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Benincasa cerifera Ameliorates Renal Ischemia/Reperfusion Injury in Hyperlipidemic Rat

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ABSTRACT

To investigate protective effect of *Benincasa cerifera* (BC) against kidney injury induced by Ischemia/Reperfusion (I/R) in hyperlipidemic (HC) rats. Hyperlipidemia was developed by cholesterol diet (2% cholesterol and 20 % coconut oil) feeding for 3 weeks. At the end of 3rd week, renal ischemia was induced by both renal arteries occlusion for 60 min followed 24 h of reperfusion. Methanolic extract of *Benincasa cerifera* (500 mg/kg/day) was administered orally 7 days before induction of ischemia. *Benincasa cerifera* treatment reversed all antioxidant parameters like superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) contents as well serum creatinine and blood urea nitrogen (BUN) levels. This data conclude the renoprotective activity of *Benincasa cerifera* against renal damage induced by ischemia/reperfusion injury in hyperlipidemic rat.

Keywords: *Benincasa cerifera;* cholesterol; oxidative stress; renal ischemia/reperfusion injury.

INTRODUCTION

In human body kidney is important organ to maintain body homeostasis and involved in excreting the toxic products of metabolism and exogenous drugs (1). Ischemia-reperfusion (I/R) injury is one of the underlying causes of acute renal failure and reactive oxygen species (ROS) play important roles in mediating cell damage during I/R injury (2,3). Imbalance between production of ROS and the antioxidant enzymes capacity leads to renal oxidative stress. Renal I/R cause tissue injury by the way of oxygen radicals and disturb balance between oxidants and antioxidants in tissue (4). Additionally, hyperlipidemic (HC) rats have an increased renal sensitivity to I/R injury (5). In renal transplant patient hyperlipidemic state is a common problem that may leads to further renal damage. I/R injury are one of the dangerous complications of this procedure. It has been demonstrated that in hyperlipidemic rats, a comparatively 30 minutes of ischemia, results in a reversible acute renal failure in rats, causes a progressive injury with end-stage renal failure (5).

Benincasa cerifera (Thunb.) cogn. (Syn: *Benincasa Hispida* (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. Various In vitro as well as In vivo study showed that *Benincasa cerifera* extract has antioxidant activity on tissues like liver and brain (7, 8, 9), but not a single study was performed on kidney tissue. 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 (10).The aim of the present study was to investigate effect of *Benincasa cerifera* (BC) against kidney injury induced by I/R in hyperlipidemic rats.

MATERIALS AND METHODS

Plant material

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

Experimental procedure

Male wistar albino rats were (150–180 g) obtained from the pharmacology laboratory of S.K.Patel College of Pharmaceutical Education and Research, Kherava, Dist Mehsana, Gujarat, India. The animals were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with a 12-h/12-h dark and light cycle. They were allowed free access to a standard pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee (IAEC). The rats were divided into five groups: control (n=6), HC (n=6), I/R (n=6), HC+I/R (n=6), BC+HC+I/R (*Benincasa cerifera*,500 mg/ kg during 07 days) (n=6). Hyperlipidemia was developed by 3 weeks feeding with cholesterol diet (2% cholesterol and 20 % coconut oil). After 07 days, the both renal arteries were occluded for 60 min followed by 24 h of reperfusion in I/R performed groups. Control animals were injected with the vehicle alone. Development of hyperlipidemia was confirmed by measuring cholesterol level in blood every week during fed cholesterol diet. At the end of third week rats were hyperlipidemic. Starting on the same day of the diagnosis of hyperlipidemic, *Benincasa cerifera* was given (500 mg/ kg/day, orally.) for one week. Serum cholesterol and triglycerides levels were measured at end of the study by the semi autoanalyzer-photometer 5010 (Nicholas India Pvt. Ltd. India) using a commercial kit (Nicholas India Pvt. Ltd. India).

Rats were anesthetized with ketamine (60 mg/kg, i.p.) and diazepam (5 mg/ kg, i.p.) before I/R operation. The both renal vascular pedicles were occluded for 60 min, followed by 24 h reperfusion.

Biochemical analysis

At the end of reperfusion period, blood sample was collected by retro orbital method under anesthesia and rats were sacrificed and both kidneys were quickly isolated to perform biochemical analysis.

Blood samples were used to measure serum cholesterol, triglyceride, creatinine and blood urea nitrogen (BUN) levels by semiautoanalyser-photometer-5010 (Nicholas India Pvt. Ltd. India) using standard diagnostic kits (Nicholas India Pvt. Ltd. India).

The renal tissues were then homogenized in cold potassium choloride solution (1.15%) with a glass homogenizer to make up 10% homogenate (w/v). After the homogenates were centrifuged, the clear supernatants were used for lipid peroxidation (LPO), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and protein analyses. LPO levels by the method of Ledwozyw (11), and GSH levels by the method of Beutler using Ellman's reagent were estimated in renal homogenates (12). The CAT activity was assayed by the method of Claiborne and the rate of decomposition of H_2O_2 was followed at 240 nm (13). The SOD activity was assessed by the method of Kono. The nitro blue tetrazolium (NBT) reduction by superoxide anion to blue formazon was followed at 560 nm (14). The protein concentration was determined by the method of Lowry using bovine serum albumin as standard (15).

Statistical analysis

The results were expressed as mean ± standard deviation (SD) and analyzed by an analysis of variance (ANOVA) followed by bonfferoni multiple comparison test using the computer based Graph pad prism5 software.

RESULTS

Serum cholesterol and triglyceride levels of HC groups were significantly higher in comparison with control groups (Table 1).

Serum creatinine and BUN levels of both I/R and HC+I/R groups had statistically higher than the control and HC groups. HC group also shows significant higher creatinine and BUN level as compared to control group. HC + I/R group shows significantly higher creatinine and BUN level as compare to I/R group. *Benincasa cerifera* treated group shows significant reduction in creatinine and BUN level in comparision to HC+I/R (Table 2).

Table 1: Effect of cholesterol diet on serum cholesterol and triglyceride levels

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control	68.8 ± 16.6	57.6 ± 16.92
HC	133.4 ± 22.47***	197.8 ± 20.19***

Data was analyzed by ANOVA followed by Bonferroni multiple comparison test. Values are expressed as mean ± SD.

***p<0.001 Vs controls*

Table 2: Effect of *Benincasa cerifera* fruit extract on levels of serum creatinine and blood urea nitrogen (BUN)

Group	Creatinine (mg/dl)	BUN (mg/dl)
Control	1.59 ± 0.18	27.42 ± 10.01
HC	2.23 ± 0.34	53.01 ± 8.58
I/R	4.29 ± 0.88***	67.13 ± 12.01***
HC + I/R	5.81 ± 0.52††	121.2 ± 18.86††
BC + HC + I/R	3.27 0.403‡‡	65.19 ± 14.83‡‡

Data was analyzed by ANOVA followed by Bonferroni multiple comparison test. Values are expressed as mean ± SD.
****p< 0.001 Vs controls,*
††p< 0.01 Vs I/R,
‡‡p< 0.01 Vs HC + I/R

The MDA levels of I/R and HC+I/R groups had statistically higher than the control and HC groups. HC group also shows significant higher MDA level as compared to control group. HC+I/R group shows significantly higher MDA level as compare to I/R group. *Benincasa cerifera* treated group shows significant reduction in MDA level in comparison to HC+I/R (Table 3).

The GSH, CAT and SOD levels were decrease after I/R in both hyperlipidemic and non-hyperlipidemic groups. HC group also shows significant reduction in GSH, CAT and SOD level in comparison to control group. HC + I/R group shows significantly reduction in GSH, CAT and SOD levels as compare to I/R group. *Benincasa cerifera* treated group shows significant rise in GSH and SOD levels in comparison to HC+I/R (Table 3).

DISCUSSION

We found high MDA, and low GSH, CAT and SOD level in kidney, and increase in serum creatinine and BUN level after renal I/R injury they may show changes take place in the renal tissue. The present results indicated that I/R injury caused renal damage. High MDA level and low GSH, CAT and SOD level are major indexes for oxidative stress. The question is what causes this distant oxidant stress stimulation. There should be sources for ROS production. One of the important sources of ROS production is activation of renin angiotensin system. During the process of I/R injury renin angiotensin system is activated, resulting in the formation of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) (16). Especially, intra renal Angiotensine-II is responsible for pro-inflammatory cytokines and stress. It was indicated that from 1 to 120 h after I/R injury intrarenal angiotensine-II level was most intense and from 12 to 24 h after I/R injury maximal tissue damage was observed (16). Thus, we decided to analyze tissue injury after 60 min ischemia and 24 h reperfusion. The acute inflammatory response is characterized by induction of proinflammatory cytokinesis, expression of different adhesion molecules and neutrophil infiltration as well as the production of cytokinesis such as TNF- α or IL1. Some author demonstrated high lipid peroxidation in renal tissue after renal I/R injury. Oxidant injury affects cellular molecules including DNA, proteins, membrane lipids (17). Demonstration of lipid peroxidation will help to better understand exact mechanism of I/R on renal tissue. Lipid peroxidation and antioxidant enzymes are important indices of oxidant injury. On one hand, the measurement of lipid peroxidation was evaluated by MDA which is brought out during the oxidative injury (18).

On the other hand, HC alter ROS production in renal tissue and these data are in good agreement with the previous works (19, 20). Their results showed that renal from the hyperlipidemic group show evidence of the occurrence of ROS. In our study high cholesterol diet for four week induce lipid peroxidation as well as alteration in antioxidant enzyme activities. Also, HC affect the serum creatinine and BUN in comparison with control group.

This is the first study to show the effect of *Benincasa cerifera* treatment on renal I/R injury in hyperlipidemic rat. The present finding shows rise in MDA level as well as low level of antioxidant enzymes in comparison to control group. Renal I/R injury in hyperlipidemic rat shows significant rise in MDA level as well as significant low level of antioxidant enzymes in comparison to I/R

Table 3: Effect of Benincasa cerifera fruit extract on renal tissue level of antioxidant enzymes and lipid peroxidation

Group	SOD (units/ mg of protein)	CAT (units/mg of protein)	GSH (nmoles/ mg of protein)	MDA (nmoles/ mg of protein)
Control	8.9 ± 1.384	7.8 ± 1.888	20.8 ± 3.52	1.9 ± 0.83
HC	5.7 ± 1.02	5.4 ± 2.74	10.5 ± 3.12	4.0 ± 1.67
I/R	4.6 ± 1.43***	3.0 ± 1.38***	9.4 ± 0.97***	5.4 ± 1.14***
HC + I/R	2.6 ± 0.578††	1.3 ± 0.58††	3.6 ± 0.94††	7.9 ± 1.68††
BC + HC + I/R	4.7 ± 1.495‡‡	1.8 ± 2.87	6.7 ± 1.66‡‡	3.9 ± 0.72‡‡

Data was analyzed by ANOVA followed by Bonferroni multiple comparison test. Values are expressed as mean ± SD.
****p< 0.001 Vs controls,*
††p< 0.01 Vs I/R,
‡‡p< 0.01 Vs HC + I/R

rats. This indicates that hyperlipidemic state enhances oxidative stress induced renal I/R injury. *Benincasa cerifera* treatment prevented renal damage induced by I/R injury in hyperlipidemic rat. The present results showed that *Benincasa cerifera* treatment caused decrease in lipid peroxidation in renal tissue after I/R injury in hyperlipidemic rats. *Benincasa cerifera* serves as ROS scavenger and an antioxidant effective agent. Antioxidant enzymes GSH and SOD activities were increased after *Benincasa cerifera* treatment in renal tissue after I/R injury in hyperlipidemic rats. The observed antioxidant effect of *Benincasa cerifera* in the present study suggests that this may be more related it's direct free radical scavenging activity. It was demonstrated that *Benincasa cerifera* had preventive effects on cochicine induced Alzheimer disease in rat via its direct and indirect antioxidant activity (21). *Benincasa cerifera* has been found to protect tissues against oxidant damage induced by diabetes (8).

CONCLUSION

I/R injury caused renal damage via oxidative stress in high cholesterol diet induced hyperlipidemic and non-hyperlipidemic rats. Also, the results showed that *Benincasa cerifera* treatment prevented renal damage induced by I/R injury in hyperlipidemic rats by the way of decreasing lipid peroxidation as well as increased antioxidant enzyme activities. The present study strengthened the oxidative role of renal damage induced by I/R injury both via high oxidant markers and prevention of this injury with antioxidants such as *Benincasa cerifera*.

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