

# Renoprotective Activity of *Benincasa Cerifera* Fruit Extract on Ischemia/Reperfusion-Induced Renal Damage in Rat

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stress

**Introduction.** Evidence suggests that reactive oxygen species play a role in the pathophysiology of renal ischemia/reperfusion (I/R) injury. This study was designed to investigate the renoprotective activity of methanolic fruit extract of *Benincasa cerifera* in I/R-induced kidney failure in rats.

**Materials and Methods.** Renal pedicles of 12 rats were occluded for 60 minutes followed by 24 hours of reperfusion. Six days prior to induction of I/R, 6 of the rats received *Benincasa cerifera*, 500 mg/kg, orally. Serum creatinine, urea, and uric acid levels were measured after the operation. At the end of reperfusion period, the rats were sacrificed. Superoxide dismutase, catalase, reduced glutathione, and renal malondialdehyde content were determined in the renal tissues. Results were compared with a group of rats with sham operation.

**Results.** Renal I/R caused significant impairment of kidney function. Six-day administration of *Benincasa cerifera*, however, minimized this effect. Rats with renal I/R only showed significantly decreased activity of superoxide dismutase, catalase, and reduced glutathione compared with the sham-operated rats. These declining trends were significantly less in the group treated with *Benincasa cerifera* compared with those in the I/R-only group ( $P = .008$ ,  $P = .07$ , and  $P < .001$ , respectively). Renal I/R produced a significant increase in malondialdehyde level, while pretreatment with *Benincasa cerifera* was associated with a significantly lower malondialdehyde level ( $P < .001$ ).

**Conclusions.** These findings imply that reactive oxygen species play a crucial role in I/R-induced kidney injury and *Benincasa cerifera* exerts renoprotective activity probably by the radical scavenging activity.

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## INTRODUCTION

Renal ischemia/reperfusion (I/R) injury is a major cause of acute renal failure (ARF),<sup>1</sup> 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.<sup>2,3</sup> 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.<sup>4,5</sup> 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).<sup>6</sup> 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.<sup>7</sup>

*Benincasa cerifera* (Thunb) Cogn (Syn: *Benincasa hispida* (T) Cogn family: *Cucurbitaceae*) is a widely used vegetable in India and other tropical countries.<sup>8</sup> Plants belonging to the *Benincasa* species have been the subjects of many investigations for their biologically active components. Various in vitro as well as in vivo studies have shown that *Benincasa cerifera* extract has antioxidant activity on tissues like the liver and brain.<sup>9-11</sup> Also, some species of *Benincasa* have been used as medicinal plants for the treatment of diabetes mellitus, urinary infection, epilepsy, peptic ulcer, and hemorrhages from internal organs.<sup>12</sup> However, not a single study has been performed on kidney tissue. In the present study, we investigated the protective effect of methanolic fruit extract of *Benincasa cerifera* on renal I/R injury in rats.

## MATERIALS AND METHODS

### Plant Material

Methanolic fruit extract of *Benincasa cerifera* was procured as a gift sample from Konark herbal and healthcare (Mumbai, India).

### Experimental Procedure

Eighteen female Wistar albino rats were procured from the animal laboratory of the Smt RB Patel Mahila Pharmacy College in Atkot, India. They were housed at a room temperature of  $25 \pm 2^\circ\text{C}$ , relative humidity of  $75 \pm 5\%$ , and 12-hour dark-light cycle. The rats were provided with basal diet in the form of pellets and water ad libitum. Approval of the Indian Animal Ethics Committee was obtained for the study, and the experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines.<sup>13</sup>

Twelve rats were anesthetized by intraperitoneal injection of ketamine, 60 mg/kg, and diazepam, 5 mg/kg. Both renal pedicles were identified through

a midline incision and occluded with a microvascular clamp for 60 minutes. The microvascular clamps were then removed and reperfusion of the kidneys was allowed. Afterwards, the abdomen was closed with continuous sutures in 2 layers. In the treatment group ( $n = 6$ ), methanolic fruit extract of *Benincasa cerifera*, 500 mg/kg, was orally administered 6 days prior to induction of ischemia. Another group of sham-operated rats ( $n = 6$ ) underwent a simple laparotomy under identical conditions and served as the operation controls. All of the rats were sacrificed after 24 hours of reperfusion period and both kidneys were harvested for antioxidant and histological analyses.

### Kidney Function Study

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, urea, and uric acid levels were measured by assay kits (Nicholas Piramal India Pvt Ltd, Mumbai, India) using semi-automatic analyzer (photometer 5010, Nicholas Piramal India Pvt Ltd, Mumbai, India).

### Preparation of Tissue Homogenates

After sacrificing the animals, their kidneys were quickly removed, perfused immediately with ice-cold 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 for 20 minutes at  $4^\circ\text{C}$  to get the postmitochondrial supernatant, which was used to assay superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation activity.

### Estimation of Antioxidant Enzymes

The antioxidant enzymes were estimated by well-established procedures. Nonprotein sulfhydryl, as a marker for reduced glutathione, was measured by the method of Jollow and colleagues,<sup>14</sup> and the yellow color developed by the reduction of Ellman's reagent by -SH groups of nonprotein sulfhydryl was read at 412 nm. Catalase activity was assayed by the method of Claiborne,<sup>15</sup> and the rate of decomposition of  $\text{H}_2\text{O}_2$  was followed at 240 nm. Superoxide dismutase activity was assessed by the method of Kono.<sup>16</sup> Nitro blue tetrazolium

reduction by superoxide anion to blue formazan was followed at 560 nm.

### Estimation of Lipid Peroxidation

Malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reacting substances.<sup>17</sup> 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.<sup>18</sup>

### Histological Analysis

The kidneys fixed in a 10% neutral-buffered formalin solution were embedded in paraffin and were used for histopathological examination. Five-micrometer-thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin-eosin. The renal sections were examined blindly for tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis in all treatments. A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes using scores on a scale of mild (1+), moderate (2+), and severe (3+) damage.

### Statistical Analyses

Values are expressed as mean ± standard deviation. One-way analysis of variance followed by Bonferroni test was applied to calculate the statistical significance between various groups. A value of *P* less than .05 was considered significant.

## RESULTS

### Kidney Function Study

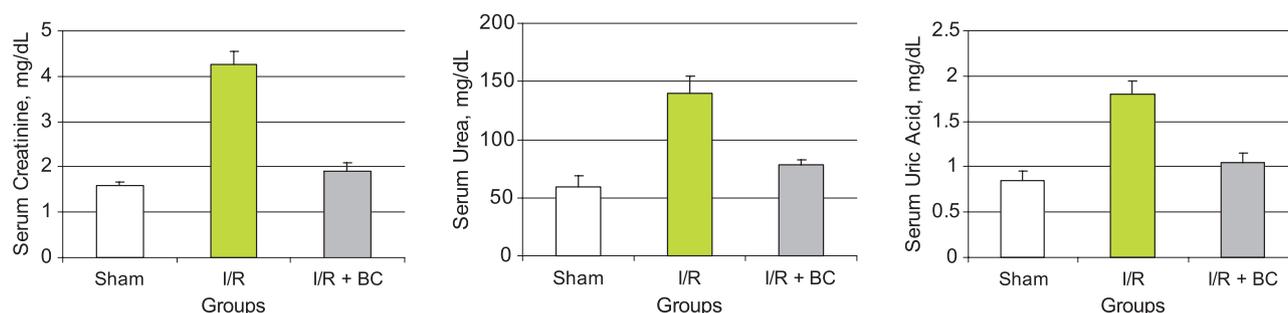
The 6 rats which underwent renal I/R exhibited a significant increase in the serum concentrations of creatinine (*P* < .001), urea (*P* < .001), and uric acid (*P* < .001) compared with the sham control animals, suggesting a significant degree of glomerular dysfunction mediated by renal I/R. Six-day treatment of the other 6 rats with methanolic fruit extract of *Benincasa cerifera* (500 mg/kg, orally) led to a significantly less increase in serum creatinine (*P* < .001), urea (*P* = .009), and uric acid (*P* < .001) levels associated with I/R compared with those in the renal I/R-only animals (Figure 1).

### Antioxidant Activity

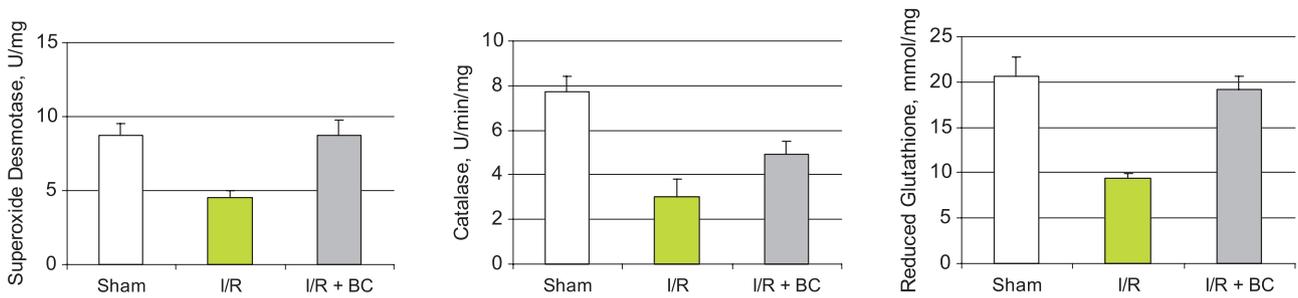
Renal I/R group of rats showed significantly decreased enzymatic activity of superoxide dismutase (*P* = .008), catalase (*P* = .004), and reduced glutathione (*P* < .001) when compared with the sham control rats. These declining trends were significantly less in the group treated with methanolic fruit extract of *Benincasa cerifera* compared with those in the I/R-only group (*P* = .008, *P* = .07, and *P* < .001, respectively; Figure 2).

### Lipid Peroxidation Activity

Renal I/R produced a significant increase in MDA levels in comparison with the sham operation in the rats (*P* < .001). Treatment with



**Figure 1.** Effect of *Benincasa cerifera* (BC) on serum creatinine, urea, and uric acid in the rats exposed to renal ischemia/reperfusion (I/R) injury. Values are expressed as mean ± standard deviation.

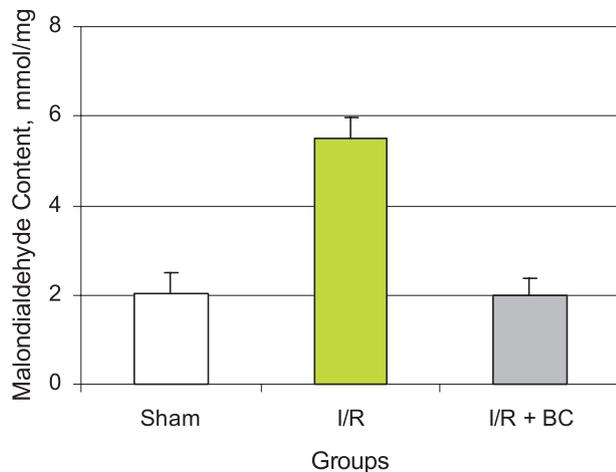


**Figure 2.** Effect of *Benincasa cerifera* (BC) on superoxide dismutase, catalase, and reduced glutathione (per milligram of protein) in the rats exposed to renal ischemia/reperfusion (I/R) injury. Values are expressed as mean ± standard deviation.

*Benincasa cerifera* before renal I/R was associated with a significantly lower MDA level than that in the rats that underwent only renal I/R ( $P < .001$ ; Figure 3).

### Histopathological Analysis

The histopathological changes were graded and summarized in the Table. 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,



**Figure 3.** Effect of *Benincasa cerifera* (BC) on lipid peroxidation measured as renal malondialdehyde content (per milligram of protein) in the rats exposed to renal ischemia/reperfusion (I/R) injury. Values are expressed as mean ± standard deviation.

whereas, *Benincasa cerifera* preserved the normal morphology of the kidney (Figure 4).

### DISCUSSION

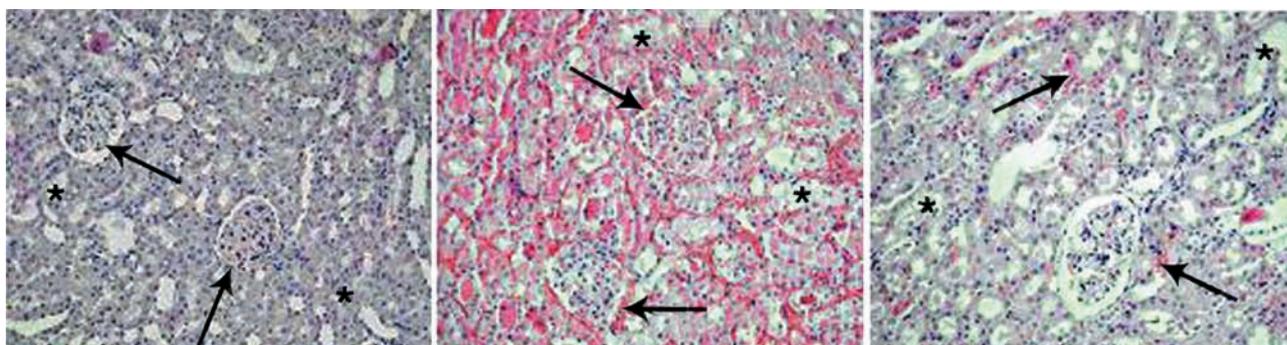
The transient discontinuation of renal blood supply is encountered in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, and elective urological operations.<sup>2,3</sup> This transient discontinuation causes renal I/R injury which results in decreased glomerular filtration and renal blood flow and increased urine output characterized by natriuresis and impaired concentrating ability. 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.<sup>19,20</sup> 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.<sup>21,22</sup>

In our study, animals subjected to renal I/R

Morphological Changes Assessed by Histopathological Examination of Kidneys of Rats Exposed to Ischemia/Reperfusion (I/R) Injury With and Without Preceded Treatment With *Benincasa cerifera* (BC) and Sham Operation\*

Rat Group	Tubular Cell Swelling	Interstitial Edema	Tubular Dilatation	Necrosis of Epithelium
Sham	-	-	-	-
I/R	+++	+++	+++	+++
I/R + BC	-	-	-	-

\*The minus sign indicates no morphological change and plus sign, some changes.



**Figure 4.** Sections of the rats' kidneys (hematoxylin-eosin,  $\times 100$ ). **Left**, kidney section of a rat in the sham operation group shows normal glomeruli (arrows) and tubuli (asterisks). **Middle**, Kidney section of a rat exposed to bilateral renal ischemia/reperfusion shows severe interstitial hemorrhage surrounding the glomeruli (arrows) and tubuli. Tubular epithelial degeneration is apparent (asterisks). **Right**, Kidney section of the rats with ischemia/reperfusion injury treated with *Benincasa cerifera*, in which slight degeneration of tubuli (asterisks) and glomeruli (arrows) are seen.

demonstrated an increase in the renal MDA and attenuated antioxidant enzymes pool. Lipid peroxidation and antioxidant enzymes are important indexes of oxidant injury.<sup>23</sup> Demonstration of lipid peroxidation as indexes for oxidative damage may help us better understand the effects of ROS on the cellular components.<sup>24</sup> 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 *Benincasa cerifera* 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 *Benincasa cerifera*. Renal I/R caused characteristic morphological changes, such as tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis. In contrast, sections of the kidneys of the rats treated with *Benincasa cerifera* showed architectural and cytological preservation of structure.

The mechanism of the protective effect of *Benincasa cerifera* on renal I/R injury can be explained by its antioxidant activity.<sup>9-11</sup> 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.<sup>6</sup> Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage.<sup>7</sup> 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.<sup>25,26</sup> 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.<sup>26</sup> 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 *Benincasa cerifera* 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. Our data support a role for *Benincasa cerifera* 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.

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## CONFLICT OF INTEREST

None declared.

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