Abstract

Background: Glucagon-like peptide-1 (GLP-1) is a hormone secreted by intestinal L cells. It is the most potent stimulator of glucose-induced insulin secretion. The incretin effect, mediated by glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), is attenuated in T2DM. Native GLP-1 has a short half-life, being degraded by dipeptidyl peptidase-4 (DPP-4), it is necessary to use a DPP-4 inhibitor to prevent its degradation.

Aim: The aim is to investigate the effect of DPP-4 inhibitor on renal injury induced by renal I/R in streptozotocin induced diabetic rats.

Design: Seventy male albino rats were divided into 7 groups, group 1- normal control, group 2- renal IR injury, group 3- DPP-4 inhibitor treatment in a dose of 10mg/kg BW for 6 weeks + renal IR injury, group 4- diabetic rats, group 5- diabetic rats + DPP-4 inhibitor, group 6- diabetic rats + renal IR injury and group 7- diabetic rats + DPP-4 inhibitor treatment + renal IR injury. In all the included animals, urea and creatinine, fasting blood glucose level, insulin level and insulin resistance test were evaluated. Oxidant stress was evaluated by measuring tissue MDA, glutathione and nitrite level. The inflammatory marker TNFα and MPO activity and DNA fragmentation were measured in the renal tissues.

Results: DPP-4 treatment improved blood glucose level, insulin and insulin sensitivity as compared to diabetic rats. MDA and nitrite levels in the renal tissue were significantly increased and glutathione was significantly decreased after I/R in diabetic rats compared to I/R in normal rats. DPP-4 inhibitor treatment significantly normalized these biochemical parameters. TNFα level, MPO activity was reduced in DPP-4 inhibitor treated I/R diabetic rats as compared to untreated group. DNA fragmentation was attenuated in the DPP-4 inhibitor treated groups compared to untreated rats. Moreover, DPP-4 inhibitor treatment preserved the function of the kidney after exposure to I/R injury.

Conclusion: This study proved that DPP-4 inhibitor protected diabetic rat kidneys from being damaged due to I/R injury through improving the glycemic states, attenuating oxidative stress, reducing inflammation, apoptosis and may be through a direct effect on the renal tissues.

Key Words: GLP-1 – DPP-4 inhibitor – Diabetes – Renal ischemia reperfusion.

Introduction

The increasing prevalence of diabetes mellitus is an acknowledged world health crisis that is both a major contributor to patient morbidity and mortality and a huge economic burden. The complex pathophysiology of type 2 diabetes makes effective treatment problematic [1].

It is now recognized that abnormalities in other hormones than insulin also contribute to the development of hyperglycemia. For example, the incretin effect, mediated by glucagon–like peptide–1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), is attenuated in T2DM [2], and although GLP-1 fasting levels are elevated in type 2 diabetes, its secretion is significantly impaired in response to meals which, in turn, is associated with the loss of ability to stimulate insulin secretion [3].

Glucagon-like peptide-1 (GLP-1) is a hormone derived from the prepro-glucagon molecule and is secreted by intestinal L cells [4]. It is the most potent stimulator of glucose-induced insulin secretion [5].

Incretin-receptor activation leads to glucose-dependent insulin secretion, induction of β-cell proliferation, and enhanced resistance to apoptosis. GLP-1 exerts glucoregulatory actions via slowing of gastric emptying and glucose-dependent inhibition of glucagon secretion. GLP-1 also promotes satiety and sustained GLP-1-receptor activation is associated with weight loss [6].
The incretin system and GLP-1 in particular, therefore offers a very appealing target for addressing many of the aspects of diabetes that are not well controlled at present, however, native GLP-1 has a short half-life (half-life less than 2 minutes). It is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), which is widely expressed in many tissues, including kidney, liver, lung, and the small intestine, so it is necessary to use a DPP-4 inhibitor such as Sitagliptin as a means of preventing its degradation [7].

DPP-4 is a ubiquitously expressed serine peptidase [3]. Initial experiments in pigs revealed that DPP-4 inhibitors prevented the degradation of both GIP and GLP-1 and this approach subsequently gained significant attention in the treatment of type 2 diabetes [8]. Thus, orally administered DPP-4 inhibitors have emerged as a new class of antihyperglycaemic agents with the ability for extending the biological effects of incretin hormones through the inhibition of their degradation [9], with the advantage of higher stability and bioavailability when compared with their mimetics.

Sitagliptin, an orally available DPP-4 inhibitor developed to be used as a once daily treatment for T2DM, has shown beneficial effects on glycaemic control, reducing HbA1c, and preventing hypoglycaemia, as well as on islet mass and function, with no relevant adverse effects [10].

Diabetics are at a higher risk of an ischemic condition caused by decreased blood flow [11]. With increasing duration and severity of ischemia, however, greater cell damage can develop, with a predisposition to a spectrum of reperfusion-associated pathologies, collectively called reperfusion injury [12]. Moreover, diabetes mellitus (DM) increases renal sensitivity to ischemia reperfusion (IR) injury. Short period of ischemia (30min) in diabetics has been demonstrated to induce reversible renal failure, leading to progressive injury with end stage renal disease [13,14].

Recently, GLP-1R was reported to be expressed in porcine proximal tubular kidney cells. Addition of GLP-1 to these cells resulted in a reduced sodium re-absorption. GLP-1 had no effect on glucose re-absorption. It was suggested that GLP-1 plays a role in the kidney most likely through a direct action via its GLP-1R [15].

The present study was designed to investigate the effect of DPP-4 inhibitor on the glycemic control, inflammation, and redox status and renal function in the renal injury induced by renal I/R in streptozotocin induced diabetic rats.

**Material and Methods**

**Experimental animal and groups:**

Seventy male albino rats, of an average weight 150-200 gm were included in this study. The rats were supplied by the animal house unit of Kasr El-Ainy, Faculty of Medicine, Cairo University, housed in wire mesh cages at room temperature (22-24°C) with normal light & dark cycle. They were fed the standard rat chow diet and had free access to water. This study was carried out in the Physiology and Biochemistry Departments, Faculty of Medicine, Cairo University. All procedures involving animals were performed in accordance with the principles and procedures of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The rats were divided into 7 groups, 10 rats each:

- **Group 1:** Normal control sham-operated (underwent all surgical procedures without I/R in normal rats).
- **Group 2:** Renal IR injury was performed (I/R control).
- **Group 3:** Dipeptidyl peptidase-4 inhibitor (DPP-4 inhibitor) treatment in a dose of 1 0mg/kg BW for 6 weeks + renal IR injury was performed on day 28 of the start of treatment (DPP-4 inhibitor + I/R).
- **Group 4:** Type II diabetes mellitus was induced and the rats were sham-operated after 4 weeks of diabetes induction (DM).
- **Group 5:** Type II diabetes was induced and the rats received dipeptidyl peptidase-4 inhibitor (DPP-4 inhibitor) treatment in a dose of 1 0mg/kg BW for 6 weeks. On day 28 the rats were sham operated (DPP-4 inhibitor + DM).
- **Group 6:** Type II diabetes was induced and the rats underwent renal IR injury, on day 28 after induction of diabetes (DM + I/R).
- **Group 7:** Type II diabetes was induced and the rats received dipeptidyl peptidase-4 inhibitor (DPP-4 inhibitor) treatment in a dose of 1 0mg/kg BW for 6 weeks. On day 28 the rats underwent IR injury (DPP-4 inhibitor + DM + I/R).

At the end of the study protocol (after 2 weeks of reperfusion), blood samples were collected. Rats were fasted overnight for at least 6 hours for fasting measurements. Blood samples were obtained via tail vein for measuring urea and creatinine, fasting blood glucose level and insulin level and HOMA test were evaluated to detect the effects of diabetes, I/R injury and DPP-4 inhibitor treatment on renal
injury in diabetic rats. The animals were killed and the kidneys were removed quickly, placed in liquid nitrogen and stored at −70°C until assayed for oxidant stress as indicated by measuring tissue MDA and reduced glutathione and nitrite levels. The inflammatory marker TNF alpha level and MPO activity and DNA fragmentation were measured in the renal tissues.

**Induction of diabetes type 2:**

Diabetes type 2 in rats was induced by a single dose of streptozotocin (STZ) (45mg/kg, i.p.) after 3 weeks of high fat diet [16]. STZ was dissolved in citrate buffer (pH 4.5). Control animals received an equal volume of vehicle. Food, water consumption, weight gain and blood glucose levels were recorded to monitor the degree of diabetes. Animals showing fasting blood glucose higher than 10mmol/L were considered as diabetic and used for the further study. A period of four weeks was maintained between induction of diabetes and ischemic injury.

**Experimental protocol:**

**Induction of renal IR injury:**

Rats were anesthetized with ketamine (60mg/kg, i.p.) and diazepam (5mg/kg, i.p.). Body temperature was maintained throughout surgery at 37°C±0.5°C. The skin on the back was shaved and disinfected with povidone iodine solution. Surgical exposure of the left and right renal pedicles was obtained in all rats via midline incision. To induce renal ischemia, both renal pedicles were occluded for 30 minutes with vascular clamps. After 30 minutes of occlusion, the clamps were removed and kidneys underwent reperfusion [17].

**Measurement of fasting plasma glucose level:**

Plasma glucose in blood samples was measured using oxidase-peroxidase method [18].

**Measurement of plasma insulin:**

Plasma insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer’s instructions [19].

**HOMA-IR test:**

To estimate insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-IR: Insulin resistance index) [20] was used, calculated as the product of fasting insulin (in µU/mL) and fasting glucose (in mmol/l) divided by 22.5. A lower index indicates greater insulin sensitivity.

**Measurement of MDA:**

To measure the MDA concentration, 100mg of kidney tissue in 1mL PBS, pH 7.0 was homogenized with micropestle in microtube. 20% TCA was added to kidney homogenate to precipitate the protein, and centrifuged. Supernatants were collected and thiobarbituric acid (TBA) solution was added to the supernatants. After boiling for 10 minutes in water bath, the absorbance was measured. Concentration of MDA in supernatants of kidney homogenate was calculated using the standard curve [21].

**Measurement of GSH:**

GSH concentration was measured from kidney homogenate in phosphate buffer pH 8.0 and then 5% TCA was added, to precipitate protein. After centrifugation, dithiobisnitrobenzoate (DTNB) solution was added to the supernatants of kidney homogenate, and incubated for 1 hour. The absorbance was measured. Concentration of GSH in kidney tissue was calculated using the standard curve [22].

**Measurement of nitrite level in renal tissues:**

Measurement of the NO end product, nitrite production was used to assess relative values of the NO. Nitrites were determined by the standard assay according to Giustarini et al. [23].

**Measurement of TNF-α:**

Serum TNF-α was measured by using ELISA (quantikine R&D system USA) according to the manufacturer’s instructions [24].

**Determination of myeloperoxidase (MPO) activity in kidney homogenate:**

Assaying of MPO activity was described by Mizutani et al. [25]. MPO activity was assayed by measuring the change in optical density at 450nm using tetramethylbenzidine, as substrate (1.5mmol/L) and H₂O₂ (0.5mmol/L). Results were expressed as MPO relative units/100mg tissue. One unit of MPO activity was defined as the quantity of enzyme degrading one mmol peroxide at 25°C. The activity of purified known human neutrophil MPO was used as the standard (Sigma Chemical Co, Egypt).

**DNA fragmentation:**

Genomic DNA was extracted from renal tissue using DNA extraction kit(promega USA), DNA ladders, an indicator of tissue apoptotic nucleosomal DNA fragmentation, were visualized under ultraviolet light.
Determination of kidney functions:

Serum creatinine and blood urea nitrogen (BUN) were determined enzymatically using commercially available kits (Bioclin, Santa Coloma, Spain).

Statistical analysis:

All the values are expressed as mean ± SD. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when \( p \) was less than 0.05.

Results

Effect of IR on kidney function and other parameters:

Ischemia reperfusion injury induced impairment of renal function as shown by significant increase in serum urea and creatinine (Table 1), (Figs. 1,2) significant increased oxidative stress (Table 2), (Figs. 3,4,5), increased MPO and inflammatory marker TNF-\( \alpha \) (Table 3), (Figs. 6,7) and induced apoptotic changes demonstrated as DNA fragmentation in rats of group 2 as compared to their control (sham operated group 1).

Effect of diabetes on kidney function and other parameters:

Diabetes resulted in significant hyperglycemia, lower level of blood insulin and increased insulin resistance (Table 4), (Figs. 8,9,10). Induction of diabetes induced a significant increase in lipid peroxidation (Table 2) and inflammation (Table 3) with apoptotic changes in kidneys of group 4 as compared with normal rats (group 1) (Fig. 11).

Effect of IR on kidneys of diabetic rats:

IR injury resulted in more severe injury in kidneys of diabetic rats as compared to non diabetic control as shown by a significant impairment in renal function (Table 1) (Figs. 1,2), more severe oxidant stress (Table 2), (Figs. 3,4,5), and inflammation (Table 3) with more DNA fragmentation observed in kidneys of group 6 as compared with both diabetic sham operated rats (group 4) and non diabetic rats exposed to IR injury (group 2) (Fig. 11).

Effect of DPP-4 inhibitor on blood glucose, insulin and insulin resistance:

The diabetic rats presented hyperglycaemia, insulin levels of STZ rats were already decreased when compared with the controls, indicating an impaired insulin secretion by the pancreatic beta-cell. DPP-4 inhibitor treatment significantly decreased blood glucose level and stimulated the release of insulin as well as increasing insulin sensitivity as shown by decreased insulin resistance (HOMA-IR test) (Table 1, Figs. 1,2,3).

Effect of DPP-4 inhibitor on lipid peroxidation and antioxidant enzymes:

The MDA level was significantly increased in diabetic and non-diabetic IR compared to normal control with significant increase in diabetic IR group compared to non-diabetic IR group. There is a significant decrease in the level of GSH in the diabetic and non-diabetic groups after IR as compared to their control groups. Diabetic group demonstrated a significant decrease in GSH level after IR compared to non-diabetic IR group (Table 2), (Figs. 3,4,5). However, DPP-4 inhibitor treatment resulted in a significant decrease in MDA level and a significant increase in GSH compared to the non treated diabetic IR group (Table 2), (Figs. 3,4,5).

Effect of DPP-4 inhibitor on nitric oxide level:

The level of NO was significantly increased in non-diabetic and diabetic IR groups in comparison with normal control. Diabetic IR group had significant higher NO level as compare to non-diabetic IR group. DPP-4 inhibitor significantly decreased the NO levels in the treated groups as compared to diabetic IR rats (Table 2), (Fig. 5).

Effect of DPP-4 inhibitor on TNF\( \alpha \) and myeloperoxidase activity:

TNF-\( \alpha \) level in kidney tissues was significantly increased in diabetic and non diabetic rats after IR injury as compared with their controls. Myeloperoxidase activity, which is accepted to be an indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the diabetic IR group than in the kidney tissue of the non-diabetic IR group. The rats in the DPP-4 inhibitor pretreated group had significantly lower TNF-\( \alpha \) and MPO activity than their control rats in the untreated groups (Table 3), (Figs. 6,7).

Effect of DPP-4 inhibitor on renal functions:

Diabetic rats showed impaired renal functions as indicated by elevated serum urea and creatinine. Ischemia reperfusion injury resulted in more significant impairment of renal function as compared to their control. DPP-4 inhibitor treatment protected the rat kidneys and preserved their functions as showed by the significant lower values of urea and creatinine in DPP-4 treated groups as compared with the untreated control (Table 1) (Figs. 1,2).
### Table (1): Comparison of kidney function (urea and creatinine) in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N+IR</th>
<th>N+IR+T</th>
<th>D</th>
<th>D+T</th>
<th>D+IR</th>
<th>D+IR+T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum Urea (mg/dl)</strong></td>
<td>18.45±2.73 a</td>
<td>57.41±3.59 b</td>
<td>19.60±1.71 a</td>
<td>49.60±3.50 c</td>
<td>19.20±1.81 a</td>
<td>84.72±5.72 d</td>
<td>18.20±1.81 a</td>
</tr>
<tr>
<td><strong>Serum Creatinine (mg/dl)</strong></td>
<td>0.16±0.03 a</td>
<td>0.46±0.04 b</td>
<td>0.17±0.03 a</td>
<td>0.45±0.05 b</td>
<td>0.15±0.02 a</td>
<td>1.60±0.14 c</td>
<td>0.16±0.02 a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SD. Number of samples in each group is 10. Means with different superscript letters differ significantly (p<0.05).

### Table (2): Comparison of oxidative stress (MDA, GSH and nitrite) in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N+IR</th>
<th>N+IR+T</th>
<th>D</th>
<th>D+T</th>
<th>D+IR</th>
<th>D+IR+T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDA (nmol/mg ptn)</strong></td>
<td>120.75±4.72 a</td>
<td>226.62±12.40 b</td>
<td>130.19±22.19 a</td>
<td>190.26±14.71 c</td>
<td>126.00±2.26 a</td>
<td>296.5±22.1 d</td>
<td>132.00±5.03 a</td>
</tr>
<tr>
<td><strong>GSH (nmol/mg ptn)</strong></td>
<td>48.95±9.55 a</td>
<td>23.76±4.02 b</td>
<td>49.36±9.11 a</td>
<td>26.8±3.15 a</td>
<td>46.8±6.70 a</td>
<td>17.23±1.42 c</td>
<td>41.30±3.06 a</td>
</tr>
<tr>
<td><strong>Nitrite (umol/mg ptn)</strong></td>
<td>0.41±0.03 a</td>
<td>0.60±0.12 b</td>
<td>0.44±0.09 a</td>
<td>0.53±0.10 b</td>
<td>0.40±0.03 a</td>
<td>0.60±0.08 b</td>
<td>0.40±0.03 a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SD. Number of samples in each group is 10. Means with different superscript letters differ significantly (p<0.05).

### Table (3): Comparison of inflammatory markers (TNF alpha and MPO) in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N+IR</th>
<th>N+IR+T</th>
<th>D</th>
<th>D+T</th>
<th>D+IR</th>
<th>D+IR+T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF alpha (pg/ml)</strong></td>
<td>106.03±7.11 a</td>
<td>185.86±10.42 b</td>
<td>111.5±7.69 a</td>
<td>157.68±11.31 c</td>
<td>115.2±6.98 b</td>
<td>204.3±10.11 d</td>
<td>121.10±7.74 a</td>
</tr>
<tr>
<td><strong>Mycoperoxidase (U/mg ptn)</strong></td>
<td>0.48±0.09 a</td>
<td>2.50±0.51 b</td>
<td>0.41±0.04 a</td>
<td>1.10±0.23 c</td>
<td>0.49±0.03 a</td>
<td>5.16±0.29 d</td>
<td>0.50±0.07 a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SD. Number of samples in each group is 10. Means with different superscript letters differ significantly (p<0.05).

### Table (4): Comparison of glucose, insulin and HOMA in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N+IR</th>
<th>N+IR+T</th>
<th>D</th>
<th>D+T</th>
<th>D+IR</th>
<th>D+IR+T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
<td>4.84±0.30 a</td>
<td>4.66±0.29 a</td>
<td>4.38±0.86 a</td>
<td>11.68±0.83 b</td>
<td>4.84±0.19 a</td>
<td>11.68±1.08 b</td>
<td>4.72±0.33 a</td>
</tr>
<tr>
<td><strong>Insulin (U/mL)</strong></td>
<td>11.23±0.83 a</td>
<td>10.94±0.52 a</td>
<td>10.60±0.52 a</td>
<td>18.90±1.52 a</td>
<td>11.12±0.43 a</td>
<td>19.41±1.21 b</td>
<td>11.10±0.27 a</td>
</tr>
<tr>
<td><strong>HOMA</strong></td>
<td>2.35±0.28 a</td>
<td>2.27±0.18 a</td>
<td>2.07±0.46 a</td>
<td>9.82±1.05 a</td>
<td>2.39±0.03 a</td>
<td>10.06±0.72 b</td>
<td>2.36±0.13 a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SD. Number of samples in each group is 10. Means with different superscript letters differ significantly (p<0.05).

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**Fig. (1):** Comparison of blood urea level (mg/dl) in the different studied groups. Means with different superscript letters differ significantly (p<0.05).

**Fig. (2):** Comparison of blood creatinine level (mg/dl) in the different studied groups.
Fig. (3): Comparison of MDA level (nmol/mg ptn) in the different studied groups.

Fig. (4): Comparison of renal GSH level (nmol/mg ptn) in the different studied groups.

Fig. (5): Comparison of renal nitrite level (umol/mg ptn) in the different studied groups.

Fig. (6): Comparison of renal TNF alpha level (pg/ml) in the different studied groups.

Fig. (7): Comparison of renal myeloperoxidase activity in the different studied groups.

Fig. (8): Comparison of blood glucose level (mmol/L) in the different studied groups.
Insulin (uU/ml) in different groups

![Graph showing insulin levels in different groups]

Fig. (9): Comparison of blood insulin level (uU/ml) in the different studied groups.

HOMA in different groups

![Graph showing HOMA in different groups]

Fig. (10): Comparison of insulin resistance in the different studied groups.

Fig. (11): DNA fragmentation an agarose gel show.

Lane M: DNA marker with 100bp.
Lane 1,3,5&7: (DNA not fragmented) in control & treated groups.
Lane 2,4&6: (DNA fragmented in ischemia, diabetic and ischemia with diabetes groups.

Discussion

In our study, rats subjected to renal I/R demonstrated impairment of renal functions in both non diabetic and diabetic rats as shown by the elevated levels of blood urea and creatinine, with a significant impairment in function in diabetic than non diabetic rats indicating that diabetes potentiates the injury induced by renal IR in rats. In fact, diabetes per se resulted in a significant impairment in renal function as compared with normal rats. Renal IR injury induced an increase in the renal MDA and attenuation of the antioxidant enzyme glutathione of both non diabetic and diabetic rats, with a significant increase in MDA and decrease in GSH in diabetic than non diabetic rats. Inflammatory markers TNF-α and MPO enzyme activity were significantly increased in diabetic than non diabetic rats and were more markedly increased after IR injury.

Lipid peroxidation and antioxidant enzymes are important indices of oxidant injury [13]. The increased oxidant stress reported might be due to ROS production via inflammatory response as inflammatory reactions are activated during the process of IR injury, resulting in the formation of inflammatory cytokines [26]. Neutrophils are the inflammatory cells, which produce abundantly ROS during IR injury. Myeloperoxidase (MPO) is found in neutrophils and catalyzes the formation of hypochlorous acid (HOCI), a toxic agent to cellular components and initiates oxidative injury. Renal IR causes tissue injury by oxygen radicals and oxidative stress caused by an imbalance between production of ROS and the antioxidant capacity [27].

In T2DM, oxidative stress is implicated both in the complications of T2DM and renal I/R. The combined oxidative stress from two sources may thus increase the total level of ROS [13]. Previous reports suggested that local and systemic low-grade inflammation and oxidative stress, which are mainly fuelled by hyperglycaemia and hyperlipidaemia, are important mediators of T2DM complications in many individuals [28].

In our study, we found severe renal injury when I/R was performed in T2DM rats, in which blood glucose level was higher than in the normal rats. Hyperglycemia, during I/R could be deleterious for the kidney.

Secondary effects of hyperglycemia such as formation of AGE, increased oxidative stress, hemodynamic alterations and differences in the response and formation of NO could also be involved [13]. In agreement with this suggestion, an increased acute sensitivity to ischemia has been demonstrated when blood glucose concentration...
was raised by dextrose infusion or intraperitoneal glucose injection in combination with renal I/R in both rats and dogs [29]. Diabetes is associated with a worse outcome after stroke in humans, and elevated blood glucose predisposes for a more severe cerebral injury even in non-DM patients [30].

These findings are in accordance with our findings that I/R injury increased inflammatory markers, TNF-α and MPO in both diabetic and non-diabetic rats. Diabetic rats showed significantly higher values associated with hyperglycaemia, decreased insulin levels with increased insulin resistance and nitrite level compared with the controls.

The nitric oxide system may be involved in the increased sensitivity to I/R in DM. Our results showed an increase in nitrite concentration in the renal tissues of diabetic rats. Renal I/R in diabetic and non-diabetic rats induced an increase production of NO.

These results are in agreement with Sudnikovich et al. [31], Stadler et al. [32] and Thorup et al. [33] who reported evidences for increased NO-production in the STZ-DM kidney, and in IR injuries. However, reduced production of NO, as well as a decreased vascular response to NO, has been observed in DM rats after renal I/R by Goor et al. [34]. Goor and co-workers suggested that endothelial injury may explain the lower NO production in DM kidneys after I/R.

These changes reported in diabetics make diabetic patients under higher risk of kidney damage induced by ischemia reperfusion injury [35,36].

Pretreatment with DPP-4 inhibitors for 4 weeks before the I/R injury prevented renal I/R-induced impairment of renal function. This effect was associated with attenuation of lipid peroxidation as evidenced by decrease MDA and increase in antioxidant enzyme GTH. Moreover, tissue nitrite level was significantly reduced. DPP-4 inhibitor pretreated kidneys demonstrated decreased inflammatory infiltration, as observed by lower values of TNFα and MPO enzyme activity in renal tissues and protected the kidneys in rats against apoptosis induced by the IR injury as demonstrated by a decrease in DNA fragmentation as compared with untreated groups.

In agreement with our results, Park et al. [37] reported that incretin (exendin-4) treatment caused a significant reduction in oxidative DNA damage and oxidative stress. Another article demonstrated that TGF-1 expression mediated by oxidant stress may be suppressible by exendin-4 [38]. Also, Sakr et al., [39] reported decreased levels of TNF-α associated with kidney protection in rats exposed to I/R.

In addition, our results showed that 6 weeks of DPP-4 inhibitor treatment in diabetic rats had improved blood glucose levels and insulin sensitivity. Restoration of normoglycemia could be one of the protective mechanisms of DPP-4 inhibitor against renal I/R in T2DM.

DPP-4 inhibitor decreases blood glucose in experimentally induced diabetic rats [40-43]. To date, the mechanisms for improved blood glucose levels are not fully understood. One of the most important functions of GLP-1 is to act as an incretin hormone [44,45]. Also GLP-1 causes an inhibition of glucagon secretion [46,47].

The present study showed that insulin levels were significantly higher in rats treated with DPP-4 inhibitor as compared with diabetic control. The increase in insulin secretion is mainly due to the insulinotropic action of GLP-1 [48] exerted via increasing exocytosis of insulin-containing granules [49]. GLP-1 also stimulates coordinated oscillations in both intracellular calcium and cAMP, and these are potentiated by glucose [50]. Furthermore, GLP-1 acts as a glucose sensitizer [51]. In addition, GLP-1 has trophic effects on beta cells [52]. Not only does it stimulate beta-cell proliferation [53,54,55], it also enhances the differentiation of new beta cells from progenitor cells in the pancreatic duct epithelium [56]. Most recently, GLP-1 has been shown to be capable of inhibiting apoptosis of beta cells including human beta cells [57]. Furthermore, GLP-1 acts as a glucose sensitizer [51].

However, in studies performed by Park et al. [58] and Kodera et al. [59] there was amelioration of renal injury without lowering blood glucose level or HbA1c concentrations in exendin-4-treated db/db mice, but it improved insulin sensitivity. Their studies were performed on a rat model of type I diabetes, however, our work was performed on rats with type II diabetes.

Another postulated mechanism is that, as shown by results in non diabetic rats, DPP-4 inhibitor reduced inflammation and oxidative stress. In our study treatment with DPP-4 inhibitor for 6 weeks protected the kidney of diabetic rats from the injury induced by IR by preventing inflammation, inflammatory cell infiltration (as indicated by decreased level of TNFα and MPO) and ROS production (as observed by reduction of renal MDA and increased glutathione). This may suggest
that suppression of lipid peroxidation and oxidative stress in diabetics has a reno-protective effect.

In agreement with our results, Kodera et al. [59] reported that exendin-4 prevented macrophage infiltration, and decreased protein levels of intercellular adhesion molecule-1 and type IV collagen in kidney tissue and they found that the GLP-1 receptor was produced on monocytes/macrophages and glomerular endothelial cells and they demonstrated that in vitro exendin-4 acted directly on the GLP-1 receptor, and attenuated release of proinflammatory cytokines from macrophages and ICAM-1 production on glomerular endothelial cells. Moreover, Liang and Tall [60] reported that exenatide prevents atheroma formation by direct suppression of inflammatory macrophage response.

Also, Park et al. [58] reported that TGF-\(\beta\) expression mediated by oxidant stress may be suppressible by exendin-4 in diabetic kidneys exposed to IR injury. The authors also demonstrated that exendin-4 treatment increased GLP-1R-positive cells in the glomeruli of db/db and db/m mice in a dosage-dependent manner. These findings suggest that exendin-4 at least acts in a renoprotective role through increasing GLP-1R expression in the glomerulus in db/db mice.

It is well known that GLP-1 signalling through GLP-1R enhances cyclic AMP as a second messenger [48]. Previous reports have revealed that an increase in activity of the cyclic AMP/protein kinase A pathway suppresses NF-\(\kappa\)B activity in THP-1 cells and HUVECs [61], and inhibits NADPH oxidase [62], and these findings could explain the modulation of the inflammatory vicious cycle in the kidney by DPP-4 inhibitor treatment.

Moreover DPP-4 inhibitor treatment inhibits apoptosis that was an obvious effect of IR injury in diabetic rats and preserves renal functions.

In agreement with our results, Vaghasiya et al. [13] reported that DPP-4 inhibitor treatment reduces nuclear oxidative stress. They suggested this reason might be responsible for protective effect of organ injury induced by IR in diabetic rats.

In another study, a significant decrease in MDA, XO activity, and increased in SOD, CAT, GSH and GSHPx was reported in rats treated with exenatide. They demonstrated reduction in nuclear oxidative stress leading to reduction in DNA fragmentation [63,64]. Furthermore, decreased cell necrosis was observed in exenatide-treated rats in comparison to untreated diabetic groups, and that confirm the finding of Timmers et al. [65] who demonstrated that incretin treatment inhibited apoptosis in myocardial cells.

Our study showed that DPP-4 inhibitor significantly reduced nitrite level in the renal tissue after IR injury in diabetic rats.

Vaghasiya et al. [13] also reported that incretin treatment of diabetic IR animals had normalized the elevated tissue NO level which might be attributed to an inhibition of inducible nitric oxide synthase (iNOS) enzyme and they stated that streptozotocin-induced diabetes caused increased in activity and expression of iNOS.

This study proved that DPP-4 inhibitor protected diabetic rat kidneys from being damaged due to IR injury through improving the glycemic states, attenuating oxidative stress, reducing inflammation, apoptosis and may be through a direct effect on the renal tissues.

References

