



# Diabetes Type-II Exaggerates Renal Ischemia Reperfusion Injury by Elevation of Oxidative Stress and Inflammatory Response

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## ABSTRACT

**Objective:** The present work was designed to investigate the role of Diabetes Mellitus Type-II (DM-II) on renal ischemia reperfusion (I/R)-associated pathophysiology in renal damage. **Materials and Methods:** DM-II in rats was induced by the administration of nicotinamide (230 mg/kg, i.p.), 15 min prior to a single dose of streptozotocin (65mg/kg, i.v.). *In vivo* renal I/R was performed in both DM-II and normal rats. **Results and Discussions:** Lipid peroxidation, xanthine oxidase activity, and nitric oxide levels were significantly increased in renal tissue after I/R in diabetic rats compared to I/R in normal rats. Levels of antioxidant enzymes such as glutathione, superoxide dismutase, catalase, and glutathione peroxidase were significantly reduced after I/R in diabetic rats compared to normal rats. Serum TNF- $\alpha$  levels, renal tissue myeloperoxidase activity, and apoptosis were also significantly increased after I/R in DM-II rats. Furthermore, DM-II rats that underwent I/R, showed severe tubular cell swelling, interstitial edema, tubular dilatation, hyaline casts, and moderate to severe necrosis. **Conclusion:** In conclusion, DM-II rats showed exaggerated renal I/R injury. These findings have a major implication in ischemic injury that is prone to develop in DM-II.

**Key words:** Diabetes Type-II, ischemia, reperfusion, kidney, inflammation, oxidative stress

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## INTRODUCTION

Diabetes mellitus (DM) type-II is one of the leading causes of end stage renal disease (ESRD).<sup>[1]</sup> Diabetic patients are at a higher risk for ischemic conditions caused by decreased blood flow.<sup>[2]</sup> Thus, ischemia is described as a decrease in oxygen supply or an increase in oxygen demand. With increasing duration and severity of ischemia, greater cell damage can develop, with a predisposition to a spectrum of reperfusion-associated pathologies, collectively called reperfusion injury.<sup>[3]</sup> A recent study demonstrated a higher incidence of nephropathy in DM type-II compared to DM type-I patients.<sup>[4]</sup> Mechanisms behind the injury in diabetic nephropathy are not fully understood despite intense research. Diabetic patients may need renal

transplantation later in life due to diabetic nephropathy; I/R injury is one of the dangerous complications of this procedure. Moreover, in DM rats, a comparatively short ischemia of 30 minutes, which in non-DM rats results in reversible acute renal failure, causes a progressive injury with end-stage renal failure.<sup>[5]</sup>

Reactive oxygen species (ROS) and nitric oxide (NO) play an important role in mediating cell damage during I/R injury.<sup>[6,7]</sup> Inflammation contributes substantially to the pathogenesis of I/R with a central role for particular cells, adhesion molecules, and cytokines.<sup>[8]</sup> Neutrophils are the inflammatory cells that produce high levels of ROS during I/R injury. Myeloperoxidase (MPO) is found in neutrophils and is found to catalyze the formation of hypochlorous

acid (HOCl), a toxic agent to cellular components, which initiates oxidative injury.<sup>[9]</sup> Renal I/R causes tissue injury via oxygen radicals and oxidative stress caused by an imbalance in the production of ROS and antioxidant capacity.<sup>[10,11]</sup> Renal I/R injury may cause oxidative stress and increase lipid peroxidation in the tissue, something that is well documented in rat tissues.<sup>[12]</sup>

Diabetic patients may need renal transplantation later in life due to diabetic nephropathy, and I/R injury is one of the dangerous complications of this procedure. Streptozotocin diabetic rats have an increased renal sensitivity to ischemia/reperfusion (I/R) injury.<sup>[13-15]</sup> Hence, the present investigation was designed to understand the role of DM-II on renal I/R-associated pathophysiology in renal damage using these diabetic rats.

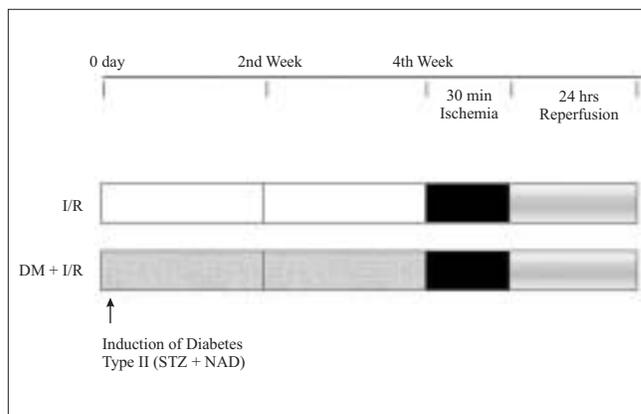
## MATERIALS AND METHODS

### 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 S.D. Fine Chemicals Mumbai, India. The diagnostic kits used for the 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 (both sexes) weighing 200-250 g were used for this experiment whose protocol described herein was approved by the Institutional Animal Ethics Committee (IAEC) of Smt. R.B.P.M.C. Atkot. Permission was obtained from the 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 assigned to three different groups ( $n = 6$ ). Group-1: Normal sham-operated (underwent all surgical procedures without ischemia reperfusion in normal rats); Group-2: Normal rats; ischemia was produced for 30 min on day 25, followed by 24 h reperfusion (I/R control); Group-3: After induction of diabetes; I/R was produced on day 25 (DM + I/R control) [Figure 1].



**Figure 1:** Scheme showing experimental protocols employed; each protocol consisted of the following periods: Ischemia 30 min, reperfusion 24 h

### Induction of diabetes type-II and measurement of blood glucose level

DM-II was induced in rats by the administration of nicotinamide (NAD) (230 mg/kg, *i.p.*), 15 min prior to the single dose of streptozotocin (STZ) (65mg/kg, *i.v.*).<sup>[16]</sup> Control animals received an equal volume of saline. The STZ solution contained STZ in saline and a sodium citrate buffer, pH 4.0. Food, water consumption, weight gain, and blood glucose levels (by using standard diagnostic kits, Beacon Diagnostics Pvt. Limited) were recorded to monitor the degree of diabetes. Four weeks were allowed to elapse 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  $37 \pm 0.5^\circ\text{C}$ . The skin on the back was shaved and disinfected with povidone iodine solution. All rats underwent 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 the kidneys observed before the 24 h reperfusion. At the end of each *in vivo* study, rats were sacrificed and the kidneys quickly removed, placed in liquid nitrogen, and then stored at  $-70^\circ\text{C}$  until assayed for oxidant and antioxidant parameters.

### Renal function

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

### Lipid peroxidation and antioxidant enzymes

The kidneys were removed and kept in cold conditions (precooled in an inverted petridish on ice). Each kidney was cross-chopped with a surgical scalpel into fine slices in chilled 0.25 M sucrose and quickly blotted on filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10% w/v) with 25 strokes of a 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 like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and Glutathione peroxidase (GSHPx). MDA formation was estimated by the method of Slater and Sawyer<sup>[17]</sup> reduced glutathione was determined by the method of Moron *et al.*,<sup>[18]</sup> and superoxide dismutase was determined by the method of Mishra and Fridovich.<sup>[19]</sup> Catalase was estimated by the method of Hugo Aebi as given by Colowick *et al.*,<sup>[20]</sup> glutathione peroxidase was determined by the method of Paglia and Valentine.<sup>[21]</sup>

### Xanthine oxidase activity

Tissue xanthine oxidase (XO) activity was measured spectrophotometrically by the increase in absorbance at 293 nm due to the formation of uric acid from xanthine.<sup>[22]</sup> The phosphate buffer (pH 7.5) and xanthine were mixed with the supernatant sample and incubated for 30 min at 37°C. The reaction was stopped at 0 and 30 min by the addition of 100% trichloroacetic acid. Then, the mixture was centrifuged at 5000 *g* for 30 min and the activity was measured at 293 nm. One unit of activity was defined as 1 mmol of uric acid formed per minute at 37°C, pH 7.5.

### Nitric oxide level

Nitrite (NO) was estimated by the method of Lepoivre *et al.*<sup>[23]</sup> To 0.5 mL of tissue homogenate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. Protein-free supernatant was used for estimating nitrite levels. To 200  $\mu$ L of the supernatant, 30  $\mu$ L of 10% NaOH was added, followed by 300  $\mu$ L of Tris-HCl buffer and mixed well. To this, 530  $\mu$ L of Griess reagent was added and incubated in the dark for 10-15 minutes 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. The standard curve was prepared by using sodium nitrite solutions with concentrations in the range of 1-100  $\mu$ M by diluting the nitrite standard solution.

### Myeloperoxidase activity

MPO activity was measured in tissues by a procedure similar to that documented by Hillegas *et al.*<sup>[24]</sup> Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged at 41,400 *g* (10 min). Pellets were suspended in 50 mM Phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze-thaw cycles with sonication between the cycles, the samples were centrifuged at 41,000 *g* for 10 min. Aliquots (0.3 mL) were added to 2.3 mL of the reaction mixture containing 50 mM Phosphate buffer, *o*-dianisidine, and 20 mM H<sub>2</sub>O<sub>2</sub> solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in the absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g of tissue.

### TNF- $\alpha$ quantification by enzyme-linked immunosorbent assay

Serum levels of TNF- $\alpha$  were determined by using an enzyme-linked immunosorbent assay (ELISA) (Endogen, mouse TNF- $\alpha$  kit, Pierce Biotech Int., Rockford, Illinois, USA) according to the manufacturer's instructions.

### DNA fragmentation

Genomic DNA was extracted from renal cortices using a 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 1-2 h. DNA ladders, an indicator of tissue apoptotic nucleosomal DNA fragmentation, were visualized under ultraviolet light and photographed for permanent records.

### Renal histopathology

Kidneys fixed in 10% neutral-buffered formalin solution were embedded in paraffin and used for histopathological examination. Sections of 5 m thickness were cut on a microtome and taken on glass slides coated with albumin. The hematoxyline-stained sections were stained with eosin for two minutes and quickly passed through ascending grades of alcohol, cleaned in xylene, and mounted on Canada Balsam. The stained sections were examined under an Olympus BX40 photomicroscope and photographed. The samples were either coded to perform a blind study or expert guidance was sought from a veteran pathologist to determine histopathological changes. A minimum of ten fields were examined for each kidney slide and assigned for the severity of changes using scores on a scale of none (-), mild (+), moderate (++) and severe (+++) damage.

## Statistical analysis

All the values are expressed as mean  $\pm$  SEM. Statistical significance was tested between more than two groups 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  $P < 0.05$ .

## RESULTS

### 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 I/R animals ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  respectively), suggesting a significant degree of glomerular dysfunction mediated by DM-II [Table 1].

### Lipid peroxidation and antioxidant enzymes

The renal tissue MDA content was elevated in the normal and diabetic groups after induction of I/R injury, compared to the normal control group ( $P < 0.01$ ,  $P < 0.001$  respectively). However, the DM + I/R group had higher renal MDA levels compared to the I/R group ( $P < 0.05$ ) [Figure 2a]. I/R in diabetic rats resulted in a significant decrease in renal tissue GSH ( $P < 0.05$ ) when compared to I/R in normal rats [Figure 2b]. Diabetic animals that underwent renal I/R had no effect on GSHPx or SOD levels when compared with I/R animals [Figures 2c and d]. The CAT activity of the DM + I/R group was decreased in comparison with the I/R group ( $P < 0.05$ ), whereas XO activity was increased in the DM + I/R group in comparison with the I/R group ( $P < 0.05$ ) [Figures 2e and f]. The levels of NO were increased in the DM+I/R group in comparison with the I/R group ( $P < 0.05$ ) [Figure 3].

### Myeloperoxidase activity

Myeloperoxidase activity, an accepted indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the DM + I/R group than in the I/R group ( $P < 0.01$ ) [Figure 4a].

### Serum TNF- $\alpha$

Serum TNF- $\alpha$  levels were significantly higher in DM + I/R rats than in the I/R control rats ( $P < 0.01$ ) [Figure 4b].

## DNA fragmentation

Apoptosis was evaluated by DNA fragmentation analysis. The typical DNA laddering activity was observed in the I/R and the DM + I/R groups, indicating cell apoptosis [Figure 5].

## Renal histology

Histological damage ranged from normal (control) to mild (I/R group) and severe (DM + I/R group), with cortical rather than medullary tubules demonstrating the most marked changes. Histopathological changes were graded and summarized in Table 2. The control group did not show any morphological changes but the kidneys of DM + I/R rats showed tubular cell swelling, interstitial edema, tubular dilatation, hyaline casts, and moderate to severe necrosis [Figure 6].

## DISCUSSION

In the present study, we used 30 minutes of ischemia as the importance of the ischemic duration is well established-it has been previously shown that a longer period of ischemia causes more severe injury.<sup>[5]</sup> The degradation of ATP to hypoxanthine and xanthine via inosine is enhanced with the duration of ischemia.<sup>[25]</sup> The restoration of ATP levels during reperfusion is slower after prolonged ischemia.<sup>[26]</sup> Temperature is a critical factor in ischemic injury-hyperthermia, especially during the ischemic phase, leads to more severe renal I/R injury. Raising the temperature from 37 to 39.5°C during ischemia leads to a 100% increase in blood urea nitrogen (BUN) in a model using 30 minutes of ischemia and uninephrectomy.<sup>[27]</sup> In order to keep the temperature constant, we used

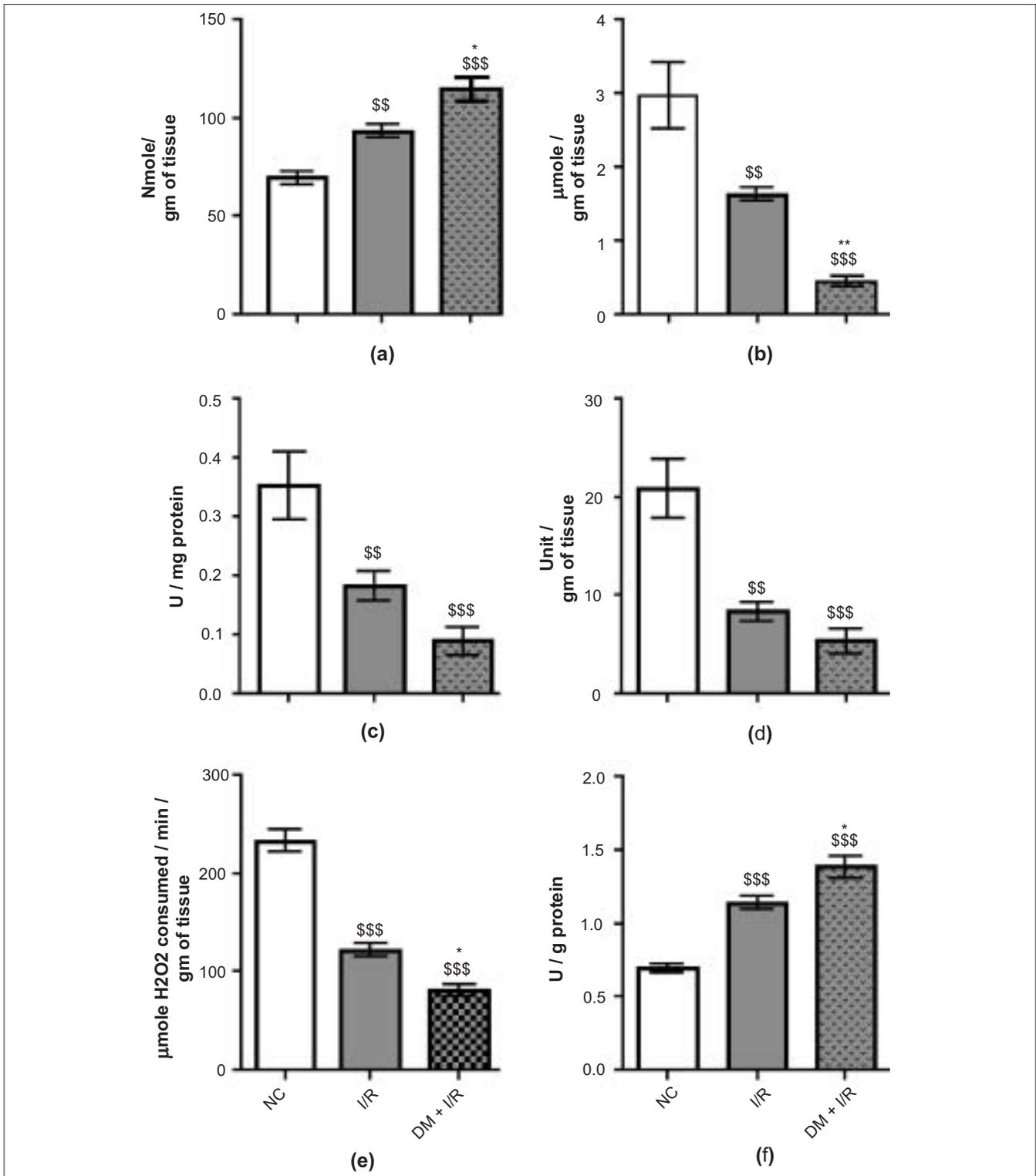
**Table 1: Blood glucose concentration and renal function during the experiments**

Groups	NC	I/R	DM + I/R
BGC (mmol/L)	4.9 $\pm$ 0.92	4.7 $\pm$ 0.84	34.48 $\pm$ 1.51 <sup>SSS,**</sup>
AST (IU/L)	98.83 $\pm$ 8.3	490.2 $\pm$ 28.92 <sup>SSS</sup>	770.5 $\pm$ 31.36 <sup>SSS,***</sup>
BUN (mg/dL)	19.17 $\pm$ 3.2	34.38 $\pm$ 4.48 <sup>SS</sup>	59.36 $\pm$ 5.259 <sup>SSS,*</sup>
Creatinine (mg/dL)	0.39 $\pm$ 0.027	0.86 $\pm$ 0.097 <sup>SSS</sup>	1.51 $\pm$ 0.18 <sup>SSS,**</sup>

Values are mean  $\pm$  SEM (n = 5), analyzed by one-way ANOVA followed by bonferroni's multiple comparison tests. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs I/R; <sup>SSS</sup> $P < 0.001$  vs normal control

**Table 2: Effect of DM-II on morphological changes of kidneys, as assessed by histopathological examination of the normal rats, and diabetic rats exposed to renal I/R**

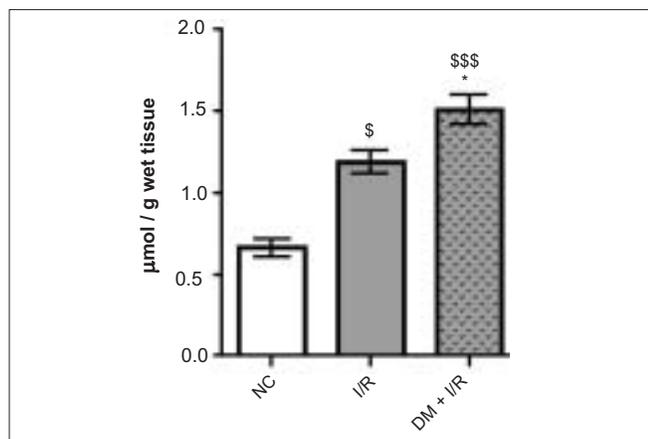
Group	Tubular cell swelling	Interstitial edema	Tubular dilatation	Necrosis of epithelium	Hyaline casts
NC	-	-	-	-	-
I/R	++	++	++	++	++
DM + I/R	+++	+++	+++	+++	+++



**Figure 2:** (a) Lipid peroxidation; (b) reduced glutathione; (c) glutathione peroxidase; (d) superoxide dismutase; (e) catalase; (f) xanthine and oxidase in renal tissue after renal I/R in normal, and diabetic rats. Values are mean  $\pm$  SEM ( $n = 6$ ), analyzed by one-way ANOVA followed by bonferroni's multiple comparison tests; \* $P < 0.05$ ; \*\* $P < 0.01$  vs I/R; \$\$\$ $P < 0.001$ ; \$\$\$ $P < 0.001$  vs normal control

a servo-controlled heating pad that kept the temperature in the rat at 37.5°C. An important question in this study is how DM-II could cause the increased sensitivity to renal I/R, which has been observed in DM animals. Several

possible explanations exist: i) the increased sensitivity to I/R could be due to hyperglycemia *per se*, ii) shortage of insulin could also be involved in the increased sensitivity to I/R, iii) secondary effects of hyperglycemia such as



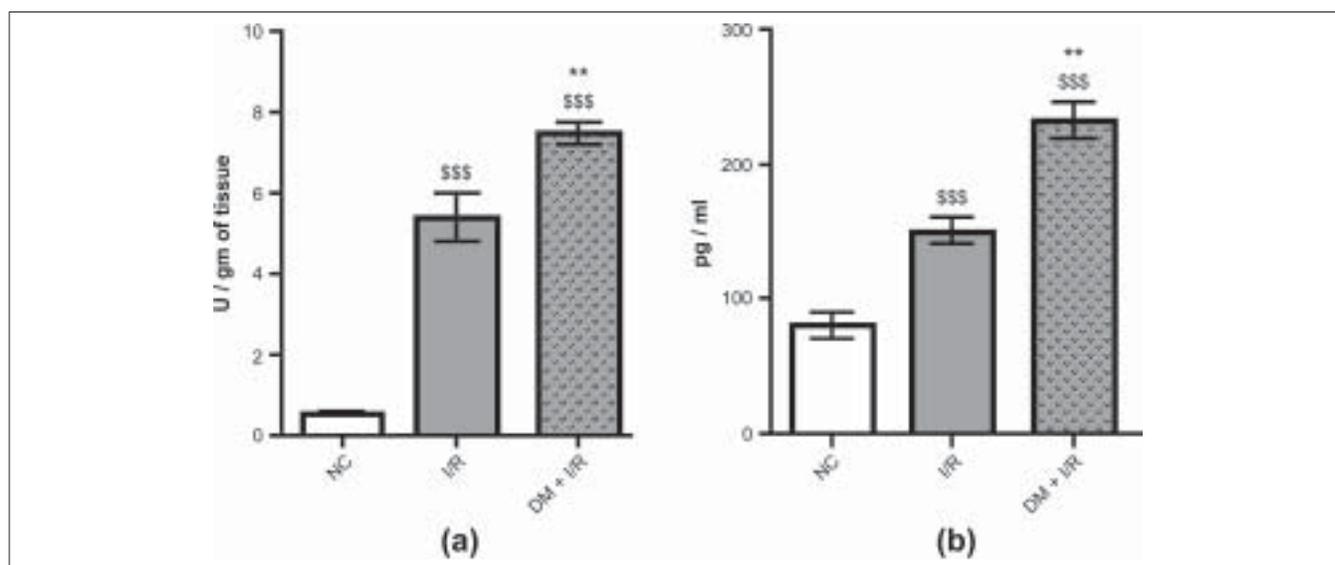
**Figure 3:** The nitric oxide in renal tissue after renal I/R in normal and diabetic rats. Values are mean  $\pm$  SEM ( $n = 6$ ), analyzed by one-way ANOVA followed by Bonfferoni's multiple comparison tests; \* $P < 0.05$ , \*\* $P < 0.01$  vs I/R; \$ $P < 0.05$ ; \$\$ $P < 0.01$ ; \$\$\$ $P < 0.001$  vs normal control

the 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 an attenuated antioxidant enzyme pool. Renal I/R-induced oxidative stress in DM-II was associated with impaired renal function, leading to a marked increase in serum creatinine, urea nitrogen and AST levels. Furthermore, histopathological evaluation of the rat kidney demonstrated the renal functional damage in the DM + I/R group of rats, which was more severe than that of I/R rats. Renal I/R in diabetic rats caused characteristic morphological changes, such as tubular cell swelling, tubular dilatation, necrosis of epithelium, and interstitial edema [Figure 6, Table 2].

Oxidative stress and inflammatory response might play a pathophysiological role in renal I/R injury in DM-II, given the knowledge that oxidative stress is implicated both in the complications of DM-II and renal I/R. Elevated oxidative stress has been demonstrated in cerebral<sup>[28]</sup> and intestinal<sup>[29]</sup> I/R in diabetic rats. The combined oxidative stress from two sources may thus increase the total level of ROS. Infiltration of inflammatory cells is one of the main features of renal I/R injury in DM-II 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.<sup>[30]</sup> After a brief ischemia of the intestine, ROS levels are also increased and the increase is more pronounced in diabetes.<sup>[29]</sup> It is likely that inflammatory cells contribute to increased oxidative stress in DM-II kidneys after I/R. According to Sakr *et al.*, pretreatment with a single injection of tacrolimus, 24 hours prior to 60minutes of ischemia was able to decrease the renal injury, an effect associated with decreased levels of TNF- $\alpha$ .<sup>[31]</sup> Thus, we decided to estimate TNF- $\alpha$  and MPO. In our finding, the serum level of TNF- $\alpha$  was higher in the DM + I/R group, a possible reason for the exaggeration of renal I/R in DM-II.

Cardiac MPO activity increased after renal I/R, consistent with leukocyte infiltration and activation. Active neutrophils show high MPO activity in the tissue as an inflammatory response.<sup>[32]</sup> The present work demonstrated that the high renal MPO activity after induction of I/R in DM-II rats, is very important because it clearly shows high leukocyte infiltration in renal tissue. Neutrophils play a major role in oxidant injury via mechanisms such as the action of



**Figure 4:** (a) Myeloperoxidase (b) TNF- $\alpha$  in renal tissue after renal I/R in normal and diabetic rats. Values are mean  $\pm$  S.E.M. ( $n = 6$ ), analyzed by one-way ANOVA followed by bonfferoni's multiple comparison tests; \* $P < 0.05$ ; \*\* $P < 0.01$  vs I/R; \$ $P < 0.05$ ; \$\$ $P < 0.01$ ; \$\$\$ $P < 0.001$  vs normal control

nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or the MPO system. Hypochlorous acid produced largely from stimulated neutrophils by MPO activity, causes oxidation of other molecules such as proteins, amino acids, carbohydrates, nucleic acids, and lipids, expanding renal tissue damage.<sup>[33]</sup>

The nitric oxide system may be involved in the increased sensitivity to I/R in DM-II. There is evidence for increased NO-production in the STZ-DM kidney;<sup>[13]</sup> the reaction of NO with O<sub>2</sub> results in peroxynitrite formation, a potent and aggressive cellular oxidant, and causes the formation of 3-nitro-L-tyrosine.<sup>[34,35]</sup> Nitrite/nitrate levels, as the end products of nitric oxide conversion, were found to be increased in blood plasma and aortic tissue in diabetic animals in comparison with nondiabetic animals,<sup>[36]</sup> something that was confirmed by elevated NO levels in our study.

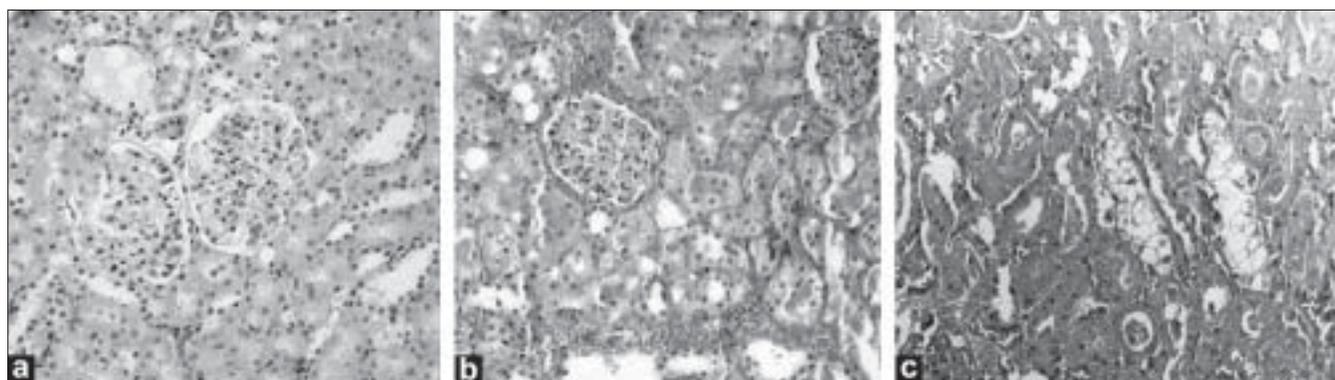
Several mechanisms might be responsible for the exaggerated renal injury seen in DM-II, for instance, the

restoration of hyperglycemia [Table 1]; previous work supports the importance of blood glucose concentration (BGC) in I/R injury. In our study, we found severe renal injury when I/R was performed in DM-II rats in whom BGC was higher than in 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.<sup>[37]</sup> 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.<sup>[38]</sup> There is conflicting evidence 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.<sup>[39]</sup> Taken together, these studies suggested a role for reperfusion in the harmful effect of hyperglycemia in ischemic injury.

In our study, increases in MDA, XO activity, and decreases in SOD, CAT, GSH, and GSHPx in DM + I/R rats demonstrated the induction in nuclear oxidative stress. In addition, the outer membrane of mitochondria becomes permeabilized in response to ROS, resulting in the translocation of Bax from cytosol to the mitochondria and the release of cytochrome C. The release of cytochrome C into the cytosol leads to the formation of the apoptosome, which stimulates the activation of procaspase-9 and procaspase-3. Active caspase-3 activates caspase-activated DNAase, leading to DNA fragmentation.<sup>[40-42]</sup> In our study, a typical laddering of fragmented DNA observed in the I/R control and DM/I/R rats. Furthermore, an increase was observed in cell necrosis in DM + I/R rats in



**Figure 5:** DNA fragmentation analysis revealed typical laddering of fragmented DNA in I/R control and DM/I/R groups; (a) Normal control; (b) Normal rat exposed to renal I/R; (c) Diabetic rat exposed to renal I/R



**Figure 6:** Microscopic observations of kidney 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 and E (×40); (a) Normal control; (b) Normal rat exposed to renal I/R; (c) Diabetic rat exposed to renal I/R

comparison to the I/R group, which confirms the finding of Timmers *et al.*<sup>[40]</sup>

## CONCLUSION

In conclusion, exaggerated renal I/R injury was seen in STZ-NAD-induced DM-II rats due to the elevation of oxidative stress and inflammation.

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