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Original Article

Comparison of the Species-Sensitive Effects of Different Dosages of Calcium and Verapamil on Gentamicin-Induced Nephrotoxicity in Rats and Rabbits

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ABSTRACT

Aim: To compare the effects of different dosages of calcium and verapamil on gentamicin-induced nephrotoxicity in rats and rabbits. **Materials and Methods:** Rabbits and rats of either sex in weight range of 1.5–2.5 kg and 175–225 g, respectively were used in study. Gentamicin 80 mg/kg i.m., calcium carbonate 0.5 g/kg/day oral, calcium carbonate 1.0 g/kg/day oral, and verapamil 7 mg/kg/day i.m. were administered for 6 days in either species containing 7 groups. Blood urea nitrogen (BUN), serum creatinine and, urine protein levels were assessed on day 0 and day 7 for kidney function. The animals were sacrificed on day 7 for histopathplogical examination and kidney superoxide dismutase levels (SOD) were measured. Statistical analysis was done using student's unpaired t-test, analysis of variance (ANOVA) and Wilcoxon Rank Sum test. P-value less than 0.05 was considered significant. **Results:** The results showed that calcium was able to reverse significantly increased BUN, serum creatinine, urine protein, and reduced kidney SOD levels in gentamicin-treated nephrotoxic rats or rabbits in a dose-dependent manner while verapamil had no protective or nephrotoxic effect. **Conclusion:** Calcium 0.5 g/kg/day and 1.0 g/kg/day were able to reverse tubular necrosis and mesangial proliferation in gentamicin-treated nephrotoxic animals. There was no species-sensitive variation in reversal of nephrotoxicity by calcium in rats and rabbits.

Key words: Calcium, gentamicin, nephrotoxicity, rabbits, rats, verapamil

INTRODUCTION

Aminoglycosides are used in the management of serious and life-threatening gram-negative bacterial infections because of their synergism with beta-lactam antibiotics, their marked post-antibiotic effect, and their rapid concentration-dependent killing effect. However, nephrotoxicity particularly on prolonged administration is observed in 10–20% of hospitalized patients who develop acute toxic renal failure.[1] Various studies carried out so far have shown that in 39% of cases of acute renal failure, prior administration of drugs was the cause of failure.[2] More alarming was the fact that gentamicin administration was responsible for as many as 89% of these cases. Nephrotoxicity is responsible for longer and more costly hospitalizations and potentiates the toxicities of other drugs.[3]

An increasing body of evidence indicates that the mechanisms involved with gentamicin-induced

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nephrotoxicity are multifaceted. The molecular and pathophysiological mechanisms of gentamicin-induced nephrotoxicity are well-characterized. Gentamicin is internalized through the giant endocytic complex that is preferentially expressed in renal proximal tubular segments S_1 and S_2.[4] In these cells, gentamicinis mainly accumulated in lysosomes, the Golgi apparatus and endoplasmic reticulum, producing lysosomal phospholipidosis, unfolded protein response and other effects, thereby turning on apoptotic and necrotic death pathways.[5]

Past decade has seen a tremendous research on agents modifying gentamicin-induced nephrotoxicity, such as use of various antioxidants like vitamin E and vitamin C,[6,7] S-allylcysteine,[8] diallysulfide,[9] herbal drugs such as *Withania somnifera,*[10] *Crocus Sativus,*[11] *Nigella sativa,*[12] and use of modified aminoglycosides such as astromicin and dactimicin which bind less tightly to phospholipid bilayers and are weaker inhibitors of lysosomal phospholipases.[13] None of the above agents has truly established itself in clinical practice. Previous studies using calcium channel blockers such as verapamil have shown contradictory results in gentamicin-induced nephrotoxicity with some showing a beneficial effect[14] while others have shown a nephrotoxicity enhancing potential.[15,16]A recent study has shown a beneficial effect of oral calcium (Ca^{+2}) administration in gentamicin-induced nephrotoxicity in rats.[17] In addition, it has been found that oral calcium loading protects against gentamicin-induced acute renal excretory failure.[18] Ca^{2+} is a competitive inhibitor of ^{125} I-gentamicin binding to isolated renal basement membranes, the initial membrane site of interaction between gentamicin and renal proximal tubule cells, thereby preventing critical cellular derangements induced by gentamicin within the renal tubular cell rather than on its cell surface. However, there are very few studies comparing the administration of different dosages of calcium and verapamil in gentamicin-induced nephrotoxicity in rats and rabbits simultaneously to see any species sensitive variation in results.

MATERIALS AND METHODS

Drugs and chemicals

Gentamicin was obtained from Ranbaxy Laboratories Limited, New Delhi, India and Verapamil from Samartha Life Sciences Pvt Ltd., Himachal Pradesh, India.

Animals

The study was conducted in two different animal species:

Rabbits: We used 42 healthy male and female New Zealand white rabbits, weighing between 1.5 to 2.5 kilograms, obtained from Haffkine bio-pharmaceuticals. They were acclimatized for 7 days prior to inclusion in study. They were housed under standard laboratory conditions and provided with free access to water and standard rabbit diet.

Rats: We used 42 Swiss albino rats, weighing between 200–300 grams, obtained from Haffkine bio-pharmaceuticals. They were acclimatized for 7 days prior to inclusion in study. They were housed under standard laboratory conditions and provided with free access to water and commercial rat feed in the form of pellets. Twelve hourly light and dark cycles were maintained.

Care of the animals was taken as per guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India and the study was approved by Institutional Animal Ethics Committee. Adequate measures were taken to avoid any pain or discomfort to the animals during handling or experimentation.

The rabbits were divided into seven groups (n = 6) and received the following treatment for six consecutive days: Normal saline and distilled water (2 ml/kg/day i.m. and 2.0 ml/kg/day per oral, group I), gentamicin (80 mg/kg/day i.m., group II), calcium carbonate (1.0 g/kg/day per oral, group III), verapamil (7 mg/kg/day i.m., group IV), gentamicin and calcium carbonate (80 mg/kg/day i.m. and 0.5 g/kg/day per oral; group V), gentamicin and calcium carbonate (80 mg/kg/day i.m. and 1.0 g/kg/day per oral, group VI), gentamicin and verapamil (80 mg/kg/day i.m. and 7 mg/kg/day i.m., group VII). The rats were also divided into seven groups (n = 6) and were treated according to same protocol as rabbits in similar doses.[15]

The following parameters were used to assess nephrotoxicity induced by gentamicin and its modification by calcium and verapamil in both rats and rabbits:

- Day 0: Blood Urea Nitrogen (BUN), Serum Creatinine, Urine protein
- Day 7: BUN, Serum Creatinine, Urine protein, Kidney Superoxide Dismutase (SOD) levels, Histopathological evaluation
- For estimating the BUN and serum creatinine, blood was collected from retro-orbital sinuses under light ether anethesia. The animals were sacrificed on day 7 and levels of kidney SOD and histopathological examination was carried out to assess tubular changes.

Blood urea nitrogen, serum creatinine, and urine protein were estimated on a semi-autoanalyser (ERBACHEM-5) using kits manufactured by Transasia Biomedicals, based on the following principles.

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BUN: Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. In the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenide dinucleotide (NADH), ammonia combines with ketoglutarate to produce L-glutamate. Adenosine diphosphate (ADP) is included as an activator and stabilizer of GLDH. The reaction is monitored by measuring the decline in absorbance at 340 nm, as NADH is converted to NAD. The value is expressed in mg/dl.

Serum creatinine: Creatinine reacts with alkaline picrate to produce an orange yellow color (Jaffe's reaction). The absorbance of the orange-yellow color formed is directly proportional to the concentration of creatinine in the sample and is measured photometrically at 510 nm. The value obtained is expressed as mg%.

Urine protein: Proteins in urine react with the copper present in Biuret reagent in alkaline medium to for a blue purple complex, which has absorption maxima at 550 nm. The values are expressed in gm%[15]

Histopathology

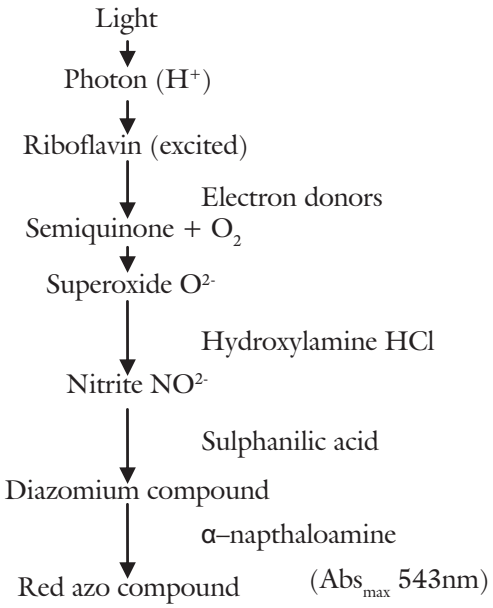
On day 7, after collection of the blood samples for estimation of blood urea nitrogen and serum creatinine, the animals were anesthetized by administration of thiopentone sodium in the dose of 50 mg/kg by intra-peritoneal route. After sacrificing the animals both the kidneys were removed and weighed. After this transverse sections were taken from both kidneys and fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 micron thickness, and mounted on slides. Duplicate slides were prepared for each kidney. One slide was stained with hematoxylin—eosin stain to observe structural changes. While the other slide was stained with periodic acid schiff (PAS). The renal injury was based on the extent of tubulo-interstitial damage, which included tubular epithelial cell swelling, necrosis and desquamation. The changes in the tubulo-interstitial area were graded as follows:

Grade 0: Normal (-)
Grade 1: Tubular damage involving <25% of cortical tubules (+)
Grade 2: Tubular damage involving 25% and <50% of cortical tubules (++)
Grade 3: Tubular damage involving 50% and <75% of cortical tubules (+++)
Grade 4: Tubular damage involving 75% of cortical tubules (++++)

Kidney superoxide dismutase levels

The SOD levels were measured in all the six groups at the end of day 7 after sacrificing the animals by using spectrophotometric method.

Principle: There is generation of superoxide dismutase radicals by photoreduction of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride at 543 nm.[19]



Statistical analysis

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) 14.0 package. The data were expressed as mean ± standard deviation (SD). Student's unpaired t-test, analysis of variance (ANOVA), and Wilcoxon Rank Sum test were used for parametric and non-parametric data, respectively. $P < 0.05$ was considered significant.

RESULTS

Rabbits

Effect on BUN, serum creatinine, urinary proteins and kidney SOD

The levels of BUN, serum creatinine, urinary proteins, and kidney SOD were compared between the seven groups, on day 7, after six days of consecutive treatment with respective drugs. The baseline values were similar in all groups on day 0. Results showed that BUN, serum creatinine, and urinary proteins were significantly elevated ($P < 0.05$) and kidney SOD levels were significantly reduced ($P < 0.05$) in group II (Gentamicin-treated group). Calcium 1.0 g/kg/day and verapamil 7.0 mg/kg/day had no significant effect on above parameters when administered alone in group III and group IV, respectively [Table 1] while calcium 0.5 g/kg/day and calcium 1.0 g/kg/day significantly reduced ($P < 0.05$) the elevated BUN, serum creatinine, urinary proteins, and significantly elevated ($P < 0.05$) the SOD levels when administered with gentamicin in group V and group VI, respectively. [Table 2] There was a significant dose-dependent relationship between group V and group VI with group VI (calcium 1.0 g/kg/day) showing more significant effect on above parameters when compared

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Table 1: Effect of normal saline, gentamicin, calcium, and verapamil on blood urea nitrogen (BUN), serum creatinine, urinary proteins, and kidney SOD in rabbits

Parameters	Mean±SD			
	Group I (control, normal saline (2 ml/kg)	Group II (gentamicin 80 mg/kg/day, i.m.)	Group III (calcium 0.5 g/kg/day, p.o.)	Group IV (verapamil 7.0 mg/kg/day, i.m.)
Blood urea nitrogen (BUN) (mg/dl)	18.30±1.2394	126.91±4.27[a]	18.233±1.1413	18.133±1.2242
Serum creatinine (mg/dl)	0.5083±0.0835	4.5817±0.61[a]	0.4950±0.10559	0.4850±0.08803
Urinary proteins (mg/dl)	0.2450±0.0437	2.0700±0.0946[a]	0.2817±0.0673	0.2333±0.0403
Kidney superoxide dismutase (SOD) (U/ml)	0.1617±0.0172	0.57±0.05[a]	0.1783±0.02317	0.17±0.02098

[a]*P*<0.05 as compared to group I. SOD = Superoxide dismutase levels, BUN = Blood urea nitrogen, SD = Standard deviation

Table 2: Effect of gentamicin, gentamicin+calcium (0.5 g/kg/day), gentamicin+calcium (1.0 g/kg/day), and gentamicin+verapamil (7 mg/kg/day) on blood urea nitrogen (BUN), serum creatinine, urinary proteins, and kidney SOD in rabbits

Parameters	Mean±SD			
	Group II (gentamicin 80 mg/kg/day, i.m.)	Group V (gentamicin 80 mg/kg/day, i.m.+ calcium 0.5 g/kg/day, p.o.)	Group VI (gentamicin 80 mg/kg/day, i.m.+ calcium 1.0 g/kg/day, p.o.)	Group VII (gentamicin 80 mg/kg/day, i.m.+ verapamil 7.0 mg/kg/day, i.m.)
BUN (mg/dl)	126.91±4.27	93.7500±5.0468[a]	21.9667±1.7603[b,c]	128.7500±4.4697
Serum creatinine (mg/dl)	4.5817±0.61	2.2033±0.4789[a]	0.4717±0.0621[b,c]	4.8033±0.1598
Urinary proteins (mg/dl)	2.0700±0.0946	1.1267±0.0294[a]	0.2467±0.0367[b,c]	1.9200±0.1210
Tissue superoxide dismutase (SOD) (U/ml)	0.57±0.05	0.3950±0.0187[a]	0.1783±0.0160[b,c]	0.4517±0.0354

[a,b]*P*<0.05 as compared to group II, [c]*P*<0.05 as compared to group V. SOD = Superoxide dismutase levels, BUN = Blood urea nitrogen, SD = Standard deviation

to group V. Concomitant administration of verapamil (group VII) however did not show any significant results when compared to group II or group V and group VI [Table 2].

Histopathological effects

The histopathological picture of animals in group I, III, and IV revealed normal architecture of glomeruli and mesangium. Basement membrane of the tubules was found to be intact. However, animals in group II (gentamicin only) showed numerous patches of focal and diffuse necrosis of tubular cells. Basement membrane breaks were found in tubule sections. Glomerular changes of grade 4 were seen with occasional infiltration. [Table 3] The renal histopathology picture in group V (calcium 0.5 g/kg/day) revealed normal architecture of glomeruli and mesangium with few areas of focal necrosis while group VI (calcium 1.0 g/kg/day) revealed normal architecture of glomeruli and mesangium. Basement membrane of the tubules was found to be intact in both groups [Table 4]. However, the animals in group VII (gentamicin + verapamil) showed patches of focal and diffuse necrosis of tubular cells. Basement membrane breaks were found in tubule sections. Glomerular changes of grade 4 were seen with occasional infiltration [Table 5].

Table 3: Effect of normal saline, gentamicin, calcium, and verapamil on histopathology in rabbit kidney

Histopathology scores	0	1	2	3	4	Mean rank
Group I (*n*=6) normal saline 2.0 ml/kg	6	0	0	0	0	6.5
Group II (*n*=6) gentamicin 80 mg/kg, i.m.	0	0	0	0	6	9.5[a]
Group III (*n*=6) calcium 1.0 g/kg, p.o.	6	0	0	0	0	6.5
Group IV (*n*=6) verapamil 7 mg/kg, i.m.	6	0	0	0	0	6.5

[a]*P*<0.05 as compared to group I

Table 4: Effect of gentamicin, gentamicin+calcium (0.5 g/kg/day), gentamicin+calcium (1.0 g/kg/day) and gentamicin+verapamil (7 mg/kg/day) on histopathology in rabbit kidney

Histopathology scores	0	1	2	3	4	Mean rank
Group II (*n*=6) gentamicin 80 mg/kg, i.m.	0	0	0	0	6	9.5
Group V (*n*=6) gentamicin 80 mg/kg/day, i.m.+ calcium 0.5 g/kg, p.o.	2	4	0	0	0	4.5[a]
Group VI (*n*=6) gentamicin 80 mg/kg/day, i.m.+ calcium 1.0 g/kg/day, p.o.	5	1	0	0	0	4.0[b]
Group VII (*n*=6) gentamicin 80 mg/kg/day, i.m.+ verapamil 7 mg/kg/day, i.m.	0	0	0	1	5	7.42

[a,b]*P*<0.05 as compared to group II

Rats

Effect on BUN, serum creatinine, urinary proteins, and kidney SOD

The levels of BUN, serum creatinine, urinary proteins, and kidney SOD were compared between the seven groups on day 7 after 6 days of consecutive treatment with respective drugs in a manner similar to those in rabbits. The baseline values were similar in all groups on day 0. The results were identical to results obtained in rabbits with group II (gentamicin only) showing a significant elevation ($P < 0.05$) of BUN, serum creatinine, urinary proteins, and significant reduction ($P < 0.05$) of kidney SOD when

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compared to control group [Table 5]. Groups III and IV showed no significant change in above parameters when compared to control group [Table 5] while groups V and VI showed a dose dependent effect on above parameters with group VI (calcium 1.0 g/kg/day) showing a more significant reduction ($P < 0.05$) of BUN, serum creatinine, and urinary proteins when compared to group V (calcium 0.5 g/kg/day) [Table 6]. The levels of kidney SOD were also raised significantly by calcium 1.0 g/kg/day (group VI) when compared to calcium 0.5 g/kg/day (group V). Concomitant administration of verapamil (group VII) had no significant effect on above parameters when compared to group V and group VI or group II [Table 6].

Histopathological effects

The renal histopathological picture results were also identical to that observed in rabbits with groups V and VI showing a significant reversal of tubular necrosis and intact basement membrane when compared to group II [Table 7] which showed a histopathological score of 4 suggestive of tubular necrosis and glomerular basement membrane damage. Concomitant administration of verapamil (group VII) failed to show any significant effect when compared to groups V and VI and the scores were almost identical to scores in group II [Table 8].

DISCUSSION

This study was conducted to compare the effects of different dosages of calcium and verapamil on gentamicin-induced nephrotoxicity in rats and rabbits to find out if there was any species-sensitive variation in results and to further enhance the evidence for protective or nephrotoxic effect of calcium and verapamil based on contradictory results in some previous studies.

Gentamicin 80 mg/kg/day i.m. for six days in rats or rabbits is known to produce morphological and biochemical alterations in kidneys similar to the manifestations in human kidney.[20] Hence, gentamicin was used in our study for induction of nephrotoxicity in rats and rabbits in similar dose and duration. In clinical settings, acute renal failure is diagnosed on the basis of BUN and serum creatinine. They are considered as the most reliable and feasible markers of renal function among some other renal parameters.[21] Therefore BUN, serum creatinine, and urinary proteins were estimated for measurement of kidney function in our study. Another hypothesis suggests that renal damage is due to various apoptotic signals activated within the renal tissue due to free radical generation.[22] This can be best assessed by measuring various lysosomal enzymes like reduced glutathione, SOD, malondialdehyde, and histopathological changes. Hence, we also assessed superoxide dismutase levels and histopathological changes as parameters for measuring renal toxicity. These parameters have been successfully used by various researchers in previous studies.[23]

The results indicated in both rats and rabbits, calcium and verapamil when administered alone for six days in groups III and IV, respectively, did not increase the BUN,

Table 5: Effect of normal saline, gentamicin, calcium, and verapamil on blood urea nitrogen (BUN), serum creatinine, urinary proteins, and kidney superoxide dismutase (SOD) in rats

Parameters	Mean±SD			
	Group I (control, normal saline, 2.0 ml/kg)	Group II (gentamicin 80 mg/kg/day, i.m.)	Group III (calcium 1.0 g/kg/day, p.o.)	Group IV (verapamil 7.0 mg/kg/day, i.m.)
BUN (mg/dl)	19.05±1.5306	131.0833±3.8654[a]	19.8167±2.0301	20.2167±1.3075
Serum creatinine (mg/dl)	0.3433±0.0445	3.9317±0.1406[a]	0.2917±0.0337	0.2750±0.0378
Urinary proteins (mg/dl)	0.1717±0.06795	1.3017±0.2185[a]	0.1567±0.0937	0.1583±0.09786
Kidney SOD (U/ml)	0.2083±0.0183	0.5283±0.0421[a]	0.2117±0.0204	0.2100±0.0253

[a]*P*<0.05 as compared to group I. SOD = Superoxide dismutase levels, BUN = Blood urea nitrogen, SD = Standard deviation

Table 6: Effect of gentamicin, gentamicin+calcium (0.5 g/kg/day), gentamicin+calcium (1.0 g/kg/day), and gentamicin+verapamil (7 mg/kg/day) on blood urea nitrogen (BUN), serum creatinine, urinary proteins, and kidney superoxide dismutase (SOD) in rats

Parameters	Mean±SD			
	Group II (gentamicin 80 mg/kg/day, i.m.)	Group V (gentamicin 80 mg/kg/day, i.m.+ calcium 0.5 g/kg/day, p.o.)	Group VI (gentamicin 80 mg/kg/day, i.m.+ calcium 1.0 g/kg/day, p.o.)	Group VII (gentamicin 80 mg/kg/day, i.m.+ verapamil 7.0 mg/kg/day. i.m.)
BUN (mg/dl)	131.0833±3.8654	95.4167±4.4803[a]	21.5167±1.8819[b,c]	126.7833±3.2220
Serum creatinine (mg/dl)	3.9317±0.1406	1.4417±0.3464[a]	0.2917±0.0292[b,c]	3.8867±0.1686
Urinary proteins (mg/dl)	1.3017±0.2185	0.7533±0.0752[a]	0.1383±0.0466[b,c]	1.4650±0.1243
Kidney SOD (U/ml)	0.5283±0.0421	0.4067±0.0196[a]	0.2317±0.0147[b,c]	0.5200±0.03688

[a,b]*P*<0.05 as compared to group II, [c]*P*<0.05 as compared to group V. SOD = Superoxide dismutase levels, BUN = Blood urea nitrogen, SD = Standard deviation

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Table 7: Effect of normal saline, gentamicin, calcium, and verapamil on histopathology in rat kidney

Histopathology scores	0	1	2	3	4	Mean rank
Group I (*n*=6) normal saline 2.0 ml/kg/day	6	0	0	0	0	6.5
Group II (*n*=6) gentamicin 80 mg/kg/day, i.m.	0	0	0	0	6	9.5[a]
Group III (*n*=6) calcium 1.0 g/kg/day, p.o.	6	0	0	0	0	6.5
Group IV (*n*=6) verapamil 7 mg/kg/day, i.m.	6	0	0	0	0	6.5

[a]*P*<0.05 as compared to group I

Table 8: Effect of gentamicin, gentamicin+calcium (0.5 g/kg/day), gentamicin+calcium (1.0 g/kg/day), and gentamicin+verapamil (7 mg/kg/day) on histopathology in rat kidney

Histopathology scores	0	1	2	3	4	Mean rank
Group II (*n*=6) gentamicin 80 mg/kg/day, i.m.	0	0	0	0	6	9.5
Group V (*n*=6) gentamicin 80 mg/kg/day, i.m.+ calcium 0.5 g/kgday, p.o.	3	3	0	0	0	5[a]
Group VI (*n*=6) gentamicin 80 mg/kg/day, i.m.+ calcium 1.0 g/kg/day, p.o.	5	1	0	0	0	4.0[b]
Group VII (*n*=6) gentamicin 80 mg/kg/day, i.m.+ verapamil 7 mg/kg/day, i.m.	0	0	0	1	5	7.42

[a,b]*P*<0.05 as compared to group II

serum creatinine, urinary proteins, or reduced kidney SOD levels, indicating that these drugs are not nephrotoxic by themselves. On the other hand, gentamicin markedly increased the BUN, serum creatinine, urinary proteins, and reduced the kidney SOD levels when administered for six days in group II in either rats or rabbits. These results were consistent with previously conducted studies in which gentamicin has been shown to be highly nephrotoxic.[24] Gentamicin markedly enhances the generation of reactive oxygen metabolites in the mitochondria. These free radicals cause lipid peroxidation and also react chemically with cellular macromolecules including proteins and nucleic acids.[25] Histopathological changes of grade 4 suggestive of tubular necrosis and glomerular damage were also seen in gentamicin-treated rats or rabbits in group II confirming the nephrotoxic potential of gentamicin observed in earlier studies.[26]

In our study we found that low doses of calcium, i.e. 0.5 g/kg/day were able to prevent nephrotoxicity induced by gentamicin in a significant manner when compared to group II animals administered gentamicin alone. There was a significant reduction in BUN, serum creatinine, urinary proteins, and elevation of kidney SOD levels as compared to rats or rabbits given gentamicin alone. Histopathological examination also showed less tubular and glomerular damage as compared to rats or rabbits given gentamicin alone. Together there was no species sensitive variation in amelioration of gentamicin-induced nephrotoxicity. There have been few studies done to compare nephrotoxic effects of gentamicin between the species[27] and the results have been indifferent to our study.

Calcium in a high dose of 1.0 g/kg/day along with gentamicin showed significant reduction in BUN, serum creatinine, urinary proteins, and elevation of kidney SOD in group V as compared to gentamicin alone in both species. The histopathological examination also showed normal architecture in animals co-treated with calcium 1.0 g/kg/day and gentamicin. The dose of calcium 1.0 g/kg/day was significantly more efficacious than calcium 0.5 g/kg/day in normalizing the deranged biochemical parameters and preventing the morphological alterations in tubules and glomerulus of kidney. These results were similar to an earlier study conducted showing a dose dependent protective effect of calcium.[15] Some workers have demonstrated that oral Ca^{2+} load markedly reduces the gentamicin-induced renal failure in humans and rats.[28] Others however have failed to document a Ca^{2+}-induced amelioration in gentamicin nephrotoxicity in rats[24] while still others have reported Ca^{2+} to potentiate acute renal failure in human beings treated with gentamicin.[29] Calcium has nephroprotective action by either alleviating functional hemodynamic alterations at the glomerular level or preventing structural cellular damage at the tubular level. Others suggest that the protective action of Ca^{2+} may be mediated either (a) by inducing metabolic changes during oral Ca^{2+} loading,[30] (b) by slowing renal cortical accumulation, or (c) by enhancing the excretion of gentamicin.[31]

In this study, we did not find any change in the BUN, serum creatinine, urinary proteins, and SOD levels in rats and rabbits administered with verapamil on gentamicin. Further histopathology showed many areas of severe focal necrosis and tubular damage in verapamil-treated nephrotoxic animals similar to gentamicin group. Thus, we did not find verapamil offering any protective effect in reducing gentamicin-induced nephrotoxicity. In addition, there was no significant increase in the toxicity on concomitant verapamil and gentamicin administration indicating that verapamil does not potentiate the nephrotoxic effects of gentamicin similar to the results of a previous study[32] but unlike the study carried out by Ali *et al*., 2002[15] which showed verapamil to enhance the effects of gentamicin. The reasons for the discrepancies are not clear but may be related to the conditions of experiment, animal strain used, dose employed, or to other reasons.

CONCLUSION

The administration of calcium 1.0 g/kg/day is more efficacious than calcium 0.5 g/kg/day in preventing gentamicin-induced nephrotoxicity in rats and rabbits. Further, there is no species sensitive variation in results that could be extrapolated to humans. Verapamil has neither nephroprotective nor nephrotoxic effect.

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