ORIGINAL ARTICLE

Lipid peroxidation and renal injury in renal ischemia/reperfusion: Effect of *Benincasa cerifera*

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To investigate the role of the methanolic fruit extract of *Benincasa cerifera* on lipid peroxidation (LPO) and renal pathology in ischemia/reperfusion (I/R).In experimental methodology, both renal pedicles were occluded for 60 min followed by 24 h of reperfusion. *B. cerifera* (500 mg/kg/day) was administered orally for 5 days prior to induction of renal ischemia and was continued for 1 day after ischemia. At the end of the reperfusion period, rats were sacrificed. Sham-operated rats followed same procedure except renal arteries occlusion. LPO and histopathological analysis were done in renal tissue. Serum creatinine and urea levels were measured for the evaluation of renal function. In ischemia/reperfusion (I/R) rats, malondialdehyde (MDA) levels were increased significantly when compared with sham-control rats. Histological changes showed tubular cell swelling, interstitial oedema, tubular dilation and moderate-to-severe necrosis in epithelium of I/R rat as compared to sham control. The methanolic fruit extract of *B. cerifera* could attenuate the heightened MDA levels. I/R-induced renal injury was markedly diminished by administration of *B. cerifera* These results indicate that the methanolic fruit extract of *B. cerifera* attenuate renal damage after I/R injury of the kidney by potent antioxidant or free radical scavenging activity.

Key words: *Benincasa cerifera*, lipid peroxidation, renal ischemia/reperfusion

INTRODUCTION

Renal ischemia/reperfusion (I/R) injury is common in several clinical situations, including renal transplantation. I/R-induced acute renal failure (ARF) is associated with decreased allograft survival in patients with transplanted kidneys and high mortality and morbidity in patients with native kidneys.[1] It was demonstrated that reactive oxygen species (ROS) increase in the areas of ischemia and reperfusion, which is responsible for renal damage.[2-10] ROS formed during oxidative stress can initiate lipid peroxidation (LPO), oxidize proteins to inactive states and cause DNA strand breaks, all potentially damaging to normal cellular function.[11] LPO is an autocatalytic mechanism leading to oxidative destruction of cellular membranes, and their destruction can lead to the production of toxic reactive aldehydic metabolites and cell death.[12,13]

Benincasa cerifera (Thunb.) cogn. (Syn: *B. Hispida* (T) cogn. Family: Cucurbitaceae) is a widely used vegetable in India and other tropical countries.[14] Plants belonging to the Benincasa species have been the subjects of many investigations for their biologically active components. Various *in vivo* as well as *in vitro* studies have been proven that *B. cerifera* having potent antioxidant activity.[15-17] Some species of Benincasa have been used as medicinal plants for the treatment of diabetes, urinary infection, epilepsy, peptic ulcer and hemorrhages from internal organs.[18]

Thus, the present study was conducted to examine the role of the methanolic fruit extract of *B. cerifera* on renal LPO status and renal pathology in an I/R model.

MATERIALS AND METHODS

Animal

Female Wistar rats, aged 4-6 months, were procured from the Laboratory of S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherava. The rats were maintained in the animal colony of the Laboratory Animal Unit under standard conditions with free access to water and chow. All animal protocols were reviewed and approved by the Animal Ethics Committee.

Plant Material

The methanolic fruit extract of *B. cerifera* was obtained as a gift sample from Konark herbal and health care laboratories, Mumbai.

Induction of Renal Ischemia/Reperfusion Injury

The rats were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and diazepam (5 mg/kg). The body

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temperature was maintained throughout experiment at 37°C using heating lamp. Both renal pedicles were identified through a midline incision and occluded with a microvascular clamp for 60 min. The microvascular clamps were then removed and the kidneys were allowed to reperfuse. Afterwards, the abdomen was closed with continuous sutures in two layers. Sham-operated rat underwent a simple laparotomy under identical conditions and served as the operation control. For analgesia, rats received topical lidocaine jelly (2%) to the wound for the first 24 h and one dose of acetaminophen (6.8 mg/kg pr) as deemed necessary by the Animal Care Staff. All rats had free access to water and food. The rats were sacrificed after 24 h of reperfusion period and the kidneys were harvested for LPO and histological analysis. Each experimental group consisted of six rats.[19]

Drug Administration

The methanolic fruit extract of *B. cerifera* (500 mg/kg/day) was administered orally for 5 days prior to induction of renal ischemia and were continued for 1 day after ischemia. The control rats were given vehicle alone on the same schedule.

Renal Function Study

Blood was collected from the rat by retro-orbital puncture at the time of sacrifice and allowed to clot for 10 min at room temperature and centrifuged at 2500 rpm for 10 min to separate the serum. Serum creatinine and urea levels were measured by assay kits purchased from Nicholas Piramal India Pvt. Ltd using semiauto analyzer (photometer 5010) manufactured by Nicholas Piramal India Pvt. Ltd, Mumbai.

Estimation of Lipid Peroxidation

After sacrificing the animals, their kidneys were quickly removed, perfused immediately with ice-cold normal saline and homogenized in chilled potassium chloride (1.17%) using a Potter Elvehjem homogenizer (Remi, Mumbai, India). The homogenate was centrifuged at 800 g for 5 min at 4°C in a refrigerated centrifuge to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 min at 4°C to get the postmitochondrial supernatant (PMS) which was used to assay LPO activity. Levels of MDA in the kidneys were determined as an indicator of LPO.[20] 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 pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% (w/v) of PMS. The mixture was made up to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of *n*-butanol: Pyridine (15: 1 v/v) were added and centrifuged. The organic layer was taken out and its absorbance measured at 532 nm. The content of LPO was reported as nmole MDA per mg protein. Tissue protein was estimated using the Biuret method[21] of the protein assay.

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 and eosin. The renal sections were examined in blindly for tubular cell swelling, interstitial oedema, 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 (+), moderate (+ +) and severe (+ + +) damage.

Statistical Analysis

The results were expressed as mean ± SEM and analysed by unpaired Student's *t*-test using the NCSS statistical computer package. The level of significance was set at $P < 0.05$.[22]

RESULTS

Effect of *B. cerifera* on Renal Function

Animals that underwent renal I/R exhibited a significant increase in the serum concentrations of creatinine and urea level when compared with sham control animals, suggesting a significant degree of glomerular dysfunction mediated by renal I/R. Treatment of rats with the methanolic fruit extract of *B. cerifera* (500 mg/kg, orally) produced a significant reduction in the serum creatinine and urea levels associated with I/R [Table 1].

Effect of *B. cerifera* on Lipid Peroxidation

Renal I/R produced a significant increase in MDA levels when compared with sham-control animals. Treatment with *B. cerifera* (500 mg/kg, orally) produced a significant reduction in MDA in renal I/R-treated animals [Table 1].

Effect of *B. cerifera* on Histopathology

The histopathological changes were graded and summarized in Table 2. The sham-control group did not show any morphological changes. In contrast, the kidneys of untreated ischemic rats showed tubular cell swelling, interstitial oedema, tubular dilatation and moderate-t- severe necrosis. *B. cerifera* (500 mg/kg, orally) preserved the normal morphology of the kidney [Figure 1].

DISCUSSION

The transient discontinuation of renal blood supply is encountered in many clinical situations such as renal transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery and elective urological operations.[23,24] This transient discontinuation causes renal

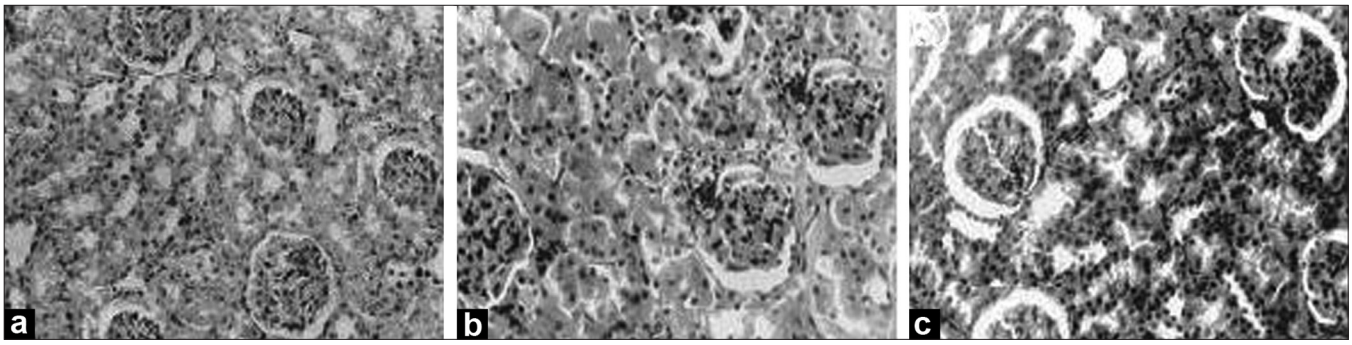

Figure 1: H and E stained section of kidney; (a) sham control rat shows normal histology; (b) I/R control; (c) I/R + *Benincasa cerifera* (500 mg/kg orally) rat

Table 1: Effect of *Benincasa cerifera* on renal function and lipid peroxidation on rats exposed to renal I/R

Group	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Renal MDA level (nmole/mg protein)
Sham control	1.598 ± 0.08212	58.68 ± 9.583	2.1 ± 0.3701
I/R control	4.292 ± 0.3942***	143.7 ± 11.49***	5.4 ± 0.5079***
I/R treated	1.898 ± 0.2041$^{\$\$\$}$	109.3 ± 15.88$^{\$\$}$	2.1 ± 0.2665$^{\$\$\$}$

Values are expressed as mean ± SEM from six animals in each group. Data are analysed by unpaired Student's *t*-test. ***$P < 0.0001$ compared with the sham control group; $^{\$\$}P < 0.0093$, $^{\$\$\$}P < 0.0001$ compared with the I/R control group

Table 2: Effect of *Benincasa cerifera* on morphological changes as assessed by the histopathological examination of kidneys of the rats exposed to renal I/R

Group	Tubular cell swelling	Interstitial oedema	Tubular dilatation	Necrosis of epithelium
Sham control	–	–	–	–
I/R control	+++	+++	+++	+++
I/R treated	–	–	–	–

I/R injury. Renal I/R injury results in decreased glomerular filtration and renal blood flow and increased urine output characterized by natriuresis and impaired concentrating ability. ARF 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.[25,26] Much of this tubular and glomerular dysfunction has been postulated to occur during the reperfusion period following anoxia, and the generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury. In I/R injury, ROS are capable of reacting with lipids leading to LPO of biological membranes, which in turn have impacts upon enzymatic processes such as ion pump activity and damages DNA, thereby inhibiting transcription and repair. If LPO remains unchecked, cell death will ultimately result.[27,28]

In our study, animals subjected to renal I/R demonstrated an increase in the renal MDA. LPO is important indice of oxidant injury.[29] Demonstration of LPO as an index for oxidative damage may help us better understand the effects of ROS on the cellular components.[30] Renal I/R-induced oxidative stress was associated with impaired renal function leading to a marked increase in serum creatinine and urea levels. *B. cerifera* prevented the renal I/R-induced LPO in rats exposed to the renal I/R. Furthermore, the renal functional damage was significantly improved by *B. cerifera*. Renal I/R caused characteristic morphological changes, such as tubular cell swelling, interstitial oedema, tubular dilatation and moderate-to-severe necrosis. In contrast, sections of the *B. cerifera*-pretreated kidneys showed architectural and cytological preservation of the structure.

The mechanism of the protective effect of *B. cerifera* on renal I/R injury can explained by its antioxidant or free radical scavenging activity.[15-17] 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. 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 the massive generation of superoxide anion in mitochondria.[31,32] 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.[32] In this study, renal I/R increased oxidative stress products like tissue MDA. *B. cerifera* prevented the renal I/R-induced LPO and protected the kidneys from severe increasing of ROS products in rats exposed to the renal I/R.

CONCLUSION

It is important to inhibit LPO to prevent renal I/R injury. Our data support a role for *B. cerifera* in attenuation of renal damage after I/R injury of the kidney, in part at least by potent antioxidant or radical scavenging activity.

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