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Research Article

EFFECT OF SIMVASTATIN IN GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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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-hypolipidemic 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. On10th 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 are concomitant administration of simvastatin significantly reduced gentamicin induced elevated levels of serum creatinine, blood urea and urea 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 nephrotoxicity[1, 2]. Gentamicin induces lysosomal phospholipidosis that disrupts normal renal function[3]. It is evidenced that the renal accumulation of gentamicin is implicated in the induction of nephrotoxicity[4]. Simvastatin has been shown in cultured renal proximal tubule cells to inhibit gentamicin accumulation and cytotoxicity[5]. 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 stress[6]. Simvastatin was reported to attenuate renal injury in a self-sensitive hypertension model[7]. Further, simvastatin has been shown to protect against renal injury in rat model of obesity and hypertension[8]. In addition, simvastatin protected diabetic mice by reversing podocyte injury[9]. Moreover, simvastatin has been shown to reverse high glucose induced loss of mesangial cells[10]. 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 known[5].

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.

Animals

Age matched young albino rats weighing about 100-150 g were employed in the present study. Rats were fed on standard chow diet and water *ad libitum.* The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. They were acclimatized in institutional animal house and were exposed to normal cycles of day and night.

Pharmacological Study[11]

Four groups were employed in the present study and each group comprised six albino rats. Gentamicin (80 mg/kg/day) intraperitonially was administered for 10 days to induce experimental nephrotoxicity in rats. Simvastatin was suspended in 0.5% sodium carboxymethylcellulose immedietly before administration. Group 1 served as normal control and received standard food and water throughout the experiment. Group 2, was injected with gentamicin (80 mg/kg/day) intraperitonially, Group 3 administered with simvastatin alone (10mg/kg/day) orally and Group 4 animals were administered gentamicin (80 mg/kg/day) intraperitonially along with simvastatin (10mg/kg/day) orally and the treatment was started 2 days prior to gentamicin administration. The treatment periods for all these groups were 10 days. On 10th day under mild ether anaesthesia, blood was collected by sinus puncture and serum was separated for the estimation of blood urea nitrogen, serum creatinine, and proteinuria. Then the rats were sacrificed and kidneys were removed for histopathological studies. Moreover, glutathione (GSH), and thiobarbituric acid relative substance (TBARS) levels were determined in renal tissues.

ASSESSMENT OF NEPHROTOXICITY

Estimation of Serum Creatinine

The serum creatinine concentration was estimated by alkaline picrate method[12] using the commercially available kit. Briefly 2.0ml of picric acid reagent in a tube was added to 0.2ml of serum for deproteinization of specimen, which was mixed well and centrifuged at 3000 rpm to obtain a clear supernatant. 100 µl of buffer reagent was added to 1.1 ml of supernatant, 0.1 ml of standard creatinine and 0.1 ml of distilled water to prepare test, standard and blank, respectively. 1.0 ml of picric acid reagent was added to blank and standard. The test tubes were mixed well and kept at room

temperature for 20 minutes. The alkaline picrate reacts with creatinine to form the orange colored complex, which was read at 520 nm spectrophotometrically.

$$\text{The serum creatinine concentration (mg/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 2$$

Estimation of Blood Urea and Urea Nitrogen

The blood urea was estimated by Berthelot method[13] using the commercially available kit. 1000 µl of working reagent-1 containing urease reagent, and a mixture of salicylate, hypochlorite and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the tubes were mixed well and incubated at 38 °C for 5 min. then 1000 µl of reagent-2 containing alkaline buffer, was added to all the test tubes, which are incubated at 38 °C for 5 min. Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia thus released reacts with a mixture of salicylate, hypochlorite and nitropruside to yield indophenol, a blue-green colored compound. The intensity of the color produced is directly proportional to the concentration of urea in the sample and is measured spectrophotometrically at 578 nm. The blood urea was calculated using the formula:

$$\text{Blood urea (mg/ml)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 40$$

Blood urea nitrogen (mg/dl) = serum urea ×0.467

Estimation of Protein in Urea

The proteinuria was assessed by pyrogallol red method[14] using the commercially available kit. 1000 µl of reagent (pyrogallol dye) was added to 10 µl of urine sample, 10 µl of standard protein and 10 µl of purified water to prepare test, standard and blank respectively. All the test tubes were mixed and incubated at 38 °C for 10 min. The absorbances of test and standard samples were noted against blank at 600 nm spectrophotometrically. When the pyrogallol red-molybedate complex binds to basic amino group of protein molecules, there is a shift in reagent absorbance. The absorbance is directly proportional to protein concentration present in the sample. The urinary protein was calculated using the formula

$$\text{Urinary protein concentration (mg/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 100$$

ASSESSMENT OF RENAL OXIDATIVE STRESS

The development of oxidative stress in the kidney was assessed by estimating renal thiobarbituric acid reactive substances (TBARS) and reduced form glutathione (GSH).

Preparation of Renal Homogenate

The kidney was dissected and washed with ice cold isotonic saline and weighed. The kidney was then minced, and a homogenate (10% w/v) was prepared in chilled 1.15% KCl. The homogenate wasused for estimating thiobarbituric acid reactive substances (TBARS), GSH and total protein.

Estimation of Thiobarbituric Acid Reactive Substances

The TBARS is an index of lipid peroxidation[15], and was estimated by mixing 0.2ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA). The reaction mixture was made upto 4.0 ml with distilled water, and then heated in water bath at 95 °C for 60 minutes. After cooling in tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) were added to reaction mixture and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. The standard curve using 1, 1, 3, 3-tetramethoxypropane was plotted to calculate the concentration of thiobarbituric acid relative substances.

Estimation of Reduced Glutathione

The GSH level in the kidney was estimated using the method described by Ellman[16]. 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 (5,5¹- dithiobis (2-nitrobenzoic acid)) dissolved in 1% w/v sodium citrate) was added. The reaction mixture was incubated for 10 min and absorbence 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 hemotoxylin and eosin and were observed under a computerized light microscope[17].

Statistical Analysis

All values were expressed as mean ± S.D. The data obtained from various groups were statistically analysed using oneway ANOVA, followed by Turkey's multiple comparison test[18]. The P value of less than 0.05 was considered statistically significant.

RESULTS

Administration of simvastain 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 administerd 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[19]. 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 diminnished glomerular filteration 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.

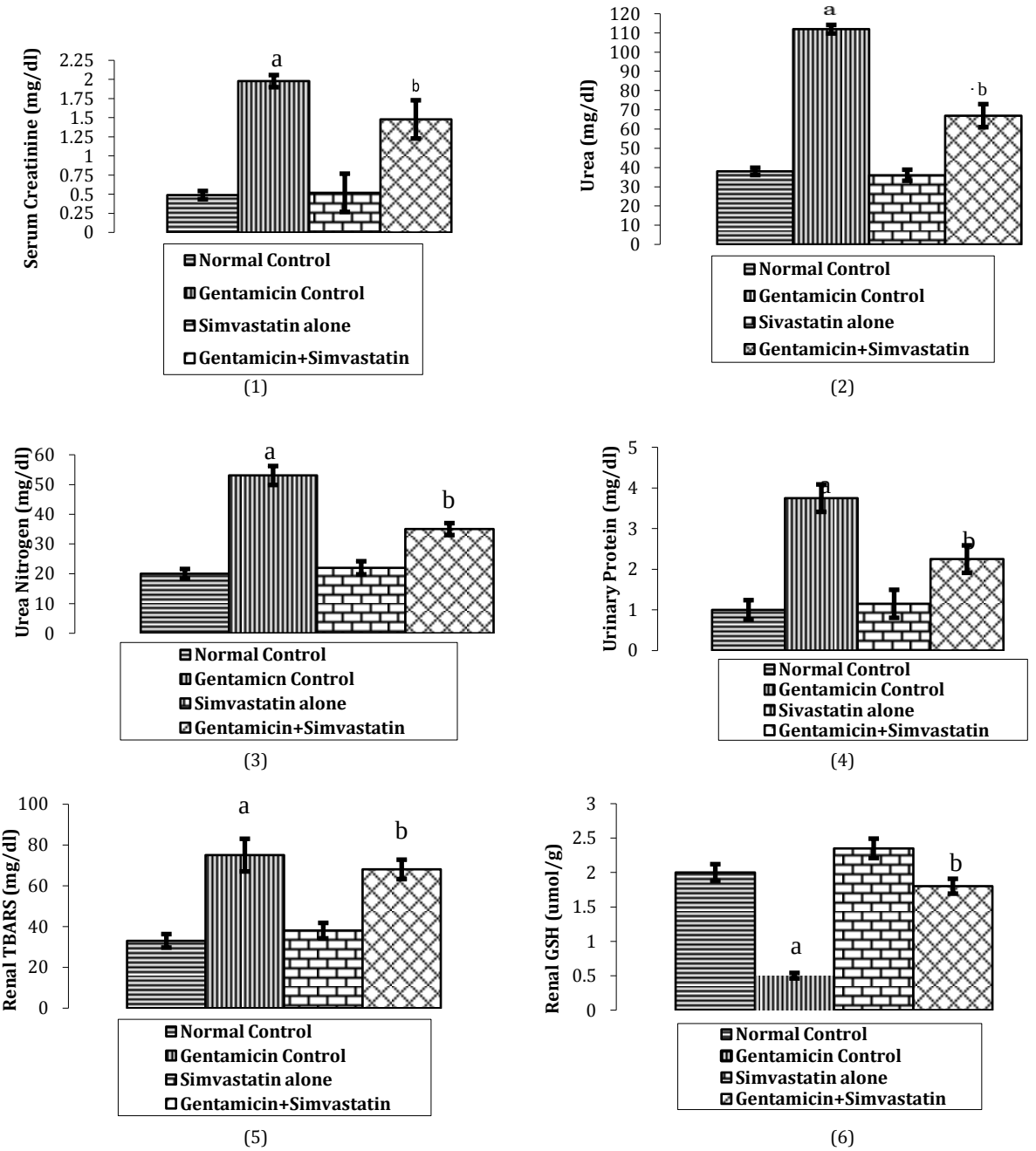

Fig: Effect of Simvastatin on Gentamicin induced increase in (1) Serum creatinine in albino rats, (2) Blood urea in albino rats, (3) Blood urea nitrogen in albino rats, (4) Urinary protein (proteinuria), (5) Renal TBARS in albino rats and (6) Renal GSH in albino rats.

All values are represented as mean ± S.D. a, P< 0.05 versus normal control; b, P<0.05 versus gentamicin control

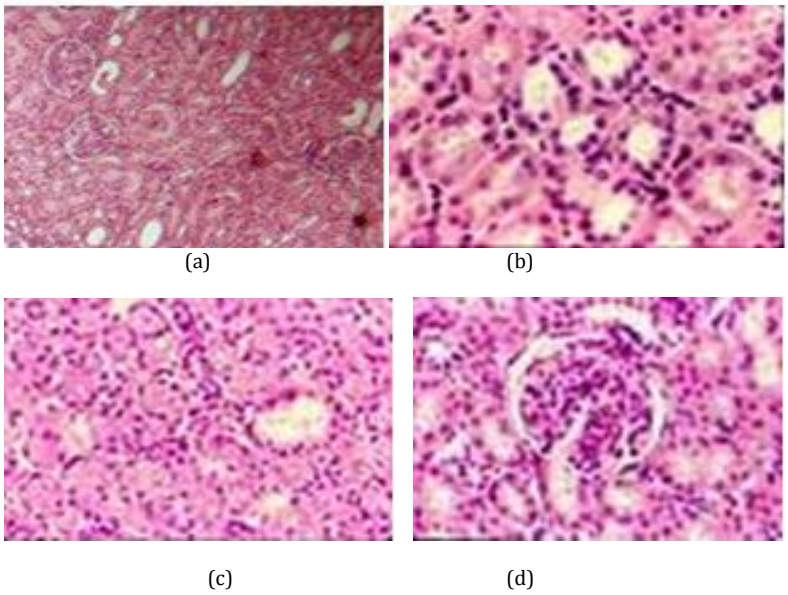

Fig 7: Histopathological studies shows the effect of simvastatin on gentamicin nephrotoxicity (a) Normal Control, (b) Gentamicin treated alone, (c) Gentamicin + Simvastatin (d) Simvastatin alone.

Oxidative stress plays a major role in gentamicin-induced nephrotoxicity[25]. 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[26]. 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.

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