



Protective role of hydroalcoholic extract of *Ficus carica* in gentamicin induced nephrotoxicity in rats

Kaksaheb J. Kore*, Rajkumar V. Shete, Babasaheb N. Kale and Amit S. Borade

Department of Pharmacology, Rajgad Dnyanpeeth's College of Pharmacy, Bor, (M.H.) - India

Abstract

Gentamicin (GM)-induced nephrotoxicity limits its long-term clinical use. Several agents/strategies were attempted to prevent GM nephrotoxicity but were not found suitable for clinical practice. Hydroalcoholic extract of *Ficus carica* (HEFC) retard the progression of certain types of cancers, cardiovascular and renal disorders. We aimed to evaluate protective effect of HEFC on GM-induced renal proximal tubular damage. The rats were pre-fed experimental diets for 8 days and then received GM (100 mg/kg body weight/day) treatment for 8 days while still on diet. Serum parameters, oxidative stress in rat kidney were analyzed. GM nephrotoxicity was recorded by increased serum creatinine and blood urea nitrogen. GM increased MDA level whereas decreased catalase, reduced glutathione. In contrast, HEFC alone increased CAT concentration, GSH content and decreased MDA level. HEFC supplementation ameliorated GM-induced specific metabolic alterations and oxidative damage due to its intrinsic biochemical/antioxidant properties.

Key-Words: *Ficus carica*, Gentamicin, Nephrotoxicity

Introduction

Nephrotoxicity can be defined as renal dysfunction that arises as a direct result if exposure to external agents such as drugs and environmental chemicals. Many therapeutic agents have been shown to induce clinically significant nephrotoxicity¹. Aminoglycoside antibiotics have been widely used for gram-negative infections. However, their nephrotoxicity and their ototoxicity are major limitations in clinical use. Among several aminoglycosides, the grade of nephrotoxicity has been reported to be in the following order, neomycin > gentamicin > tobramycin.² GM nephrotoxicity, which occurs in about 15-30% of treated subjects, is manifested clinically as nonoliguric renal failure, with a slow rise in serum creatinine and hypoosmolar urinary output developing after several days of treatment³. Gentamicin is filtered through glomeruli into tubular urine, binds with anionic phospholipids, such as phosphatidylinositol or phospholipidylserine, in brush border membrane of proximal tubular cells reabsorbed actively via pinocytosis process into tubular cells, taken by lysosomes and thereafter produces phospholipidosis².

The drug enters cells by adsorptive/receptor mediated endocytosis after binding to acidic phospholipids and megalin and is found essentially in lysosomes. Animals treated with low, therapeutically relevant doses of aminoglycosides show both lysosomal phospholipidosis and apoptosis in proximal tubular cells⁴.

The fig is acts as antipyretic, tonic, purgative, alexiteric, aphrodisiac, lithonriptic, useful in inflammation, weakness, paralysis, thirst, diseases of the liver and spleen, pain in the chest, cures piles, stimulate growth of hair, expectorant, diuretic. Usefull in vata disease of the head and blood, leprosy, nose bleeding, root is tonic, useful in leucoderma and ringworm⁵. The fig is emollient, demulcent, cooling, laxative, nutritive. The fruit is also use in constipation⁵. Figs are given to cure piles, in the treatment of gout, milky juice is applied to cure ulcer in mouth. The fresh figs form a nice tonic to weak people who suffer from cracks in lips, tongue and mouth⁶ (Nadkarni, 1982). Fruit is useful in anemia; latex is anthelmintic, act as anticancer compound⁷. The fruit is acts as expectorant, analgesic and anti-inflammatory⁸.

Fig is mainly recommended for people suffering from constipation, for pregnant women and for mental and physical exhaustion. They are also used for treatment on pharyngitis, gastritis, bronchitis and irritative

* Corresponding Author:

E-mail: amitborade1@gmail.com

Mob.: +91-9657227402

cough⁹. Therefore, this experimental study was designed to investigate the possible protective effects of *ficus carica* on nephrotoxicity induced by GEN in a rat model, and to clarify the association between body weight, kidney weight, malondialdehyde (MDA), catalase (CAT) activities, glutathione (GSH) content, Cr, urea, BUN levels and GEN-induced nephrotoxicity.

Material and Methods

Plant Collection and authentication of Fruit

The dried ripe fruits of *Ficus carica* was procured from Pune region in the month of September-October (2008-09) and air-dried at room temperature ($28 \pm 2^{\circ}\text{C}$) for a one week. The plant specimens were authenticated by Dr. A. M. Mujumdar at Agharkar research institute, Pune, India. Authentication number Auth.08-167.

Preparation of crude extract

The plant material was rendered free from soil and adulterated materials coarsely ground by electrical device. The powdered material was soaked into aqueous ethanol (80%) for 72 hrs with occasional shaking. The soaked material was rendered free plant debris by passing through a muslin cloth and fluid portion was filtered through a fine filter paper. The above mentioned extraction procedure was repeated twice on plant debris and filtrate were subsequently combined before subjecting to evaporation under reduced pressure on a rotary evaporator to thick paste like mass of dark brown colour¹⁰.

Experimental design

In this investigation, 36 healthy adult male Wistar rats weighting between 190 and 250 g were used. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature ($24 \pm 3^{\circ}\text{C}$) during the experimental period. All experimental procedures were conducted in accordance with the guide to the care and use of laboratory animals. The rats were provided ad libitum with tap water and fed with standard commercial rat chow (Pranav agro industries, Sangali). Thirty-six rats were randomly assigned to five groups equally: (1) Normal control; injected intraperitoneal (i.p.) saline for 8 days, (2) GM treated group; injected intraperitoneal (i.p.) GM (100 mg/kg) for for 8 days, (3) GM + HEFC250; treated group; injected intraperitoneal (i.p.) GM (100 mg/kg) and HEFC (250 mg/kg) for for 8 days, 4) GM + HEFC500; treated group; injected intraperitoneal (i.p.) GM (100 mg/kg) and HEFC (500 mg/kg) for for 8 days, 5) GM + HEFC750; treated group; injected intraperitoneal (i.p.) GM (100 mg/kg) and HEFC (750 mg/kg) for for 8 days. After the experimental period, blood samples were collected from animals of each treatment group by retro orbital puncture under light ether anesthesia.

Serum was separated by centrifugation at 10000 x g for 10 min. Serum was analyzed for biochemical estimation. The rats were then sacrificed by cervical dislocation, the abdomen was opened and kidney was removed, decapsulated and divided equally into two longitudinal sections. One of these was placed in formaldehyde solution for routine histopathologic examination by light microscopy. The other half was placed in KCL and stored for assayed of MDA, CAT activities, and GSH content.

Animals

Swiss albino mice and albino rats were used in this study was obtained from the Yash farm and National Toxicological Center, Pune.

Weight: Wistar rats - 150-250 gm

Biochemical determination

The methods used for biochemical determinations, described briefly below, have been used and reported in our previous studies¹¹⁻¹². MDA levels in the kidney tissue were determined by the method described¹³ Mihara and Uchiyama (1978). Kidney tissue (200 mg) was homogenized with ice-cold 1.15% KCl to form a 10% homogenate. Then, 0.5 ml of this homogenate was pipette into a 10ml centrifuge tube and 3.0 ml of 1% w/v H_3PO_4 and 1.0 ml of 0.6% v/v aqueous thiobarbituric acid solution were added. The tubes were heated for 45 min in a boiling water bath and the reaction mixture was then cooled in an ice-bath. This was followed by the addition of 4.0 ml of n-butanol. The contents were mixed for 40 s using a vortex mixer, centrifuged at 1200g for 10 min and the absorbance of the organic layer was measured at wavelengths of 535nm. GSH was determined by a spectrophotometric method, based on the use of Ellman's reagent¹⁴. Tissue homogenates were mixed with 50% trichloroacetic acid in distilled water in glass tubes and centrifuged at 3000 rpm for 15 min. The supernatants were mixed with 0.4M Tris buffer, pH 8.9, and 0.01M 5,50-dithio-bis (2-nitrobenzoic acid) (DTNB) was added. After shaking the reaction mixture, its absorbance was measured at 412nm. The absorbance values were expressed as mmol/g tissue. CAT activity was determined according to the method of Aebi¹⁵. Briefly, 10 ml of kidney tissue supernatant was added to 2.99 ml of phosphate-buffered saline (PBS) and the absorbance was read at 240 nm using a UV spectrophotometer. Serum levels of Cr, urea and BUN were determined using the Autoanalyser (Chem Master LabLife Instruments) according to manufacturers' instructions

Statistical analysis

Data obtained for each set of anti-inflammatory model was expressed as means \pm SEM and analysed by one-way ANOVA followed by Dunnett's test.

Results and Conclusion

In normal control, serum creatinine level was found 0.563 ± 0.011 whereas in gentamicin group was 1.409 ± 0.043 significantly ($p < 0.001$) increased as compared to normal control. The animal treated with HEFC (250 mg/kg, 500 mg/kg and 750 mg/kg) showed significantly ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) decreased serum creatinine and value were 1.273 ± 0.496 , 0.821 ± 0.023 and 0.634 ± 0.008 respectively as compared to gentamicin group.

In normal control, serum urea and blood urea nitrogen level were found 19.52 ± 0.17 and 9.11 ± 0.10 respectively. The serum urea and blood urea nitrogen in gentamicin group were 50.61 ± 0.62 and 23.63 ± 0.26 showed significantly ($p < 0.001$) increased as compared to normal control. The animal treated with HEFC (500 mg/kg and 750 mg/kg) showed significantly ($p < 0.01$ and $p < 0.001$ respectively) decreased serum urea and blood urea nitrogen and value were 31.38 ± 0.98 , 21.46 ± 0.64 and 14.65 ± 0.51 , 10.02 ± 0.35 respectively, as compared to gentamicin group; whereas, HEFC 100 was not significant in this regards.

The gentamicin toxicity was reversed by treatment groups and an optimum creatinine, urea and blood urea nitrogen level was observed. The results thus indicated HEFC effective in reducing serum creatinine, urea and blood urea nitrogen level in gentamicin toxicity.

Table 1: Effect of HEFC on serum creatinine, urea and blood urea nitrogen in gentamicin induced nephrotoxicity

Treatment group	Serum creatinine (mg/dl) mean \pm SEM	Serum urea (mg/dl) mean \pm SEM	Serum BUN (mg/dl) mean \pm SEM
Normal control	0.563 ± 0.011	19.52 ± 0.17	9.11 ± 0.10
GM	$1.409 \pm 0.043###$	$50.61 \pm 0.62###$	$23.63 \pm 0.26###$
HEFC 250	$1.273 \pm 0.046^*$	44.02 ± 1.2	20.55 ± 0.69
HEFC 500	$0.821 \pm 0.023^{**}$	$31.38 \pm 0.98^{**}$	$14.65 \pm 0.51^{**}$
HEFC 750	$0.634 \pm 0.008^{***}$	$21.46 \pm 0.64^{***}$	$10.02 \pm 0.35^{***}$

Data are expressed as mean \pm SEM., Data by one way ANOVA followed by Dunnett's test, n=6. ### $p < 0.001$

as compared to normal control; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared to GM group.

In normal control, the concentration of MDA was 194.8 ± 40.23 whereas in gentamicin group the MDA concentration was significantly ($p < 0.01$) increase to 498.4 ± 76.3 as compared to normal control. The animal treated with HEFC (500 mg/kg and 750 mg/kg) showed significantly ($p < 0.05$ and $p < 0.01$ respectively) decreased MDA concentration and value were 276 ± 54.25 and 203.7 ± 52.44 respectively, as compared to gentamicin group respectively; whereas HEFC 250 was not significant in this regards.

In normal control, GSH concentration was found 3.617 ± 0.367 , whereas in gentamicin group the GSH concentration was 0.9067 ± 0.256 showed significant ($p < 0.001$) decreased as compared with normal control. The animal treated with HEFC (500 mg/kg and 750 mg/kg) showed significantly ($p < 0.05$ and $p < 0.01$ respectively) decreased GSH concentration and value were 2.250 ± 0.380 and 2.806 ± 0.498 respectively, as compared to acetaminophen group respectively, whereas HEFC 250 was not significant in this regards.

In normal control, the concentration catalase was 2.999 ± 0.465 whereas in gentamicin group the catalase concentration was 0.6313 ± 0.185 showed significantly ($p < 0.001$) decreased as compared with normal control. The animal treated with HEFC (500 mg/kg and 750 mg/kg) showed significantly ($p < 0.05$ and $p < 0.01$ respectively) decreased catalase concentration and value were 1.458 ± 0.180 and 2.367 ± 0.255 respectively, as compared to gentamicin group respectively; whereas HEFC 250 was not significant in this regards. The result indicate that, gentamicin toxicity significantly increase the MDA concentration and decreased GSH and catalase concentration due to increase in oxidative stress, while on other hand, the HEFC significantly reduced the elevation of MDA levels and increased GSH and catalase concentration indicating a nephroprotective effect.

Table 2: Effect of HEFC on lipid peroxidation (MDA), reduced glutathione (GSH), catalase (CAT) concentration in acetaminophen induced nephrotoxicity

Treatment group	MDA (nmole/g tissue) mean \pm SEM	GSH (μ g/g tissue) mean \pm SEM	CAT (μ g/sec/g tissue) mean \pm SEM
Normal control	194.8 ± 40.23	3.617 ± 0.367	2.999 ± 0.465
APAP	$498.4 \pm 76.3##$	$0.9067 \pm 0.256###$	$0.6313 \pm 0.185###$
HELI 250	$408.5 \pm$	$1.134 \pm$	$0.8965 \pm$

	61.42	0.370	0.276
HELI 500	276 ± 54.25*	2.205 ± 0.380*	1.458 ± 0.180*
HELI 750	203.7 ± 52.44**	2.806 ± 0.498**	2.367 ± 0.255**

Data are expressed as mean ± SEM., Data by one way ANOVA followed by Dunnett's test, n=6. ## p<0.01, ### p<0.001 as compared to normal control; *p<0.05 and **p<0.01 as compared to GM group.

The kidney is a common target for toxic xenobiotics, due to its capacity to extract and concentrate toxic substances, and to its large blood flow share (about 20% of cardiac output)¹⁶. Development of nephrotoxicity can further increase load on the kidney leading to serious complications. This requires either stoppage of drug therapy or change over in the therapy. This raises a question whether any kind of nephroprotection is possible that can handle this problem. Screening of either substances from synthetic origin or herbal origin for nephroprotection can answer this question probably. Several pharmacological uses of the *Ficus carica* have been documented but the leaves of the *Ficus carica* have not been subjected to the scientific study for its nephroprotective activity. Hence, it was thought worthwhile to test the hydroalcoholic extract of fruits of *Ficus carica* for the nephroprotective activity against GM induced nephrotoxicity. Gentamicin, an amino-glycoside antibiotic with a wide spectrum of activities against Gram-positive and Gram-negative bacterial infections but with high preference for the latter, is equally associated with nephrotoxicity as its side-effect¹¹. Thus, gentamicin-induced nephrotoxicity are well established experimental models of drug-induced renal injury¹⁷.

Administration of gentamicin (100 mg/kg i.p.) reported to cause nephrotoxicity^{11,18,10}. In agreement in the study decrease in body weight, GSH and catalase concentration; increase in kidney weight, serum creatinine, urea, BUN and MDA as well as marked histopathological changes in kidney tissue of GM treated nephrotoxic rats was observed. Protective effect of HEFC was assessed by evaluating serum parameter, biochemical parameter and histopathological study of kidney as end point of renal damage. GM reduces the glomerular filtration rate which is shown by an increase serum creatinine. The impairment in glomerular function was accompanied by an increase in blood urea¹⁹. The administration of GM showed significant increase in serum creatinine, serum urea and serum BUN as compared to normal control. Administration of HEFC 250 mg/kg, 500 mg/kg and 750 mg/kg has showed significant decrease in serum

creatinine whereas HEFC 500 mg/kg and 750 mg/kg was showed significant decrease serum urea and serum BUN as compared to GM group.

In the complex pathogenesis of GM nephrotoxicity oxidative stress is probably the most common pathogenic²⁰. The exact mechanism of GM which induces the renal damage is unknown. Recently, ROS were considered to be important mediators of GM-induced nephrotoxicity²¹. It has been found that the GM-treatment increases H₂O₂ production and it is known that H₂O₂ and O₂⁻ induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate (GFR). O₂⁻ this radical can react with nitric oxide (NO, a vasodilator) to form peroxynitrite, cytotoxic oxidant radical species. The inactivation of NO by O₂⁻ could also lead to a decrease in the GFR. It has been suggested that the oxidative stress induces tubular damage. It is known that the increase in ROS levels induces cytotoxicity due to a concerted action of oxygen and nitrogen-derived free radicals. GM (100 mg/kg) has given alone significant increased in MDA level while CAT activities and GSH content were reduced in the kidney tissue. GM nephrotoxicity was associated with low activity of CAT and GSH content in the renal cortex. This decreased renal antioxidant enzymatic defense could aggravate the oxidative damage in these rats. The exaggerated production of ROS in GM-induced nephrotoxicity could induce inactivation of antioxidant enzymes¹¹.

In the current study GM (100 mg/kg) has significant increased in MDA level while decrease GSH content and CAT activities as compared to normal control. The administration of HEFC 500 mg/kg and 750 mg/kg showed significant decrease in MDA level whereas increase the GSH content and CAT activities as compared to GM group. Thus agents having strong antioxidant and cellular anti-inflammatory properties may have ability to halt gentamicin-nephrotoxicity²². From this discussion it was strongly indicates that the hydroalcoholic extract of the *Ficus carica* protecting the kidney from GM-induced toxicity, through improvement in oxidant status and a possible antioxidant activity.

The present studies conclude that hydroalcoholic extract of *Ficus carica* fruits has nephroprotective activity, these effects of hydroalcoholic extract of *Ficus carica* leaves may be due to presence of phytochemicals like flavonoids also its ability of anti-inflammatory activity and antioxidant status which may act individually or synergistically.

References

1. Maliakel D.M., Kagiya T.V., Nair C.K., (2008). Prevention of cisplatin-induced nephrotoxicity by glucoside of ascorbic acid and α -tocopherol. *Experimental and Toxicologic Pathology*, **60**: 521-527.
2. Suzuki S.S., Takamura J., Yoshida Y., Shinzawa Niwat and Tamatani S R. (1995). Comparison of gentamicin nephrotoxicity between rats and mice. *Camp. Biochem. Physiol.* **1**: 15-28.
3. Ali B.H. (2003). Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: some recent research. *Food and Chemical Toxicology*, **41**: 1447-1452.
4. Servais H., Ortiz A., Devuyt O., Denamur S., Tulkens P.M., and Mingeot-Leclercq M.P. (2008). Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. *Apoptosis*, **13**: 11-32.
5. Kirtikar K.R. and Basu B.D. (2005). *Indian Medicinal Plants*. Text vol. III, International book distributors book sellers and publishers dehradun, India, 2329-2330
6. Nadkarni K.M. (1982). *Indian Materia Medica*, Vol. 1. Popular Book Depot, Bombay, India, p 545- 547.
7. Khare, C.P., (2007). *Indian Medicinal Plants an, illustrated dictionary*. Springer reference, 265.
8. Roger P. (2000). *Encyclopedia of medicinal plant*, Vol. 2, 708-709.
9. Harlalka G.V., Patil C.R. and Patil M.R., (2009). Protective effect of *Kalanchoe pinnata pers.* (*Crassulaceae*) on gentamicin-induced nephrotoxicity in rats. *Indian Journal of Pharmacology*, **39**: 201-205.
10. Parlakpınar H., Polat A., Tasdemir S. and Bay- Karabulut A. (2005). Protective role of caffeic acid phenethyl ester (CAPE) on gentamicin-induced acute renal toxicity in rats. *Toxicology*, **207**: 169-177.
11. Polat A., Parlakpınar H., Tasdemir S., Colak C., Vardi N., Ucar M., Emre M. and Acet A. (2006). Protective role of aminoguanidine on gentamicin-induced acute renal failure in rats. *Acta histochemica*, **108**:365-371.
12. Mihara M. and Uchiyama M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem.*, **86**: 271-8.
13. Gupta A., Gupta A., Nigam D., Shukla G.S. and Agarwal A.K. (1999). Profile of reactive oxygen species generation and antioxidative mechanisms in the maturing rat kidney. *Journal Applied Toxicology*, **19**: 55-9.
14. Aebi H. (1984). *Catalase methods of enzymatic analysis*. New York, London: Academic Press, 673-77.
15. Salgado C.M., Francisco J., Lopez H.J., and Lopez N. (2007). Glomerular nephrotoxicity of aminoglycosides. *Toxicology and Applied Pharmacology*, **223**: 86-98.
16. Ali B.H. (1995). Gentamicin nephrotoxicity in humans and animals: some recent research. *Gen. Pharma*, **26**:1477-148.
17. Karahan I., Atessahin A., Yilmaz S., Ceribasi O.A. and Sakin F. (2005). Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. *Toxicology*, **215**: 198-204.
18. Al-Majed A.A., Mostafa A.D., Al-Recabi A.C. and Shabanah O.A. (2002). Protective effects of oral arabic gum administration on Gentamicin induced nephrotoxicity in rats. *Pharmacological Research*, **5**: 445-451.
19. Stojiljkovic N., Mihailovic D., Veljkovic S., Stojiljkovic M. and Jovanovic I. (2008). Glomerular basement membrane alterations induced by gentamicin administration in rats. *Experimental and Toxicologic Pathology*, **60**: 69-75.
20. Priyamvada S., Priyadarshini M., Arivarasu N.A., Farooq N., Khan S., Khan S.A., Khan W. and Yusufi A.N.K. (2008). Studies on the protective effect of dietary fish oil on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Prostaglandins, Leukotrienes and Essential Fatty Acids **7**: 369-381.
21. Balakumar P., Rohilla A. and Thangathirupathi A. (2010). Gentamicin-induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? *Pharmacological Research*.