Indian Journal of Animal Sciences **80** (10): 965–68, October 2010

Protective effect of *Mangifera indica* Linn. on gentamicin induced nephrotoxicity in rats

K J BIBU[1] and *A D JOR*[2]

Kerala Agricultural University, Mannuthy, Thrissur, Kerala 680 651 India

Received: 14 July 2009; Accepted: 3 July 2010

ABSTRACT

Nephroprotective effect of stem bark of *Mangifera indica (M. indica)* against gentamicin-induced nephrotoxicity in male Sprague Dawley rats was assessed. Rats administered with gentamicin daily @ 80 mg/kg i/p, for 8 days showed an increase in lipid peroxidation and a decrease in superoxide dismutase, catalase and reduced glutathione. Significant ($P<0.05$) increase in serum creatinine and urea levels was also observed. Histopathological examination of kidney revealed extensive proximal tubular necrosis with marked glomerular changes. Ethanolic extract of *M indica* at a dose rate of 100 mg/kg p.o. and 500 mg/kg p.o. showed a significant ($P<0.05$) reduction in lipid peroxidation and an increase in the activities of superoxide dismutase, catalase and reduced glutathione, which suggest its efficacy in scavenging free radical-induced renal damage. The ethanolic herbal extract also showed a reduction in serum creatinine and serum urea levels. These effects were predominant with 500 mg/kg p.o. Histopathological examination of tissue sections revealed tubular vacuolar degeneration and necrosis in a dose dependent manner. Thus, the study revealed that treatment with ethanolic extract *M indica* inhibited gentamicin-induced proximal tubular necrosis though the dose rate was not sufficient to provide complete protection.

Key words: Gentamicin, *Mangifera indica*, Nephroprotection, Rats, Toxicity

Gentamicin (GM) remains dependable antibactericidal agents for severe Gram-negative infection. Despite nephrotoxicity and ototoxicity, GM is continuously used in clinical practice because of their bactericidal efficacy, synergism with ß-lactamases, low cost, limited bacterial resistance and a post-antibiotic effect. Proximal tubular injury leading to cell necrosis underlines nephrotoxicity. The stem bark of *Mangifera indica (M indica)* Linn., has been reported to have several pharmacological activities including antispasmodic, analgesic, antipyretic, antioxidant, antitumor, antiviral, antidiabetic, anti-bone resorption and immunomodulatory effects (Kumar *et al.* 2009). Hitherto no research work has been published to explore the nephroprotective effect of stem bark of *M indica.* Thus, the present study would elucidate the nephroprotective effect of ethanolic extract of *M indica* stem bark against gentamicin induced nephrotoxicity in rats.

MATERIALS AND METHODS

Plant materials and preparation of extracts: The stem bark of *M indica* was identified, authenticated, air-dried, coarsely powdered and the powders were extracted using a soxhlet apparatus with 95% ethanol. The ethanolic extract was then concentrated in a rotary vacuum evaporator under reduced pressure and temperature (55°C). The yield of the extract was 25.71% on dry matter basis.

Experimental design: The study was conducted in 32 adult male Sprague Dawley rats weighing 200 - 250g, purchased from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India. The rats were maintained under identical feeding and management practices in the laboratory. GM-induced oxidative stress in kidney has also been documented in experimental animals (Kozat *et al.* 2007). The animals were randomly divided into 4 groups comprising eight animals each. The experiment was conducted for a period of 30 days.

Group 1 - normal control administered with vehicle (0.125% Tween 80) p.o. for 30 days.

Group 2 - received GM @ 80 mg/kg i.p. for 8 consecutive days. This dose was proved to be nephrotoxic by Vijaykumar *et al.* (2000).

Group 3 - GM @ 80 mg/kg i.p. for 8 days + ethanolic extract of *M indica* @ 100 mg/kg p.o. from 9th day to day 30.

Group 4 - GM @ 80 mg/kg i.p. for 8 days + ethanolic

Present address: [1]Research Associate, ICAR Network Project on Ethnoveterinary Medicine, Department of Clinical Medicine, (e mail: bibujohn@rediffmail.com), [2]Professor, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences.

extract of *M indica* @500 mg/kg p.o. from 9th day to day 30.

Observations: Blood was collected from the retro-orbital plexus from all the rats on days 0, 9, 15 and 30 under mild ether anaesthesia for the assay of serum creatinine and urea using standard kits. On 30th day, animals were sacrificed by cervical dislocation. Right and left kidneys were immediately removed, processed for the histological examination as per the standard procedures and for the estimation of superoxide dismutase (SOD) (Mimami and Yoshikawa 1979), lipid peroxidation (Fraga *et al.* 1988), catalase (CT) (Cohen *et al.* 1970) and reduced glutathione (RG) (Ellman 1959).

Statistical analysis: Data are expressed as mean ± SE. The results obtained were analysed using analysis of variance (ANOVA) and the comparisons of parameters between different groups were evaluated by Duncan's multiple range test (Snedecor and Cochran 1985).

RESULTS AND DISCUSSION

Administration of GM caused an increase III lipid peroxidation and decrease in SOD, CT and RG (Table 1). The biochemical parameters like serum creatinine and urea were also elevated (Table 2). Similar observations have also been reported by Kozat *et al.* (2007). Many investigators have described the role of reactive oxygen species (ROS) including hydroxyl radicals in gentamicin-induced nephrotoxicity (Abdel-Raheem *et al.* 2009). Reactive oxygen species (ROS) generated by the toxic effect of GM might have caused lipid peroxidation elevation and SOD, CAT and RG depletion, which are indicators of tissue damage. Serum creatinine and urea were elevated because GM at a dose rate of 80 mg/kg i/p affected glomerular filtration and elevated levels were found in blood.

Administration of ethanolic extract of *M. indica* could reverse the nephrotoxic effect of GM through its antioxidant potential in a dose dependent manner which was revealed by significant increase in the SOD, CT, RG levels and by significant ($P<0.05$) reduction in the lipid peroxidation (Table 1). Kozat *et al*. (2007) came to a conclusion that administration of antioxidant substances in GM-induced nephrotoxicity could ameliorate the toxicity. Mangiferin has been reported to be the major polyphenol component in the stem bark (Rastogi *et al*. 2007). The significant nephroprotective effect was found with higher dose of ethanolic extract of *M. indica.* The nephrotoxicity produced by gentamicin was reversed by the plant extracts under study due to the action of mangiferin, indicating the antioxidant effect of these plant extracts.

Serum creatinine and serum urea, serum markers of kidney function, have been considered the most important manifestations of severe tubular necrosis of kidney. The results of the present study indicated that ethanolic extract of *M. indica* showed a considerable reduction in serum creatinine and serum urea values on day 15 of the treatment. At the end of the experiment a significant reduction in these values were observed when compared to the GM alone treated group (Table 2). These results correlates with the findings of Singh *et al.* (2009) where they observed that nephroprotection

Table 1. Effect of ethanolic extracts of stem bark of *M. indica* on superoxide dismutase, lipid peroxidation, catalase and reduced glutathione levels of kidney tissue. Values are expressed as mean±SE of 8 animals

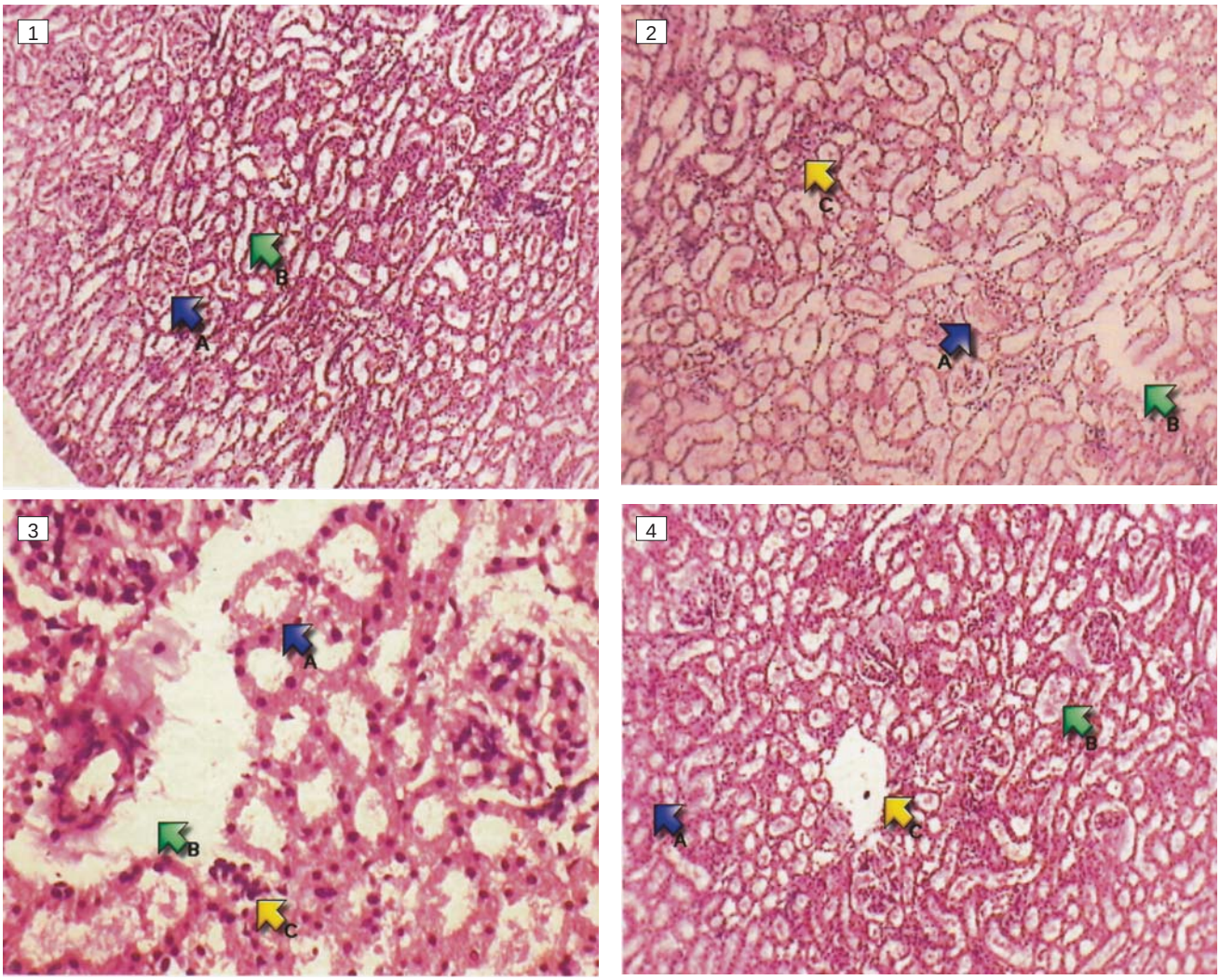
Groups	SOD (units/mg of protein)	Lipid peroxidation (mM/100 mg of tissue)	CT (units/assay mixture) (250 mg)	RG (mg/100 g of tissue)
1	2.494±0.12^{a}	24.367±0.43^{a}	0.988±0.02^{a}	133.525±3.23^{a}
2	1.612±0.05^{a}	36.400±0.95^{b}	0.722±0.01^{b}	81.337±2.97^{b}
3	11.826±0.53^{b}	22.138±0.47^{c}	2.278±0.01^{c}	159.672±3.68^{c}
4	20.616±0.69^{c}	17.600±0.89^{d}	4.883±0.01^{d}	229.212±3.45^{d}

Means bearing the same superscript within a column do not differ significantly at $P<0.05$; Group 1 – control group; group 2, gentamicin control; Group 3 and group 4, treated group.

Table 2. Effect of ethanolic extract of stem bark of *M. indica* on serum creatinine and serum urea levels (mg/dl). Values are expressed as mean±SE of 8 animals

Group	Creatinine				Urea			
	0th day	9th day	15th day	30th day	0th day	9th day	15th day	30th day
1	0.54±0.05	0.67±0.03^{c}	0.52±0.04^{c}	0.58±0.05^{c}	46.88±1.90	50.25±2.02^{c}	48.50±2.81^{d}	50.38±1.73^{d}
2	0.45±0.03	2.81±0.36^{a}	2.13±0.23^{a}	1.56±0.18^{a}	28.63±1.92	125.63±4.74^{a}	67.63±2.62^{a}	56.63±2.54^{b}
3	0.44±0.03	2.63±0.16^{b}	0.92±0.02^{b}	0.63±0.04^{b}	27.88±1.22	105.00±2.21^{b}	58.50±2.35^{b}	53.13±1.98^{a}
4	0.45±0.03	2.21±0.21^{b}	0.59±0.07^{c}	0.59±0.06^{b}	29.50±0.91	106.63±2.83^{b}	34.13±2.33^{c}	48.13±1.39^{c}

Means bearing the same superscript within a column do not differ significantly at $P<0.05$. Group 1, Control group; group 2, gentamicin control; group 3; group 4, treated group.



Figs 1–4. **1.** Normal group (H & E×100) A : intact glomerulus B : intact proximal convoluted tubules; **2.** gentamicin group (H & E×100) A : marked glomerular changes B : extensive tubular necrosis C : mononuclear cell infiltration in the interstitium; **3.** ethanolic *M. indica* (100 mg/kg) treated group (H & E×400) A : vascular changes in the proximal convoluted tubules B : perivascular oedema C : focal collection of mononuclear cells in the interstitium; **4.** ethanolic *M. indica* (500 mg/kg) treated group (H & E×100) A : mild tubular degenaration in subcapsular area B : hyaline cast in distal convoluted tubule C : dilated lumen of distal convoluted tubule.

could be achieved by reduction in the serum creatinine and urea levels in GM-induced nephrotoxicity. The glomerular filtration process affected by GM administration was reversed by the ethanolic extract of *M. indica* in a dose-dependent manner due to the presence of the active polyphenol component, mangiferin. Histopathologically, in GM group (group 2), there were extensive proximal tubular necrosis and loss of the lining epithelium and these features were predominantly subcapsular. Besides, there were interstitial oedema, perivascular oedema and multiple focal collections of mononuclear cells in the interstitium. The glomerular changes were quite marked (Fig. 2). The results can be correlated with the works of Abdel-Raheem *et al.* (2009). In the ethanolic extract of *M. indica* 500 mg/kg treated group, mild tubular degeneration and necrosis could be observed (Fig. 4) whereas in the ethanolic extract of *M. indica* 100 mg/kg treated group, tubular vacuolar degeneration and necrosis with mononuclear infiltration and dilated distal convoluted tubules (DCT) with hyaline casts could be seen (Fig. 3). The histopathological examination of kidney tissues reveals that the doses of *M. indica* were not sufficient enough to provide complete nephroprotection.

Thus the plant extracts under study provided nephroprotection by the scavenging of the free radicals that were generated by gentamicin administration mediated by the action of phytoconstituents especially mangiferin, present in the plant extracts. But the dose which was used for the present study was not sufficient to provide complete nephroprotection.

ACKNOWLEDGEMENT

The authors are thankful to Dr A J Mammen and Mrs K A Mercey and Dr K T Prasanna, alumni of Kerala Agriculture

University for their proximal professional assiatance in succeeding the experiment. The financial support provided by the Kerala Agricultural University with grant number G5/5502/06 is also acknowledged.

REFERENCES

Abdel-Raheem I T, Abdel-Ghany A A, and Mohamed G A. 2009. Protective effect of quercetin against gentamicin induced nephrotoxicity III rats. *Biological and Pharmaceutical Bulletin* **32**: 61–67.

Cohen G, Dembree D and Marcus J. 1970. An assay to analyse the catalase level in tissues. *Analytical Biochemistry* **34**: 30–38.

Ellman G L. 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* **82**: 70–77.

Fraga C G, Leiboritz B E and Toppel A L. 1988. Lipid peroxidation measured as TBARS in tissue slices. Characterisation and comparison with homogenates and microsomes. *Free Radical Biology & Medicine* **4**: 155–61.

Kozat S, Mert H, Mert N, Demir H and Dogan I. 2007. Gentamicin induced acute renal failure on biochemical parameters in rats. *Indian Veterinary Journal* **84**: 138–40.

Kumar B D, Mitra A and Manjunatha M. 2009. *In vitro* and *in vivo* studies of antidiabetic Indian medicinal plants: A review. *Journal of Herbal Medicine and Toxicology* **3**: 9–14.

Mimami M and Yoshikawa H. 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta* **92**: 337–42.

Singh P, Srivastava M M and Khemani L D. 2009. Nephroprotective activities of root extracts of *Andrographis paniculata* (*Burm f.*) *Nees* in gentamicin induced renal failure in rats: A time-dependent study. *Archives of Applied Science Research* **1**: 67–73.

Snedecor G Wand Cochran W G. 1985. *Statistical Methods.* 8th edition. Oxford and IBH Publishing Company, Calcutta.

Vijaykumar K, Naidu M U R, Shifow A A and Ratnakar K S. 2000. Probucol protects against gentamicin-induced nephrotoxicity in rats. *Indian Journal of Pharmacology* **32**: 108–13.