EFFECT OF SIMVASTATIN IN GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

V. CHINNAPA REDDY1*, V. AMULYA, CH. ANUSHA LAKSHMI1, D. BALA PRAVEEN KUMAR REDDY1, D. PRATIMA, A. THANGA THIRUPATHI 1, K. PAVAN KUMAR2, S. SENGOTTUVELU2

1Sankaralingam Bhuvaneshwari College of Pharmacy, Department of Pharmacy, Sivakasi-626 130, TamilNadu, India, 2Nandha College of Pharmacy and Research Institute, Department of Pharmacy, Erode -638 052, TamilNadu, India. E-mail: chinnapreddy2020@gmail.com

Received: 25 August 2011, Revised and Accepted: 3 October 2011

ABSTRACT
Gentamicin (GM) is an aminoglycosidal antibiotic widely used in treating severe gram-negative infections. However, its limited uses due to renal dysfunction. The aim of the present work was to investigate the possible protective effect of the simvastatin, an anti-hyperlipidemic drug on gentamicin induced nephrotoxicity. For this purpose, albino rats were selected and divided into four groups, each group comprised of six albino rats. Group 1 served as normal control received vehicle, Group 2 was injected with gentamicin (80 mg/kg/day) intraperitoneally. Group 3 administered with simvastatin alone (10 mg/kg/day) orally and the group 4 animals received gentamicin (80 mg/kg/day) intraperitoneally and simvastatin (10mg/kg/day) orally. All the test drugs were administered for 10 days. On 10th day blood was collected and serum was separated for the estimation of blood urea nitrogen, serum creatinine, and protein contents. Then the rats were sacrificed and kidneys were removed for histopathological studies. Moreover, glutathione (GSH), and thiobarbituric acid relative substances (TBARS) levels activities were determined in renal tissues. The results showed that concomitant administration of simvastatin significantly reduced gentamicin induced elevated levels of serum creatinine, blood urea and nitrogen in albino rats. There was significant decrease in GSH levels and increase in TBARS levels, indicated that GM-induced nephrotoxicity was mediated through oxidative stress reactions. Histopathological examination of GM-treated rats revealed degenerative changes in glomeruli and tubules. On the other hand, simultaneous administration of simvastatin plus gentamicin protected kidney tissues against nephrotoxic effects of gentamicin as evidenced from amelioration of histopathological changes and normalization of kidney biochemical parameters.

Key Words: Gentamicin, Simvastatin, Nephrotoxicity, Albino rats.

INTRODUCTION
Gentamicin an aminoglycoside antibiotic is used against Gram negative bacteria. However, the use of gentamicin is associated with nephrotoxicity that limit its frequent use. The gentamicin induced nephrotoxicity involves renal oxidative stress, which is accompanied with reduction in renal antioxidant defence mechanisms. In addition, induction of acute tubular necrosis, glomerular damage and renal inflammation are the major events implicated in gentamicin nephrotoxicity1,2. Gentamicin induces lysosomal phospholipidosis that disrupts normal renal function3. It is evidenced that the renal accumulation of gentamicin is implicated in the induction of nephrotoxicity4. Simvastatin has been shown in cultured renal proximal tubule cells to inhibit gentamicin accumulation and cytotoxicity5. Numerous studies demonstrated that simvastatin has an ability to protect renal structure and function due to its additional properties such as anti-inflammatory, anti-fibrotic and anti-oxidant effects. Oxidative stress plays a key role in gentamicin induced nephrotoxicity. It has been reported that simvastatin reduced renal nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) dependent superoxide production and oxidative stress6. Simvastatin was reported to attenuate renal injury in a self-sensitive hypertension model7. Further, simvastatin has been shown to protect against renal injury in rat model of obesity and hypertension8. In addition, simvastatin protected diabetic mice by reversing podocyte injury9. Moreover, simvastatin has been shown to reverse high glucose induced loss of mesangial cells10. Though the in vitro potential of protecting renal cells against gentamicin-induced renal toxicity has been demonstrated the in vivo renoprotective effect of simvastatin against gentamicin induced nephrotoxicity is not known11.

Therefore the present study has been designed to investigate the effect of simvastatin, a lipophilic statin, in gentamicin induced nephrotoxicity in albino rats.

MATERIALS AND METHODS
Materials
Gentamicin and Simvastatin was obtained as gift samples from Alembic Laboratories Pvt. Ltd., Vadodara, and Ranbaxy Laboratories, Gurgaon, India respectively. All other chemicals used in this study were of analytical grade which was obtained from HIMEDIA, Mumbai, India.
Estimation of Reduced Glutathione

The GSH level in the kidney was estimated using the method described by Ellman. Briefly, the renal homogenate was mixed with 10% w/v trichloroacetic acid in ratio of 1:1 and centrifuged at 4°C for 10 min at 5000 rpm. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of distilled water. Then 0.25 ml of 0.001 M freshly prepared DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) dissolved in 1% w/v sodium citrate) was added. The reaction mixture was incubated for 10 min and absorbance of yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using reduced form of glutathione.

**Histopathological Examination**

The kidneys were sectioned longitudinally into two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. These sections were stained with hematoxylin and eosin and were observed under a computerized light microscope.

**Statistical Analysis**

All values were expressed as mean ± S.D. The data obtained from various groups were statistically analysed using one way ANOVA, followed by Turkey’s multiple comparison test. The P value of less than 0.05 was considered statistically significant.

**RESULTS**

Administration of simvastatin did not produce any significant per se effect on various parameters assessed in normal albino rats.

**Effect of Simvastatin on Serum Creatinine, Blood Urea and Urea Nitrogen**

FIG. 1, 2 and 3 demonstrates the effect of simvastatin about the serum creatinine, blood urea and urea nitrogen. There was marked increase in serum creatinine was noted in gentamicin administered albino rats as compared to normal albino rats. In addition, the blood urea and nitrogen urea were noted to be increased in gentamicin administered albino rats. However, concomitant administration of simvastatin significantly reduced gentamicin-induced elevated levels of serum creatinine and blood urea and urea nitrogen in albino rats.

**Effect of Simvastatin on Proteinuria**

FIG. 4 shows the effect of Simvastatin in gentamicin administered albino rats and it showed marked induction of proteinuria as compared to normal albino rats. However, the concurrent administration of simvastatin significantly reduced the incidence of proteinuria in albino rats administered gentamicin.

**Effect of Simvastatin on Renal Oxidative Stress**

FIG. 5 and 6 suggests about the marked increase in TBARS was noted in gentamicin administered albino rats as compared to normal albino rats. In addition, the renal concentration of GSH was noted to be decreased in gentamicin administered albino rats as compared to normal albino rats. The concomitant administration of simvastatin significantly prevented gentamicin-induced increase in renal TBARS and decrease in renal GSH.

**Histopathological studies**

In histopathological study kidneys of normal control albino rats showed normal tubular epithelial cells and glomeruli (FIG. 7 a). In GM group, 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 (FIG. 7 b). In kidneys of GM and simvastatin treated rats, there was mild proximal tubular necrosis were observed which indicates simvastatin has reversed nephro toxicity induced by Gentamycin (FIG. 7 c). In simvastatin alone treated animals, the normal integration of cellular structures were maintained which reveals that simvastatin is safer to kidneys (FIG. 7 d).
DISCUSSION

The elevated levels of serum creatinine, urea and urea nitrogen, and the urinary excretion of protein (proteinuria) have been suggested to be an index of renal damage and dysfunction\(^9\). The ability of the kidney to filter creatinine (a non-protein waste product of creatinine phosphate metabolism) is reduced during renal dysfunction as a result of diminished glomerular filtration rate. Thus, the increase in serum creatinine level is an indication of renal dysfunction\(^20\).

Moreover, the elevated levels of blood urea and urea nitrogen occur during renal dysfunction. The incidence of proteinuria is associated with glomerulosclerosis and tubulointerstitial fibrosis\(^21\). In the present study, the gentamicin administration in albino rats increased the level of serum creatinine. In addition, the blood urea and urea nitrogen levels were increased in gentamicin administered albino rats as compared to normal albino rats. Furthermore, the elevated level of urea protein was noted in gentamicin-administered albino rats as compared to normal albino rats. These results suggest the development of renal damage and renal dysfunction in albino rats administered gentamicin, which was consistent with earlier studies of others\(^22\)-\(^24\).

The concurrent administration of simvastatin prevented the elevated level of serum creatinine in albino rats administered gentamicin. In addition, simvastatin treatment markedly reduced gentamicin-induced increase in blood urea and urea nitrogen in albino rats. Moreover, the elevated level of urinary protein was significantly reduced by simvastatin treatment in albino rats administered gentamicin. These results suggest that simvastatin has an ability to prevent gentamicin-induced nephrotoxicity in albino rats.

![Graphs showing the effect of simvastatin on gentamicin-induced increase in various parameters](image-url)

Fig: Effect of Simvastatin on Gentamicin induced increase in (1) Serum creatinine in albino rats, (2) Blood urea in albino rats, (3) Blood urea nitrogen in albino rats, (4) Urinary protein (proteinuria), (5) Renal TBARS in albino rats and (6) Renal GSH in albino rats.

All values are represented as mean ± S.D. a, P< 0.05 versus normal control; b, P<0.05 versus gentamicin control.
Oxidative stress plays a major role in gentamicin-induced nephrotoxicity. The increase in lipid peroxidation as assessed in terms of estimating TBARS and consequent decrease in reduced form of glutathione are the indicators of oxidative stress. In the present study, the renal TBARS has been noted to be increased in gentamicin-administered albino rats. In addition the reduction in renal GSH in gentamicin-administered rats was noted. These results suggest the induction of renal oxidative stress in albino rats administration gentamicin. However, treatment with simvastatin markedly prevented renal oxidative stress induced by gentamicin in albino rats by reducing renal TBARS and increasing renal GSH. Accordingly, it may be suggested that the pleiotropic anti-oxidant effect of simvastatin may have played a key role in preventing the gentamicin-nephrotoxicity.

CONCLUSION

The present study investigated the effect of simvastatin, a lipophilic statin, in gentamicin-induced nephrotoxicity in albino rats. It suggests that simvastatin has an ability to halt the development of gentamicin induced nephrotoxicity in albino rats. Simvastatin may prevent gentamicin nephrotoxicity in albino rats by reducing renal oxidative stress.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Sankaralingam Bhavaneshwari College of Pharmacy, Sivakasi and Nandha College of Pharmacy and Research Institute, Erode, Tamilnadu, India.

REFERENCES

19. Finco DR, Duncan JR. Evaluation of blood urea nitrogen and serum creatinine concentrations as indicators of renal...


