ORIGINAL ARTICLE

Effect of Silymarin on Gentamicin Induced Nephrotoxicty in Albino Rats

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ABSTRACT

Aim: To observe morphological and biochemical changes in the kidneys of gentamicin treated rats and their treatment with silymarin

Design: Experimental study

Place and duration of study: Experimental research laboratory, University of Health sciences, Lahore. Duration of study was one year.

Methods: 24 albino rats 6-8 weeks old weighing 175-200 grams were divided into three groups having 8 animals each. Group A served as control and was given 1ml distilled water intraperitoneally for 8 days. While group B was given 100mg/kg body weight of gentamicin dissolved in 1ml distilled water intraperitoneally for 8 days and group C served as the experimental group and was given 100mg/kg body weight of gentamicin dissolved in 1ml distilled water intraperitoneally for 8 days followed by 200mg/k/day silymarin suspended in 1ml distilled water for 15 days At the end of experimental period; animals were anesthetized. Blood samples were drawn by cardiac puncture for biochemical analysis, and the kidneys were removed for histological examination.

Results: The histological picture of kidneys showed loss of brush border, pyknosis, karyolysis, and presence of protein casts in lumen of proximal convoluted tubules along with a significant rise in serum urea and creatinine levels indicating decline in kidney function. On treatment with silymarin there was significant improvement in the above mentioned parameters

Conclusion: Silymarin reversed the damage caused to kidneys by gentamicin, as observed by histological and biochemical parameters.

Keywords: Gentamicin, proximal convoluted tubule, urea creatinine.

INTRODUCTION

Aminoglycosides are a class of antimicrobial agents which are protein synthesis inhibitors. Streptomycin, neomycin, kanamycin, amikacin, gentamicin, tobramycin and netilmicin belong to this class[1].

Gentamicin is an aminoglycoside antibiotic derived from a bacterium, Micromonospora purpurea[2]. It is commonly used to treat infections caused by aerobic gram negative bacteria. It acts by irreversibly inhibiting protein synthesis. It passes through the outer membrane of bacteria by passive diffusion through porin channels. It is then transported to the cytoplasm passing through the cell membrane by an oxygen dependent process[1]. Once inside the cell, gentamicin binds to the 30S subunit of bacterial ribosomes and interferes with protein synthesis by making the ribosomes misread the genetic code, preventing the process of polysome disaggregation and assembling[3].

Gentamicin is poorly absorbed from the gastrointestinal tract; if administered orally it is entirely excreted in feces. The preferred route of administration is intramuscular, in which case peak concentration is reached in 30–90 minutes; it can also be given intravenously. Gentamicin shows bactericidal effect directly related to its blood concentration levels. When administered as a single large dose it showed strong bactericidal effect as compared to when given in small divided doses[1].

Due to its polar nature Gentamicin is not uniformly distributed in most tissues of the body and is reported to be present in significant quantity in body secretions. However it tends to accumulate in the renal cortex and in the endolymph and perilymph of inner ear, where high concentrations of gentamicin may be found (4). Half-life of gentamicin in serum of normal individuals is 2–hours. It might increase to 24–48 hours if the renal function is significantly impaired as it is mainly excreted by the kidneys[1].

Gentamicin penetrates the cell membranes poorly; however the proximal tubular cells of kidneys tend to accumulate the drug many fold as compared to the plasma. Gentamicin binds to anionic phospholipids of the cell membrane and enters the cell. Once inside the cell it binds to organelles and accumulates in the lysosomes. Thus the

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accumulation of gentamicin within the renal cortex is reported to be related intimately to its nephrotoxicity[5]. Gentamicin acts as an iron chelator. The drug iron complex leads to the generation of reactive oxygen species causing cell damage[6]. It damages membranes of cells and organelles causing increase in their permeability. Damage to lysosomal membranes results in release of acid hydrolases in the cytoplasm leading to cell damage[7]. Accordingly acute renal failure ensues following necrosis of proximal tubular cells[8]. Serum urea and creatinine levels rise due to the failure of kidney to function properly[9].

MATERIALS AND METHODS

This study was approved from Ethical Review Committee of the University of Health and Sciences, Lahore. Sixteen albino rats, 6-8 weeks of age were procured from University of Health Sciences Lahore. They were weighed and their blood samples were collected, from tail vein, for estimation of serum urea and creatinine levels before starting the experiment. They were housed under controlled room temperature (23±2°C), humidity (50±5%) and light and dark cycles of 12 hours each. The animals were fed on standard rat diet and water ad- libitum and allowed to acclimatize for four days before start of experiment.

Animals were randomly divided into two groups, having 8 animals each. Group A served as control and was given 1 ml distilled water intraperitoneally for 8 days. Group B was given gentamicin 100mg/kg/day dissolved in 1ml distilled water intraperitoneally for 8 days[10].

On the 9th day each rat was anaesthetized using chloroform and a blood sample from each rat was collected by cardiac puncture for serum urea and creatinine estimation. Each animal was then sacrificed. The kidney of each animal was removed for gross and histological examination. The details of macroscopic features including color, lobularity and weight of kidneys were recorded. Kidneys were cut longitudinally into two pieces and fixed in 10% formalin solution for 48 hours. Each kidney piece was then placed separately in a single tissue cassette that was labeled for identification. The kidney pieces were processed in an automatic tissue processor for 18 hours and were dehydrated by passing them through ascending grades of alcohol using 50%, 70%, 90% and absolute ethanol, cleared in xylene and infiltrated with paraffin wax of melting point 56-58^{0}C. The tissue cassettes were removed from processor and tissues pieces were taken out of the cassettes for embedding. Paraffin block was prepared by placing the tissue piece in a metal trough. The blocks were transferred to the freezer 15 minutes before sectioning. Tissue blocks were trimmed to the size, keeping upper and lower margins parallel. These were then fixed firmly in the microtome chuck. Five micron thick sections were obtained using Leica RM 2125 rotary microtome and stained with hematoxylin and eosin stains. Microscopic parameters included brush border, cytoplasmic vacuolation, presence of intraluminal protein casts, desquamation of epithelial cells, pyknosis and karyolysis and were recorded for proximal convoluted tubules.

At least 10 randomly selected non-overlapping fields per section were used. The results were graded as the percentage of the damage, as previously described by Erdem et al[5]. The tubules were regarded as normal (-) which showed no damage to the tubules, mild (+) when less than quarter of the total number of tubules showed damage (<25 %). The damage was considered moderate (++) when less than half of the tubules showed damage (26-50 %) and severe (+++) when more than half of the tubules showed damage (>50 %).

Data was analyzed using SPSS version 21. Mean±SD was given for quantitative data and frequency and percentages were given for qualitative data. One way ANOVA and Post Hoc Tukey's test were performed for quantitative data and Fisher's Exact test was performed for qualitative data. P value of ≤0.05 was considered statistically significant.

RESULTS

On gross examination the kidneys of both groups appeared reddish brown with smooth surface.

Mean weight of animals of group A at the start and end of experiment was 179.63±61.6 gm and 203 ± 5.39 gm respectively and of group B was 184.13±9.35 gm and 158.75±4.20 gm respectively. There was a significant decrease in mean body weight (p value <0.001) in group B as compared to group A at the end of experimental period.

When mean weight of both right and left kidneys combined was compared among groups A and B, the difference was statistically insignificant. P value = 0.654

In groups A and B, the values of mean diameter of renal corpuscles were 63.66±2.37, 82.52±2.68 respectively. There was a statistically significant increase in size of renal corpuscle (p value <0.001) in group B when compared to group A.

The proximal convoluted tubules of kidneys in animals of group A showed normal epithelium with a prominent brush border. Fig. 1. While the proximal convoluted tubules in group B exhibited, patchy loss of brush border, pyknotic and karyolitic nuclei, intraluminal protein casts and desquamated epithelial

cells in their lumina. Fig. 2. Fisher's exact test showed statistically significant association between groups regarding percentages of above mentioned histological parameters ($p < 0.001$).

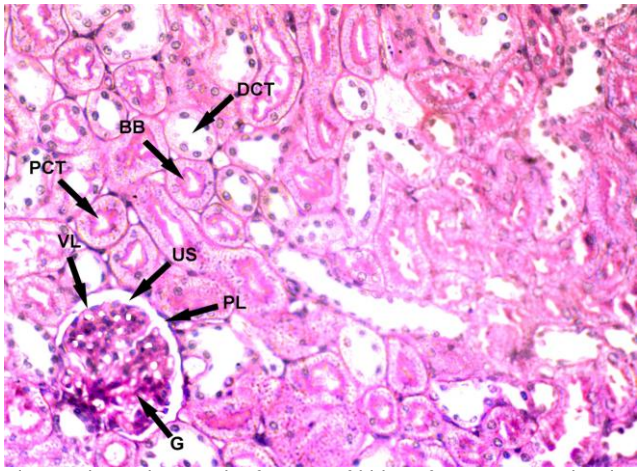

Fig 1: Photomicrograph of cortex of kidney from group A showing glomerulus (G) enclosed in Bowman's capsule containing visceral (VL) and parietal layers (PL) separated by urinary space (US). Proximal convoluted tubules (PCT) are lined by cuboidal cells with prominent brush border (BB) and distal convoluted tubules (DCT) are lined by simple cuboidal epithelium**.** H&E stain X400.

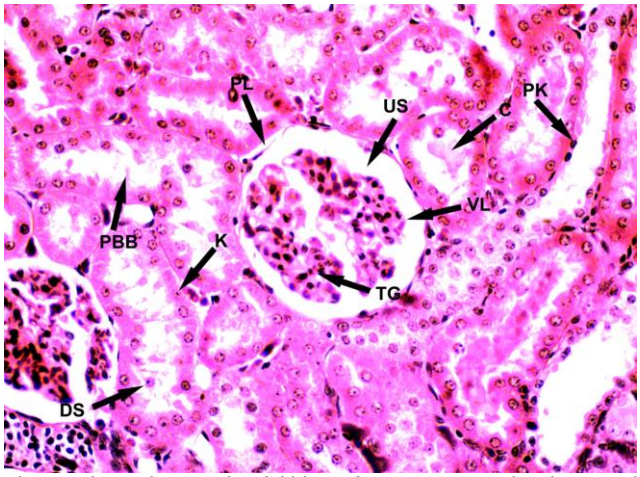

Fig 3: Photomicrograph of kidney from group B showing renal corpuscle having Bowman's capsule with visceral (VL) and parietal layers (PL) separated by a urinary space (US), glomerulus with thickened capillary walls (TG). The tubules are showing intraluminal protein casts (C), patchy loss of brush border (PBB), desquamating epithelial cell (DS), nuclear pyknosis (PK) and karyolysis (K). H&E stain X400.

In group A the mean values of serum urea at the start and end of experimental period were 30.62 ± 5.12 mg/dl and 29.25±3.99 mg/dl respectively. While in group B the mean values of serum urea at the start and end of experimental period were 29.37±5.12 mg/dl and 122.62 ± 8.78 mg/dl respectively. There was a statistically significant rise in the mean values of serum urea (p value <0.001) in group B when compared with those of group A.

In group A the mean values of serum creatinine at the start and end of experimental period were 0.50±0.07mg/dl and 0.50±0.10mg/dl respectively. While in group B the mean values of serum creatinine at the start and end of experimental period were 0.48±0.08 mg/dl and 2.67±0.48mg/dl respectively. There was a statistically significant rise in the mean values of serum creatinine (p value <0.001) in group B when compared to those in group A.

The rise in mean values of serum urea and creatinine in group B, as compared to group A, indicate a decline in kidney function in animals of group B.

DISCUSSION

In the current study, on comparing the mean body weights of animals in groups A and B it was observed that at the end of experimental period the animals of group B showed decrease in body weight as compared to control group A. A similar decrease in body weight after gentamicin administration was observed by kumar et al[11], Tavafi et al[12] and Nivetha and Prasanna[3].

When the mean weights of both right and left kidneys together were compared between groups A and B statistically insignificant difference was observed showing that gentamicin treatment did not produce significant effect in the weight of kidneys. This finding is in accordance with the findings of Singroha et al[14]. Our findings however differed from the findings of Nivetha and Prasanna[13] who observed a decrease in weights of kidneys after treatment with gentamicin and Noorani et al[15] who observed increase in kidney weight after gentamicin treatment.

Kidneys of animals in groups A and B showed smooth surface and reddish brown color. These findings differed from those observed by Alarifi et al. [16] who observed whitish spots on the surface of the kidneys.

The mean size of renal corpuscle was significantly increased in gentamicin treated group B when compared with those from group A. These findings were also observed by Noorani et al[15]. Who also reported swelling of renal corpuscles after administration of 80mg/kg/day gentamicin intraperitoneally for 7 days.

Current study showed significant loss of brush border and presence of intraluminal protein casts in proximal convoluted tubules in group B when compared to group A. Guzmán et al[18] and Azab et al. (17) also observed loss of brush border and presence of protein casts in proximal convoluted tubules upon treatment with gentamicin. Protein casts in lumina of proximal tubules were also observed by Frazier et al[19], Kang et al[20] and Lakshmi and Sudhakar[21].

Gentamicin treated group B showed significant desquamation of epithelial cells when compared with group A. these findings are similar to the findings of Padmini and Kumar[22] and Lakshmi and Sudhakar[21], who also observed tubular epithelial cell desquamation.

The present study showed significant Pyknosis and Karyolysis in group B indicating tubular necrosis. Comparable findings were also observed by Kang et al[20], Lakshmi and Sudhakar[21] and Qadir et al[23], on treatment of animals with Gentamicin.

A statistically significant increase in serum urea and creatinine was observed in group B when compared to control group. Similar findings were also reported by Nivetha and Prasanna[13], Kumar et al[11] and Erdem et al[5].

CONCLUSION

The Current study showed gentamicin induced nephrotoxicity in rats as manifested by histological and functional changes. There was significant damage to the proximal convoluted tubules as evidenced by the loss of brush border, cytoplasmic vacuolation, pyknosis, karyolysis and presence of protein casts in the lumina of proximal tubules. The renal corpuscles also showed glomerular swelling and increased glomerular capillary wall thickening. The values of urea and creatinine were elevated indicating damage to kidney function.

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