

GENTAMYCIN INDUCED NEPHROTOXICITY IN ALBINO MICE

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ABSTRACT

Twelve, male albino mice, aged 6-8 weeks, were injected intraperitoneally, aqueous solution of gentamicin (80 mg / kg / day) for fifteen days and the effects observed on the kidney structure and function. Group A served as control while Group B was given gentamicin. At the end of the experiment, blood was drawn from each animal by cardiac puncture for renal function tests and kidneys were fixed for histological studies. In group B, values of serum urea (66.40 ± 0.54 mg/dl) and serum creatinine (1.41 ± 0.08 mg/dl) were significantly increased ($p < 0.001$) when compared with control group A (34.73 ± 0.84 and 0.53 ± 0.04 mg/dl respectively). Both body weight ($p < 0.001$) and kidney weight ($p < 0.05$) decreased significantly in gentamicin treated groups. In histological preparations from group B, the proximal convoluted tubules in cortex were dilated and their epithelial cells showed hydropic changes with cytoplasmic vacuolations in some areas. Loss of brush border, patchy necrosis, presence of cellular debris and accumulation of inflammatory exudates within lumina of proximal convoluted tubules were also observed. The renal medulla from group B, showed an increase in intra-luminal tubular protein casts. Chi-square test showed statistically significant ($p < 0.001$) association between tubular necrosis and tubular casts. It is concluded that gentamicin is nephrotoxic in albino mice.

Key Words: Gentamycin, nephrotoxicity, hydropic changes, necrotic tubules, protein casts, serum urea, serum creatinine

INTRODUCTION

Gentamicin, an amino – glycoside, synthesized by *Micromonospora*, is used for the treatment of various bacterial infections including both gram – negative and gram – positive bacteria.¹ Gentamicin is a heat – stable antibiotic that remain active even after autoclaving, thus making it useful in the preparation of certain microbiological growth media. After oral administration, gentamicin is not very effective due to the fact that it is not absorbed to an appreciable extent from the intestinal tract; the drug, however, avidly binds to certain tissues. It appears to be eliminated unchanged primarily in the urine. The recommended route of administration of gentamicin is intravenous, intra-muscular, intraperitoneal or topical.² It inhibits protein synthesis by binding with 30S subunit of the bacterial ribosome.³ Its use is now limited due to its toxic effects, mainly on kidney and vestibular system.^{4,5} Nephrotoxic effects are produced in 10 – 15% of cases due to over dosage or its accumulation in renal cortical tubular epithelial cells and necrosis of cells in the proximal tubule leading to acute renal failure.⁶ Gentamicin stimulates the generation of reactive oxygen species⁷ and by forming iron – drug complex,⁸ that leads to renal damage. Blood urea nitrogen (BUN) and serum creatinine are reported to increase significantly in gentamicin – induced nephrotoxicity.⁹ Gentamicin acts by binding to anionic phospholipids of plas-

malemma and altering its biophysical properties and functions by decreasing the permeability of the glycerol moiety of phosphatidylinositol, membrane fluidity and promoting membrane aggregation. Membranous structures that can be damaged by gentamicin include lysosomes, mitochondria, microsomes and probably the Golgi apparatus. Lyses of lysosomes containing gentamicin may release both potent acid hydrolases and high concentrations of the drug into the cytoplasm, disrupting critical intracellular processes including mitochondrial respiration, electron transport chain, and microsomal protein synthesis.¹⁰ In the present study nephrotoxic effects of gentamicin on functional derangement and structure of the kidneys are reported in a correlative study.

MATERIAL AND METHODS

This study was an Experimental Randomized Control Trial (ERCT) conducted at the University of Health Sciences Lahore. Twelve male albino mice, 6–8 week old, weighing 20 – 25 gm each were procured from National Institute of Health, Islamabad. The animals were kept under controlled temperature ($23 - 25^{\circ}\text{C}$), humidity (60%) and light and dark cycles of 12 hours each and were acclimatized for one week; they were fed on standard mouse diet and water *ad libitum*, and weighed to the nearest mg at the start of experiment. The animals were randomly di-

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vided into two groups, having six mice each. Group A served as control and were given 1 ml distilled water per day by mouth, in addition to water *ad libitum*. Group B was given 80 – mg / kg / day of gentamicin intraperitoneally dissolved in 1 ml of distilled water for fifteen days. The body weight of each animal was recorded twice weekly and at the end of the experimental period, when each animal was taken out of cage and was euthanized under chloroform before 2ml of blood was taken in 5 ml disposable syringe by cardiac puncture. The blood sample was allowed to stand for one hour and centrifuged at 3000 rpm for 10 minutes. The serum was collected in Eppendorf tubes and stored in freezer at -20°C . Serum urea and serum creatinine were measured by using commercially available kits of "Human Company".

Each animal was then sacrificed. The kidney of each animal was removed and examined for gross changes; 2 mm² pieces were taken from different sites of kidney; fixed in 10% formaldehyde for 48 hours and processed for routine histology.¹² Five micron thick sections were obtained using Leica RM 2125 rotary microtome and stained with H&E.

The data were analyzed using SPSS version 17.0. Mean \pm SE is given for quantitative variables. Chi-Square test was applied to observe association between qualitative variables. Differences between groups were considered to be statistically significant, if p value was < 0.05 .

RESULTS

All animals of group A (controls) were healthy and active; however, the animals of group B (gentamicin treated) showed irritable behaviour. In group A, the mean body weight of the animals at the start and at the end of the experiment was 23.66 ± 0.33 and 25.50 ± 0.50 gm respectively; whereas, in group B, these parameters were 24.33 ± 0.42 and 17.16 ± 0.30 gm respectively. There was a statistically significant decrease in the mean body weight of animals of group B at the end of the experiment ($p < 0.05$).

In – group A, the mean values of serum urea and serum creatinine were 34.73 ± 0.84 and 0.53 ± 0.04 mg/dl respectively; whereas in group B, serum urea and serum creatinine were 66.40 ± 0.54 and 1.41 ± 0.08 mg/dl respectively. These values were significantly higher ($p < 0.001$) in the experimental group B.

Kidneys of group A was reddish brown with smooth and shiny surface with thin glistening capsule which was not adherent to adjoining organs; however, in group B, kidneys of all animals were brownish in colour. The mean weight of the kidneys in group A was 0.39 ± 0.001 gm. A significant ($P < 0.05$) decrease in kidney weight was seen in group B.

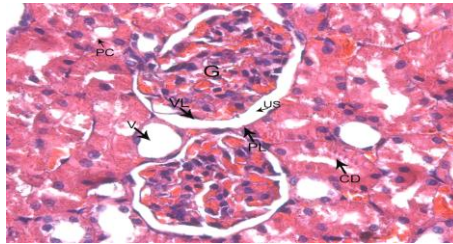


Fig. 1: Photomicrograph of histological section of kidney from group A, showing renal corpuscle with glomerulus (G) surrounded by Bowman's capsule having parietal (PL) and visceral layers (VL) containing urinary space (US), proximal convoluted tubule lined by cuboidal epithelium (PC), vessel (V) and collecting duct (CD). Stain H&E X400.

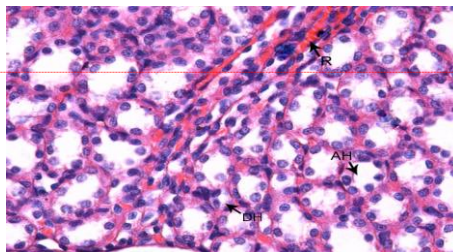


Fig. 2: Photomicrograph of histological section of kidney from group A, showing thin descending limb of loop of Henley's (DH) having simple squamous epithelium, ascending loop of Henley's (AH) having wider lumen with simple cuboidal epithelium and red blood cells in peritubular capillaries (R). Stain H&E X400.

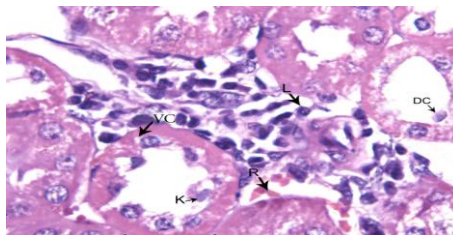


Fig. 3: Photomicrograph of histological section of kidney from group B, showing damaged and dilated tubule with desquamating epithelium having cytoplasmic vacuolations (VC), dropping out cell (DC), karyolytic nuclei (K), infiltration of lymphocyte (L) and red blood cells (R). Stain H&E X630.

Histological Examination of Kidneys

In group A (controls), the renal corpuscles appeared

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as dense rounded structure comprising of glomeruli, surrounded by double walled epithelial Bowman's capsule and lined by simple squamous cells, having an outer parietal and inner visceral layers with a urinary space in between the two layers (Fig. 1). Numerous nuclei in glomerulus were those of capillary endothelial cells, mesangial cells and podocytes. Proximal convoluted tubules (PCT) were lined by simple cuboidal epithelium, prominent brush borders and acidophi-

Table 1: Comparison of proximal tubular necrosis and tubular casts between control and experimental groups.

Percentage of Tubular Necrosis	Group A (Control)		Group B (Experimental)	
	Tubular Necrosis	Tubular Casts	Tubular Necrosis	Tubular Casts
No Change (-)	6 (100%)	6 (100%)	0 (0.0%)	0 (0.0%)
Mild (<25% +)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (33.33%)
Moderate (26 – 51% ++)	0 (0.0%)	0 (0.0%)	1 (16.66%)	3 (50.00%)
Severe (>51% +++)	0 (0.0%)	0 (0.0%)	5 (83.33%)	1 (16.66%)
TOTAL	6	6	6	6
Fisher Exact Test			P < 0.001	P < 0.001

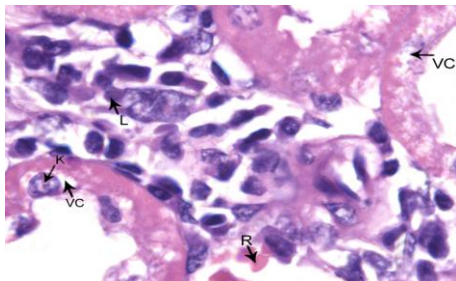


Fig. 4: Photomicrograph of histological section of kidney from group B, showing cytoplasmic vacuolations (VC), karyolysis (K), lymphocyte (L) and red blood cells (R) Stain H&E X1000.

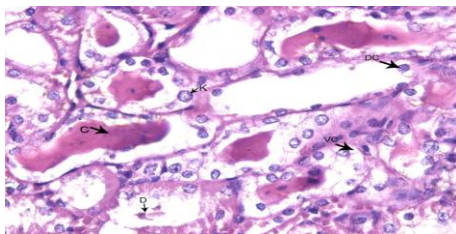


Fig. 5: Photomicrograph of histological section of kidney from group B, showing intra luminal protein casts (C), cytoplasmic vacuolations (VC), karyolysis (K), intra luminal cellular debris (D) and drooping out cell (DC). Stain H&E X400.

lic cytoplasm. Distal convoluted tubules (DCT) had simple cuboidal epithelium, clearly defined wider lumen, than those of the PCT and closely packed nuclei per section (Fig. 1). Collecting tubules, lined with low cuboidal epithelium, were also seen (Fig.1).

Thin descending and thick ascending limbs of loop of Henley, vasa recta and collecting ducts are shown in Fig. 2.

In experimental group B, the proximal convoluted tubules in cortex were dilated and showed patchy necrosis, loss of brush border, and presence of cellular debris and accumulation of inflammatory exudates within their lumina. The lining epithelial cells of proximal convoluted tubules showed hydroptic changes with cytoplasmic vacuolations at some areas. Some of the tubules exhibited desquamation of epithelial cells in their lumina. The nuclei of these cells were swollen and karyolytic (Fig. 3). A cellular infiltration of lymphocytes was also evident particularly around necrotic tubules (Fig. 4). The renal medulla, showed an increase in intra-luminal tubular protein casts (Fig. 5). Student "t" test showed statistically significant ($p < 0.001$) increase in proximal tubule luminal diameter in group B ($60.71 \pm 1.20 \mu\text{m}$) as compared to group A ($41.45 \pm 0.04 \mu\text{m}$).

Chi-Square test showed statistically significant association between groups regarding percentages of tubular necrosis and tubular casts ($p < 0.001$; Table 1).

DISCUSSION

Gentamicin produced statistically significant loss of kidney weight in treated group B. This can be a manifestation of anorexia caused by the drug as reported earlier by Houghton^{1et} and Ali². These authors also observed that gentamicin produced renal failure that resulted in acidosis associated with anorexia, leading to decrease in body weight. We also found a decrease ($p < 0.001$) in the mean body weight of treated animals showing nephrotoxicity of gentamicin. The drug treatment also significantly increased the mean serum urea and creatinine in group B, presumably due to gentamicin induced oxidative injury causing tubular damage and renal impairment. This finding is in accord with that of

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Lipsky¹⁴ who also reported similar results.

Our study also showed that in group B, there was a statistically significant ($p < 0.001$) association between percentage of renal tubules exhibiting necrosis, showing cytoplasmic vacuolations and loss of brush borders. Kacew reported that gentamicin caused tubular necrosis and loss of brush borders. This author further reported that gentamicin accumulated in renal cortex due to its reabsorption in proximal convoluted tubules causing degeneration and necrosis of the epithelial cells. Further, Lipsky observed accumulation of inflammatory exudates and hyaline casts within the lumen of gentamicin treated tubules. We also observed intra-luminal protein casts in renal medulla.

It is concluded that Gentamicin treated albino mice showed a fair degree of derangement of renal functions with concomitant changes in the histological structure of the organ.

REFERENCES

- Gilbert, D.N., Mandell, G.L., Bennett, Dolin, R. Aminoglycosides in principles and practice of infectious diseases. 5th ed. New York: Churchill Livingstone; 2000: 307-36.
- Ali, M.Z., Goetz, M.B. A meta-analysis of the relative efficacy and toxicity of single daily dosing versus multiple daily dosing of aminoglycosides. Clin. Infect. Dis. 1997; 24: 796-809.
- Buss, W.C., Piatt, MK. Gentamicin administered in vivo reduces protein synthesis in microsomes subsequently isolated from rat kidney but not from rat brain. J. Antimicrob. Chemother. 1985; 15: 715-21.
- Humes, D.H., Weinberg, JM., Knauss, TC. Clinical and pathophysiologic aspects of aminoglycoside nephrotoxicity. Am. J. Kidney Dis. 1982; 2: 5-29.
- Giurgea, M.L., Toubeau, G., Laurent, G., Heuson, J.A., Tulkens, P.M. Impairment of lysosome-pinosome vesicle fusion in rat kidney proximal tubules after treatment with gentamicin at low doses. Toxicol. Appl. Pharmacol. 1986; 86: 271-85.
- Leehey, D.J., Braun, B.I., Tholl, DA. Can pharmacokinetic dosing decrease nephrotoxicity associated with aminoglycoside therapy. J. Am. Soc. Nephrol. 1993; 4: 81-90.
- Kumar, S.V., Walker, PD. Reactive oxygen metabolites in toxic acute renal failure. Renal failure 1992; 14: 363-70.
- Priuska, E.M., Schacht, J. Formation of free radical by gentamicin and iron and evidence for an iron gentamicin complex. Biochem. Pharmacol. 1995; 55: 1749-54.
- Michiels, L.M., Anzola, K., Amaya, G., Solano, M. Quantitative and qualitative scintigraphic measurement of renal function in dogs exposed to toxic doses of Gentamicin. Vet. Adiol. Ultrasound. 2001; 42: 553-61.
- David, P.S., Ruben, S., Bruce, A.M. Gentamicin inhibits renal protein and phospholipid metabolism in rats : Implications involving intracellular trafficking. J. Am. Soc. Nephrol. 2001; 12: 114-23.
- Bacroft, J.D., Gamble, M. Theory and Practice of histological techniques, 5th ed., Edinburgh, Churchill Livingstone, 2002: pp. 125-37.
- Houghton, D.C., Harnet, M., Cambellm M., Porter, G., Bennet, W. A light and electron microscopic analysis of gentamicin nephrotoxicity. Am. J. Pathol. 1975; 82: 589-612.
- Ali, B.H., Gayoum, AA., and Bashir, AA. Gentamicin nephrotoxicity in rat: some biochemical Correlates. Pharmacol. Toxicol, 1992; 70: 419-23.
- Lipsky, J.J., Cheng, L., Sacktor, B., Leitman, PS. Gentamicin uptake by renal brush border membrane vesicles. J. Pharm. Clin. Ther. 1980; 215: 390-3.
- Kacew, S. Inability of nitrendipine to protect against gentamicin nephrotoxicity in rats. Bio. Med. Environ. Sci. 1989; 2: 160-6.

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