Ameliorative Effect of Metformin and Vildagliptin on Rat Model Diabetic Kidney Disease

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Abstract
Diabetic kidney disease (DKD) serves as the leading factor in the development of chronic kidney disease, which Progress to end-stage renal failure. Metformin is the first-line therapy indicated for diabetes but it has many side effects including gastrointestinal tract (GIT) disturbance. Researchers have developed various antidiabetic drugs that can reveal hypoglycemia, such as dipeptidyl peptidase 4 (DDP-4) inhibitors such as vildagliptin. This study shows the therapeutic value of metformin and vildagliptin in rat models of STZ-induced DKD. Diabetes was produced in the experimental rats by feeding on a high-fat-high fructose diet (HFHF) and intraperitoneal (I.P) injection of single low-dosage streptozotocin (35 mg/kg.BW). As soon as the development of diabetes, metformin (100 mg/kg/day) and vildagliptin (6 mg/kg/day) were administered orally for eight weeks. The biochemical parameters of blood glucose, serum insulin, creatinine, urea, serum albumin, and lipid profile were evaluated. The levels of serum oxidant/antioxidant markers Malondialdehyde (MDA) and total antioxidant capacity (TAC) were determined. Treatment with metformin and vildagliptin dramatically improved the biochemical parameters of serum glucose, insulin, urea, creatinine, serum albumin, lipid profile, and oxidative stress via oxidant/antioxidant activity (MDA and TAC) showed that vildagliptin outperformed metformin in almost all of the assayed parameters.

Keywords: Diabetic kidney disease, Metformin, Vildagliptin.

Introduction
Diabetes mellitus (DM) is a widespread global health condition; it is a group of metabolic and inflammatory disorders characterized by hyperglycemia, a condition caused by partial or complete insulin insufficiency [1, 2].

It is classified into two types: Type 1 diabetes mellitus (T1DM) is a persistent autoimmune disorder identified by raised blood glucose levels above normal (hyperglycemia) caused by insulin insufficiency due to loss of pancreatic islet β-cells, and Type 2 Diabetes Mellitus (T2DM) is a chronic and progressive metabolic disorder that developed mainly due to combination of main two factors: deficiency in the secretion of insulin by pancreatic β-cells and insulin-sensitive tissues are unable to respond to insulin, Characterized by hyperglycemia, dyslipidemia, and insulin resistance [3, 4].
Diabetic kidney disease is classified as chronic kidney disease with diabetes. It typically initiates with microalbuminuria, progresses to macroalbuminuria, and ultimately leads to a gradual decline in kidney function which leads to the advance of end-stage renal disease (ESRD) and represents about 30-40% of diabetic patients worldwide [5, 6]. Many risk factors can be developed as susceptibility factors such as age, and gender, initiation factors such as hyperglycemia, and progression factors such as hypertension, oxidative stress, and obesity. It is characterized by albuminuria, decreasing glomerular filtration rate, glomerular hypertrophy, glomerular basement membrane thickening, glomerulosclerosis, tubulointerstitial inflammation, and fibrosis which leads to ESRD [7-9].

Oxidative stress (OS) is a condition when excessive amounts of free radicals and antioxidant mechanisms cannot degrade them effectively. In T2DM, OS is a common and essential mechanism relating to prolonged hyperglycemia to vascular problems through metabolic changes in target tissue molecules which enhance the creation of free radicals, reactive nitrogen species (RNS), and reactive oxygen species (ROS). Prolonged untreated OS in T2DM causes endothelial dysfunction (ED), insulin resistance (IR), damaged pancreatic β-cells, and lipid peroxidation which lead to the development of microvascular complications in the kidney which causes podocyte injury, endothelial cell dysfunction, mesangial cell injury, microalbuminuria, and glomerular apoptosis, as well as promotes the development of ESRD [10-12]. OS-induced lipid peroxidation can result in more than only oxidative indicators in chronic kidney disease (CKD); Malondialdehyde is the byproduct of polyunsaturated fatty acid peroxidation. It has been demonstrated to cause malfunctioning high-density lipoprotein (HDL) molecules [13].

Effective T2DM management is important. The main objectives are to lower and maintain blood glucose levels, which reduce the occurrence and severity of microvascular and macrovascular complications, enhance quality of life, and decrease mortality [14]. Several synthetic antidiabetic drugs are used for the management of diabetes, we focused on metformin and dipeptidyl peptidase 4 (DPP-4) inhibitors [15].

Metformin is a biguanide and hypoglycemic drug, with over 100 million patients worldwide using it as their first line of medication for T2DM [16]. These drugs are limited by side effects including GIT disturbance, and some cannot be administered in patients with severe chronic renal impairment [17]. As mentioned in previous studies metformin improves by reducing renal inflammation, oxidative stress, and fibrosis [18, 19].

Vildagliptin is one of the DPP-4 inhibitor drugs that reduce the breakdown of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin-releasing polypeptide (GIP), leading to increase active incretins hormone levels, the response of pancreatic β- cells to glucose, insulin secretion, sensitivity, lipoprotein metabolism, at the same time, reduces the glucagon secretion, and improved diabetic symptoms [20]. Vildagliptin is more safe and more effective than metformin in patients with impaired renal function, while causing an increase in the possibility of heart failure, acute pancreatitis, and pancreatic cancer [21, 22]. DDP-4 reduced tubulointerstitial and glomerular fibrosis, as well as
albuminuria by affecting the signaling pathway of advanced glycation end products (AGE) and their receptor, oxidative stress, inflammation, and the endothelial activity of nitric oxide, by elevating the levels of the DPP-4 substrates stromal cell-derived factor 1 and GLP-1 [23]. The present work aims to investigate the ameliorative effects of vildagliptin and metformin in diabetic kidney disease of type 2-induced diabetic rats.

**Materials and Method**

**Chemicals**

Streptozotocin (STZ) was purchased from Sigma Aldrich, USA. Synthetic antidiabetic drugs (metformin and vildagliptin) were purchased from a local pharmacy. Fructose obtained from El gomhorya for drugs trade & medical supplies.

**Preparation of STZ and other different anti-diabetic drugs**

STZ was dissolved in a 0.1M cold citrate buffer with a pH of 4.5 at a dose (35 mg/kg.BW) [24]. Metformin (Glucophage® 500mg tablets) was obtained from a local pharmacy and dissolved in phosphate buffering saline and the rats received it orally using 18-gauge soft gastric gavage tubes at a dose of (100mg/kg/day) [25]. Vildagliptin (Galvus® 50mg tablets) was obtained from a local pharmacy and dissolved in phosphate buffering saline and the rats received it orally using 18-gauge soft gastric gavage tubes at a dose (6mg/kg/day) [26].

**Animal model and treatment protocols**

40 male albino rats (approximately 8 weeks old) weighing between 200 and 250 g were sourced from animal houses at the Faculty of Veterinary Medicine, Zagazig University, Egypt. They were housed under standard laboratory conditions for two weeks. They were kept in an environment with temperatures ranging from 20 to 25°C, 60% relative humidity, following a 12-hour cycle of light and darkness, and given unlimited access to both water and food. The study approach has been approved by Zagazig University's Institutional Animal Care and Use Committee (ZU-IACUC/2/F/127/2023). The diabetic rat model has been developed by feeding on HFHF with an I.P injection of a single low-dose STZ (35 mg/kg.BW) according to a previous study [27].

HFHF consisted of 60% lard (350g) and fructose 17% (170g) obtained from El gomhorya for drug trade & medical supplies, Egypt then added (480g) of chow to complete (1kg). 10% sucrose(100g) was obtained from the local market and mixed with the drinking water. The control group was provided with a regular diet, whereas the remaining groups were administered a high-fat diet. Following four weeks of feeding, the diabetic group administered a single low dosage of STZ (35 mg/kg.BW, dissolved in 0.1 M sodium citrate buffer, pH 4.5), whereas the normal control group received citrate buffer (1 ml/kg.BW), both intraperitoneally.

Following 5 days of STZ administration, fasting blood glucose level was monitored, and rats with blood glucose levels > 200 mg/dl were diagnosed as diabetic rats. Following this, diabetic rats were given an HFHF diet for an additional 4 weeks. Subsequently, The diabetic rats were grouped randomly into three groups, each consisting of ten rats, alongside a normal control group. The control normal group comprised normal rats given distilled water orally by oral gavage. The diabetic group comprised
diabetic rats given distilled water orally by oral gavage. The metformin group included diabetic rats treated daily with orally administered metformin (100 mg/kg/day). The vildagliptin group included diabetic rats treated daily with orally administered vildagliptin (6 mg/kg/day). Treatment lasted for another 8 weeks. Fasting blood glucose level was measured biweekly.

**Blood samples**

The rats were euthanized using the decapitation technique. Blood samples were gathered in centrifuge tubes without anticoagulant, left to stand for thirty minutes, and then centrifuged at 3000 rpm for 15 minutes. The serum samples were then transferred to Eppendorf tubes and promptly frozen at -20°C until needed for subsequent biochemical analysis.

**Biochemical analysis**

**Serum glucose, insulin, HOMA-IR, and HOMA-B**

Glucose level was measured by the glucose oxidase method (Spinreact, Girona, Spain), and serum insulin level was evaluated using a rat insulin ELISA kit (Bio Vendor Laboratory Medicine, Brno, Czech Republic) according to the manufacturer’s protocol. The homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = fasting blood glucose (mg/dl) × fasting insulin (ng/ml) /405 [28]. HOMA of β-cell function (HOMA-B) = [20 ×fasting insulin (µU/mL)]/[fasting glucose (mmol/L)-3.5], and HOMA of insulin sensitivity (HOMA-S) = 1/HOMA-IR × 100 [29].

**Lipid profile markers**

Serum lipid profile including Serum triacylglycerol (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using commercially available kits (Spinreact, Spain).

**Kidney function markers**

Kidney function markers including urea were measured by using the Enzymatic Colorimetric method by N.S. BIO-TEC (Alexandria, Egypt), serum creatinine was measured by using the Colorimetric Kinetic Method by bio Reactivos GPL kits (Barcelona, España), and Uric acid (UA) was measured by using Enzymatic, the colorimetric method by Vitro Scient (Alsharkia, Egypt).

**Oxidant/Anti-oxidant markers**

Malondialdehyde (MDA) and serum total antioxidant capacity (TAC) using bio-diagnostic colorimetric kits (Giza, Egypt).

**Statistical analysis**

The obtained results for biochemical parameters are represented as mean ± standard error (S.E.). Statistical comparisons were performed using a one-way analysis of variance (ANOVA) test, and results with P ≤ 0.05 were considered significantly different. The results were statistically analyzed using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, United States).

**Results**

**Effect on serum glucose, insulin, and albumin levels**

As shown in Figure 1, pancreatic β-cell function declined rapidly in diabetic rats during the study period. This was shown via the significant (p < 0.0001) elevation in glucose, a significant reduction in serum insulin (p < 0.001), and serum albumin (p < 0.05) in comparison with the control group. On the contrary, metformin administration significantly
reduced glucose (p < 0.0001) compared with the diabetic group. However, vildagliptin administration showed a significant (p < 0.0001) decrease in and elevation in serum albumin (p < 0.01) in comparison with the diabetic group.

Figure 1. Effect on serum glucose, insulin, and albumin levels (A.-C.) A. mean value of serum glucose level (mg/dL), B. serum insulin (mIU/mL), and C. Serum Albumin (g/dl) in control and diabetic male albino rat groups. All Data are confirmed as means ± SEM. N = 5. * (p < 0.05), ** (p < 0.01), *** (p < 0.001), **** (p < 0.0001) reveal a significant difference.

**Effect on HOMA-B%, HOMA sensitivity, and HOMA-IR**

As shown in Figure 2, the diabetic group demonstrated a significant (p < 0.0001) decrease in the HOMA B% and a significant (p < 0.0001) increase in HOMA-IR in comparison with the control group. While the metformin group showed a significant (p < 0.001) increase in HOMA B% and a significant (p < 0.01) decrease in HOMA-IR compared with the control group, and a significant (p < 0.0001) decrease in HOMA-IR in comparison with the diabetic group. At the same time, the vildagliptin group showed a significant (p < 0.01) increase in HOMA B%, a significant (p < 0.01) decrease in HOMA-IR compared with the control group, and a significant (p < 0.01) decrease in HOMA-IR in comparison with the diabetic group.

Figure 2 Effect on HOMA-IR and HIMA-B% (A.-C.) A. mean value of HOMA B%, B. HOMA sensitivity, and C. HOMA-IR in control and diabetic male albino rat groups. All Data are
confirmed as means ± SEM. N = 5. * (p < 0.05), ** (p < 0.01), *** (p < 0.001), **** (p < 0.0001) reveal significant difference.

**Effect on lipid profile.**

As shown in Figure 3, there is a significant (p < 0.0001) elevation in serum TC, TAG, LDL, and a significant (p < 0.0001) reduction in HDL in the diabetic group in comparison with the control group. At the same time, the metformin group showed a significant (p < 0.01) reduction in LDL in comparison with the diabetic group (figure 3D.). However, the vildagliptin group showed a significant (p < 0.0001) reduction in TC, LDL, HDL (p < 0.001), and a significant (p < 0.05) elevation in TAG in comparison with the diabetic group, and showed a significant decrease in (p < 0.05) TC, (p < 0.001) TAG, and (p < 0.01) LDL compared with metformin group. The most significant result was represented in the vildagliptin group.

**Figure 3** Effect on lipid profile (A.-C.) A. total serum cholesterol (mg/dl), B. serum triacylglycerol (mg/dl), C. serum HDL (mg/dl), and D. serum LDL (mg/dl) in control and diabetic male albino rat groups. All Data are confirmed as means ± SEM. N = 5. * (p < 0.05), ** (p < 0.01), *** (p < 0.001), **** (p < 0.0001) reveal a significant difference.
Effect on kidney function

As shown in Figure 4, the diabetic group showed a significant (p< 0.0001) elevation in UA, urea, and creatinine levels in comparison with the control group. On the contrary, the metformin group showed a significant reduction in UA (p < 0.05), urea (p < 0.001), and creatinine (p < 0.0001) in comparison with the diabetic group. At the same time, the vildagliptin group showed a significant (p < 0.0001) reduction in urea, creatinine, and UA (p < 0.01) in comparison with the diabetic group, and a significant (p< 0.001) reduction in creatinine in comparison with metformin group.

Figure 4 Effect on kidney function (A.-C.) A. serum uric acid (mg/dl), B. urea (mg/dl), and C. serum creatinine (mg/dl) in control and diabetic groups. All Data are confirmed as means ± SEM. N = 5. * (p < 0.05), ** (p < 0.01), *** (p < 0.001), **** (p < 0.0001) reveal a significant difference.

Effect on the serum oxidant/antioxidant (MDA and TAC) levels.

As shown in Figure 5, diabetes enhances oxidative stress, as evidenced by a significant (p < 0.0001) elevation in MDA and a significant reduction in TAC (p < 0.0001) in comparison with the control group. However, the metformin group showed a significant decrease in MDA (p < 0.001) and a significant (p < 0.0001) increase in TAC in comparison with the diabetic group. At the same time, the vildagliptin group showed a significant (p < 0.0001) decrease in MDA, and a significant (p < 0.0001) increase in TAC compared with the diabetic group, while showed a significant (p< 0.05) decrease in MDA in comparison with the metformin group.

Figure 5 Effect on the serum oxidant/antioxidant (MDA and TAC) levels. A. serum MDA (nmol/g) and B. TAC (mg/ml) in control and diabetic groups. All Data are confirmed as means ± SEM. N = 5. * (p < 0.05), **** (p < 0.0001) reveal a significant difference.
Figure 5 Effect on serum oxidant/antioxidant (A.-B.) A. serum MDA (nmol/g), and B. serum TAC (mg/ml) in control and diabetic male albino rat groups. All Data are confirmed as means ± SEM. N = 5. * (p < 0.05), ** (p < 0.01), *** (p < 0.001), **** (p < 0.0001) reveal a significant difference.

Discussion

Diabetic kidney disease is one of the diabetic complications affecting 56.7% of diabetic patients caused by many factors including advancing age, increased duration since diabetes diagnosis, obesity, hypertension, stroke, insulin use (either alone or combined with oral glucose-lowering medications), and poorer hemoglobin A1C (HbA1c) control [30]. The present study's outcomes supported the development of DKD by an elevation in levels of urea, uric acid, and serum creatinine. These outcomes were in keeping with those previously reported by [31-33]. This correlation can be explained by the impact of hyperglycemia on the function and viability of both podocytes and tubular cells. According to Liu and Tang [34] hyperglycemia-induced apoptosis of podocytes has been related to proteinuria, renal fibrosis, and permanent renal impairment [34, 35]. This research proposes to investigate the benefits of metformin and vildagliptin in the treatment and management of oxidative stress, glycemic management, lipid profile, and renal function in diabetic rats. In this study, the HFHF diet and STZ (35 mg/kg.BW) were used for the induction of type II diabetes and DKD in rats. This is in agreement with [36-38]. As HFHF leads to insulin resistance, whereas STZ is toxic to islet β cells, Large doses of STZ can destroy most islet β cells and produce T1DM. However, our researchers found that a low-dose STZ plus HFHF diet can be utilized to produce T2DM models by inducing moderate damage of the pancreatic β-cells, which leads to insulin resistance and hyperinsulinemia, as well as hyperglycemia [39, 40].

Treatment with metformin and vildagliptin show an improvement in hyperglycemia such as glucose, HOMA-IR, and insulin level [39, 41-43] and these results are in agreement with the current study as compared with the diabetic group; glucose level and HOMA-IR in metformin and vildagliptin-treated groups declined significantly, with no significant effect on the insulin level of treated groups.

Metformin acts on the liver and causes an impairment in hepatic glucose production and intestinal glucose absorption by inhibiting the glucagon action and the activation of activated
protein kinase (AMPK) which reduces glucose synthesis in the liver, improves insulin sensitivity by modifying lipid metabolism and stimulates glucose absorption in skeletal muscles [41, 44].

The mechanism of DPP-4 inhibitors is still unknown but in some studies, it is related to GLP-1 by promoting insulin secretion and inhibiting the secretion of glucagon by increasing endogenous GLP-1 and GIP levels without causing hypoglycemia. It also improves β-cell function by improving β-cell proliferation and inhibiting apoptosis, decreasing glucagon levels, decreasing hepatic glucose production, decreasing intestinal glucose absorption, increasing peripheral glucose uptake, and increasing glycolysis [45, 46].

In T2DM there is a disturbance in glucose and lipid metabolism that usually happen together leading to Dyslipidemia which causes an increase in the production of AGEs. According to previous studies, there is a change in lipid profile characterized by an increase the levels of TC, TAG, and LDL-C and a decrease in the level of HDL-C [47, 48]. The result of this study represents an improvement in the lipid profile in diabetic rats treated either with metformin or vildagliptin with an outperforming effect for the vildagliptin group and this is in the same line with [38, 49-51].

The effect mentioned above could be owed to Vildagliptin can reduce postprandial hyperlipidemia and enhance the lipid profile by enhancing early insulin secretion, hepatic insulin resistence, and maintaining GLP-1 and GIP levels which inhibit fasting lipolysis in adipose tissue and reduce accumulated TAG in liver, muscle, and pancreas [49, 52].

The effect of metformin on lipid profile can be explained by many mechanisms, one of them the direct effect of metformin on genes associated with intestinal lipid homeostasis by reducing its mRNA expression which affects lipoprotein production in diabetic rats' intestines, and an indirect effect through delaying gastric emptying and improving GLP-1 secretion [53, 54]. Another mechanism through regulating AMPK, Metformin activates AMPK and suppresses hepatic expression of Acetyl-CoA carboxylase, resulting in an anti-lipogenic action. Acetyl-CoA carboxylase catalyzes the production of malonyl-CoA, a precursor substrate for de novo fatty acid synthesis. It also inhibits the rate-limiting step for fatty acid oxidation. Metformin's direct phosphorylation of acetyl-CoA carboxylase could stimulate fatty acid oxidation and decrease TAG production. AMPK downregulates the expression of sterol regulatory element binding proteins in hepatocytes, resulting in a reduction in lipogenesis and lipid formation [55].

In the STZ-induced DKD model, the onset of DKD was confirmed via assaying kidney function through increasing serum uric acid, ura, and creatinine levels. Hyperglycemia destroys the glomerular filtration barrier, resulting in glomerular injury that causes urine protein or albumin leakage, elevated urea and blood glucose levels indicate that hyperglycemia might cause kidney damage so urea typically appears as the most accurate measure of renal function. Increased urea levels are common in kidney injury or malfunction [56-59]. In the current study, the treatment with different antidiabetic drugs causes an improvement in kidney function markers than the diabetic rats. The most significant result appears in the vildagliptin-treated group. This meets with [60-62] who described the effect of different anti-
diabetic drugs in improving renal functions.

These findings might be owed to the anti-inflammatory, anti-oxidant, hypoglycemic effect of metformin that improves renal damage and protects renal tubular cells, which delays renal progression in diabetic kidney injury through modulating the AMPK/mammalian target of rapamycin (mTOR) signaling pathway by upregulating klotho which is an anti-aging gene that produces the Klotho protein in the distal convoluted tubules. Its expression in diabetic kidney disease decreases as the illness progresses, and this reduction can activate the mTOR signaling pathway and increase kidney damage [63, 64].

The actual mechanism of Vildagliptin remains unclear, but it acts as an anti-hyperglycemic, anti-apoptotic, anti-inflammatory, and anti-oxidant agent through inhibiting DPP-4 which restores GLP-1 signaling pathways, improves pancreatic β-cell response to glucose, insulin sensitivity, and lipoprotein metabolism. Additionally, it decreases abnormal secretion of glucagon, glucose, HbA1c, and postprandial glucose levels [65, 66].

Numerous studies suggest that oxidative stress is identified as an established pathway in the progression of diabetic complications, including diabetic kidney disease and diabetic nephropathy. It is caused by an imbalance between the oxidant and antioxidant system, and destroys cellular proteins, membrane lipids, and nucleic acids, consequently leading to cell death [67]. Diabetes increases biomarkers of oxidative stress, including AGEs and MAPK, leading to lipid peroxidation and increased production of free radicals such as ROS and Nitric oxide (NO) radicals. High levels of ROS can alter the production and activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), lipid peroxidation as MDA, and a decrease in the antioxidant defense mechanisms as TAC [68, 69].

In the recent study, there was a significant reduction in MDA levels and a significant enhancement in TAC levels in treated groups compared to the diabetic group. The effect of vildagliptin in the diabetic group was more significant than the effect of metformin in the diabetic group. These findings align with [70-73] who represent the ameliorative effect of vildagliptin in oxidative stress.

These findings may be attributed to the anti-oxidant effect of Metformin due to its anti-hyperglycemic effect which reduces free radicals resulting from glucose auto-oxidation, Inhibit protein kinase C activity which protects mitochondria from TGF-β1-induced damage, reducing ROS generation and activating the mitochondrial antioxidant system [74, 75].

Vildagliptin improves pancreatic β-cell function, and insulin sensitivity, and reduces lipid peroxidation. It also protects pancreatic β-cells from oxidative stress by inhibiting the DPP-4 effect, which increases the activity of circulating GLP-1. This has anti-oxidant and anti-inflammatory effects and can protect against mitochondrial damage [49, 76].

**Conclusion**

Vildagliptin could potentially offer superior therapeutic benefits compared to metformin for managing type 2 diabetes and related kidney complications, as it may effectively improve renal oxidative stress, hyperglycemia, and hyperlipidemia.

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**Conflict of Interest**

The authors don’t have a conflict of interest.

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