



Protective role of *Benincasa hispida* (Thunb.) Cogn. fruit extract against gentamicin induced nephrotoxicity in rats

Hyma Sara Varghese^{1*} & Gunti Gowtham Raj²

¹Department of Pharmacology, The Oxford College of Pharmacy,
Bangalore, Karnataka-560 068, India

²Department of Pharmacology, Gautham College of Pharmacy,
Bangalore, Karnataka-560 068, India

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Drug induced nephrotoxicity is an important cause of renal failure. Gentamicin, an aminoglycoside antibiotic, is a recognized nephrotoxicant in human beings. Use of plant based natural products in the treatment of such renal disorders caused by toxins is not uncommon. In this study, we investigated the nephro-protective potential of hydroalcoholic whole fruit extract of Ash guard, *Benincasa hispida* (Thunb.) Cogn. (HABH) against the toxicity caused by gentamicin in albino Wistar rats. Animals were divided into four groups consisting of six animals in each treatment like normal control (Gr. I), gentamicin 80 mg/kg, *i.p.* (Gr. II), gentamicin 80 mg/kg, *i.p.* + HABH 200 (Gr. III) and 400 mg/kg, *p.o.* (Gr. IV). The degree of protection was assessed by estimating body weight, kidney weight, urine volume of the animals, biochemical parameters in urine and blood and histopathological examination of kidneys. The *in vivo* antioxidant activity was assessed by estimating the levels of GSH and lipid peroxidation in kidney tissues. The treatment with HABH (200 and 400 mg/kg, *p.o.*) markedly reduced gentamicin induced elevation of urinary sodium, potassium electrolytes, urinary glucose, blood urea and creatinine levels. It also increased the relative body weight of animals, urinary creatinine along with preservation of antioxidant glutathione. The comparative histopathological study of kidneys showed almost normal architecture as in normal control group. The results demonstrated beneficial nephroprotective effects of hydro-alcoholic whole fruit extract of *B. hispida* by potentially reversing the conditions of kidney damage induced by gentamicin.

Keywords: Antioxidant, Ash guard, Kidney, Renal failure, Winter melon

Nephrotoxicity can be defined as renal dysfunction that arises as a direct result of exposure to external agents such as drugs and environmental chemicals. Many therapeutic agents have been shown to induce clinically significant nephrotoxicity¹. The kidney is a

central organ which maintains homeostasis, regulating water and electrolyte balance and acid-base maintenance, among other critical functions it also has an endocrine function. Clinical syndromes of nephrotoxicity can be defined according to the predominant regions of the kidney affected by toxin and reversibility of the injury is likely related to the severity and nature of the injury and also to the duration of toxin exposure². Many evidences indicates that free radicals are responsible for the birth of many disorders like inflammation, atherosclerosis, diabetes, ageing and renal toxicities and may induce renal vasoconstriction either by direct effect on vascular smooth muscle cells or via action in the juxtaglomerular apparatus that enhance the vasoconstriction induced by the tubulo-glomerular feedback response³. Modern medicines have certain serious side effects and there is an urgent need to systematically evaluate traditional healers for their activities. Medicinal plants have curative properties due to the presence of various complex chemical substances. Early literatures have prescribed various herbs for the cure of renal disorders⁴⁻⁶. Co-administration of various medicinal plants possessing nephron protective activities along with different nephrotoxic agents may attenuate its toxicity. In response to this, the medicinal potential of a lot of plants have been explored⁷⁻¹².

Benincasa hispida (Thunb.) Cogn. belongs to cucurbitaceae family is commonly known as 'ash gourd' and locally, 'Chalkumra' or 'Kusmanda'. It is a large climbing or trailing herb with stout hispid stems. Fruits are 30 to 45 cm long broadly cylindrical, not ribbed hairy, ultimately covered with a waxy bloom¹³. Most of the peoples usually take its fruits as vegetable. It contains β -sitosterol, asparagines, manitol, proline, arginine, aspartic acid, glucose and vitamin B1. Moreover, the fruit of *B. hispida* has been used in India for centuries in various ailments such as gastrointestinal problems, respiratory diseases, heart diseases, diabetes mellitus and urinary diseases¹⁴. In the present study, we tried to evaluate whether the hydroalcoholic whole fruit extract of *Benincasa hispida* (HABH) could decrease the intensity of toxicity caused by gentamicin in albino Wistar rats.

*Correspondence:

Phone: +91 9945483876 (Mob.)

E-Mail: hymavarghese@gmail.com

Materials and Methods

Chemicals and reagents

Gentamicin (Sigma-Aldrich, Bangalore, India) was used as a nephrotoxicant, biochemical diagnostic kits (Transasia diagnostics, Bangalore, India) were used to study biochemical parameters while other chemicals, reagents and solvents (Reachem laboratory, Chennai, India) (Sd fine chemicals, Navi Mumbai, India) used in the study were of analytical grade with highest purity.

Collection, authentication and extract preparation of *B. hispida*

The fruits of *Benincasa hispida* were collected from vegetable market near yelahanka, Bangalore, Karnataka during mid-winter season at 2017. The fruits were identified, confirmed and authenticated by Prof. MD Rajanna, Head, Department of Botany (No.3/proj/B-Garden), University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India. A voucher specimen was deposited for future reference.

The whole fruit was cut into small pieces and shade dried at room temperature $25\pm 2^\circ\text{C}$. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then extracted directly with 70% v/v ethanol, using soxhlet extraction apparatus. The solvents were evaporated using rotary vacuum evaporator (Yamato RE 300, Japan) at 50°C and dried in dessicator. The yield of the hydroalcoholic extract of *B. hispida* (HABH) was found to be 7.7 % w/w.

Experimental animals

Albino Wistar rats weighing 180-250 g were procured from biogen, Bangalore, Karnataka. They were maintained in the animal house of Gautham College of Pharmacy for experimental purpose. The animals were maintained under controlled conditions of temperature $23\pm 2^\circ\text{C}$, relative humidity 30-70% and 12 h light-dark cycle. They had free access to standard pellets and water was allowed *ad libitum*. The study conducted was approved by the Institutional Animal Ethics Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF-IAEC/02/05/2011) according to prescribed guidelines of CPCSEA (Reg No: 491/01/c/CPCSEA), Govt. of India.

Determination of acute toxicity (LD_{50})

Acute oral toxicity study was performed according to OECD 423 (Organization for Economic Co-operation and Development - Acute toxic class method)¹⁵. Female albino rats (n=6/each dose) were used for this study, doses of 300, 2000 and 5000 mg/kg of HABH were selected through random sampling technique, to

evaluate any toxic effects. The general behaviour such as motor activity, tremors, convulsions, straub reaction, aggressiveness, pilo erection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhoea and skin colour were observed for 30 min post dose of HABH and periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter for a total of 14 days.

Experimental design

The albino wistar rats weighing 200-240 g were divided in to four groups and each group contains six animals and treatment would be as follows. Gr. I, normal saline at 10 mL/kg, *i.p.*; Gr. II, gentamicin @80 mg/kg, *i.p.* for 8 days (Proved to be nephrotoxic¹⁶); Gr. III & IV, gentamicin @80 mg/kg, *i.p.* for 8 days + hydroalcoholic whole fruit extract of *Benincasa hispida* (HABH) @200 and 400 mg/kg, *p.o.* three days prior to gentamycin till post 8 days, respectively. After the last treatment, the animals were kept individual in metabolic cages for 24 h urine collection. On the 12th day, the animals were sacrificed under mild isoflurane anaesthesia and the kidney tissues, urine and blood samples were collected and assessed.

Physical parameters

Body weight, kidney weight and urine volume

The weight of the animals before starting and at the end of the treatment was measured and percentage change in body weight was calculated. Body weight, weight of kidneys and urine volume of the animals at the end of the treatment were measured as physical parameters.

Estimation of biochemical parameters¹⁷

The urinary parameters estimated were sodium, potassium, creatinine and glucose, blood parameters such as urea, creatinine and total protein.

Estimation of antioxidant activity

Glutathione estimation

Tissue samples were homogenized in ice cold trichloroacetic acid (1.0 g tissue plus 10 mL 10% TCA) using tissue homogenizer. Briefly, after centrifugation at 3000 rpm for 10 min, 0.5 mL supernatant was added to 2.0 mL of 0.3 M disodium hydrogen phosphate solution and 0.2 mL solution of dithio-bisnitrobenzoate (0.4 mg/mL in 1% sodium citrate), the absorbance at 412 nm was measured immediately after mixing. Percentage increase in OD is directly proportional to the increase in the levels of

glutathione. Hence, percentage increase in OD is calculated^{18,19}.

Lipid peroxidation

Stock solution of TCA-TBA-HCl reagent containing 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 N hydrochloric acid was prepared. This solution was mildly heated to assist dissolution of thiobarbituric acid. Combine 1.0 mL of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 μ M of lipid phosphate) with 2.0 mL of TCA-TBA-HCl and mix thoroughly. 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 sample was determined at 535 nm against a blank that contains all the reagents minus the lipid. Percentage decrease in OD is directly proportional to the decrease in the levels of lipid peroxidation, and hence, percentage decrease in OD was calculated¹⁸.

Histopathological evaluation

Representative samples of kidney were obtained from dissected animals and were fixed in 10% formalin. They were then processed and paraffin embedded. The sections were stained with haematoxylin and eosin²⁰. The sections were examined in detail under light microscope.

Statistical analysis

The values are expressed as Mean \pm SEM. The data was analysed by using one-way ANOVA followed by Dunnett's test using Graph pad prism 7 software. P value <0.05 was considered statistically significant.

Results and Discussion

Acute toxicity study

In acute toxicity studies, the high dose of the extract (5 g/kg) did not produce any signs of toxicity and mortality. At the doses tested, the plant extract did not significantly affect the body weights of the treated rats or the weights of the kidneys relative to the body weights. This suggests that the extract, at the doses used, caused no adverse effects on feed intake or metabolism. Therefore, the approximate LD50 should be above 5 g/kg.

Physical parameters

Effect of HABH on change in body weights, urine volume and kidney weights

There was found to be decrease in percent body weight and urine volume in gentamicin treated group. However, there was dose dependent increase of

body weights significantly in animals treated with HABH 200 mg/kg ($P <0.001$) and 400 mg/kg ($P <0.0001$) (Fig. 1) and there was significant dose dependent increase in urine volume in animals treated with HABH 200 & 400 mg/kg ($P <0.001$). There was increase in kidney weights in gentamicin treated group. However, there was significant dose dependent decrease of kidney weights in animals treated with HABH 200 and 400 mg/kg ($P <0.01$) when compared with toxicant control as indicated in Table 1.

Biochemical parameters

Effect on urinary sodium, potassium, glucose and creatinine

Levels of urinary sodium were found increased in gentamicin treated group when compared with normal control group. However HABH 200 mg/kg showed slight non-significant increase in the levels of sodium and in the case of HABH 400 mg/kg the levels of sodium decreased significantly ($P <0.01$) in urine samples when compared with toxicant control. Potassium and glucose levels in gentamicin treated group were increased when compared with normal control group. However HABH 200 and 400 mg/kg decreased the levels of potassium and glucose significantly ($P <0.001$) in urine samples when compared with toxicant group. Urinary creatinine levels in gentamicin treated group were decreased

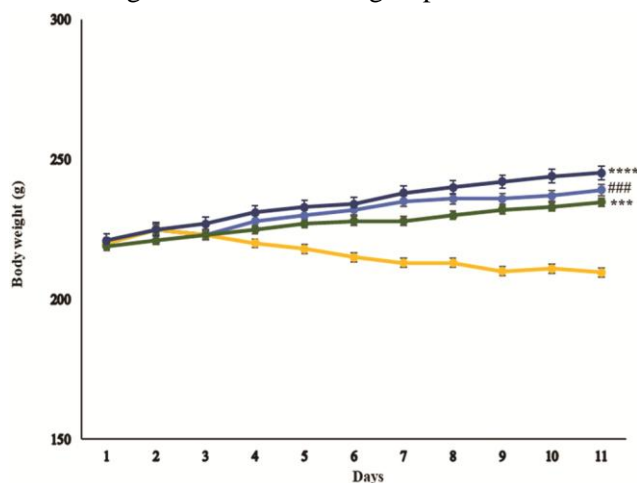


Fig. 1 — Effect of HABH on body weight in gentamicin induced nephrotoxic rats. [Values are expressed as Mean \pm SEM (n=6) and analyzed by one way ANOVA followed by Dunnett's test, **** $P <0.0001$, ***/#### $P <0.001$. All values are compared with toxicant control. HABH: Hydro alcoholic extract of *Benincasa hispida* (Thunb.) Cogn. — G1: Vehicle — G2: Gentamicin, 80 mg/kg, i.p. — G3: Gentamicin, 80 mg/kg, i.p. + HABH, 200 mg/kg, p.o. — G4: Gentamicin, 80 mg/kg, i.p. + HABH, 400 mg/kg, p.o. There was found to be decrease in body weight in gentamicin treated group. However, there was significant dose dependent increase in body weights in animals treated with HABH 200 mg/kg ($P <0.001$) and 400 mg/kg ($P <0.0001$)]

Table 1 — Effect of HABH on physical, biochemical and free radical scavenging capacity in gentamicin induced nephrotoxic rats

| Parameters | Units | Groups | | | |
|-------------------------------------------|-------|-----------------------------|--------------------------------------|-------------------------------------------------------------------|-------------------------------------------------------------------|
| | | G1:Vehicle | G2: Gentamicin 80 mg/kg, <i>i.p.</i> | G3: Gentamicin 80 mg/kg, <i>i.p.</i> +HABH 200 mg/kg, <i>p.o.</i> | G4: Gentamicin 80 mg/kg, <i>i.p.</i> +HABH 400 mg/kg, <i>p.o.</i> |
| Physical parameters | | | | | |
| Change in body weight | % | 8.85±0.37 ^{####} | -2.47±0.81 | 4.33±0.54 ^{***} | 7.99±0.18 ^{****} |
| Urine volume | mL | 6.33±0.32 ^{###} | 4.16±0.45 | 5.36±0.20 ^{***} | 6.41±0.35 ^{***} |
| Kidney weight | gm | 0.64±0.83 ^{##} | 0.98±0.35 | 0.74±0.27 ^{**} | 0.69±0.09 ^{**} |
| Biochemical parameters | | | | | |
| Urinary sodium levels | mM/L | 144.50±5.16 ^{####} | 174.70±3.65 | 182.05±1.74 ^{ns} | 154.20±1.62 ^{**} |
| Urinary potassium levels | mM/L | 5.21±0.40 ^{##} | 7.23±0.25 | 4.51±0.37 ^{***} | 3.97±0.37 ^{***} |
| Urinary glucose levels | mg/dL | 15.31±0.49 ^{***} | 86.22±2.10 | 26.46±1.25 ^{***} | 15.14±1.13 ^{***} |
| Urinary creatinine levels | g/dL | 2.64±0.26 ^{####} | 1.02±0.16 | 2.95±0.45 ^{****} | 3.56±0.33 ^{****} |
| Blood urea | mg/dL | 31.70±1.08 ^{###} | 77.92±2.34 | 49.03±1.18 ^{**} | 21.49±2.30 ^{***} |
| Blood creatinine | mg% | 1.94±0.27 ^{####} | 6.89±0.72 | 4.50±0.38 ^{***} | 1.70±0.26 ^{****} |
| Blood total protein | mg/dL | 6.40±0.52 ^{##} | 3.20±0.38 | 5.60±0.44 ^{**} | 6.74±0.35 ^{***} |
| <i>In vivo</i> antioxidant studies | | | | | |
| Lipid peroxidation | Au | 0.39±0.01 ^{###} | 0.64±0.01 | 0.42±0.02 ^{***} | 0.33±0.02 ^{***} |
| Inhibition (LP) | % | - | - | 56.41 | 68.54 |
| Glutathione | Au | 1.34±0.04 ^{###} | 0.88±0.05 | 1.16±0.05 ^{**} | 1.31±0.06 ^{***} |
| Increase (GSH) | % | - | - | 20.89 | 32.08 |

[Values are expressed as Mean ± SEM (n=6) and analyzed by one way ANOVA followed by Dunnett's test, ****/####P <0.0001, ***/###P <0.001, **/##P <0.01, */#P <0.05 and ns represents not significant. All values are compared with toxicant control. HABH: Hydro alcoholic extract of *Benincasa hispida* (Thunb.) Cogn.]

when compared with normal control group. However, HABH 200 and 400 mg/kg reverted the levels of creatinine significantly ($P < 0.0001$) when compared with toxicant group (Table 1).

Effect on blood urea, creatinine and total protein

Blood urea levels increased in gentamicin treated group when compared with normal control group. However, HABH 200 ($P < 0.01$) and 400 mg/kg ($P < 0.001$) decreased urea levels significantly when compared with toxicant group. Blood creatinine levels increased in gentamicin treated group when compared with normal control group. However HABH 200 and 400 mg/kg decreased creatinine levels significantly ($P < 0.001$ and $P < 0.0001$) when compared with toxicant group. Blood total protein levels decreased in gentamicin treated group when compared with normal control group. However HABH 200 and 400 mg/kg increased total protein levels significantly ($P < 0.01$ and $P < 0.001$) when compared with toxicant group (Table 1).

In vivo antioxidant studies

Effect of HABH on tissue lipid peroxidation (LP) and glutathione (GSH)

The free radicals, such as superoxide anions, hydroxyl radical, lipid peroxy, and lipid peroxide, are unstable and highly reactive molecules, attacking molecules such as proteins, lipids and DNA²¹. Lipid peroxidation produces oxidative degradation of lipids. The products of lipid peroxidation damage the membranes, cells and even tissues²². This results in an increase in membrane permeability, destruction of cell

surface receptors and ligands for vital messengers causing toxic effects and decreased functions of the renal and hepatic cells²³. GSH protects the biomolecules from oxidative tissue damage by scavenging ROS. Decreased GSH levels in the renal tissues further perpetuates cisplatin-induced renal damage. The depletion of GSH seems to be a prime factor that permits lipid peroxidation. Glutathione is one of the essential compounds for regulation of variety of cell functions. It has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione (GS-SG) and other disulfides. Glutathione S-transferase (GST) and Glutathione peroxidase (GSH-Px) are GSH-dependent antioxidant enzymes²⁴.

There was dose dependent inhibition of *in vivo* LP by both the doses of HABH 200 and 400 mg/kg of 56.41% and 68.54% inhibition, respectively. There was a marked depletion of GSH levels in tissues of gentamicin treated animals (Table 1). HABH showed remarkable increase in the levels of GSH. However HABH 200 mg/kg showed 20.89% increase in GSH levels, while HABH 400 mg/kg showed 32.08% increase in GSH levels.

Histopathological study of kidneys in gentamicin induced nephrotoxicity

Figure 2 depicts the alterations caused by the gentamicin induced toxicity in the various groups of experimental albino Wistar rats and the ameliorative

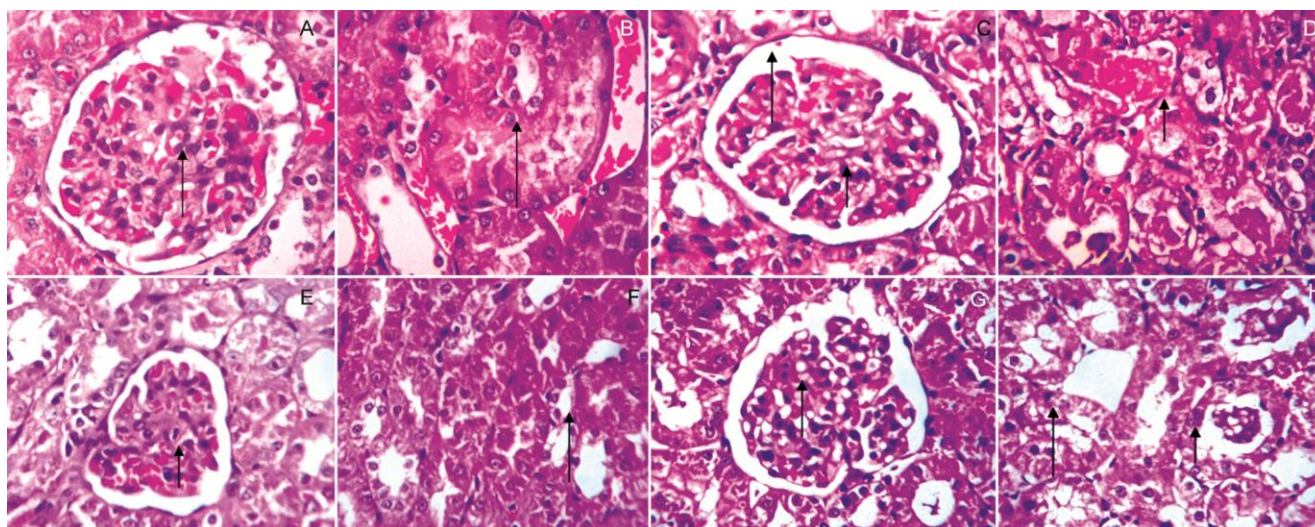


Fig. 2 — Effect of HABH on histopathological study of kidneys in gentamicin induced nephrotoxic rats. (A & B) Normal control; (C & D) Gentamicin 80 mg/kg, *i.p.*; (E & F) Gentamicin 80 mg/kg, *i.p.* + HABH 200 mg/kg, *p.o.*; and (G & H) Gentamicin 80 mg/kg, *i.p.* + HABH 400 mg/kg, *p.o.* [Toxicant control showed many degenerative changes in the architecture of renal tubules which was reverted back to intact architecture with minimal alterations up on administration of HABH 200 and 400 mg/kg, *p.o.*]

changes influenced by the hydroalcoholic fruit extract of Ash guard *B. hispida* (HABH). Animals in Gr. I (normal control) showed Renal parenchyma: Intact architecture; Glomerulus (Fig. 2A): Intact bowman's space and mesangial cells; Renal tubules: Intact tubules (Fig. 2B); and Blood vessels and interstitium: Unremarkable. Gr. II animals treated with gentamicin @80 mg/kg, *i.p.* exhibited the following changes: Renal parenchyma: Intact architecture; Glomerulus (Fig. 2C, Short arrow): Intact bowman's space and mesangial cells; Renal tubules: Most of the tubules show necrosis and degenerative changes (Fig. 2D); and Blood vessels and interstitium: Unremarkable. Gr. III rats treated with gentamicin @80 mg/kg, *i.p.* + HABH 200 mg/kg, *p.o.* showed Renal parenchyma: Intact architecture; Glomerulus (Fig. 2E, Short arrow): Intact bowman's space and mesangial cells, extravasation of erythrocytes; Renal tubules: some of the tubules show degenerative changes (Fig. 2F); and Blood vessels and interstitium: Unremarkable. In Gr. IV treated with gentamicin @80 mg/kg, *i.p.* + HABH 400 mg/kg, *p.o.* also showed intact architecture of renal parenchyma as observed in other groups however, glomerulus (Fig. 2G, and blood vessels and interstitium; did not show any remarkable change. In renal tubules, while few showed degenerative changes (Fig. 2H, Long arrow) few others showed hyaline casts (Thyroidization, Fig. 2H, Short arrow).

Kidneys help in maintaining the homeostatic balance of body fluids by filtering and secreting toxic metabolites from the blood. They have vital role in

controlling blood pressure, erythropoiesis and glucose metabolism. Therefore, to conserve the kidney functions from various toxic agents is foremost²⁵. Aminoglycoside-induced nephrotoxicity is typically characterized by tubular necrosis with marked decrease in glomerular filtration rate and alters intraglomerular dynamics^{26,27}. Previous *in vivo* and *in vitro* studies strongly suggested the mediation of reactive oxygen species (ROS) in the tubular and glomerular effects of gentamycin. In *In vivo*, ROS have been identified as mediators of proximal tubular necrosis and acute renal failure caused by gentamycin²⁸⁻³⁰. Reported reasons for stimulation of contractile and proliferative effects on mesangial cells by gentamycin to induce apoptosis in renal glomeruli and mesangial cells were by the increase of cytosolic free calcium concentration in mesangial cells, by mediation of platelet-activating factor, by activation of phospholipase A₂ for the release of eicosanoids in mesangial cells³¹.

The present study has demonstrated that gentamicin administered rats suffered acute kidney dysfunction as evidenced by elevation in kidney weight, urinary sodium, urinary potassium, urinary glucose, blood urea, blood creatinine and decrease in body weight, urine volume, urinary creatinine and blood total protein levels with multiple histological damages. It also depleted the levels of GSH and increased the levels of lipid peroxidation.

Treatment with HABH at the dose levels of 200 and 400 mg/kg significantly lowered the blood urea, blood

creatinine, urinary glucose, urinary sodium, urinary potassium with a significant weight gain and increased urine volume, blood total protein, urinary creatinine levels when compared with the toxicant control group. The histological changes in HABH treated group were minimal in contrast to the gentamicin intoxicated group. The HABH has shown significant and dose dependant antioxidant activity and prevented the depletion of GSH and decreased the extent of lipid peroxidation. The nephroprotective activity of extract may be due to the antioxidant potential of *Benincasa hispida*.

Conclusion

It can be concluded that gentamicin, when administrated at a dose of 80 mg/kg, *i.p.* for 8 days induced renal damages as evidenced by the physical, histological and biochemical alterations. On the other hand, prophylactic treatment of the hydroalcoholic fruit extract of Ash guard *Benincasa hispida* (HABH) at 200 and 400 mg/kg, *p.o* is beneficial in gentamicin-induced renal dysfunction and organ damage in rats, presumably via prevention of lipid peroxidation and preservation of antioxidant glutathione and also observed that all the physical, histological and biochemical parameters were brought back nearly to the normal levels revealing its nephroprotective potential.

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Conflict of interest

Authors declare no competing interests.

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