Vol 2 | Issue 1 | Jan –Jun 2011 | 30-37. **Print ISSN – 2229 7502**

Research Article

EFFECT OF SOME SYNTHETIC AND HERBEL DRUGS ON TUMER NECROSIS FACTOR ALPHA IN RENAL REPERFUSION INDUCED RENAL DAMAGE IN TYPE 2 DIABETIC RATS

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ABSTRACT

Present study was designed to evaluate effect some synthetic drugs and some herbal compound on Tumor necrosis factor in renal Ischemia/Reperfusion induced renal damage in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Tumor necrosis factor-alpha (TNF-α) has been established as an important mediator in renal ischemia/reperfusion (I/R) injury. Diabetic rats manifest abnormal renal hemodynamic responses, with persistent renal vasodilation at reduced renal perfusion pressures. 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, p.o), Glimepiride (0.5 mg/kg, p.o), Nobivolol (2 mg/kg, p.o), Valsartan (8 mg/kg, p.o) and Hesperidin (100 mg/kg, p.o) were administered for 15 days. On the 16th day, ischemia was induced in contra lateral kidney for 45 min, followed by reperfusion for 24 hr. TNF- α was estimated at the end of 24 hr reperfusion. Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose and glycosylated hemoglobin (HbA1c). At the end of experimental period the level of TNF-α in kidney tissue was significantly increased. Treatment with Pioglitazone and Hesperidin significantly ($P<0.05$) decreased TNF- α but treatment with Nobivolol and Valsartan significantly ($p<0.001$, $p<0.01$) decreased and treatment with Glimepiride no change. This study concluded that Nebivolol may better reduce renal complication in Ischemia/Reperfusion induced renal damage in type 2 diabetic rats.

KEYWORDS: Streptozotocin, Nicotinamide, TNF-α

INTRODUCTION

Ischemia/reperfusion (I/R) is an important cause of organ dysfunction, often causing high mortality Ischemic cell injury in the kidney occurs during cardiovascular surgery, renal transplantation, as well as the early allograft rejection subsequent to renal transplantation [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].

Hyperglycaemia is most probably a contributing factor in the development of ischemic an ARF in many patients. Both clinical and experimental data suggest that hyperglycaemia increases the risk of ARF [7-9]. Hyperglycaemia also worsens the outcome in renal transplantation [10]. Conversely, I/R combined with hyperglycaemia could also be important in the development of diabetic nephropathy.

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Organ injury as a consequence of ischemia followed by reperfusion is a major clinical problem. I/R injury is the most common cause of acute renal failure as seen after renal transplantation, major abdominal and vascular surgery, coronary bypass surgery, and in trauma and sepsis [11].

Recently, a protective effect of Pioglitazone against oxidative stress in liver and kidney of diabetic rabbits [12] 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 [13]. Pioglitazone lowers blood pressure and restores blunted endothelium-dependent vasodilatation in fructose-fed rats [14], insulin-resistant rheus monkey [15], SHR [16] and sucrosefed SHR [17].

Nebivolol 1-(6-fluorochroman-2-yl)-2-[[2-(6-fluorochroman- 2-yl)-2-hydroxy-ethyl] amino] ethanol is a third generation β-blocker having highly selective β1 adrenergic receptor blockade [18]. It is reported to possess antihypertensive, anti-oxidant activity, and also reduces renal fibrosis and prevents endothelial dysfunction [19, 20].

Glimepiride (GLI) an oral blood glucose lowering drug of the sulfonylurea class is reported to have pancreatic and extra pancreatic effects as well. The blockages of K_{ATP} channels of pancreatic cells by sulphonylurea are critical in the regulation of glucose regulated insulin secretion.

Recent evidence suggest that blockade of the rennin-angiotensin system ameliorates diabetes induced renal dysfunction. Because activation Valsartan (VAL) - Angiotensin II receptor (AT 1) blocker blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscles and the adrenal gland.

Hesperidin (HES) is an abundant and inexpensive byproduct of Citrus cultivation and isolated from the ordinary orange Citrus aurantium and other species of the genus Citrus (family: Rutaceae). It is reported to have anti-allergic, radio protective, immunomodulator, anti-hypertensive and anti-oxidant properties.

So far the effect of PIO, GLI, NOB, VAL and HES on Tumor necrosis factor in experimentally induced renal damage in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to investigate the effect of PIO, GLI, NOB, VAL and HES on TNF-α in I/R induced renal damage in diabetic rats.

MATERIALS AND METHOD

Drugs and Chemicals

Nobivolol was obtained as a gift sample from Torrent Pharmaceuticals Pvt. Ltd., Ahmadabad, India. Hesperidin was obtained from ACROS Lab, US. Pioglitazone hydrochloride and Valsartan 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 libitium*.

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 or mice 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 [21]. Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study.

Experimental Protocol

Animals were divided into following groups, each group containing 10 animals and the treatment period for whole study was 4 weeks.

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

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 24 hr (**I/R + PIO**).

Group 4: After right nephrectomy on day 1, Glimepiride (0.5 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 24 hr (**I/R + GLI**).

Group 5: After right nephrectomy on day 1, Nobivolol (2 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 24 hr (**I/R + NOB**).

Group 6: After right nephrectomy on day 1, Valsartan (8 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 24 hr (**I/R + VAL**).

Group 7: After right nephrectomy on day 1, Hesperidine (100 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 24 hr (**I/R + HES**).

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.

BIOCHEMICAL ESTIMATIONS

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 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Measurement of TNF- α by ELISA:

Serum levels of TNF-α 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.

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

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

Effect of drug and herbal on Tumor necrosis factor- α

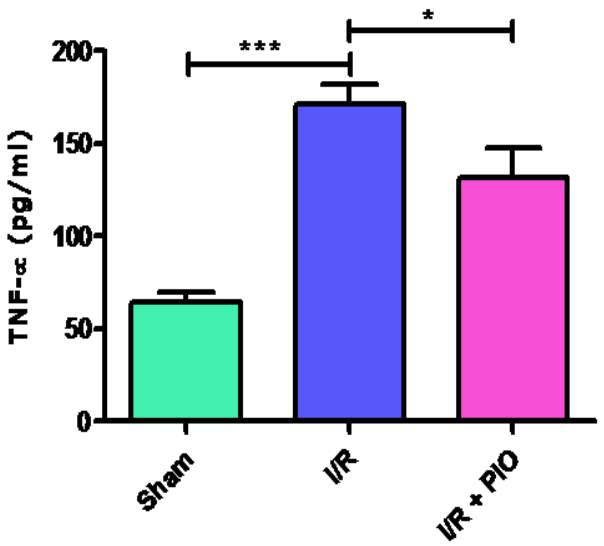
Renal I/R group of diabetic rats showed significantly ($P<0.001$) increased of TNF –α when compared with the sham control rats. Treatment of PIO and HES in STZ-NIC diabetic rats (I/R + PIO, I/R + HES) significant ($p<0.05$) decrease levels of TNF- α as compared to Sham (Fig. 1, 5). Treatment of VAL and NOB in I/R diabetic rats significant ($p<0.01$, $p<0.001$) decreased levels of TNF-α as compared to respective Sham group (Fig. 4, 3) but treatment of GLI in I/R diabetic rats (Fig. 2).

Table 1 Effect of Streptozotocin and Nicotinamide on Glucose in rats.

Groups	Glucose (gm)				
	Before Treatment	**1 weeks**	**2 weeks**	**3 weeks**	**4 weeks**
ND	102.7±5.43	103.3±6.16	103.3±5.34	102.5±6.22	101.8± 6.799
D	371.5±9.54***	375.7±7.25***	370.8±10.33***	340.6±8.41***	332.8± 9.16***

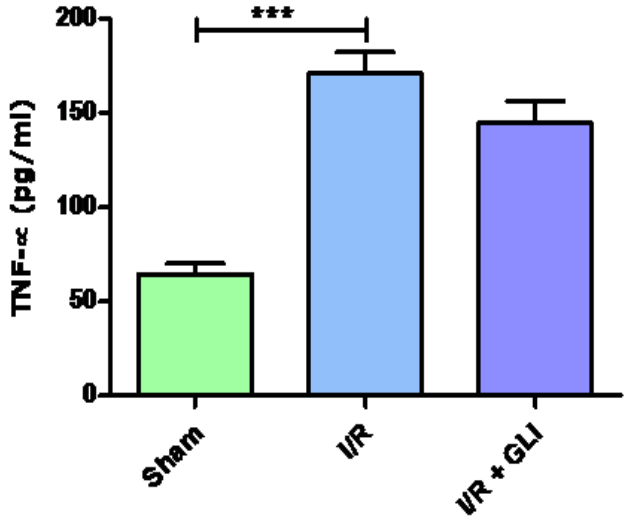
Values are expressed as mean ± SEM (n=10). ***P<0.001 compared to ND group. ND = nondiabetic, D = Diabetic

Figure 1. Effect of Pioglitazone (10 mg/kg/day, p.o) on serum TNF-α in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



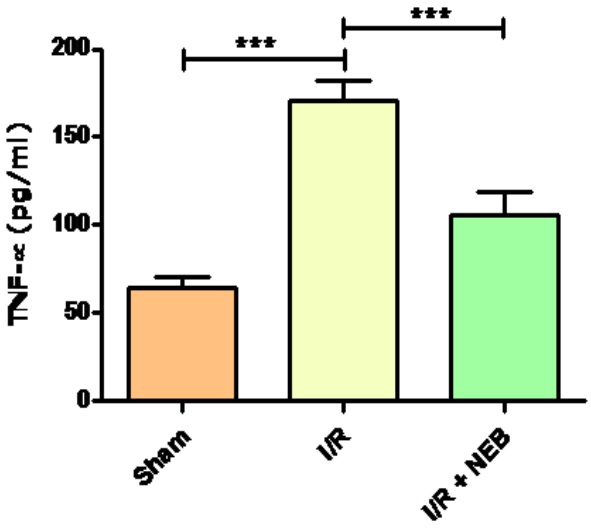
Values are expressed as mean ± SEM for ten animals in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

Figure 2. Effect of Glimepiride (0.5 mg/kg/day, p.o) on serum TNF-α in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



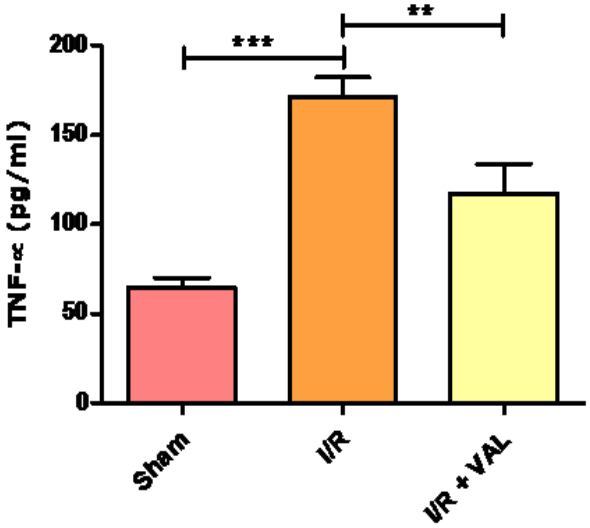
Values are expressed as mean ± SEM for ten animals in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

Figure 3. Effect of Nebivolol (2 mg/kg/day, p.o) on serum TNF-α in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



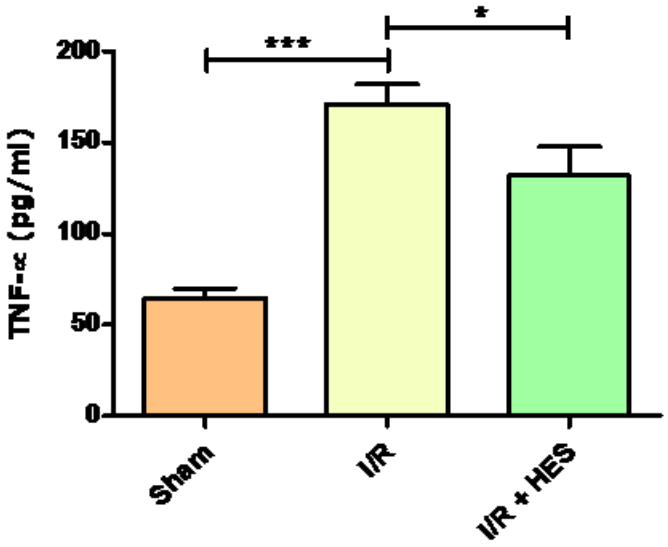
Values are expressed as mean ± SEM for ten animals in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

Figure 4. Effect of Valsartan (8 mg/kg/day, p.o) on serum TNF-α in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



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

Figure 5. Effect of Hesperidine (100 mg/kg/day, p.o) on serum TNF-α in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



Values are expressed as mean ± SEM for ten 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 the Pioglitazone, Glimepiride, Nobivolol, Valsartan and Hesperidin on Tumor necrosis tumor alpha in experimentally induced renal damage in diabetic rats.

Acute Tubular Necrosis (ATN) and the ensuing renal failure induced by ischemia and reperfusion injury or sepsis remains a major cause of morbidity and mortality among patients in the intensive care unit [22]. Ischemia-induced ATN has an attendant 30% mortality rate, and many survivors require dialysis [22]. Indeed, this common clinical entity occurs during cardiopulmonary bypass [23], kidney transplantation [24-26], aortic bypass surgery [27], accidental or iatrogenic trauma [28], sepsis [29], hydronephrosis [30], and elective urological operations [31].

This 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, like tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and arachidonic acid metabolites [32]. Cyclooxygenase (COX)-2 is induced in response to proinflammatory cytokines and it catalyzes the metabolism of arachidonic acid. It reported that from 3 to 5 h after IR injury COX-2 expression was most intense and from 12 to 24 h after IR injury maximal tissue damage was observed. Thus, we decided to analyze tissue injury after 30 min of ischemia and 24 h of reperfusion [32].

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 [33] and intestinal [34] I/R in diabetic rats.

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, hydrogen peroxide and TNF-α that leads to kidney damage [35]. 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 [36, 37].

Significant renal dysfunction and cellular degeneration occurs after short periods of renal ischemia [25, 26, 38-40]. Although the exact mechanisms of ischemia/reperfusion injury remain undefined, accumulating evidence suggests that local, early TNF production may play a role in the pathogenesis of this injury [25, 41-43]. In the present study, an increase in the levels of serum TNF-alpha in I/R induced renal damaged in diabetic rats. Treatment of Pioglitazone and hesperidin were improving TNF-α

in diabetic rats. Nobivolol and Valsartan treatment more improved TNF-α as compared to diabetic control so renoprotective in diabetic rats, but treatment of Glimepiride was no any change on tumor necrosis factor alpha in diabetic rats

Pioglitazone, Hesperidin, Nebivolol and Valsartan treatment reduced Tumor necrosis factor alpha in experimentally induced renal damaged in diabetic rats which suggest renoprotective activity and Sham control diabetes. This study concluded that PIO at 10 mg/kg, HES at 100 mg/kg and VAL at 8 mg/kg may show reduced TNF-α which suggest all drug better effects on renal complication in diabetic rats and NOB at 2 mg/kg may show more reduced TNF-α same as sham control, so Nebivolol may reduced renal complication in Ischemia/Reperfusion induced renal damage in type 2 diabetic rats.

ACKNOWLEDGEMENT

The authors would like to thank the principal of Dharmaj Degree Pharmacy College of Pharmacy for providing facilities for work.

AUTHOR'S STATEMENTS

Competing interests. The authors declare no conflict of interest.

ANIMAL RIGHTS

The institutional and (inter) national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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