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Combination of empagliflozin and curcumin ameliorates the induced diabetic nephropathy in male albino rats, through their synergistic anti-diabetic and antioxidative effects

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ABSTRACT

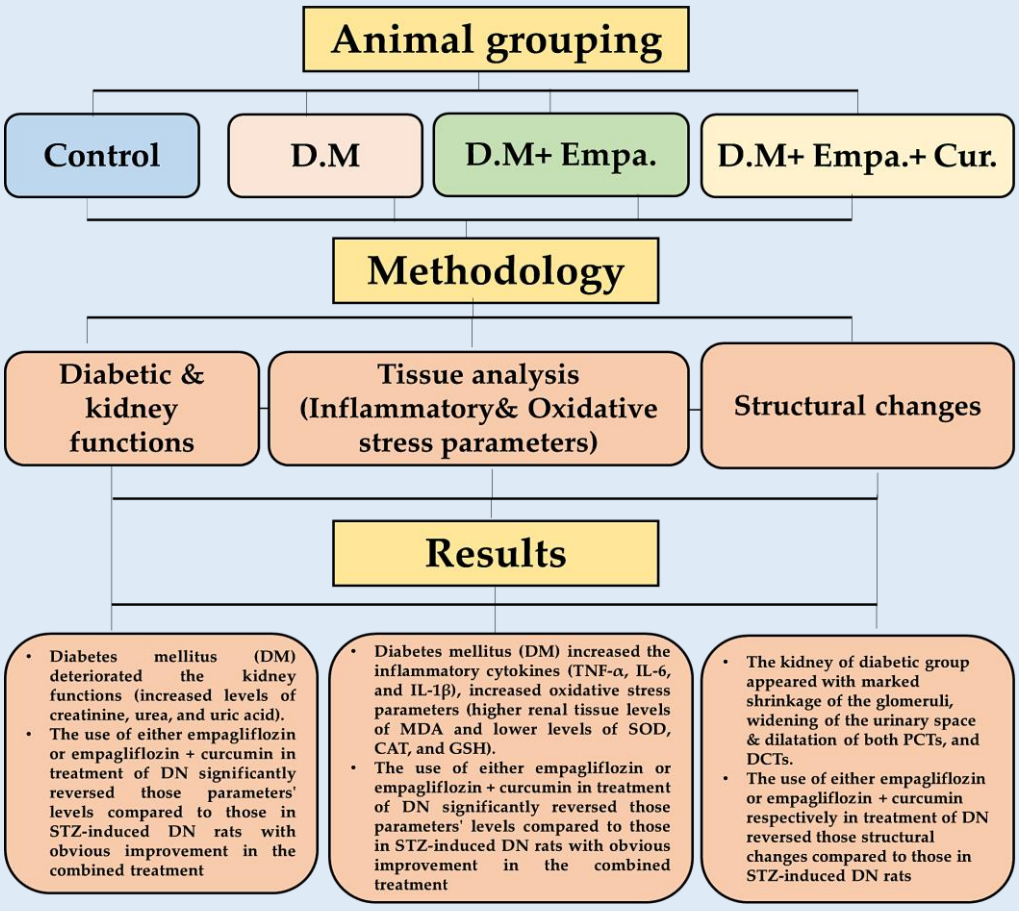
Background: Diabetic nephropathy is considered a global public health burden. We aimed to examine the combined effects of empagliflozin (a conventional drug) and curcumin (a natural compound) in the alleviation of nephropathic changes in diabetic rats, which could be a unique therapeutic strategy.

Materials and Methods: Forty adult male albino rats were randomly assorted into 4 equal groups (10 rats/group): control group, diabetic induction group by streptozocin, empagliflozin group, and empagliflozin plus curcumin group. After the rat's scarification, blood samples were taken to test levels of blood glucose and kidney functions. Fresh kidney specimens were removed and processed to further analyze oxidative stress and inflammatory markers. Additionally, histological and immunohistochemical studies were conducted by different stains to detect structural and apoptotic changes. An image-analyzing microscope was used for the assay of the optical density.

Results: Diabetes mellitus (DM) deteriorates kidney functions (increased levels of creatinine, urea, and uric acid) and increases oxidative stress, leading to pathological structural changes in the kidney (glomerular dystrophy, tubular affection, and apoptosis). The use of empagliflozin in treating DM alleviates most of those changes, but the combined treatment with empagliflozin plus curcumin enhanced those pathological changes more significantly than empagliflozin alone.

Conclusion: Combination of empagliflozin and curcumin ameliorates diabetic nephropathy in rats through their anti-diabetic and antioxidative effects.

Keywords: Diabetic nephropathy, Curcumin, Nephropathy, Empagliflozin; Streptozocin.



Graphical Abstract: Combination of empagliflozin and curcumin ameliorates the induced diabetic nephropathy in male albino rats, through their synergistic anti-diabetic and antioxidative effects

INTRODUCTION

Globally, DM is among the most prevalent non-communicable diseases and considered as a primary cause of death and disability [1]. By 2030, it is predicted that 7.7% of adults worldwide will have diabetes mellitus [2].

Individuals with diabetes mellitus are more likely to experience macrovascular and microvascular problems affecting large and small blood vessels. Prolonged diabetes increases the severity of microvascular complications, such as renal, retinal, and organ neuropathy [3-4].

Diabetic nephropathy (DN), one of the primary effects of diabetes mellitus, can progress to end-stage renal disease (ESRD) and require costly renal replacement therapy, such as dialysis or transplantation [5]. Some hallmarks of diabetic DN, defined by structural and functional kidney damage caused by chronic high blood glucose levels through different inflammatory and oxidative stress mechanisms, include a slow fall in glomerular filtration rate, presence of proteinuria, and increase in deterioration of renal function [6].

When diabetes mellitus is treated early with diet, exercise, and weight loss, medication use can be avoided. However, antidiabetic drugs are necessary to reach ideal blood glucose levels to prevent further problems [7].

From a therapeutic perspective, treating DM early can reduce the loss of renal function and lessen unfavorable outcomes. Hence, finding new treatment of DM and associated DN is crucial to stopping or slowing the development of DM to DN [8]. Many strategies have been proposed to mitigate the effects of diabetic kidney disease, such as those that disrupt oxidative stress, apoptotic pathways, and inflammatory response. Empagliflozin is an antidiabetic with significant anti-inflammatory, antioxidant, and antiapoptotic effects, it is an inhibitor of sodium-glucose co-transporters-2 (SGLT2) [9]. Because of its various effects (antioxidative, anti-inflammatory, and antiapoptotic), empagliflozin has been demonstrated in experimental studies to lessen renal tubular dysfunction and nephropathic effects in diabetic rats and mice [10-11].

Functional food products (FFPs) and their ingredients, known as food bioactive compounds (FBCs), are a potential substitute for traditional medication. These products have unique qualities that may reduce the risk of certain diseases [12].

Curcumin, a natural extract from Curcuma longa roots, is regarded as a functional food component [13]. Curcumin, a bioactive phenolic molecule, has numerous pharmacological and biological characteristics, including healing wounds, lowering blood sugar, inducing anti-inflammatory, anti-cancer, and antioxidant properties, reducing inflammation, and having antibacterial effects [14-15].

We designed this study to investigate the possible protective effects of the novel combined therapeutic strategy of empagliflozin and curcumin on rat's kidney exposed to induced diabetic nephropathy.

Materials and methods

Approval Ethics

The Research Ethical Committee, Damietta Faculty of Medicine, Al-Azhar University approved the protocol of this experimental research (DFM-IRB 00012367-24-10-007).

Drugs and chemicals

Streptozotocin: A fresh solution of streptozotocin (Sigma Aldrich, St. Louis, MO, USA) was prepared using 0.9% ice-cold sterile saline.

Empagliflozin: Empagliflozin (Empaglimax 10mg tablet) was purchased from Hikma Pharmaceutical Co, Giza, Egypt. Rats were given the precise dose at the designated time [16].

Curcumin: Curcumin was suspended in 0.5-riboximethyl cellulose after being acquired from Acros Organics

Product in the United States, and the fresh suspension of curcumin was prepared at the time of experiment. Rats were given the precise dose at the designated time [17].

Animals: 40 adult male albino rats (average weight 120g) that appeared to be in good health were utilized in this study. The animals were purchased and raised in the Veterinary Serum & Vaccine Research Institute's Animal House in Abbasya, Cairo, Egypt. The rats were housed in well-ventilated stainless-steel covers on polypropylene cages in a controlled environment with a 12-hour light/dark cycle, 24°C (±3°C) temperature, and 50–70% humidity. They were acclimated for a week, given a normal diet and unrestricted access to tap water.

Induction of diabetes: To induce diabetes, rats were given a fasting diet for 12 hours before receiving an intraperitoneal injection of streptozocin (STZ) at a dose of 60 mg/kg BW. Then rats were given a 5% sucrose solution mixed with 0.01 M sodium citrate buffer, pH 4.5 using a glucometer (Glucodoctor, China). 1 week after the STZ injections, blood samples were taken from the tail vein, and the rat's glucose level was assessed to confirm induction of diabetes. The STZ-induced rats were classified as diabetic when their fasting blood glucose levels were more than 250 mg/dL. [18, 19].

Design of experimental animals: Four equal groups (10 rats/group) were randomly assigned from among the animals: the first group received normal rat chow bullets (control group), the second group received one intraperitoneal injection of STZ of concentration 60 mg/kg b.w. (diabetic group). The third group received oral doses of empagliflozin (10 mg/kg/day) after 1 week of induction of D.M with intraperitoneal injection of STZ of concentration 60 mg/kg b.w. (Empagliflozin group), and the fourth group received oral doses of empagliflozin (10 mg/kg/day) plus curcumin (150 mg/kg/day) by oral gavage after 1 week of induction of D.M with intraperitoneal injection of STZ of concentration 60 mg/kg b.w. (Empagliflozin plus curcumin group). 8 weeks were dedicated to the administration of either Empagliflozin therapy or combination therapy of Empagliflozin plus curcumin.

Blood collection, Serum biochemical analyses: Blood samples from the retro-orbital plexus were taken at the end of the study. The serum was separated by centrifugation at 1200 g for 15 minutes and stored at -20°C for additional biochemical analyses. The rat insulin ELISA Millipore kit was used to measure the serum insulin level in accordance with the supplier's instructions. Using standard kits (Biodiagnostic, Giza, Egypt), serum parameters of kidney function (creatinine, uric acid and urea) and serum sodium & potassium levels were measured calorimetrically in accordance with the manufacturer's instructions.

Evaluation of Histological Alterations and oxidative stress parameters: Fresh kidney specimens were preserved in 10% formalin for 24 hours, then dehydrated in alcohol, cleared in xylol, embedded in paraffin, sectioned using a microtome, and stained with H&E for structural analysis. PAS staining was used to detect mucopolysaccharide content, while the anti-apoptotic marker Bcl-2 was applied to assess apoptotic changes. Using a light microscopy (Raywild, Germany) to examine the produced slides, images were captured. The area density % of PAS stain for mucopolysaccharide contents and B cell lymphoma 2 (BCL-2) expression for apoptosis were calculated using an image analyzing microscope.

Another fresh specimen of kidney tissues was removed from the various experimental groups, cleaned, homogenized in saline, and centrifuged at 4000 rpm for 5 minutes. The resultant suspension was gathered and kept for later analysis at -80°C. Using the manufacturer's instructions, the renal tissue levels of inflammatory

cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1β), were investigated. Kidney reduced glutathione (GSH) and malondialdehyde (MDA) levels were estimated using bio diagnostic kits from Egypt. The primary method of analyzing the activity of catalase (CAT) and superoxide dismutase (SOD) activity is blocking adrenaline auto-oxidation to adrenochrome in an alkaline media were measured [20].

Statistics: The mean ± SE is used to represent data. The statistical software program SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data using the one-way ANOVA and two-tailed Student's test. Duncan's post hoc test was then used to compare data among groups. $P < 0.05$ was considered statistically significant.

RESULTS

Blood Glucose Levels: Rats with STZ-induced DN showed increased glucose levels and decreased serum insulin levels. The use of either empagliflozin or empagliflozin plus curcumin in treatment of DN significantly increased the insulin levels. It significantly decreased the glucose level compared to those in STZ-induced DN rats (Table 1).

Table 1. Assay of glucose and insulin levels in different groups.

Variables	Control	D.M	D.M + Empa.	D.M + Empa.+ Cur.
F.B.G (mg /dl)	82 ± 3.47	318 ± 20.29 *	105 ± 4.74 #	89 ± 2.41#
F.I.L (ug/l)	2.54 ± 0.27	0.84 ± 0.18 *	1.97 ± 0.35 #	2.18 ± 0.38#

F.B.G: fasting blood glucose; F.I.L.: Fasting Insulin level; D.M: Diabetes mellitus; Empa: Empagliflozin; Cur. Curcumin

*Significant change compared to the control group

#Significant change compared to the diabetic group.

Kidney functions:

Rats with STZ-induced DN showed elevated urea, creatinine, and uric acid levels and decreased serum sodium and potassium levels compared to the control group. Using either empagliflozin or empagliflozin plus curcumin in treatment of DN significantly decreased renal parameter levels and increased serum sodium & potassium levels compared to those in STZ-induced DN rats. An obvious improvement in the positive effects was seen in the combined treatment (Table 2) compared to the treatment of just empagliflozin.

Table 2. Assay of kidney functions in different groups.

Variables	Control	D.M	D.M + Empa.	D.M + Empa.+ Cur.
Creatinine (mg /dl)	0.41 ± 0.34	1.67 ± 0.28 *	0.85 ± 0.34#	0.74 ± 0.16#
Urea (mg /dl)	27.74 ± 5.14	50.28 ± 6.17 *	33.71 ± 4.74#	5.15 ± 0.24#
Uric acid (mg /dl)	4.34 ± 0.64	7.24 ± 0.67 *	5.35 ± 0.74#	5.15 ± 0.24#
Na+ ions (mEq/L)	137 ± 0.47	80.41 ± 4.16*	109.5 ± 5.69#	134.7 ± 2.61#
K+ ions (mEq/L)	4.24 ± 0.41	8.17 ± 0.49*	6.08 ± 0.32#	4.91 ± 0.45#

*Significant change compared to the control group

#Significant change compared to the diabetic group.

Effects on inflammatory cytokines in the studied group: The rats in the DN group had higher renal tissue levels of (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β). The use of either empagliflozin or empagliflozin plus curcumin in the treatment of DN significantly reversed those parameters' levels compared to those in STZ-induced DN rats with obvious improvement in the combined treatment (**Table 3**).

Table 3. Assay of inflammatory cytokines in different groups.

Variables	Control	D.M	D.M + Empa.	D.M + Empa.+ Cur.
TNF-α (pg/mg protein)	0.41 ± 0.34	1.67 ± 0.28 *	0.85 ± 0.34#	0.74 ± 0.16#
IL-1β (pg / mg protein)	27.74 ± 5.14	50.28 ± 6.17 *	33.71 ± 4.74#	5.15 ± 0.24#
IL-6 (pg / mg protein)	4.34 ± 0.64	7.24 ± 0.67 *	5.35 ± 0.74#	5.15 ± 0.24#

TNF-α: tumor necrosis factor-α; IL-1β: Interleukin-1β; IL-6: Interleukin-6

*Significant change compared to the control group

#Significant change compared to the diabetic group.

Effects on oxidative stress and antioxidant system in rats' kidney: The rats in the DN group had higher renal tissue levels of MDA and lower levels of SOD, CAT, and GSH. The use of either empagliflozin or empagliflozin plus curcumin in the treatment of DN significantly reversed those parameters' levels compared to those in STZ-induced DN rats with obvious improvement in the combined treatment (Table 4).

Table 4. Assay of oxidative stress and antioxidant system in different groups.

Variables	Control	D.M	D.M + Empa.	D.M + Empa.+ Cur.
MDA (µmol/g tissue)	0.427 ± 0.067	1.361 ± 0.248*	0.371 ± 0.053#	0.403 ± 0.016#
GSH (µmol/g tissue)	1.635 ± 0.091	0.739 ± 0.125*	1.34 ± 0.092#	1.409 ± 0.149#
CAT (U/g protein)	3.284 ± 0.381	0.814 ± 0.093*	2.836 ± 0.421#	3.103 ± 0.387#
SOD (U/g protein)	3.254 ± 0.257	0.782 ± 0.036*	3.012 ± 0.314#	3.149 ± 0.091#

MDA: malondialdehyde; GSH: reduced glutathione; CAT: Catalase: SOD: superoxide dismutase.

*Significant change compared to the control group

#Significant change compared to the diabetic group.

Structural changes

Effects on Kidney Structures in Hx. &E-stained sections. The kidneys of the diabetic group appeared with marked shrinkage of the glomeruli, widening of the urinary space, and dilatation of both PCTs and DCTs. The use of either empagliflozin or empagliflozin plus curcumin in the treatment of DN reversed those structural changes compared to those in STZ-induced DN rats (Figure 1).

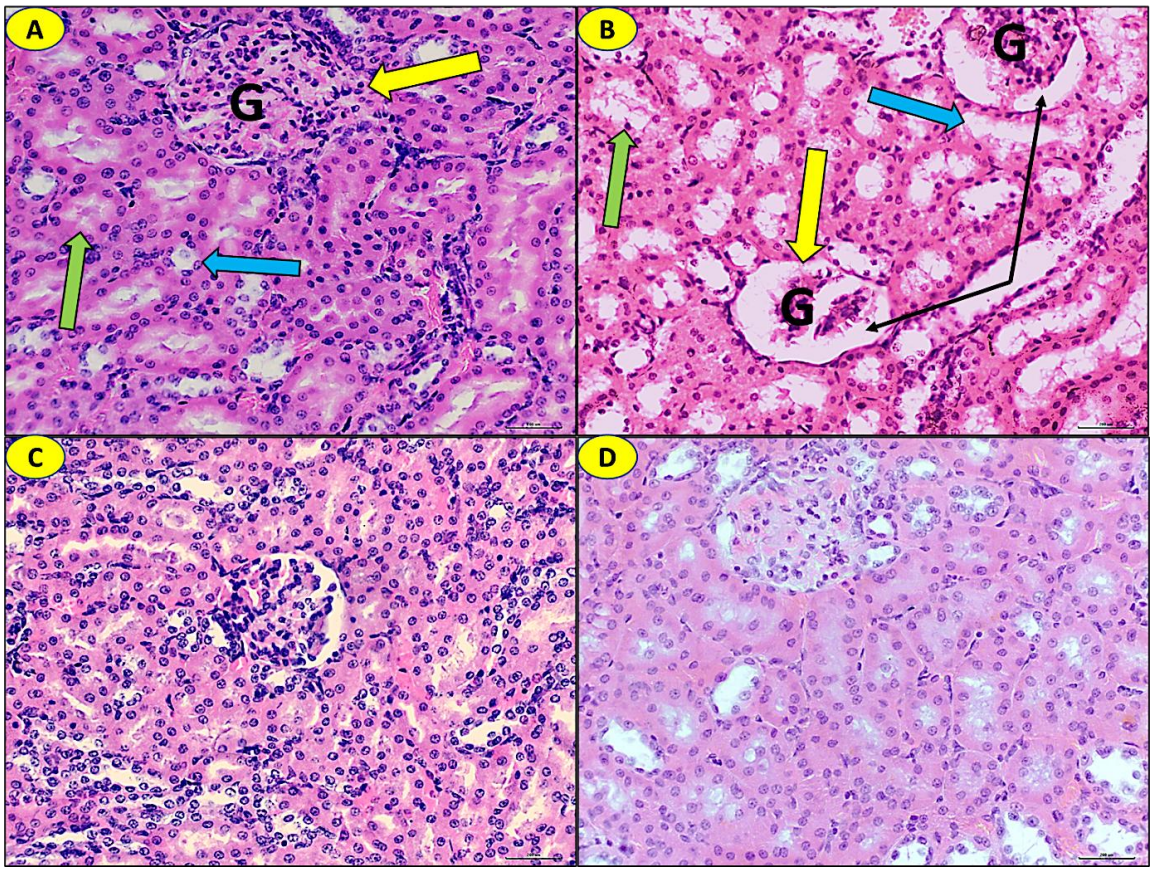


Figure 1. (A) The kidney from the control rat group displayed normal renal corpuscle (yellow arrow), with its glomerulus (G) composed of a capillary tuft enclosed in a Bowman's capsule, Typical narrow lumina and simple cubical epithelium lining proximal convoluted tubules with a prominent brush border (green arrow) and the distal convoluted tubules (blue arrow) appeared with wider lumen. **(B)** The kidney of the diabetic group appeared with marked shrinkage of the glomeruli, widening of the urinary space (double-sided arrow) & dilatation of PCTs with loss of its brush border, and DCTs (green & blue arrows respectively). **(C&D).** Treatment of DN with empagliflozin or empagliflozin + curcumin respectively reversed those structural changes compared to those in STZ-induced DN rats (Hx. &E. X400) Scale bars, 200 µm.

Assay of apoptotic, and Mucopolysaccharide contents in rats' kidney: The diabetic rats exhibited a significant decrease in Bcl-2 immunohistochemical expression and increase in the P.A.S reaction in the kidney compared to that of the control group. However, when empagliflozin or empagliflozin plus curcumin in the treatment of DN were administered simultaneously to the diabetic rats, those levels were reversed in comparison to that of the diabetic group with obvious improvement in the combined treatment (Table 5 and Figures 2&3).

Table 5. Assay of apoptotic, and Mucopolysaccharide contents in rats, kidney in the studied groups:

Variables	Control	D.M	D.M + Empa.	D.M + Empa.+ Cur.
Bcl-2 density (mm3)	1.823 ± 0.101	0.464 ± 0.084*	1.348 ± 0.073#	1.512 ± 0.065#
P.A.S density (mm3)	2.381 ± 0.360	4.015 ± 0.208*	3.107 ± 0.129#	2.82 ± 0.527#

*Significant change compared to the control group

#Significant change compared to the diabetic group.

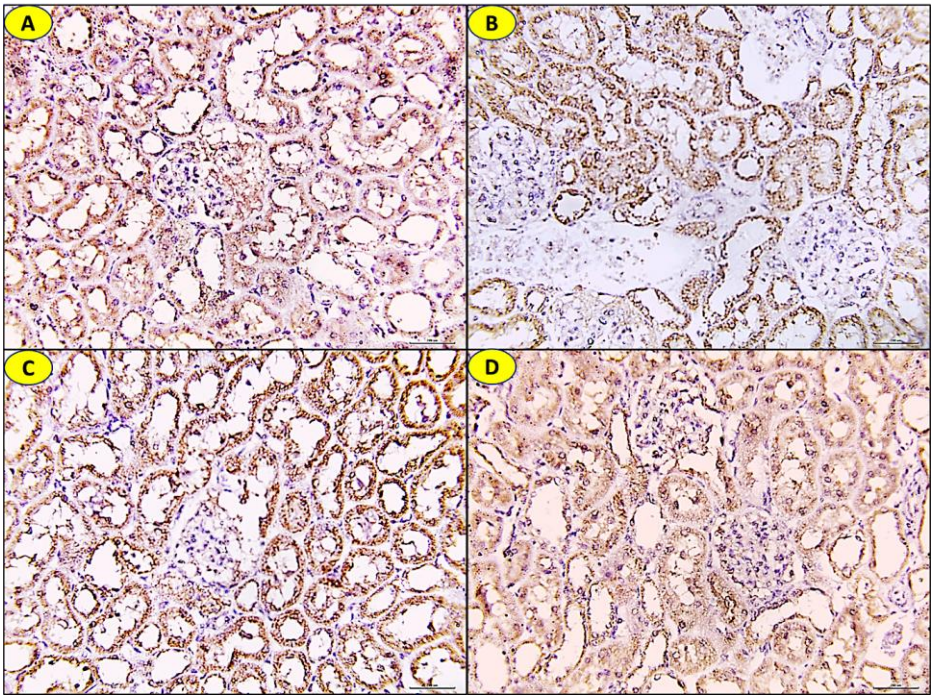


Figure 2: The diabetic rats exhibited a significant decrease in the Immuno stained kidney sections for Bcl-2 density compared to that of control group, however when empagliflozin or empagliflozin + curcumin in treatment of DN were administered simultaneously to the diabetic rats, those levels were increased in comparison to that of diabetic group (Bcl-2 immunohistochemical stain. X400) Scale bars, 200 µm.

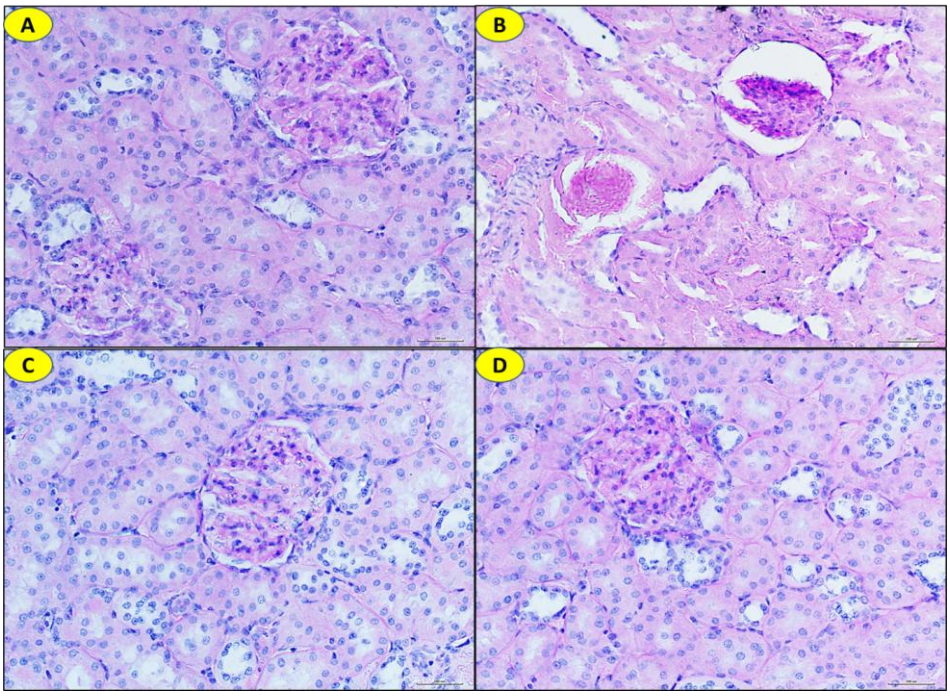


Figure 3: The glycogen deposition in the control group was found to be in the thin basement membrane of Bowman's capsule, the thin tubular basement membrane, and the brush border of the proximal tubule. The diabetic rats exhibited a significant increase in the glycogen contents in the kidney compared to that of the control group. The significant glycogen deposition was noticed within the glomeruli and the thick basement membrane of Bowman's capsule, with Loss of the brush border of the PCT of diabetic rats. When empagliflozin or empagliflozin + curcumin in treatment of DN were administered simultaneously to the diabetic rats, the glycogen deposition was decreased, and the brush border of proximal tubules was restored in comparison to that of diabetic group (P.A.S stain. X400) Scale bars, 200 µm.

DISCUSSION

This study aimed to investigate the efficacy of the combination of empagliflozin and curcumin in ameliorating induced diabetic nephropathy in male albino rats.

Induction of D.M.: In the present study, DM was usefully induced in rats receiving a single intraperitoneal injection of streptozocin (STZ) at a dose of 60 mg/kg BW.

Similarly to our results in biomedical research, STZ was frequently used as the preferred method to create animal models of diabetes mellitus by specifically destroying the islet β-cells. The cytotoxic activity on pancreatic β-cells affects endogenous insulin release and/or action leading to an increase in fasting blood glucose levels [21-22].

Effect of D.M on the kidney structures and functions: In this study, we found that induction of D.M by streptozocin in albino rats caused functional kidney impairment as evidenced by elevated serum levels of creatinine and uric acid and decreased sodium and potassium levels. This coincides with the previous studies that investigated the effect of induction of D.M on the rat's kidney and revealed deterioration of kidney functions in SZ-induced diabetic rats [17-18, 23].

In earlier research, elevated blood levels of albumin, creatinine, and urea and decreased sodium and potassium levels have been suggested to function as clinical markers of increased glomerular filtration rate due to DN [22, 24].

In the present study, the presence of renal injury was further supported by the observation of histological lesions in the renal cortex and medulla in diabetic rats. Alterations in renal structural changes in the form of tubular and glomerular dystrophy with widening of the tubular and urinary space of the glomeruli, loss of brush border of the proximal convoluted tubules, and thickening of tubular membranes and bowman's membrane were found. Similar findings were conducted by previous studies on the induced diabetes in rats by either SZ or alloxan, where they found dramatic alteration in their kidneys' glomerular and tubular histological features [25-27].

This could confirm that untreated D.M induces DN, and the elevated blood glucose levels induce macro- and microvascular problems, end-stage renal disease, and long-term diabetic consequences globally [28].

Oxidative stress: Oxidative stress has been proposed as the primary factor causing DN. Decreased antioxidant activity and increased prooxidant generation may contribute to the pathophysiology of DN [29].

In this study, we found an increase in oxidative markers MDA levels in the kidneys of diabetic rats, indicating increased production of free radicals and related lipid peroxidation. This confirmed that persistent hyperglycemia produced oxidative stress and excess ROS.

Similar to the results of this study, earlier studies on diabetic rats have found that streptozotocin significantly increases oxidative stress and may also cause polyunsaturated fatty acids to peroxide, which is crucial for the synthesis of MDA, a byproduct of lipid peroxidation [30].

Prolonged hyperglycemia is the primary factor believed to be responsible for the formation of intracellular ROS. Some cells cannot maintain intracellular glucose homeostasis, which accelerates glycolysis and releases excessive ROS. As a result, significant amounts of glucose are transported into the glomerular endothelium, mesangial cells, and tubular epithelial cells [31].

Antioxidant system: In our study, we found decreased levels of antioxidative enzymes like reduced glutathione (GSH), CAT, and SOD. The previous investigation supported this attribution because the STZ-induced diabetic rats showed inhibition of GSH and catalase activities [21].

Similarly, a human study that assessed the plasma activity of the antioxidant enzymes in expecting DN development revealed that the levels of Superoxide dismutase (SOD) and glutathione peroxidase (GPX) plasma activity were considerably lower in the diabetic patients than in the controls [32].

The current study's observation of a drop in antioxidative enzymes (GSH, CAT and SOD) levels in the untreated diabetic group may have resulted from the enzymes excessive use to scavenge free radicals under oxidative stress caused by diabetes [33].

Apoptotic changes: Along with oxidative stress's effect in causing nephropathy, inflammation and apoptosis may also play a significant part in causing kidney failure and histological deteriorations, as demonstrated by STZ-induced diabetic rats.

In this study, the anti-apoptotic mediator Bcl-2 levels were lower than the control group. These findings are consistent with the earlier research, where it was found that the kidney of STZ-induced diabetic rats had a higher level of the apoptotic mediator Bax and a lower level of Bcl-2, as determined by immunofluorescence and immunohistochemical methods [34-35]. Hyperglycemia-induced apoptosis in the kidney of STZ-induced diabetic rats, may be mediated by the mitochondrial route [36].

Inflammatory changes: The present study revealed that the rats in the DN group had higher renal tissue levels of inflammatory cytokines (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1β) compared to the normal group. In accordance with the results, a recent study demonstrated an elevation of inflammatory cytokines, TNF-α, IL-6, and IL-1β, in the kidney of diabetic rats compared to the normal group [37]. One of the main causes of the onset and progression of DN is inflammation. Symptoms of the inflammatory process include increased levels of inflammatory cytokines, presence of pyroptosis, and activation of the inflammasome complex [38].

Based on the above findings, our research found that chronic hyperglycemia causes DN, which is characterized by abnormal renal histology, higher levels of ROS, the presence of apoptotic markers, decreased antioxidant levels, inflammation of cytokines, and impaired kidney function.

Empagliflozin alone or in combination with curcumin: In the current study, it was found that the use of Empagliflozin alone or in combination with curcumin in treatment of diabetic rats enhanced the nephropathic changes induced by diabetes as evidenced by the remarkable changes in kidney function parameters (serum levels of creatinine, uric acid and increased serum levels of sodium and potassium and the structural changes that occurred in the kidney of diabetic rats were also enhanced. This coincides with the previous research on Empagliflozin alone or curcumin alone, which was used in treating diabetic rats [39-40].

In this study, the apoptotic changes in the kidneys of diabetic rats were also enhanced under the effect of Empagliflozin alone or in combination with curcumin in the treatment of diabetic rats, as evidenced by the slower rise in levels of the antiapoptotic marker Bcl-2. In accordance with the results, Empagliflozin alone or curcumin alone were found to enhance the apoptotic changes induced by diabetes in the testis and kidney of albino rats in several studies [41-42].

The obvious improvement in the combination therapy coincides with a previous study that revealed that treatment of DM necessitates the use of greater doses or combination therapy to maintain glycemic control because it is a progressive disease marked by increasing hyperglycemia [43].

Thus, it was postulated that giving diabetic rats Empagliflozin alone or combined with curcumin would lessen the production of free radicals and lipid peroxidation, minimizing oxidative damage to cellular structures. This was confirmed by the increased oxidative

markers, MDA levels, and the rise in levels of the antioxidative enzymes like reduced glutathione (GSH), CAT, and SOD in the diabetic groups treated with Empagliflozin alone and in combination with curcumin. There was an extra increase in these effects in the combined trial.

In agreement with the results of this study, several experimental studies found that Empagliflozin or curcumin alone alleviated diabetic nephropathy in rats through their antioxidative effects [39-40].

The obvious improvement in the combination therapy coincides with a previous study that revealed that treating DM necessitates the use of greater doses or a combination therapy to maintain glycemic control. This is because DM is a progressive disease marked by increasing hyperglycemia [18].

In addition, it was also postulated that giving diabetic rats Empagliflozin in combination with curcumin would significantly reverse diabetic nephropathy changes by decreasing the production of free radicals, lipid peroxidation, inflammatory markers, and oxidative damage to cellular structures.

Finally, this study's main finding was that Empagliflozin alone or in combination with curcumin treatment significantly suppressed all molecular-cellular perturbations, including oxidative stress, inflammation, and apoptotic biomarkers that occurred in diabetic animals.

CONCLUSION

Combining empagliflozin and curcumin could be a novel therapeutic strategy for ameliorating diabetic nephropathy in rats through their anti-diabetic, anti-inflammatory, and antioxidative effects.

Abbreviations: DM: Diabetes mellitus, DN: Diabetic nephropathy, ESRD: end-stage renal disease, SGLT2: sodium-glucose co-transporters-2, FFPs: functional food products, FBCs: food bioactive compounds, STZ: streptozocin: BCL-2: B cell lymphoma 2, TNF-α: tumor necrosis factor-alpha, IL-6: interleukin-6, IL-1β: interleukin-1β, GSH: reduced glutathione, MDA: malondialdehyde, CAT: catalase, SOD: superoxide dismutase, Empa.: empagliflozin, Cur.: curcumin, ROS: reactive oxygen species.

Authors Contribution: OR, RT, FA and MA involved in supervision of the experiment, collection of data for the investigation, and result interpretation. All authors contributed to designing the experimental protocol, the implementation of the overall study, the statistical analysis of the study, researched the data, result interpretation, and wrote the manuscript. All authors contributed to the revision of the manuscript and approval of the final manuscript.

Competing Interests: None

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