



## RENOPROTECTIVE ACTIVITY OF PIOGLITAZONE ON ISCHEMIA/REPERFUSION INDUCED RENAL DAMAGE IN DIABETIC RATS

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### Abstract

Present study was designed to evaluate in Renoprotective activity of Pioglitazone on Ischemia/reperfusion (I/R) induced renal damage in diabetic rats. Ischemia/reperfusion injury, which is commonly seen in the field of renal surgery or transplantation in diabetic condition, is a major cause of acute renal failure. Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. After right nephrectomy, Pioglitazone (10 mg/kg/day, p.o) was administered for 15 days. On the 16th day, ischemia was induced in contra lateral kidney for 45 min, followed by reperfusion for 24 hr. Renal function marker and oxidative parameter were estimated at the end of 24 hr reperfusion. At the end of experimental period the level of malondialdehyde formation/ lipid peroxidation (LPO) in kidney tissue and serum marker Creatinine, Urea and Uric acids were significantly increased. Whereas, the activity of biomarkers of oxidative stress such as reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were found to be decreased significantly compared to control rats. Pioglitazone improved the renal dysfunction and oxidative stress after renal ischemia/reperfusion injury in diabetic rats. In conclusion, Pioglitazone shows potent may improve renal function marker and oxidative stress in kidney in I/R induced renal damage in type 2 diabetic rats.

**Keywords:** Pioglitazone; renal ischemia reperfusion injury; oxidative stress; type 2 diabetes

### Introduction

Ischemic injury to brain, heart, and kidney is associated with high morbidity and mortality. Improving the ability of these organs to tolerate ischemic injury would have important implications. Ischemic insults are often recurrent in diabetic patients. In the setting of loss of renal blood flow autoregulation that characterizes the post-ischemic kidney [1], Renal ischemia/reperfusion (I/R) injury is a major cause of acute renal failure (ARF) [2], which is faced in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery, angioplasty, aortic aneurysm surgery, and elective urological operations. In these conditions, I/R injury initiates a complex and interrelated sequence of events, resulting in injury to and the eventual death of renal cells [3, 4]. Several factors have been implicated in the pathophysiological changes occurring while renal I/R injury including vascular or microvascular injury, endothelial dysfunction, accelerated cell necrosis, granulocyte activation, and modulation of nitric oxide/angiotensin II axis [5, 6].

The rennin-angiotensin system plays a pivotal role in regulation of blood pressure. Renin acts on angiotensinogen to form angiotensin I, which is converted to angiotensin II with the help of

angiotensin-converting enzyme (ACE) [7]. Angiotensin II is an important mediator in kidney injury. Accumulating evidence suggests that angiotensin II stimulates intracellular formation of reactive oxygen species (ROS) such as the superoxide anion and hydrogen peroxide that leads to kidney damage [8].

Recently, a protective effect of Pioglitazone against oxidative stress in liver and kidney of diabetic rabbits [9] has been reported. Pioglitazone (PIO) hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. PIO showed dose dependant beneficial effects in many of the pathological conditions including reduction in blood glucose, lowering blood pressure and restoring endothelial functions in animals [10]. Pioglitazone lowers blood pressure and restores blunted endothelium-dependent vasodilatation in fructose-fed rats [11], insulin-resistant rhesus monkey [12], SHR [13] and sucrosefed SHR [14].

The present study, we investigated the protective effect of Pioglitazone on renal I/R injury in diabetic rats and other word effect of Pioglitazone on renal marker and oxidative stress of kidney tissues in I/R induced renal damage in diabetic rats.

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## Materials and Methods

### Drugs and Chemicals

Pioglitazone hydrochloride was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

### Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats (210 ± 15 g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitum*. The animal experiment was approved by Animal Ethical Committee of the Institute (1163/a/08/CPCSEA).

### Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin

(65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose [15]. Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study.

### Experimental Protocol

The rats were divided into three groups each consisting of six animals:

**Group 1:** Animals served as sham-operated (underwent all surgical procedures without ischemia reperfusion).

**Group 2:** After right nephrectomy on day 1, vehicle (0.5 % sodium CMC) was administered for 15 days; on day 16, ischemia was produced in the left kidney for 45 min, followed by reperfusion of 24 hr (I/R control).

**Group 3:** After right nephrectomy on day 1, Pioglitazone (10 mg/kg/day, p.o.) was administered for 15 days; on day 16, ischemia was produced in the left kidney for 45 min, followed by reperfusion of 24hr(I/R+PIO).

### Surgical Procedure

| The progress of the experiment |                                   |
|--------------------------------|-----------------------------------|
| Day 1                          | Unilateral right nephrectomy      |
| Day 15                         | Treatment                         |
| Day 16                         | 45 minutes ischemia (left kidney) |
| Day 17                         | 24 hr reperfusion                 |

Right nephrectomy was performed through a right flank incision (2 cm) under general anesthesia, ketamine (100 mg/kg, i.p.). After right nephrectomy, several treatments were given as mentioned previously for 15 days. On day 16, ischemia was produced in the left kidney by performing a left flank incision and dissecting the left renal pedicle to expose the renal vessels. Non traumatic vascular clamps were used to stop blood flow (in artery and vein) for 45 min. Reperfusion was established by removing the clamp after 45 min ischemia. The abdominal wall (muscular layer and skin) was closed with 4.0 mononylon suture. At the end of reperfusion period (after 24 hr), blood samples were collected and used for the estimation of renal function (BUN and creatinine). The abdomen was opened, and the kidneys were harvested for the biomarkers of oxidative stress.

### Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After seven day, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

### Estimation of kidney function marker

Blood was collected from the rats by retro-orbital puncture at the time of sacrifice and was allowed to clot for 10 minutes at room temperature. Clots were centrifuged at 2500 rpm for 10 minutes to separate the serum. Serum creatinine and urea levels were measured by assay kits (SPAN Diagnostics Pvt. India) and Serum Uric acid levels were measured by assay kits (Crest Biosystems Ltd. India).

**Preparation of Tissue Homogenate**

After sacrificing the animals, their kidneys were quickly removed, perfused immediately with ice cold hypertonic saline solution, weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×g at 0°C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of following antioxidant parameters. The levels of Lipid peroxidation (LPO) formation and the activities of endogenous antioxidant enzymes such as catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) were estimated by the method of Slater and Sawyer [16] Hugo Aebi as given by Hugo [17] Moron et al [18] and Mishra and Fridovich [19].

**Statistical Analysis**

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when p < 0.05.

**Results**

**Characterization of Type 2 Diabetes**

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals (Table 1).

Table 1. Effect of Streptozotocin (65mg/kg/day, p.o) and Nicotinamide (110 mg/kg/day, p.o) on serum glucose and HbA1c changes level in rats.

| Groups    | Glucose          | HbA1c             |
|-----------|------------------|-------------------|
| CON       | 101.8 ± 6.799    | 5.455 ± 0.3729    |
| STZ + NIC | 332.8 ± 9.167*** | 9.900 ± 0.6323*** |

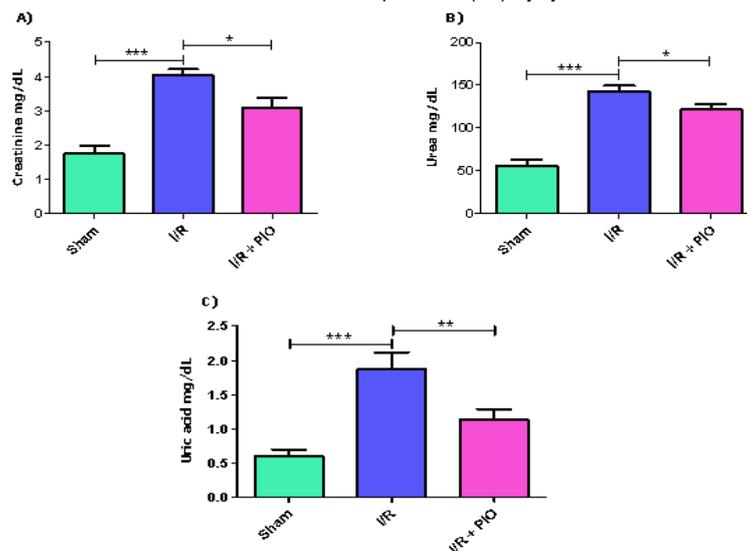
Values are expressed as mean ± SEM for six animals in the group. \*\*\*P<0.001 considered statistically significant as compared to respective Control group.

**Effect of PIO on kidney function marker**

The six rats which underwent renal I/R exhibited a significant increase in the serum concentrations of creatinine (P < 0.001), urea (P < 0.001), and uric acid (P < 0.001) compared with the sham control animals, suggesting a significant

degree of glomerular dysfunction mediated by renal I/R. In I/R+PIO treated diabetic rats, serum creatinine, urea and uric acid levels were significantly (p< 0.05, n = 6) higher as compared to I/R control group alone (Fig.1).

Figure 1. Effect of Pioglitazone (10 mg/kg/day, p.o) on serum Creatinine (A), Urea (B), and Uric acid (C) in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



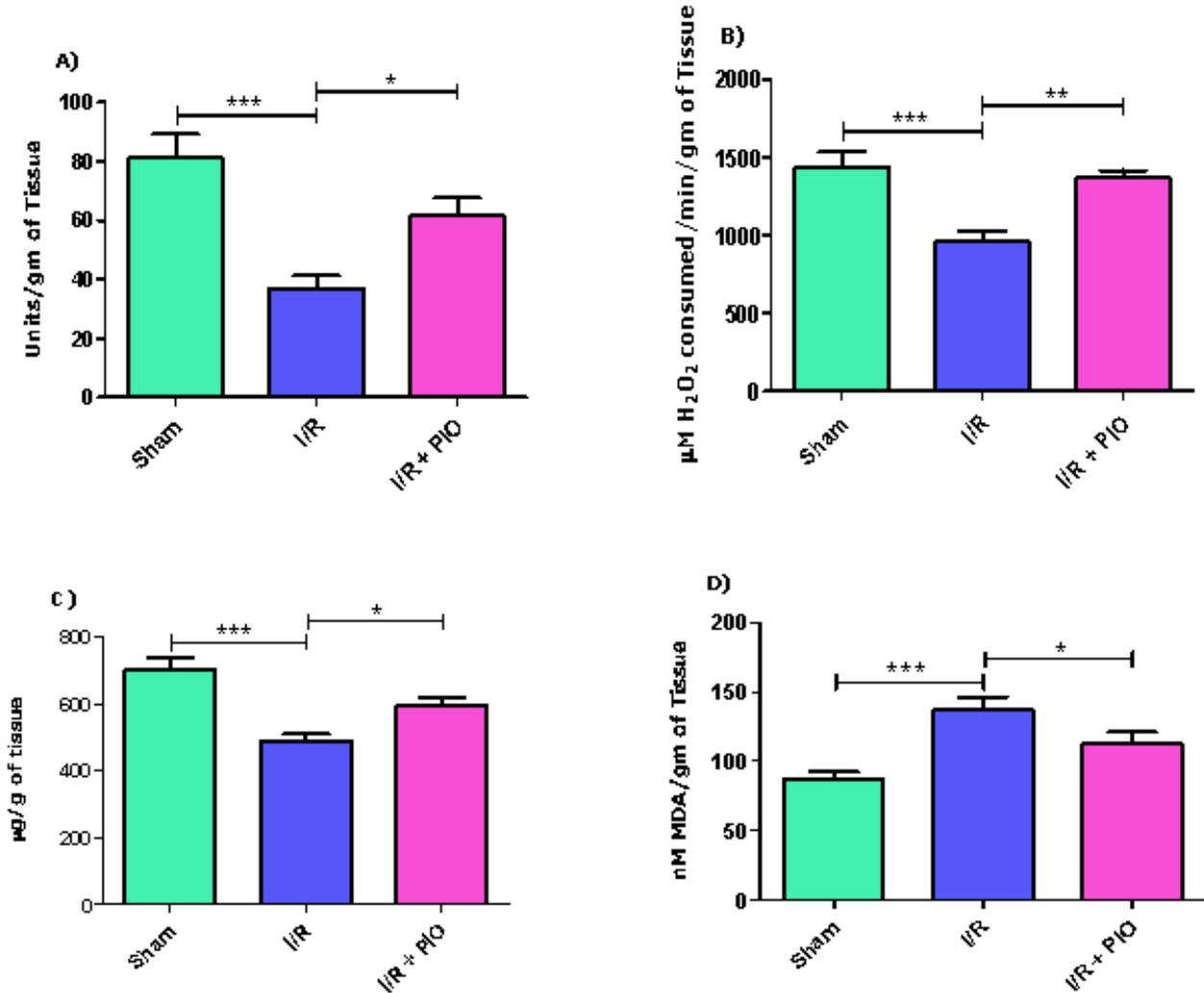
Values are expressed as mean ± SEM for six animals in the group. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 considered statistically significant as compared to respective Sham group.

**Effect of PIO on antioxidant activity**

Renal I/R group of diabetic rats showed significantly decreased enzymatic activity of superoxide dismutase ( $P < 0.001$ ), catalase ( $P < 0.001$ ), and reduced glutathione ( $P < 0.001$ ) when compared with the sham control rats. These declining trends were significantly ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.05$ , respectively Sham control Group)

decreased in the group treated with PIO compared with those in the I/R-only group (Fig. 2). Renal I/R produced a significant ( $P < 0.001$ ) increase in MDA levels in comparison with the sham operation in the rats. Treatment with PIO before renal I/R was associated with a significantly ( $P < 0.05$ ) lower MDA level than that in the rats that underwent only renal I/R (Fig. 2).

Figure 2. Effect of Pioglitazone (10 mg/kg/day, p.o) on Superoxide dismutase (A), Catalase(B), Reduced glutathione (C) and lipid peroxidation (D) in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



Values are expressed as mean  $\pm$  SEM for six animals in the group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  considered statistically significant as compared to respective Sham group.

**Discussion**

The present study was under taken with the objective of exploring evaluate Pioglitazone on renal marker in I/R induced renal damage in diabetic rats.

The transient discontinuation of renal blood supply is encountered in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, and elective urological operations [3, 4]. This transient

discontinuation causes renal I/R injury which results in decreased glomerular filtration and renal blood flow and increased urine output characterized by natriuresis and impaired concentrating ability. Much of this tubular and glomerular dysfunction has been postulated to occur during the reperfusion period following anoxia, and generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury. In renal I/R injury, ROS are capable of reacting with lipids leading to lipid peroxidation of biological membranes, which in turn impacts enzymatic processes, such as ion pump activity, inhibiting transcription and repair of DNA. If lipid peroxidation remains unchecked, it will ultimately result in cell death [20, 21].

In our study, animals subjected to renal I/R demonstrated an increase in the renal MDA and attenuated antioxidant enzymes pool. Lipid peroxidation and antioxidant enzymes are important indexes of oxidant injury [22]. Demonstration of lipid peroxidation as indexes for oxidative damage may help us better understand the effects of ROS on the cellular components [23]. Renal I/R-induced oxidative stress was associated with impaired kidney function, leading to a marked increase in serum creatinine, urea, and uric acid levels.

Pretreatment with Pioglitazone prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe attenuation of antioxidant enzymes activity in rats exposed to the renal I/R. Furthermore, the impaired kidney function was significantly improved by Pioglitazone. The mechanism of the protective effect of Pioglitazone on renal I/R injury can be explained by its antioxidant activity [9].

The rennin-angiotensin system plays a pivotal role in regulation of blood pressure. Renin acts on angiotensinogen to form angiotensin-I, which is converted to angiotensin-II with the help of angiotensin-converting enzyme. Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage [8]. Generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury. Oxidative stress can result from increased ROS production, and/or from decreased ROS scavenging capability. The ROS attach to the polyunsaturated fatty acids in the membrane lipids and result in peroxidation, which may lead to disorganization of cell structure and function. After reperfusion and reoxygenation, the imbalance between restoration of oxygen supply and mitochondrial respiratory function results in massive generation of superoxide anion in

mitochondria [22, 23]. Under these conditions, the defensive system, which is known as antioxidant or antioxidant enzymes, cannot prevent the escape of ROS, especially in mitochondria, and their effects on other intracellular sites. This cascade of events is known as reperfusion injury [23]. In this study, renal I/R increased oxidative stress products including tissue MDA and depleted the antioxidant enzymes pool, as is evident from the declined activity of superoxide dismutase, catalase, and reduced glutathione. It can be speculated that pretreatment with Pioglitazone prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe increasing of ROS products and depletion of superoxide dismutase and reduced glutathione in rats exposed to the renal I/R.

## Conclusions

It is important to inhibit oxidative stress to prevent renal I/R injury in diabetic condition. Our data support a role for Pioglitazone in attenuation of kidney damage after I/R injury of the kidneys in an animal model, in part at least by antioxidant or free radical scavenging activity.

## Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

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