

**ANTIOXIDANT ACTIVITY OF *BENINCASA HISPIDA* ON
RENAL ISCHEMIA/REPERFUSION INJURY**

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Summary

There is increasing evidence to suggest that reactive oxygen species play a pilot role in the pathophysiology of renal ischemia/reperfusion injury. This study was designed to investigate the antioxidant activity of *Benincasa hispida* on renal ischemia/reperfusion injury in rat. In experimental methodology, both the renal pedicles were occluded for 60 min followed by 24 h of reperfusion. *Benincasa hispida* was administered orally for five days prior to induction of renal ischemia and were continuing for one day after ischemia. At the end of the reperfusion period, rats were sacrificed. Sham operated rats followed same procedure except renal arteries occlusion. Lipid peroxidation and antioxidant enzymes were measured in renal tissue. Serum creatinine, urea and uric acid levels were measured for the evaluation of renal function. In ischemia/reperfusion rats, malondehyde content were increased significantly when compared with sham control rats. Antioxidant enzymes like superoxide dismutase, catalase and reduced glutathione level were decrease significantly as compared to sham control rat. *Benincasa hispida* could attenuate the heightened malondehyde levels. Ischemia/reperfusion induced antioxidant enzymes depletion was markedly increase by administration of *Benincasa hispida*. These results indicate that *Benincasa hispida* attenuate renal damage after Ischemia/reperfusion injury of the kidney by potent antioxidant activity.

Keywords: Antioxidant; *Benincasa hispida*; Renal ischemia/reperfusion

Introduction

Acute renal failure (ARF) continues to be associated with significant morbidity and mortality (1), renal ischemia/reperfusion (I/R) injury is a major cause of ARF, which has to be faced in many clinical situations like renal transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery and elective urological operations that initiating a complex and interrelated sequence of events, resulting in injury to, and the eventual death of renal cells (2,3). Several factors have been implicated in the pathophysiological changes of renal I/R injury including vascular/ microvascular injury, endothelial dysfunction, accelerated cell necrosis and granulocyte activation (4). The renin–angiotensin system plays a pilot role in the regulation of blood pressure. Angiotensin-II (Ang-II) is an important mediator in renal injury. Accumulating evidence suggests that Ang-II stimulates intracellular formation of reactive oxygen species (ROS) such as the superoxide anion and hydrogen peroxide leads to renal damage (5).

Benincasa hispida (Thunb.) cogn. (Syn: *Benincasa cerifera* (T) cogn. Family: Cucurbitaceae) is a widely used vegetable in India and other tropical countries (6). Plants belonging to the *Benincasa* species have been the subjects of many investigations for their biologically active components. It has been proven that *Benincasa hispida* has an ACE inhibitor and antioxidant activity (7). *Benincasa hispida* extract shows potent antioxidant activity on various tissues like liver (8) and brain (9). Some species of *Benincasa* have been used as medicinal plants for the treatment of diabetes, urinary infection, epilepsy, peptic ulcer, hemorrhages from internal organs (10). In the present work we studied the antioxidant activity of methanolic fruit extract of *Benincasa hispida* on renal I/R injury in rats.

Methods

Plant material

Methanolic fruit extract of *Benincasa hispida* was obtained as a gift sample from Konark herbal and health care laboratories, Mumbai.

Animal

Female wistar albino rats (150–200 g) maintained in the Experimental Animals Laboratory of the S.K.Patel college of pharmaceutical Education and Research, Ganpat University, Kherva were used for the experiments. They were housed at a room temperature of 25 ± 2 °C, relative humidity of $75 \pm 5\%$ and 12 h dark–light cycle and provided basal diet in the form of pellets and water ad libitum. Necessary permission from the Departmental Ethical Committee was obtained for the study and the experiments were conducted in accordance with the principles prescribed for laboratory animal use.

Induction of renal I/R injury

The rats were anesthetized by intraperitoneal (i.p) injection of Ketamin (60 mg/kg) and diazepam (5 mg/kg). The 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. The rats were sacrifice after 24 hrs of reperfusion period and the kidneys were harvested for antioxidant analysis. Each experimental group consisted of six rats.

Drug administration

Methanolic fruit extract of *Benincasa hispida* (500 mg/kg/day) was administered to the rat by oral gavage for 5 days prior to the induction of I/R and continue for one 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 minutes at room temperature and centrifuged at 2500 rpm for 10 minutes to separate the serum. Serum creatinine, urea and uric acid levels were measured by assay kits purchased from Nicholas Piramal India Pvt. Ltd using semiauto analyzer-photometer 5010 (Nicholas Piramal India Pvt. Ltd, Mumbai).

Preparation of Tissue homogenates

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 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 10500 g for 20 min at 4°C to get the postmitochondrial supernatant (PMS) which was used to assay superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and Lipid peroxidation (MDA) activity.

Antioxidant enzymes assay

The antioxidant enzymes (AOEs) were estimated by the well-established procedures. The nonprotein sulphhydryl (NPSH) as a marker for GSH, was measured by the method of Jollow *et al.* (1974) and the yellow color developed by the reduction of Ellman's reagent by -SH groups of NPSH was read at 412 nm (11). The CAT activity was assayed by the method of Claiborne A. (1985) and the rate of decomposition of H₂O₂ was followed at 240 nm (12). The SOD activity was assessed by the method of Kono Y. (13). The nitro blue tetrazolium (NBT) reduction by superoxide anion to blue formazon was followed at 560 nm.

Lipid peroxidation assay

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reacting substances (TBARS) (Ohkawa H *et al.*, 1979). 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) was added and centrifuged. The organic layer was taken out and its absorbance measured at 532 nm. The renal MDA content expressed as nanomoles of MDA per milligram of protein (14). Tissue protein was estimated using the Biuret method of protein assay (15).

Statistical analysis

Values are expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Bonferroni's test was applied to calculate the statistical significance between various groups. A value of P < 0.05 was considered to be statistically significant.

Results

Renal function study

Animals that underwent renal I/R exhibited significant increase in the serum concentrations of creatinine, urea and uric acid level when compared with sham control animals, suggesting a significant degree of glomerular dysfunction mediated by renal I/R. *Benincasa hispida* (500 mg/kg, orally) treatment produced a significant reduction in the serum creatinine, urea and uric acid levels associated with I/R (Table 1).

Group	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Serum uric acid (mg/dl)
Sham	1.68 ± 0.18	58.72 ± 21.43	0.84 ± 0.18
I/R	4.38 ± 0.88 ***	143.83 ± 25.69 ***	1.82 ± 0.26 ***
BH	1.98 ± 0.45 ###	109.44 ± 10.54 ##	1.04 ± 0.18 ###

Table: 1 Effect of *Benincasa hispida* on renal function [Values expressed as mean ± SD, (n=6)]. *** P < 0.001 compared with sham control group; # # p < 0.01, # # # p < 0.001 compared with I/R group. I/R, ischemia/reperfusion; BH, *Benincasa hispida*

Antioxidant activity

Renal I/R significantly decreased the enzymatic activity of SOD, CAT and GSH. This reduction was significantly improved by 500 mg/kg, orally treatment with methanolic fruit extract of *Benincasa hispida* (Table 2).

Group	SOD (units/mg of protein)	CAT (units/min/mg of protein)	GSH (nmoles/mg of protein)	MDA content (nmole/mg protein)
Sham	8.9 ± 1.38	7.8 ± 1.88	20.8 ± 3.52	2.2 ± 0.82
I/R	4.6 ± 1.43 **	3.1 ± 1.38 **	9.4 ± 0.96 ***	5.5 ± 1.13 ***
BH	8.8 ± 1.81 ##	4.9 ± 1.33 NS	19.2 ± 2.82 ###	2.2 ± 0.59 ###

Table: 2 Effect of *Benincasa hispida* on antioxidant enzymes and lipid peroxidation. [Values expressed as Mean ± SD, (n=6)]. ** p < 0.01, *** P < 0.001 compared with sham control group; # # p < 0.01, # # # p < 0.001 compared with I/R group. I/R, ischemia/reperfusion; BC, *Benincasa hispida*;

Lipid peroxidation activity

Renal I/R produced a significant increase in MDA levels when compared with sham control animals. Treatment with *Benincasa hispida* (500 mg/kg, orally) produced a significant reduction in MDA in renal I/R-treated animals (Table 2).

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 (2,3). This transient discontinuation causes renal 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. In I/R injury, ROS are capable of reacting with lipids leading to lipid peroxidation of biological membranes, which in turn impacts upon enzymatic processes such as ion pump activity and damages DNA, thereby inhibiting transcription and repair. If lipid peroxidation remains unchecked, cell death will ultimately result (16,17).

In our study, animals subjected to renal I/R demonstrated an increase in the renal MDA and attenuated the antioxidant enzymes pool. Lipid peroxidation and antioxidant enzymes are important indices of oxidative injury (18). Demonstration of lipid peroxidation as index for oxidative damage may help us better understand the effects of ROS on the cellular components (19). Renal I/R-induced oxidative stress was associated with impaired renal function leading to a marked increase in serum creatinine, urea and uric acid levels. *Benincasa hispida* treatment prevents the 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 renal functional damage was significantly improved by *Benincasa hispida*.

The mechanism of the protective effect of *Benincasa hispida* on renal I/R injury can be explained by its antioxidant activity (7,8,9). 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. 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 (20,21). Under these conditions, the defensive system, which is known as 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 (21). 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 SOD, CAT and GSH. Pretreatment with *Benincasa hispida* prevented the renal I/R-induced lipid peroxidation and protected the kidneys from severe increasing of ROS products and depletion of SOD, CAT and GSH in rats exposed to the renal I/R.

Conclusion

In conclusion, it is important to inhibit oxidative stress to prevent renal I/R injury. Our data support a role for *Benincasa hispida* in attenuation of renal damage after I/R injury of the kidney, in part at least by antioxidant activity. This could be useful for prevention or early treatment of renal damage. Further studies are in progress to isolate, identify and characterize the active principles.

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