ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Volume - 01 Issue - 03 MAY-JUN 2013

website: www.ajprd.com
editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

FREE RADICAL SCAVENGING AND NEPHROPROTECTIVE ACTIVITY OF *BENINCASA HISPIDA* (THUNB) LINN. AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN RATS.

Zulfkar Latief Qadrie*[1], R. Anandan[2], Humaira Ashraf[3], Md. Mushtaque[4], Shakil U Rehman[1], Zahoor Wafai[1]

[1] Department of Cl. Pharmacology, SKIMS, Srinagar, J&K, India
[2]Department of Pharmacology, Aditya BIPER, Bangalore, India
[3]Department of Animal Nutrition, SKUAST-K, J&K, India
[4]Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Salem, India

Received: 20 March 2013, ***Revised and Accepted: 24 April 2013***

ABSTRACT

The seeds of Benincasa hispida (Thunb) COGN. (Family: Cucurbitaceae) was extracted with ethanol and was used to study acute toxicity, nephroprotective effects. Gentamycin was used to induce nephrotoxicity in rats. The extract was non lethal to the rats up to the dose of 5000 mg/kg b.w. At doses of 250 and 500 mg/kg b.w, the extract significantly (P<0.05) increased the nephroprotective effect in a dose dependent manner in rats. Furthermore, the extract was studied for free radical scavenging potential to correlate its nephrprotective activity. These results indicate that ethanolic extract of Benincasa hispida possesses potent nephroprotective effect and thus pharmacologically justifying its folkloric and traditional use in the management of nephrotoxicity conditions.

Key words: *Gentamicin, chemically induced toxicity, nephrotoxicity, Benincasa hispida*

INTRODUCTION

The term renal failure primarily denotes failure of the excretory function of kidney, leading to the retention of nitrogenous metabolites in body. In addition, there is failure of regulation of fluid and electrolyte imbalance along with liver dysfunction [1, 2]. Gentamicin is a commonly used amino-glycoside antibiotic that is effective against most of gram negative microorganisms. It has high antibacterial efficacy, rapid onset of action, low rate of true resistance, synergy with β-lactam antibiotics, and low cost; however, therapeutic doses of gentamicin can cause nephrotoxicity, and it is among the most common causes of acute kidney injury [3]. Nephrotoxicity caused by gentamicin is characterized by non-oliguric acute kidney failure. It decreases renal blood flow rate and urinary concentrating ability, and eventually leads to renal insufficiency. After endocytosis, aminoglycosides accumulate in lysosomes. These lysosomes eventually swell up with excessive lipid debris, giving the classic electron-microscopic appearance of myelin figures [4]. Pathologically, gentamicin induced nephrotoxicity is characterized by tubular damage, which is localized predominantly in the proximal tubules [5]. This is due to extensive accumulation of gentamicin in the kidney cortex. Although the polyanionic

For correspondence
Zulfkar Latief Qadrie,
Department of Cl. Pharmacology,
SKIMS, Srinagar, J&K, India
E-mail: zulfkarzulfi@gmail.com

inositol phospholipids are believed to be the major anionic–membrane binding sites for aminoglycosides, *megalin*, a member of the low-density lipoprotein receptor family of glycoproteins, has been shown to mediate, at least in part, the proximal tubular uptake of aminoglycosides [6, 7].

Benincasa hispida (Thunb) COGN. is employed traditionally to treat disorders such as dry-cough, fever, urethral discharges, biliousness, thirst. It acts as brain tonic and also possesses anti-helminthic property. In China, it is used in the treatment of appendicitis. Oil from seeds is soporific, good for the brain and liver and effective in the treatment of syphilis. Seed ash is a prized remedy for gonorrhea; ash is applied to painful wounds and swellings [8, 9]. 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 [10].

The present study was carried out to scientifically prove some of the folkloric use of this plant in conditions of nephrotoxicity by exploring nephroprotective.

MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The seeds of *Benincasa hispida* were collected in the month of December 2006 and the seeds were authenticated by Dr. Marimuthu, Professor, Department of Botany, Government Arts and Science College, Salem and the specimen of *Benincasa hispida* bearing reference number 106/col./219 was kept in the museum of Vinayaka Mission's College of Pharmacy, Salem for future reference [11].

Extraction procedure

The dried powdered seeds of *Benincasa hispida* were defatted with petroleum ether (60-80^{o}C) in Soxhlet apparatus. The defatted powder material thus obtained was further extracted with chloroform, acetone, ethanol and water. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator [11].

Preliminary phytochemical screening

The ethanolic extract of *Benincasa hispida* were tested for the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, gums and mucilages, saponins, proteins and free amino acids, phenolic compounds, tannins and flavonoids [11].

Experimental animals

Studies were carried out using male *Wistar* Albino rats (150-200 g). They were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25±2 ^{o}C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Bangalore, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee. [11]

In vitro ANTIOXIDANT STUDIES

Reduction of 1, 1- Diphenyl- 2- Picryl Hydrazyl (DPPH) Free Radical [12]

To 1ml each of various concentrations of ethanolic and aqueous extract in ethanol, 1ml of solution of DPPH (0.1 mM) was added and incubated at room temperature for 20 min. Absorbance's of the solutions were then measured at 517 nm. Ascorbic acid was used as the standard for comparison. Experiment was performed in triplicate.

Nitric Oxide Scavenging Activity. [13]

Nitric oxide scavenging assay was performed as described by Sreejayan. Briefly, to 1 ml

each of various concentrations of the extract, 0.3 ml of sodium nitroprusside (5 mM) was added. The test tubes were then incubated at 25°C for 5hr. After 5hr, 0.5ml of Griess reagent (Equal volume of 1% Sulphanilamide in 5% ortho Phosphoric acid and 0.01% Napthyl ethylenediamine in distilled water. Used after 12 h of preparation) was added. The absorbance was measured at 546 nm. The experiment was performed in triplicate.

ABTS – scavenging activity [14]

To 0.5 ml of various concentrations of extract, 0.3 ml of ABTS radical solution and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract methanol for alcoholic extract and water for aqueous extract was taken. The absorbance was measured at 734 nm. The experiment was performed in triplicate.

Superoxide dismutase scavenging activity. [15, 16]

To 0.5 ml of different concentrations of extract, 1 ml alkaline DMSO and 0.2 ml NBT (20 mM) was added. The absorbance was measured at 560 nm. The experiment was performed in triplicate.

Lipid peroxidation. [17]

Stock TBA – TCA – HCl reagent:
This solution was mildly heated to assist the dissolution of TBA. 0.5ml of rat brain homogenate was added to the 1 ml of various concentrations of the drug. Then the mixture was incubated for 30 min. The peroxidation was terminated by the addition of 2 ml of TBA-TCA –HCl reagent (15% w/v Trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25N hydrochloric acid.). The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm. The experiment was performed in triplicate.

Reduction of ferric ions by o-Phenanthroline colour method. [12]

The reaction mixture consisting of 1ml ortho-Phenanthroline , 2 ml ferric chloride (200 µM) & 2 ml of various concentrations of the extract was incubated at ambient temperature for 10 min. Then the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate.

Nephroprotective Activity

After acclimatization, the animals were divided randomly into three groups (8 rats / each group), and placed in metabolic cages separately for collecting 24-hour urine samples. After collecting the first urine samples, the rats in group 1 received saline 1 ml/kg for 6 days, those in group 2 received gentamicin 80mg/kg/day for 6 days**,** [18] and the rats in group 3 & 4 received ethanolic *Benincasa hispida* seed extract 250 mg/kg and 500 mg/kg, *orally*, respectively plus gentamicin 80 mg/kg/day after 1 hour for 6 days. Urine samples were collected daily. All gentamicin injections were intraperitoneal. Twenty-four hours after the last injection, urine samples were collected for measuring glucose and protein, using enzymatic method glucose oxidase that catalyses the oxidation of glucose to gluconic acid, and turbidimetry (trichloroacetic acid), [19] respectively. Then the animals were euthanized under ether anesthesia. Blood samples were collected by cardiac puncture for measuring urea and creatinine as an indicator of kidney damage, using urease (a nickel-metallo enzyme that catalyzes the degradation of urea to ammonia and carbon dioxide), [20] and Jaffé (the combined use of creatinine, amidohydrolase, and alkaline sodium picrate) methods, respectively [21].

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). The differences among treated groups were analyzed by one-way

ANOVA followed by Turkey test. P<0.05 was considered statistically significant.

Results and Discussion

The present study was undertaken to establish the free radical scavenging and nephroprotective activity of the *Benincasa hispida*. From our study it was observed that gentamicin induced renal injury was evidenced by decrease in renal function in experimental animals.Concentrations of urea, creatinine, glucose, and protein, as indicators of kidney damage, are shown in table-1. Concentration of urea in gentamicin-treated group was significantly higher than the control and EEBHS-treated groups (P<0.001). Concentration of creatinine, in gentamicin-treated group was also higher (P<0.05) than the control and EEBHS-treated groups. Concentration of urinary glucose in gentamicin- treated group was significantly higher than the control (P<0.001) and EEBHS treated groups (P<0.01). And concentration of urinary protein in gentamicin- treated group was significantly higher than the control (P<0.01) and EEBHS-treated groups (P<0.05). There was no significant difference between the control and EEBHS-treated groups. Our findings indicated that EEBHS, is able to protect kidneys against gentamicin-induced nephrotoxicity in rats. Concentration of blood urea, creatinine, and urinary glucose and protein were used as indicators of damage to kidney.Both the ethanolic extracts at dose levels of 500mg/kg and 250mg/kg showed significant free radical scavenging effect on DPPH, ABTS, Super oxide, Nitric oxide, and TBARS and Ferric ion free radicals shown in Table 2.Histopathological studies shows shown in table 3:Control Group (a): Normal mice kidney tubular epithelial cells and glomeruli by a section through kidney.Gentamicin treated Group (b): Treated with gentamicin (80mg/kg) showed glomerular atrophy, infiltration of cells, tubular congestion. Cystone Group (c): Treated with gentamicin and Cystone (500mg/kg) Showed regenerative changes in glomeruli and tubules.EEBHS Group (d): Treated with gentamicin and EEBHS (250mg/kg) showed regenerative changes in glomeruli and tubules.EEBHS Group (e): Treated with gentamicin and EEBHS (500mg/kg) showing normalcy regaining of tubular epithelial cells and glomeruli.Overall Ethanolic extract of *Benincasa hispida* at the dose level of 500mg/kg were more significant nephroprotective and free radical scavenging as compared to lower dose.In conclusion, findings of the present study show that EEBHS is a protective agent against gentamicin-induced nephrotoxicity in rat. However, the exact protective mechanism(s) of EEBHS is unknown and need to be more investigated.

Table No. 1: Nephroprotective effect of ethanolic extract of *Benincasa hispida* seeds against gentamicin induced Nephrotoxicity in albino rats.

Animal group	Blood urea (mg/dl)	Blood creatinine (mg/dl)	Urinary glucose (mg/dl)	Urinary protein (mg/dl)
Control	33.75 ± 1.6***	0.53 ± 0.02*	7 ± 0.71***	1.68 ± 0.09**
Gentamicin (80mg/kg)	85 ± 7.6	1.95 ± 0.49	15.75 ± 0.85	3.83 ± 0.17
Cystone (500mg/kg) + Gentamicin				
EEBHS (250 mg/kg) + Gentamicin	30.95 ± 3.7***	0.91±0.12*	11.23±1.32**	3.10±0.20**
EEBHS (500 mg/kg) + Gentamicin	27.34±2.8	0.59 ± 0.08*	9.88 ± 0.89**	2.45 ± 0.35*

* P<0.05 **P<0.01 ***P<0.001 compare with gentamicin-treated group

EEBHS- Ethanolic Extract of *Benincasa hispida* seeds

Table No. 2: Effect of free radical scavenging activities of Ethanolic Extract of *Benincasa hispida* seeds

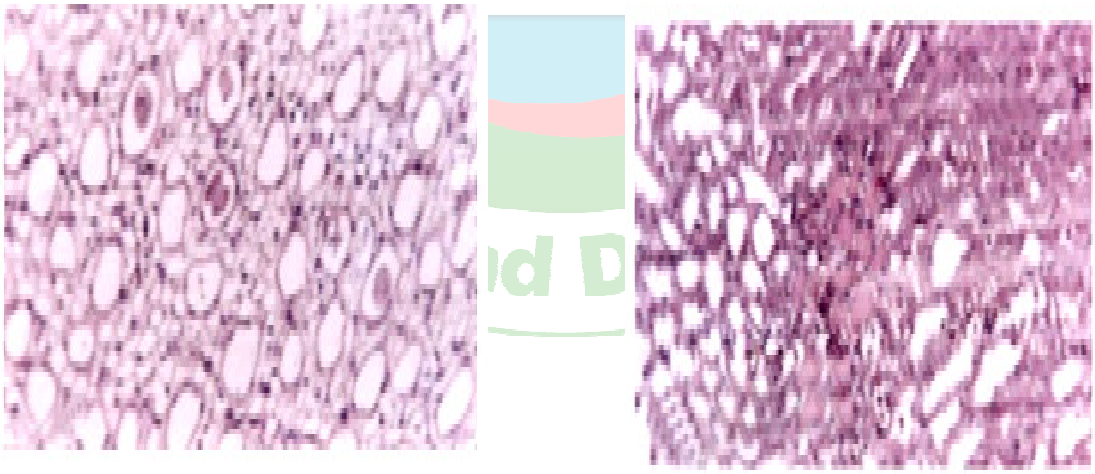
Conc. µg/ml	% inhibition					
	DPPH	Nitric Oxide	ABTS	Super oxide	Lipid Peroxidation	Fe^{++}
5	7.00	9.25	6.21	15.25	24.6	1.02
10	8.22	15.66	8.25	42.33	28.96	5.26
15	8.89	20.82	9.36	52.26	38.32	5.82
25	11.43	22.01	10.24	62.75	42.19	16.33
50	19.25	24.04	12.85	63.30	43.48	19.64
100	22.3	27.28	16.98	68.54	46.28	34.45
250	43.26	33.46	30.74	72.32	55.43	57.78
500	75.32	38.85	56.60	73.48	57.85	68.87
1000	80.57	50.60	88.77	78.99	62.80	85.27

Table No. 3: Histopathological features of the kidney

Groups	Glomerular Congestion	Blood vessel congestion	Intestinal Edema	Inflammatory Cells	Necrosis	Tubular casts
I	-	-	-	-	-	-
II	+++	++++	+++	+++	++	+++
III	++	+	-	++	-	-
IV	+	-	+	-	+	+
V	-	-	+	-	-	+

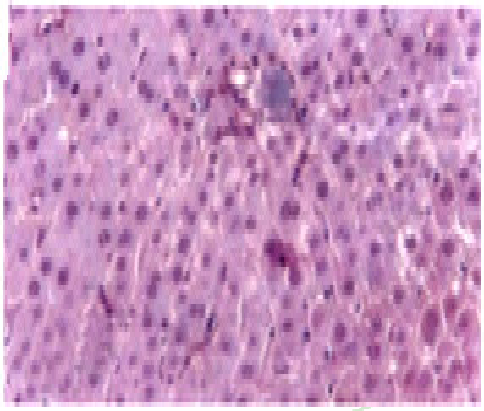
++++ = very high, +++ = High, ++ = medium, + = low, - = negative.

Histopathological Slides:

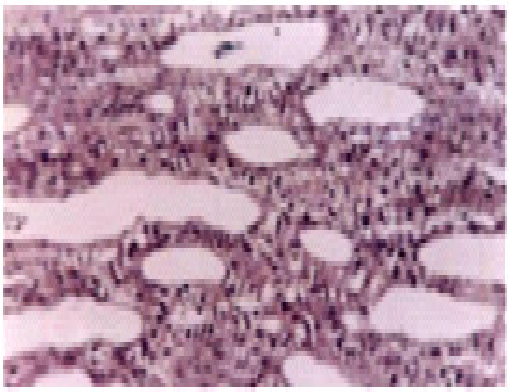


A. Normal TS of Kidney

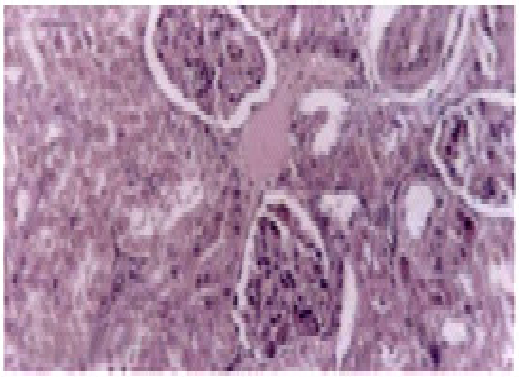
B. Gentamicin Treated



C. Cystone Treated



D. Ethanolic Extract Treated 250mg/kg



E. Ethanol Extract Treated 500mg/kg

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The animal experiment procedures were conducted in accordance with the institutional and (inter)national principles for the care and use of laboratory animals, approved by a local ethics committee in the Vinayaka Mission's University.

REFERENCES

1. *Herfindal, Gourley; Text book of therapeutic drug and disease management. 7th ed. London: Chirchill Livingstone; 2000; 425-36*
2. *Barry M, Brenner, Floyd C. Rector.The Kidney. 6th ed. Volume I, Philadelphia: WB Saunders Company; 2000; 3-67*
3. *Kahlmeter G, Dahlager JI. Aminoglycoside toxicity – a review of clinical studies published between 1975 and 1982. J Antimicrob Chemother. 1984; 13 Suppl A:9-22.*
4. *Kosek JC, Mazze RI, Cousins MJ. Nephrotoxicity of gentamicin. Lab Invest. 1974; 30:48-57.*
5. *Wiland P, Szechcinski J. Proximal tubule damage in patients treated with gentamicin or amikacin. Pol J Pharmacol. 2003; 55: 631-7.*
6. *Sastrasinh M, Knauss TC, Weinberg JM, Humes HD. Identification of the aminoglycoside binding site in rat renal brush border membranes. J Pharmacol Exp Ther.1982; 222:350-8.*
7. *Moestrup SK, Cui S, Vorum H, Bregengård C, Bjørn S E, Norris K, Gliemann J, Christensen E I. Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. J Clin Invest. 1995; 96:1404-13.*
8. *Nadkarni's. Indian Materia Medica. 1995; 1: 185-186.*
9. *Kirtikar and Basu. Indian Medicinal Plants. 1985; 2: 1127-1128.*
10. *Him-Che Y. Handbook of Chinese herbs and formulas. Los Angeles: Institute of Chinese Medicine; 1985; 120-201.*
11. *Zulfkar Latief Qadrie, Najat Tayeb Hawisa, Mohd.Wajid Ali Khan, Moses Samuel and R Anandan. Antinociceptive and Anti-pyretic activity of Benincasa hispida (THUNB.)*

COGN. IN wistar albino rats. Pak. J. Pharm. Sci.; 2009; 22: 3; 287-290.

12. *Sreejayan, N., Rao M. N. A. Free radical scavenging activity of curcuminoids, Drug Research. 1996; 46; 169-171*
13. *Sreejayan, N., Rao, M. N. A. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacology.; 1997; 49; 105.*
14. *Miller, N.J., Rice, C.A. Total antioxidant status in plasma and body fluids. Methods in enzymology. 1994; 234-279.*
15. *Sanchez-Moreno C, Larrauri J and Saura-Calixto F. Free radical scavenging capacity of selected red and white wine. J. Sci.Food. Agri.; 1999; 79: 1301-1304.*
16. *Henry LEA, Halliwell B and Hall DO. The Superoxide dismutase activity of various Photosynthetic organisms measured by a new and a rapid assay technique, FEBS Lett.; 1976; 66: 303-305.*
17. *Rajakumar, D.V., Rao M.N.A. Antioxident properties of Dehydrozingerone and Curcumin in rat brain homogenates. Molecular and Cellular Biochemistry.; 1994; 140: 73-79.*
18. *Ali BH, Al-Qarawi AA, Mousa HM. The effect of calcium load and the calcium channel blocker verapamil on gentamicin nephrotoxicity in rats. Food Chem Toxicol.; 2002; 40: 1843-1847.*
19. *Burtis CA, Ashwood ER, Bruns DE. Textbook of clinical chemistry and molecular diagnostics. 4th ed. Washington, Sanders; 2006; 215.*
20. *Guilbault GG, Montalvo JG Jr. A ureaspecific enzyme electrode. J Am Chem Soc 1969: 91: 2164-2165*
21. *Masson P, Ohlsson P, Björkhem I. Combined enzymic-Jaffé method for determination of creatinine in serum. Clin Chem.; 1981; 27: 18-21.*