ROLE OF OCIMUM CANUM IN PREVENTION OF REPERFUSION-INDUCED RENAL ISCHEMIA IN WISTAR ALBINO RATS

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Abstract
Acute renal failure is defined as rapid loss of renal function and has been associated with a high mortality rate. Ischemia and reperfusion (I/R) injury of the kidney is the most prominent cause of intrinsic acute renal failure. Activation of reactive oxygen species is implicated in renal ischemia/reperfusion (I/R) injury. This study investigated the anti-ischemic effect of hydro-alcoholic leaf extract of Ocimum canum (OC) against renal I/R injury by its effect on reactive oxygen species. Wistar albino rats were administered different doses of hydro-alcoholic leaf extract of Ocimum canum (OC) before renal ischemia, followed by reperfusion for 24 hours. Serum creatinine, Serum cystatin C, serum oxaloacetate transaminase (SGOT), serum pyruvate transaminase (SGPT), blood urea nitrogen (BUN) and lactate dehydrogenase (LDH) were measured for renal dysfunction. Serum and tissue malondialdehyde (MDA), reduced glutathione (GSH) levels, catalase (CAT), and superoxide dismutase (SOD) levels were measured. Renal sections were analyzed for histological grading of renal injury. Hydro-alcoholic leaf extract of Ocimum canum (OC) significantly reduced increased creatinine, cystatin C, serum oxaloacetate transaminase (SGOT), serum pyruvate transaminase (SGPT), blood urea nitrogen (BUN) and Lactate dehydrogenase (LDH) levels. Ocimum canum also increased kidney superoxide dismutase activity, catalase and reduced glutathione levels and reduced the malondialdehyde levels. Hydro-alcoholic leaf extract of Ocimum canum reduced histological renal damage. These results suggest that the hydro-alcoholic leaf extract of Ocimum canum reduces renal dysfunction and injury caused by renal I/R.

Keywords: Ocimum canum (OC) hydro-alcoholic leaf extract; Ischemia/ reperfusion injury; Oxidative stress

1. Introduction
Acute renal failure (ARF) is defined as a rapid loss in renal function. Traditionally, the mortality rate of people with intrinsic acute renal failure is quite high and this report has not improved over the past several decades. Renal I/R injury are common in several clinical situations, including renal transplantation and shock. Renal ischemia/reperfusion (I/R) injury is the most prominent cause of intrinsic acute renal failure, a primary contributor in delayed graft function, allograft nephropathy and post-transplant hypertension in transplant patients. The term “I/R injury” represents the total damage caused by the initial ischemic episode coupled to the subsequent reperfusion period in which blood flow is reinitiated into the tissue. Therefore, the damage induced by I/R cannot be limited to the ischemic stage, since reperfusion plays an essential role in the process. At present, there is a paucity of data regarding the mechanisms involved in I/R injury, but complex interactions of distinct signaling cascades are known to be involved, resulting in cellular, inflammatory and immune responses. It was demonstrated that reactive oxygen species (ROS) and reactive nitrogen species (RNS) increase in the areas of ischemia and reperfusion, which are responsible for renal damage. Inflammation also plays an important role in the pathogenesis of renal I/R injury, through leukocyte activation and expression of adhesion molecules and cytokines1-6. Free radicals and pro-inflammatory cytokines can damage cellular membrane and subcellular structures, which contain large amounts of phospholipids and protein, resulting in lipid peroxidation and sequentially structural and
metabolic alterations, leading to cell apoptosis and necrosis. Free radical ablation for the treatment of reperfusion injury has found its first clinical application in the prevention of post ischemic tissue injury following organ transplantation. Thus, agents proposed to be useful in the clinical settings of I/R damage include free radical scavengers and antioxidants. An extract of the leaves of Ocimum canum Sims (Kala Tulsi), mainly composed of volatile oils, flavonoid glycoside, carbohydrates, phytosterols, tannins and fixed oils has been shown to exhibit a variety of pharmacological actions. Ocimum canum leaf extract has been reported to be a potent free radical scavenger and an antioxidant. Ocimum canum has significant antioxidant properties and can partly prevent the consequences of ethanol-induced toxicity and to some extent reverse the consequences of ethanol toxicity.

Based on these reports, the present study was designed to determine the possible protective effect of Ocimum canum leaves against oxidative stress during I/R injury of the kidney, by determining biochemical parameters and histological examination. To the best of our knowledge, no scientific data regarding the anti-ischemic effect of O. canum leaves are available except in the treatise of Ayurvedic medicine. Therefore, the present study investigated the possible therapeutic effects of Ocimum canum hydro-alcoholic leaf extracts on renal I/R injury in rats.

2. Materials and Methods

2.1 Animals: Adult Wistar albino rats weighing 200–220 g were given free access to normal rat diet and tap water and maintained in a temperature-controlled room with a 12:12-h light/dark cycle (lights on at 06:00 h). All procedures were performed in accordance with the approval of the Indian Animal Ethics Committee of Royal College of Pharmacy and Health Sciences. The experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines.

2.2 Ocimum canum hydro-alcoholic leaf extract: Leaves of Ocimum canum were collected in the month of December 2011 from its natural habitat from nearby Mohuda village, Berhampur, Ganjam district of Odisha. The plant was authenticated by Dr. Prafulla Kumar Pattanyak, Reader in Botany, from Department of Botany, Science College, Hinjlikatu, Berhampur, Odisha (Voucher specimen no: CNH/13/2011/Tech.I/460). The leaves were cleaned and dried under the shade to avoid degradation of volatile oil. The leaves were dried in hot air oven at 55°C for 3 days and at 40°C for the next 4 days. The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation. Further the extracts were subjected for the pharmacological screening.

2.3 Renal Ischemia/Reperfusion: The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) before the surgical procedure and placed in a supine position. The abdominal region of rats were shaved and sterilized with povidone iodine solution. Following surgery preparation, the rats were placed on a heated table to maintain constant temperature between 36° and 37°C. A midline incision was made, and the renal pedicles were isolated. After laparotomy and dissection of both renal pedicles, bilateral ischemia was induced by occluding the renal pedicles withatraumatic micro-vascular clamp for 45 min followed by reflow to the kidneys was reestablished with visual verifications of blood return. After the surgical procedures, the midline incision was sutured followed by the local application of povidone iodine solution. At the end of the reperfusion period, the animals were euthanized by cervical dislocation.

2.4 Experimental Groups: Animals were divided into four groups consisting of six rats each:

- **Group-I**: NAIVE Normal control-rats in this group did not undergo ischemia or reperfusion and served as the control group.
- **Group-II**: SHAM Sham operated (animals subjected to the identical procedure of surgery without ischemia-reperfusion injury) plus physiologic saline treatment.
- **Group-III**: I/R Animals subjected 45 minutes of renal ischemia, followed by reperfusion for 3 hours and served as untreated experimental control.
- **Group-IV**: OC control Sham operated plus Ocimum canum control (400 mg/kg body wt. treatment up to 8 weeks).
2.6 Preparation of Tissue Homogenates: Secunderabad, India) Evolusion, Tulip Diagnostics (p) Ltd, Goa, India) using semiautomatic analyzer (3000 kits (Crest Biosystems, Bambolim Complex. pyruvate transaminases were measured by assay cystatin C, urea, serum oxaloacetate and separate the serum. Serum creatinine, serum centrifuged at 2500 rpm for 10 minutes to clots were collected from the rats by retro-orbital puncture at the time of sacrificing and was allowed to clot immediately before induced death. All of the rats were sacrificed after 24 hours of reperfusion period and both kidneys were harvested for antioxidant and histological analyses. The blood was collected, and was spun at 1000 rpm for 15 min and serum samples were collected. The serum samples were stored at −20°C until serum level determinations were completed for blood serum samples were stored at 20°C until serum urea nitrogen (BUN), serum creatinine, serum cystatin C, lactate dehydrogenase (LDH), serum oxaloacetate and pyruvate transaminases (SGOT & SGPT). The renal tissue samples were cut in two and immediately placed in Bouin’s solution for histological evaluation and for subsequent determination of malondialdehyde (MDA), reduced glutathione (GSH) levels, catalase and super oxide dismutase (SOD).

2.5 Kidney Function Study: Blood was collected from the rats by retro-orbital puncture at the time of sacrificing 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, serum cystatin C, urea, serum oxaloacetate and pyruvate transaminases were measured by assay kits (Crest Biosystems, Bambolim Complex. Goa, India) using semiautomatic analyzer (3000 Evolution, Tulip Diagnostics (p) Ltd, Secunderabad, India)

2.6 Preparation of Tissue Homogenates: After sacrificing the animals, their kidneys were quickly removed, perfused immediately with icecold hypertonic saline solution, and homogenized in chilled potassium chloride (1.17%) using a Potter Elvehjem homogenizer (Remi, Mumbai, India). The homogenate was centrifuged at 10500 g or 10500 rpm for 20 minutes at 4°C to get the post-mitochondrial supernatant, which was used to assay superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation activity.

2.7 Assessment of Renal Function and Serum Lactate Dehydrogenase Levels: Serum samples were assayed for BUN, serum creatinine, serum LDH using standard diagnostic kits (Crest Biosystems, Bambolim Complex. Goa, India). Serum concentration of LDH was used as a marker of necrosis in tissues. Problems with creatinine (varying muscle mass, recent meat ingestion, etc.) have led to evaluation of alternative agents for estimation of renal function, one of these is cystatin C, a ubiquitous protein secreted by most cells in the body (it is an inhibitor of cysteine protease). cystatin C is freely filtered at the glomerulus. After filtration, cystatin C is reabsorbed and catabolized by the tubular epithelial cells, with only small amounts excreted in the urine. cystatin C levels are therefore measured not in the urine, but in the bloodstream. cystatin C concentration was determined with a particle-enhanced nephelometric immunoassay. Renal function was assessed by serum creatinine, BUN concentration, cystatin C and LDH levels

2.8 Determination of serum oxaloacetate and pyruvate transaminases (SGOT & SGPT): Serum GOT and GPT were determined by the method of Reitman and Frankel, 12. Each substrate (0.5 ml) (2mM α-ketoglutarate and either 200 mM α-L-Alanine or L-Aspartate was incubated for 5 min at 37°C in a water bath. Serum (0.1 ml) was then added and the volume was adjusted to 1.0 ml with sodium phosphate buffer. The reaction mixture was incubated for exactly 30 min and 60 min for GPT and GOT, respectively. Then to the reaction mixture, 0.5 ml of DNPH (1mM) was added and left for another 30 min at room temperature. Finally, the colour was developed by addition of 5.0 ml of NaOH (0.4 N) and product read at 505 nm.

2.9 Estimation of Antioxidant Enzymes: The antioxidant enzymes were estimated by well-established procedures. Nonprotein sulphydryl, as a marker for reduced glutathione (GSH), was measured by the method of Jollow and colleagues, 13 and the yellow color developed by the reduction of Ellman’s reagent by -SH groups of nonprotein sulphydryl was read at 412 nm. Catalase activity was assayed by the method of Claiborne, 14 and the rate of decomposition of H2O2 was followed at 240 nm. Superoxide dismutase (SOD) activity was assessed by the method of Kono. 15 Nitro blue tetrazolium reduction by superoxide anion to blue formazan was followed at 560 nm.

2.10 Estimation of Lipid Peroxidation: Malondialdehyde (MDA) content, a measure of
lipid peroxidation, was assayed in the form of thiobarbituric acid-reacting substances. In brief, the reaction mixture consisted of 0.2 mL of 8.1% sodium lauryl sulphate, 1.5 mL of 20% acetic acid solution adjusted to a pH of 3.5 with sodium hydroxide, and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid added to 0.2 mL of 10% (w/v) of postmitochondrial supernatant. The mixture was made up to 4.0 mL with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of n-butanol: pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. The renal MDA content was expressed as nanomoles of MDA per milligram of protein. Tissue protein was estimated using the Biuret method of protein assay.

2.11 Histological Analysis: The kidneys was isolated immediately after sacrificing the rats and washed with ice-cold saline. Thereafter, it was fixed in a Bouin’s solution and embedded in paraffin wax. Five micrometer-thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin-eosin. The renal sections from all treatments were examined in blind fashion for tubular cell swelling, tubular dilatation, interstitial edema, and moderate to severe necrosis. A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes using scores on a scale of mild (+), moderate (++), and severe (+++) damage.

2.12 Statistical Analyses: Results are presented as the mean ± SEM. All statistical analyses were performed using Graph Pad Prism Software program (version 5). Data were analyzed using analysis of variance followed by Bonferroni’s post-test. The Kruskal-Wallis 1-way analysis of variance by ranks was used to simultaneously test the pathologic score for the I/R and I/R ± Ocimum canum groups. A P value of < 0.05 was considered statistically significant.

3. Results
3.1 Kidney Function Study: Animals subjected to renal ischemia exhibited significant increases in serum urea, creatinine cystatin C and lactate dehydrogenase levels compared with the naive, sham and OC treated groups, suggesting a significant decrease in glomerular function due to renal I/R injury (P < 0.01). However, the rats treated with OC before I/R had significantly lower levels of serum urea, creatinine, cystatin C and lactate dehydrogenase compared with the I/R group (P<0.05) (Table 1).

### Table 1: Level of BUN, Creatinine, Cystatin C and LDH in Serum

| GROUP          | TREATMENT          | BUN (mg/dl) (Mean ± SEM) | Creatinine (mg/dl) (Mean ± SEM) | Cystatin C (mg/dL) (Mean ± SEM) | LDH (U/l) (Mean± SEM) |
|----------------|--------------------|--------------------------|-------------------------------|---------------------------------|-----------------------|
| GROUP I        | Naive              | 13.56±3.69               | 0.58±0.09                     | 0.13±0.02                       | 358.05±9.04           |
| GROUP II       | Sham -operated     | 16.12±4.51               | 0.64±0.10                     | 0.15±0.03                       | 356.03±8.56           |
| GROUP III      | Ischemia/reperfusion(I/R) | 73.67±16.4a          | 1.89±0.41a                    | 0.38±0.11a                     | 586.27±9.31a          |
| GROUP IV       | OC control         | 14.05±4.62               | 0.52±0.08                     | 0.14±0.06                       | 347.14±9.68           |
| GROUP V        | OC 100mg/kg + I/R  | 50.67±12.56b             | 1.28±0.29b                    | 0.27±0.11b                     | 428.13±8.64b          |
| GROUP VI       | OC 200mg/kg + I/R  | 40.43±15.68              | 1.01±0.22                     | 0.21±0.12                      | 398.76±9.45           |
| GROUP VII      | OC 400mg/kg + I/R  | 30.27±9.94               | 0.76±0.15                     | 0.17±0.10                      | 357.52±9.85           |

*p < 0.01 vs. naive, sham and OC groups.

*p < 0.05 vs. I/R

I/R, ischemia/reperfusion; OC, Ocimum canum; BUN, Blood Urea Nitrogen; LDH, Lactate dehydrogenase

**Fig 1**: Level of BUN, Creatinine, Cystatin C and LDH in Serum
I/R resulted in a significant (p<0.001) rise in the levels of SGOT, SGPT in serum. Pretreatment with *Ocimum canum* hydro-alcoholic extract showed a marked dose dependent alleviation of SGOT and SGPT. Down regulation of release of GPT and GOT in serum was observed and is shown in Table 2.

**Table 2**: Level of serum transaminases

| GROUP    | TREATMENT                        | SGPT (IU/L) (Mean ± SEM) | SGOT (IU/L) (Mean ± SEM) |
|----------|----------------------------------|--------------------------|--------------------------|
| GROUP I  | Naive                            | 18.66±0.454              | 25.67±0.965              |
| GROUP II | Sham                             | 46.35±0.287              | 63.74±1.255              |
| GROUP III| Ischemia/reperfusion (I/R)       | 70.92±0.410a             | 78.62±2.342a             |
| GROUP IV | OC control                       | 15.56±0.375              | 22.92±0.808              |
| GROUP V  | OC 100mg/kg + I/R                | 39.78±0.448b             | 61.78±1.656b             |
| GROUP VI | OC 200mg/kg + I/R                | 31.75±0.364              | 54.83±1.498              |
| GROUP VII| OC 400mg/kg + I/R                | 24.94±0.429              | 38.97±0.875              |

\(^a_p < 0.01 \text{ vs. naive, sham and OC groups} \)

\(^b_p < 0.05 \text{ vs. I/R + OC} \)

I/R, ischemia/reperfusion; OC, *Ocimum canum*; SGPT, serum pyruvate transaminase; SGOT, serum oxaloacetate transaminase

**Fig 2**: Level of serum pyruvate transaminase and serum oxaloacetate transaminase

*Ocimum canum* (OC)-Induced Changes in the Antioxidant and Oxidant Pool in Rat Kidney Tissue: After renal I/R, the serum enzymatic activities of SOD and GSH significantly decreased in the I/R group. This reduction was significantly improved by treatment with OC in the I/R + OC group (P<0.01 and P< 0.05, respectively) (Table 3). The I/R process also resulted in significant decreases in tissue enzymatic activity of CAT, when compared with rats treated with OC before the process (P<0.01). Additionally, tissue SOD and GSH activities decreased after I/R and GSH activity improved with OC treatment; however, the difference in SOD was not statistically significant between the I/R and I/R+OC groups (Table 4).
Table 3:- Serum SOD and GSH of the groups

| GROUP | TREATMENT                          | GSH (U/g protein) (Mean ± SEM) | SOD (U/mg protein) (Mean ± SEM) |
|-------|------------------------------------|-------------------------------|---------------------------------|
| GROUP I | Naive                              | 2128±147                      | 4.33±0.51                       |
| GROUP II | Sham -operated control             | 1886±319                      | 3.67±0.47                       |
| GROUP III | Ischemia/reperfusion (I/R)      | 1569±206<sup>a,b</sup>       | 2.65±0.45<sup>ab</sup>          |
| GROUP IV | OC control                        | 2114±356                      | 4.19±0.75                       |
| GROUP V | OC 100mg/kg + I/R                 | 1708±345                      | 3.16±0.45                       |
| GROUP VI | OC 200mg/kg + I/R                 | 1907±245                      | 3.85±0.56                       |
| GROUP VII | OC 400mg/kg + I/R               | 2003±247                      | 4.05±0.47                       |

<sup>a</sup>P < 0.01 vs. naive, sham and OC groups.
<sup>b</sup>P < 0.05 vs. I/R + OC

I/R, ischemia/reperfusion; OC, Ocimum canum; SOD, super oxide dismutase; GSH, reduced glutathione

Fig 3:- Serum SOD and GSH

Table 4:- The renal tissue oxidant and antioxidant enzyme levels of the groups

| GROUP | TREATMENT                          | GSH (U/g protein) (Mean ± SEM) | SOD (U/g protein) (Mean ± SEM) | CATALASE (k/ mg protein) (Mean ± SEM) |
|-------|------------------------------------|-------------------------------|---------------------------------|--------------------------------------|
| GROUP I | Naive                              | 0.19±0.05                     | 0.13±0.03                      | 0.65±0.13                            |
| GROUP II | Sham -operated control             | 0.17±0.04                     | 0.09±0.02                      | 0.44±0.09                            |
| GROUP III | Ischemia/reperfusion (I/R)      | 0.12±0.03<sup>a,b</sup>       | 0.04±0.02<sup>ab</sup>         | 0.29 ±0.07<sup>ab</sup>             |
| GROUP IV | OC control                        | 0.17±0.03                     | 0.11±0.03                      | 0.60±0.19                            |
| GROUP V | OC 100mg/kg + I/R                 | 0.14±0.05                     | 0.08±0.02                      | 0.39±0.17                            |
| GROUP VI | OC 200mg/kg + I/R                 | 0.16±0.04                     | 0.10±0.01                      | 0.45±0.11                            |
| GROUP VII | OC 400mg/kg + I/R               | 0.19±0.02                     | 0.12±0.02                      | 0.57±0.19                            |

<sup>a</sup>P < 0.01 vs. naive, sham and OC groups
<sup>b</sup>P < 0.05 vs. I/R + OC

I/R, ischemia/reperfusion; OC, Ocimum canum; SOD, super oxide dismutase; GSH, reduced glutathione

Fig 4:- The renal tissue oxidant and antioxidant enzyme levels of the groups
In addition, renal ischemia and reperfusion produced a significant increase in MDA in serum and tissue compared with the naive, sham-operated and OC groups. Treatment with OC resulted in a significant reduction in MDA (P<0.05 for tissue and serum levels) (Tables 5).

**Table 5:** Lipid Peroxidation Activity

| GROUP       | TREATMENT                          | MDA (In Serum) (Mean± SEM) | MDA (In Renal tissue) (Mean± SEM) |
|-------------|------------------------------------|----------------------------|----------------------------------|
| GROUP I     | Naive                              | 0.21±0.03                  | 2.58±0.47                        |
| GROUP II    | Sham-operated control              | 0.33±0.07                  | 3.46±0.60                        |
| GROUP III   | Ischemia/reperfusion (I/R)         | 0.54±0.11<sup>a,b</sup>    | 4.79±0.72<sup>a,b</sup>         |
| GROUP IV    | OC control                          | 0.24±0.07                  | 3.04±0.57                        |
| GROUP V     | OC 100mg/kg + I/R                  | 0.41±0.08                  | 4.00±0.69                        |
| GROUP VI    | OC 200mg/kg + I/R                  | 0.32±0.09                  | 3.29±0.58                        |
| GROUP VII   | OC 400mg/kg + I/R                  | 0.28±0.07                  | 3.04±0.59                        |

<sup>a</sup>P < 0.01 vs. naive, sham and OC groups  
<sup>b</sup>P < 0.05 vs. I/R + OC

**Fig 5:** Lipid Peroxidation Activity

Although the activity of antioxidant enzymes (CAT, SOD and GSH) decreased in the I/R group compared with the naive, sham and OC groups, pretreatment with OC improved the levels of CAT, SOD and GSH compared with the I/R group. There were statistically significant increases in renal tissue MDA levels in the I/R group compared with the naive, sham and OC groups. Pretreatment with OC significantly improved these levels compared with the I/R group.

### 3.2 Histopathological Analysis:

The histopathological changes were graded and summarized in the Table 6. The sham control group of rats did not show any morphological changes. By contrast, the kidneys of the rats with I/R only showed tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis, whereas, *Ocimum canum* preserved the normal morphology of the kidney (Figure 6).
Table: - 6 Morphological changes assessed by Histopathological examination of kidneys of Rats Exposed to Ischemia/Reperfusion (I/R) Injury With and Without Preceded Treatment with Ocimum canum (OC) and Sham Operation*

| Rat group       | Tubular cell swelling | Interstitial edema | Tubular dilatation | Necrosis of Epithelium |
|-----------------|-----------------------|--------------------|--------------------|------------------------|
| Naive           | -                     | -                  | -                  | -                      |
| Sham            | -                     | -                  | -                  | -                      |
| IR              | +                     | +                  | +                  | +                      |
| IR + OC 100mg/kg| +                     | +                  | -                  | +                      |
| IR + OC 200mg/kg| +                     | +                  | -                  | -                      |
| IR + OC 400mg/kg| -                     | -                  | -                  | -                      |

*The minus sign indicates no morphological change and plus sign indicates some change

Figure 6: - Light photomicrograph of rat’s kidneys (hematoxylin-eosin stained sections × 400)

A - Kidney sections from naive  
B - Sham-operated group showing normal corpuscles and tubules  
C, D - Ischemia-reperfusion group showing marked tubular epithelial degeneration (arrowhead) and intraluminal tubular eosinophilic casts (thin arrows)  
E - Group received Ocimum canum, showing normal renal corpuscles and tubular cells  
F, G & H - Ischemia-reperfusion group treated with Ocimum canum (100, 200 and 400 mg/kg body wt. respectively) showing marked improvement of tubular epithelium (arrowheads) and decline of luminal casts (arrows)

We studied the effects of OC on ischemia-induced tissue damage. Representative examples of these experiments are presented in Figure 6. I/R injury resulted in severe tissue damage in the S3 segment of the proximal tubules, and the outer medullary stripe exhibited loss of the brush border and detachment of epithelial cells from the basement membrane. This effect resulted in naked basement membranes (Figure 6C and Figure 6D) and tubular obstruction. In the naive group, renal tissue sections had a normal morphology (Figure 6A). No significant
morphological damage was observed in the OC-only and sham operated groups; most tubules were intact and demonstrated normal brush borders (Figures 6B and 6E). Renal sections obtained from rats treated with OC before the I/R process demonstrated a marked reduction in the histological features of renal injury, mainly consisting of focal and mild tubular necrosis (Figures 6F, 6G and 6H).

4. Discussion
ARF, which is increasing in prevalence, is associated with high mortality in humans. Ischemic ARF frequently occurs in hospitalized patients. The pathophysiology after renal I/R injury is not well established. The mechanisms are mostly multifactorial and interdependent, involving hypoxia, inflammatory responses and free radical damage. 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. This transient discontinuation causes renal I/R injury which results in decreased glomerular filtration and renal blood flow and increased urine output is characterized by natriuresis and impaired concentrating ability. Acute renal failure produced by ischemia and reflow is histopathologically characterized by extensive tubular damage, tubular cell necrosis, glomerular injury, and signs of tubular obstruction with cell debris. 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. Recently, studies have focused on the role of ROS in I/R injury, and oxidative stress has been implicated in the pathogenesis of ischemic ARF. A number of drugs or chemicals have been used to prevent I/R kidney injury, vitamin E, montelukast, angiotensin-converting enzyme inhibitor, cyclosporine and lefunomide and an endothelin-A receptor antagonist have been found to be effective in the prevention of lipid peroxidation and general damage. Ischemia is also a stimulus for the release of chemotactic factors for neutrophils. During the reperfusion phase, renal tissue is further destroyed by the release of free radicals and toxic enzymes by neutrophils that have adhered to and traversed the endothelium.

Several reports indicate that Ocimum canum may exert antioxidant effects. In our earlier study, we showed that Ocimum canum can provide protection against injury caused by hydrogen peroxide. Ocimum canum reduces ROS activity and protects vascular smooth muscle cells from injury. In agreement with that study, the results of this study confirm that Ocimum canum protects kidneys against I/R injury; however, the novel findings from this study are its effects on oxidative stress, the antioxidant system.

Oxidative stress can result from increased ROS production and/or from decreased ROS scavenging capability. The cells natural protective system against the devastating actions of ROS includes protective enzymes, including SOD, CAT and GSH. SOD, CAT, GSH and other antioxidants are believed to play important roles in reversing the pathological damage caused by I/R injury. Increased serum levels of SGPT and SGOT confirmed renal reperfusion injury (Figure 2). The SGPT and SGOT are not only specific to the liver but also found in other organs such as kidney and smooth muscle. These enzymes are especially elevated after renal tubular injury in rat. Because SGPT and SGOT are present within the proximal tubules and are regarded as a nonspecific marker of extensive cellular damage, we have used serum SGPT and SGOT in this study as markers of reperfusion injury. Furthermore, evidence of tubular injury was supported by the histopathological scoring of renal injury as there was marked tubular injury. More studies have now established the ability of Ocimum canum to inhibit free radical generation and act as free radical scavengers and antioxidants.

In our study, animals subjected to renal I/R demonstrated an increase in the renal MDA and attenuated the antioxidant enzymes pool (Figures 5, 3 and 4). Lipid peroxidation and antioxidant enzymes are important indexes of oxidant injury. This study shows that pretreatment with OC protects rat kidneys against I/R injury, as demonstrated by improved renal function, normalized renal histopathology, improvement in antioxidant enzyme status.
(increased levels of GSH and activity of SOD and CAT), reduced oxidation products (reduced MDA levels). Our study also showed that tissue SOD and GSH activities and serum CAT, SOD and GSH levels were significantly decreased in the I/R group, when compared with the naive group (Figures 4). Furthermore, OC prevented depletion of SOD, GSH and CAT activity after I/R. Demonstrations of lipid peroxidation as index for oxidative damage may help us better understand the effects of ROS on the cellular components. Renal I/R-induced oxidative stress was associated with impaired kidney function, leading to a marked increase in serum creatinine, urea, cystatin C and LDH levels.

Increased serum LDH concentration is usually a good marker of necrosis in tissues and is regarded as an index of generalized tissue damage. In the present study, serum creatinine, Cystatin C, BUN, and serum LDH levels were significantly increased with the application of I/R to the kidney. However, pretreatment with OC resulted in a decrease in serum creatinine, cystatin C, BUN, and LDH levels was reduced by Ocimum canum treatment (p<0.001) most likely as a result of Reno protective effect by inhibition of ROS (Figure 1).

Conclusion

The results of our study allow us to conclude that OC protects kidneys against I/R injury. Administration of Ocimum canum hydro-alcoholic leaf extract attenuates the increase in markers of renal injury and oxidative damage. These findings provide a basis for the development of novel therapeutic strategies. Ocimum canum leaves have antioxidant properties and may be used in human studies in the future.

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