21, 22). In the present study, diabetic non-treated rats showed a considerable increase in the fibrotic cytokine TGF-β1 and the inflammatory cytokines TNF-α and IL-6 compared to control rats. Those were similar to the results of other studies (23, 24). This could be explained by the accumulation and activation of macrophages in diabetic kidneys, which is linked to the deposition of glomerular immune complexes, elevated cytokine production, and progressive fibrosis (25).

Our study assessed kidney fibrosis by detecting collagen fibers in renal tissue with a Masson trichrome stain. We found that diabetes caused an increase in collagen fiber deposition in renal tissue compared to control nondiabetic rats. Similar to our results (26, 27)The increased collagen production in diabetic kidneys could be due to increased levels of TGF-β, which is essential for glomerulosclerosis and interstitial fibrosis; it also promotes tissue fibrosis by decreasing collagenase synthesis and extracellular matrix formation. (28).

From the above findings, D.M. induced nephropathy through increased production of oxidative stress and inflammatory and fibrotic markers.

In this study, we used Empagliflozin to alleviate the nephropathic diabetes-induced changes. We found that after Empagliflozin treatment of diabetic rats, the glycated hemoglobin level decreased compared to that of untreated diabetic rats. Similar to our results, multiple researchers found that Empagliflozin use has been linked to decreased glycosylated hemoglobin levels in type 2 diabetic patients, particularly those with chronic renal disease. (6, 29).

Empagliflozin has been shown to lower hyperglycemia in diabetic individuals by decreasing glucose reabsorption through the kidneys, which raises urine glucose excretion. (5).

Also, we found that using Empagliflozin significantly improved kidney function, including a decrease in serum levels of creatinine, BUN, and uric acid compared to the diabetic group. These findings coincide with the findings of other studies, which found that early signs of diabetic nephropathy are lessened with Empagliflozin in the form of decreased serum levels of creatinine, BUN, and uric acid. (6, 8, 29).

The improvement in renal functions after treatment of diabetic rats with Empagliflozin in our study was associated with

improvement of kidney structure compared to diabetic rats. In agreement with our results, several studies found that treatment of diabetic rats with Empagliflozin notably ameliorated tissue injury. (30, 31). Multiple mechanisms cause Empagliflozin's nephroprotective effects, but its direct renovascular effects are the main ones. (32).

Also, Empagliflozin was found to have antioxidant effects in this study, which ameliorate the oxidative stress effects produced by D.M. in the form of decreased oxidative enzyme MDA and enhanced the antioxidant markers (GSH & SOD). Similar results regarding the oxidative stress markers were recorded in previous studies, showing that treatment of diabetic rats with Empagliflozin decreased the renal tissue levels of MDA compared to diabetic animals, along with significantly increased levels of antioxidant enzymes, GSH, and SOD. (5, 31).

The antioxidant effects of Empagliflozin in this study were associated with anti-inflammatory and antifibrotic effects in the form of a considerable decrease in the fibrotic cytokine TGF-β1, decreased deposition of collagen in kidney tissue, and the inflammatory cytokines TNF-α and IL-6 compared to diabetic rats. Those were similar to the findings of other studies, which indicated that Empagliflozin alleviates renal fibrosis caused by D.M. (the main pathological feature of DN) through its antioxidative, antifibrotic, and anti-inflammatory effects. (5, 33).

Hence, Empagliflozin protects diabetic nephropathy through antioxidative, anti-inflammatory, and anti-fibrotic pathways.

Conclusion

Empagliflozin could ameliorate diabetic nephropathy in albino rats through its antioxidative and anti-inflammatory pathways.

Declaration of Competing Interests

The authors declare no competing interests

Data sharing plans

Data will be available upon request

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