Exenatide protects renal ischemia reperfusion injury in type 2 diabetes mellitus

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Background: In view of the reported efficacy of glucagons-like peptide-1 (GLP-1) on ischemia reperfusion (I/R) injury, this study was designed to assess the effect of exenatide (GLP-1 receptor agonist) on renal I/R in type 2 diabetes mellitus (T2DM). Materials and Methods: T2DM in rats were induced by administration of nicotinamide (230 mg/kg, i.p.), 15 min prior to the single dose of streptozotocin (65 mg/kg, i.v.). In vivo renal I/R were performed in both T2DM and normal rats. Results: The lipid peroxidation, xanthine oxidase activity, and nitric oxide level in renal tissue were significantly increased after I/R in diabetic rats compared to I/R in normal rats. Antioxidant enzymes such as glutathione, superoxide dismutase, catalase, and glutathione peroxidase were significantly reduced after I/R in diabetic rats compared to normal rats. Exenatide treatment significantly normalized these biochemical parameters compared to diabetic I/R rats. Serums TNF-α level and myeloperoxidase activity in renal tissue and apoptosis were also significantly normalized after administration of exenatide. Furthermore, treatment with exenatide (10 mcg/kg) had preserved the normal morphology of the kidney compared to I/R performed in T2DM rats. Conclusion: In conclusion, exenatide protects exaggerated renal I/R injury in T2DM. These findings have major implication in the treatment of ischemic injury that prone to develop in T2DM.

KEY WORDS: Type 2 diabetes mellitus, exenatide, inflammation, ischemia reperfusion, kidney, oxidative stress

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Introduction

Type 2 diabetes mellitus (DM) is one of the leading causes of end-stage renal disease (ESRD). Diabetics at a higher risk of an ischemic condition caused by decreased blood flow. 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. The recent study have demonstrated higher incidence of nephropathy in type 2 DM than in type 1 DM patients. The mechanisms behind the injury in diabetic nephropathy are not fully understood despite intense research. Diabetic patients may need renal transplantation in their later life due to diabetic nephropathy. I/R injury is one of the dangerous complications of this procedure.

The short period of ischemia (30 min) in diabetes has been demonstrated to lead to reversible renal failure, leading to progressive injury with ESRD. Reactive oxygen species (ROS) and nitric oxide (NO) play an important role in mediating cell damage during I/R injury. Inflammation contributes substantially to the pathogenesis of I/R with a central role for particular cells, adhesion molecules, and cytokines. Neutrophils are the inflammatory cells, which produce abundantly ROS during I/R injury. Myeloperoxidase (MPO) is found in neutrophils, which catalyze the formation of hypochlorous acid (HOCl), a toxic agent to cellular components, and initiates oxidative injury. Renal I/R causes tissue injury by oxygen radicals, and oxidative stress caused by an imbalance between production of ROS and the antioxidant capacity. Renal I/R injury may cause oxidative stress and increase lipid peroxidation in the tissue, and the rat tissue decreased shows antioxidant enzyme activities after renal I/R is well reported.

Exenatide, a synthetic version of Exe-4 is presently the
first GLP-1 receptor agonist approved for clinical use by the Federal Drug Administration in the treatment of type 2 diabetes mellitus. Exenatide exerts insulinotropic and insulominimetic properties via the G-protein-coupled GLP-1 receptor, which has also been reported to be expressed in various tissues like kidney. \[13,14\] GLP-1 have reported efficacy in ischemia reperfusion (I/R) injury. \[15-17\] So far, no study has proved that treatment with exenatide could protect renal I/R in T2DM, and therefore, this study was designed to investigate the effect of exenatide on renal I/R in diabetic rats.

**Materials and Methods**

**Chemicals**

Superoxide dismutase (SOD), crystalline beef liver catalase (CAT), 1,1,3,3-tetrahydroxy-propane, glutathione (GSH), and epinephrine hydrochloride were purchased from Sigma Aldrich, USA. A 1 kb DNA marker was obtained from Fermentas, Germany. RNase A, ethidium bromide, and agarose were procured from Himedia Lab, Mumbai. Tris buffer, thiobarbituric acid, and trichloroacetic acid were purchased from Himedia Lab. Folin's phenol reagent was procured from SD Fine Chemicals Mumbai, India. The diagnostic kits used for estimation of BUN, Creatinine and AST were purchased from Span Diagnostic Pvt. Ltd, India. All other chemicals used in the study were of laboratory grade.

**Experimental groups and animals**

Healthy adult Wistar rats (either sex) weighing 200–250 g were used. The experiment and protocol described in this study were approved by the Institutional Animal Ethics Committee (IAEC) of Smt. R.B.P. M.C. Atkot and with permission from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The rats were divided into five different groups \((n = 6)\). **Group-1**: Normal sham-operated (underwent all surgical procedures without I/R in normal rats); **Group-2**: Diabetic sham-operated (underwent all surgical procedures without I/R in diabetic rats); **Group-3**: In normal rats; on day 25, ischemia was produced for 30 min, followed by reperfusion of 24 h (I/R control); **Group-4**: After induction of diabetes; on day 25, I/R was produced (DM + I/R control), and **Group-5**: In diabetic rats; on day 14, exenatide (Sigma, St. Louis, MO) was injected in a dose of 10 mcg, subcutaneous, twice a day for 2 weeks, on day 24, I/R was produced (Exn + DM + I/R) [Figure 1].

**Induction of T2DM and measurement of blood glucose level**

T2DM in rats were induced by administration of nicotinamide (NAD) (230 mg/kg, i.p.), 15 min prior to the single dose of streptozotocin (STZ) (65 mg/kg, i.v.). \[18\] Control animals were received an equal volume of saline. The STZ solution contained STZ in saline with a sodium citrate buffer, pH 4.0. Food, water consumption, weight gain, and the blood glucose levels (by using standard diagnostic kits, Beacon Diagnostics Pvt. Ltd.) were recorded to monitor the degree of diabetes. Four weeks elapsed in between the induction of diabetes and ischemic injury.

**Renal I/R injury**

Diabetic and normal rats were anesthetized with ketamine (60 mg/kg i.p.) and diazepam (5 mg/kg i.p). Body temperature was maintained throughout surgery at 37 ± 0.5°C. The skin on back was shaved and disinfected with povidone–iodine solution. All rats were undergoing surgical exposure of the left and right renal pedicles via midline incision. To induce renal ischemia, both renal pedicles were occluded for 30 min with vascular clamps. After 30 min of occlusion, the clamps were removed, and kidneys observed to undergo reperfusion for 24 h. At the end of each in vivo study, rats were killed, and the kidney quickly removed, placed into liquid nitrogen, and then stored at -70°C until assayed for oxidant and antioxidant parameters.

**Renal function**

Serum samples were assayed for blood urea nitrogen (BUN) (Jaffe’s method), creatinine (DAM method), and aspartate aminotransferase (AST) by using standard diagnostic kits (Span Diagnostics, Gujarat, India).

![Figure 1](http://www.ijddc.com)
Lipid peroxidation and antioxidant enzymes

The kidney was removed and kept in cold conditions (precooled in inverted petridish on ice). It was cross-chopped with surgical scalpel into fine slices in chilled 0.25 M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris–HCl buffer, pH 7.4 (10%w/v) with 25 strokes of tight Teflon pestle of a glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for assays of lipid peroxidation (MDA content) and endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GSHPx). The MDA formation was estimated by the method of Slater and Sawyer.[19] Reduced GSH was determined by the method of Moran et al.[20] SOD was determined by the method of Mishra and Fridovich.[21] Catalase was estimated by the method of Hugo Aebi as given by Colowick et al.[22] GSH peroxidase was determined by the method of Paglia and Valentine.[23]

Xanthine oxidase activity

Tissue xanthine oxidase (XO) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm.[24] The phosphate buffer (pH 7.5) and xanthine were mixed with supernatant sample and then incubated for 30 min at 37°C. The reaction was stopped at 0 and 30 min by addition of 100% trichloroacetic acid. Then, the mixture was centrifuged at 5000g for 30 min. The activity was measured as 1 mmol of uric acid formed per minute at 37°C, pH 7.5.

Nitric oxide level

The nitrite (NO) was estimated by the method of Lepoivre et al.[25] To 0.5 mL of tissue homogenate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 min. The samples were then centrifuged at 5000g for 15 min. The protein-free supernatant was used for estimating nitrite levels. To 200 μL of the supernatant, 30 μL of 10% NaOH was added, followed by 300 μL of Tris–HCl buffer and mixed well. To this, 530 μL of Griess reagent was added and incubated in the dark for 10–15 min, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained. Standard curve was prepared by sodium nitrite solutions with concentrations in range 1–100 μM by diluting the nitrite standard solution.

Myeloperoxidase activity

MPO activity was measured in tissues in a procedure similar to that documented by Hillegas et al.[26] Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged at 41,400g (10 min); pellets were suspended in 50 mM phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles with sonication between cycles, the samples were centrifuged at 41,000g for 10 min. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50 mM phosphate buffer, o-dianisidine, and 20 mM H₂O₂ solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g of tissue.

TNF-α quantification by ELISA

Levels of TNF-α in serum were determined by using an enzyme-linked immunosorbent assay (ELISA) (Endogen, mouse TNF-α kit, Pierce Biotech Int., Rockford, Illinois, USA) according to the manufacturer’s instructions.

DNA fragmentation

Genomic DNA was extracted from renal cortices using DNA extraction kit (DNeasy kit, Axygen). Ten micrograms of DNA were loaded into 1.5% agarose gel containing 0.5 mg/mL ethidium bromide. DNA electrophoresis was carried out at 80 V for 1 to 2 h. DNA ladders, an indicator of tissue apoptotic nucleosomal DNA fragmentation, were visualized under ultraviolet light, and photographed for permanent records.

Renal histopathology

The kidneys fixed in 10% neutral-buffered formalin solution were embedded in paraffin, and were used for histopathological examination. Sections of 5 μm thickness were cut on a microtome and taken on glass slides coated with albumin. The hematoxyline-stained sections were stained with eosin for 2 min and were then quickly passed through ascending grades of alcohol, cleaned in xylene and mounted on Canada Balsam. The stained sections were examined under Olympus BX40 photomicroscope and photographed. Either samples were coded to perform blind study or expert guidance from the veteran pathologist was sought to determine histopathological changes. A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes using scores on a scale of none (−), mild (+), moderate (++), and severe (+++) damage.
Statistical analysis
All the values are expressed as mean ± SEM. Statistical significance between more than two groups were tested using one-way ANOVA followed by the Bonferroni multiple comparisons test using computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when \( P < 0.05 \).

Results

Effect of exenatide on renal function
Diabetic animals that underwent renal I/R exhibited significant increase in the serum concentrations of creatinine, urea nitrogen, and AST when compared with DM animals (\( P < 0.001 \)), suggesting a significant degree of glomerular dysfunction mediated by renal I/R. Serum concentrations of urea nitrogen, creatinine, and AST were also significantly different in between I/R group and DM + I/R group (\( P < 0.05 \), \( P < 0.01 \), \( P < 0.001 \), respectively). Pretreatment of rats with exenatide (10 mcg/kg) produced a significant reduction in the serum levels of creatinine, urea nitrogen, and AST compared to DM + I/R group (\( P < 0.001 \)) [Table 1].

Effect of exenatide on lipid peroxidation and antioxidant enzymes
The renal tissue MDA content in the normal as well as in diabetic group was elevated after induction of I/R injury, compared to normal control (\( P < 0.01 \), \( P < 0.001 \), respectively); however, exenatide treatment in Exn + DM + I/R group, significantly decreased the I/R-induced elevation in renal MDA level compare to DM + I/R group (\( P < 0.001 \)) [Figure 2A]. In accordance with that, I/R in diabetic rats demonstrated a significant decrease in renal tissue GSH (\( P < 0.001 \)) and GSHPx (\( P < 0.001 \)) level when compared to DM, while in the exenatide-treated Exn + DM + I/R group, renal GSH, and GSHPx content were found to be preserved (\( P < 0.05 \), \( P < 0.001 \)), being not significantly different from that of the DM and NC [Figure 2B and C]. Diabetic animals that underwent renal I/R exhibited significant decrease in the SOD activity when compared with DM animals (\( P < 0.01 \)), and I/R animals (\( P < 0.01 \)). However, the activity of SOD in renal tissue was increased in the pretreatment with exenatide, in comparison with the DM + I/R (\( P < 0.05 \)) [Figure 2D]. The CAT activity of DM + I/R group was decreased in comparison with the DM (\( P < 0.001 \)), and I/R group (\( P < 0.05 \)), whereas it was higher in the pretreatment with exenatide group than in the DM + I/R group (\( P < 0.001 \)) [Figure 2E]. The XO activity was increased in I/R and DM + I/R groups in comparison with the NC group (\( P < 0.001 \)). However, the XO activity was also found to be significantly different in between I/R and DM + I/R (\( P < 0.05 \)). The XO activity was normalized by treatment with exenatide compared to DM + I/R (\( P < 0.001 \)) [Figure 2F]. The levels of NO were increased in I/R and DM + I/R groups in comparison with NC group (\( P < 0.001 \)). Exenatide treatment to DM + I/R groups demonstrated

Table 1: Effect of exenatide on blood glucose concentration and renal function during the experiments

<table>
<thead>
<tr>
<th>Groups</th>
<th>NC (mmol/L)</th>
<th>DM (mmol/L)</th>
<th>DM + I/R (mmol/L)</th>
<th>Exn + DM + I/R (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG (mg/dL)</td>
<td>4.8 ± 0.94</td>
<td>34.26 ± 1.25</td>
<td>32.58 ± 2.51</td>
<td>6.4 ± 1.21</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>90.83 ± 8.20</td>
<td>84.47 ± 17.51</td>
<td>503.2 ± 28.92</td>
<td>796.5 ± 29.36</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13.17 ± 2.63</td>
<td>16.33 ± 2.801</td>
<td>36.33 ± 4.485</td>
<td>56.33 ± 6.259</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.375 ± 0.027</td>
<td>0.475 ± 0.057***</td>
<td>0.963 ± 0.097$$</td>
<td>1.433 ± 0.12$$</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. (n = 6), analyzed by one-way ANOVA followed by Bonferroni’s multiple comparison tests. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \).

Figure 2: Effect of exenatide on lipid peroxidation (A); reduced glutathione (B); glutathione peroxidase (C); superoxide dismutase (D); catalase (E); and xanthine oxidase (F) in renal tissue after renal I/R in normal, and diabetic rats. Values are mean ± SEM. (n = 6), analyzed by one-way ANOVA followed by Bonferroni’s multiple comparison tests. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \). *Compared with normal control, *compared with I/R, and + compared with DM + I/R group.
a significant decrease in NO level in comparison with DM + I/R group \( (P < 0.01) \) [Figure 3].

**Effect of exenatide on myeloperoxidase activity**
Myeloperoxidase activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the DM + I/R group than that of the DC \( (P < 0.001) \) group and I/R group \( (P < 0.01) \). On the other hand, exenatide treatment significantly decreased renal tissue MPO level \( (P < 0.001) \) compared to DM + I/R, which was found to be not different than that of the NC and DM groups [Figure 4A].

**Effect of exenatide on serum TNF-α**
The serum TNF-α level was significantly increased in the I/R control as compared to NC group \( (P < 0.001) \). In DM + I/R rats, serum TNF-α level was significantly higher as compared to I/R control \( (P < 0.01) \). Exn + DM + I/R group had significantly lower serum TNF-α level as compared to the DM + I/R control \( (P < 0.001) \) [Figure 4B].

**Effect of exenatide on DNA fragmentation**
Apoptosis was evaluated by DNA fragmentation analysis. The typical DNA laddering activity was observed in the I/R control and in DM + I/R groups, which indicates cell necrosis. Treatment with exenatide in DM + I/R had decreased DNA fragmentation and necrosis in comparison to the DM + I/R group [Figure 5].

**Effect of exenatide on renal histology**
Histological damage ranged from normal (control and exenatide treatment groups) to mild (I/R group) and severe (DM + I/R group), with cortical rather than medullary tubules demonstrating the most marked changes. The histopathological changes were graded and summarized in Table 2. The control group did not show any morphological changes. By contrast, the kidneys of untreated I/R rats showed tubular cell swelling, interstitial edema, tubular dilatation, hyaline casts, and moderate-to-severe necrosis. Treatment with exenatide (10 mcg/kg) preserved the normal morphology of the kidney, and shows normal glomeruli with slight edema of the tubular cells [Figure 6].

**Discussion**
In this study, we used 30 min of ischemia, the importance of the ischemic duration is well established,
and it has been previously shown that a longer period of ischemia causes a more severe injury.\(^{[15]}\) The degradation of ATP to hypoxanthine and xanthine via inosine is enhanced with the duration of ischemia.\(^{[27]}\) The restoration of ATP levels during reperfusion is slower after prolonged ischemia.\(^{[28]}\) Temperature is a critical factor in ischemic injury. Hyperthermia, especially during the ischemic phase, leads to a more severe renal I/R injury. Raising the temperature from 37 to 39.5°C during ischemia leads to a 100% increase in BUN in a model using 30 min of ischemia and uninephrectomy.\(^{[29]}\) To keep the temperature constant, we used a servo-controlled heating pad that kept the temperature in the rat at 37.5°C. An important question in this work is how T2DM could cause the increased sensitivity to renal I/R, which observed in DM animals. Several possible explanations exist. The increased sensitivity to I/R could be due to hyperglycemia per se. Shortage of insulin could also be involved in the increased sensitivity to I/R. Secondary effects of hyperglycemia such as formation of advanced glycosylated end products, increased oxidative stress, hemodynamic alterations, and formation of NO could also be involved.

In our study, animals subjected to renal I/R demonstrated an increase in the renal tissue MDA levels, and attenuated the antioxidant enzyme pool. Renal I/R-induced oxidative stress in DM was associated with impaired renal function leading to a marked increase in serum creatinine, urea nitrogen, and AST levels. Pretreatment with exenatide prevented the renal I/R-induced lipid peroxidation and protected the kidneys from severe attenuation of antioxidant activity in rats exposed to the renal I/R in diabetes. Furthermore, histopathological evaluation of rat kidney demonstrated the renal functional damage in DM + I/R group of rats, which was significantly improved by exenatide treatment. Renal I/R in normal as well as in diabetic rats caused characteristic morphological changes, such as tubular cell swelling, tubular dilatation, necrosis of epithelium, and interstitial edema. In contrast, sections of the exenatide pretreated kidneys demonstrated architectural and cytological preservation of structure.

Oxidative stress and inflammatory response might play a pathophysiological role in renal I/R injury in T2DM given the knowledge that oxidative stress is implicated both in the complications of T2DM and renal

### Table 2: Effect of exenatide (10 mcg/kg) pretreatment on morphological changes of kidneys, as assessed by histopathological examination of the normal rats, and diabetic rats exposed to renal I/R

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular cell swelling</th>
<th>Interstitial edema</th>
<th>Tubular dilatation</th>
<th>Necrosis of epithelium</th>
<th>Hyaline casts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I/R</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>DM + I/R</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Exn + DM + I/R</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

### Figure 6: Microscopic observations of kidneys tissue sections of normal and diabetic rats subjected to 30-min ischemia and 24 h reperfusion with BIOXL light microscope showing morphological changes. Images were taken under light microscopy using (H&E, ×40): (A) Normal control; (B) diabetic control; (C) normal rat exposed to renal I/R; (D) diabetic rat exposed to renal I/R; (E) exenatide (10 mcg/kg) pretreated diabetic rat exposed to renal I/R.
Numerous studies have investigated the NO production in the STZ-DM kidney, and increased in SOD, CAT, GSH and GSHPx in the treatment with exenatide, and nuclear oxidative stress was normalized in treatment with exenatide might be one of the main features of renal I/R injury in T2DM rats. The infiltrate mainly consists of cells identified as macrophages/monocytes and T-lymphocytes. The inflammatory response is increased acutely after I/R of the intestines in diabetic animals. After a brief ischemia of the intestine, ROS are also increased and the increase is more pronounced in diabetes. It is likely that inflammatory cells contribute to increased oxidative stress in T2DM kidneys after I/R. According to Sakr et al., pretreatment with a single injection of tacrolimus, 24 h prior to 60 min of ischemia was able to decrease the renal injury, and the effect was associated with decreased levels of TNF-α. Thus, we decided to estimate TNF-α and MPO. In our finding, the serum level of TNF-α was normalized in treatment with exenatide might be one reason of exenatide to protect against renal I/R in T2DM.

The cardiac MPO activity increased after renal I/R, consistently with leukocyte infiltration and activation. The active neutrophils show high MPO activity in the tissue as an inflammatory answer. This study demonstrated that the high renal MPO activity after induction of I/R in T2DM rats is very important because it clearly shown high-leukocyte infiltration in the renal tissue. The neutrophils play a major role in oxidant injury via the mechanisms such as the action of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or MPO system. HOCl is produced largely from stimulated neutrophils by MPO activity. HOCl causes oxidation of other molecules such as proteins, amino acids, carbohydrates, nucleic acids, and lipids, expanding renal tissue damage. In this study, increase in MPO activity was inhibited by exenatide treatment. This might also result in reduced lipid peroxidation and thus less accumulation of MDA, because activation of neutrophils might lead to the generation of more oxygen-reactive metabolites.

The NO system may be involved in the increased sensitivity to I/R in DM. There is evidence for increased NO-production in the STZ-DM kidney, the reaction of NO with O2· results in peroxynitrite formation, a potent and aggressive cellular oxidant and causes the formation of 3-nitro-l-tyrosine. Nitrite/nitrate levels, as the end products of NO conversion, were increased in blood plasma and aortic tissue in diabetic animals comparison with nondiabetic animals, which was confirmed by elevated NO level in our study. Exenatide treatment with diabetic I/R animals had normalized the elevated tissue NO level that might be attributed to protective effect of exenatide against renal I/R in T2DM.

Several mechanisms might be responsible for the renoprotective effects of exenatide against renal I/R. First, the restoration of normoglycemia is the explanation for the beneficial effects of exenatide treatment in the present model [Table 1], because glucose metabolism is stimulated over fatty acid metabolism, which is more efficient with respect to oxygen consumption for adenosine triphosphate production and which might therefore reduce oxygen demand. Some previous works support the importance of BGC in I/R injury. In our study, we found severe renal injury when I/R performed in T2DM rats, in which blood glucose concentration (BGC) was higher than in the normal rats. Hyperglycemia, the elevated BGC during I/R could be deleterious for the kidney. An increased acute sensitivity to ischemia has been demonstrated when BGC was raised by dextrose infusion or intraperitoneal glucose injection in combination with renal I/R in both rats and dogs. Numerous studies have investigated the influence of hyperglycemia and diabetes in cerebral ischemia. 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. There are conflicting evidences regarding the influence of hyperglycemia and diabetes on the degree of injury in experimental cerebral ischemia. DM or hyperglycemia in non-DM animals caused increased cerebral injury in most studies, especially when models with reperfusion were used. Taken together, these studies suggested a role for reperfusion in the harmful effect of hyperglycemia in cerebral ischemic injury. In our study, BGC controlled by exenatide pretreatment could be a protective effect of exenatide against renal I/R in T2DM [Table 1].

Additionally, exenatide might reduce apoptosis and oxidative stress. Timmers et al. demonstrated myocardial expression of pAkt was increased after exenatide treatment and expression of active caspase 3 were reduced. Also, activity of the antioxidant enzymes SOD and catalase were higher in animals treated with exenatide, and nuclear oxidative stress was reduced. In our study, decreased in MDA, XO activity, and increased in SOD, CAT, GSH and GSHPx in the treatment with exenatide demonstrated the reduction in nuclear oxidative stress. In addition, in response to
ROS, the outer membrane of mitochondria becomes permeabilized, resulting in the translocation of Bax from cytosol to the mitochondria and the release of cytochrome c occurs. The release of cytochrome c into the cytosol leads to form the apoptosome, which stimulates the activation of procaspase-9 and procaspase-3. Active caspase-3 activates the caspase-activated DNAase, leading to DNA fragmentation.[43,44] In this study, severe cell necrosis was observed in the I/R control and DM + I/R groups. Furthermore, decreased cell necrosis was observed in exenatide-treated rats in comparison to DM + I/R groups, and that confirm the finding of Timmers et al.[43]

In conclusion, T2DM had exaggerated renal I/R in STZ-NAD induced T2DM. Also, the exenatide treatment attenuated renal I/R in diabetic rats by modification in oxidative stress and inflammation. The treatment with exenatide for at least 2 weeks before I/R was a very efficient way to decrease the degree of renal injury in T2DM.

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