

# Protective Effect of Vitamin E (A Tocopherol Acetate) on Gentamicin-induced Nephrotoxicity in Adult Albino Rats

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## ABSTRACT

**Objective:** To determine the preventive role of Vitamin E on renal parenchyma after given of gentamicin in adult albino rats.

**Study Design:** Experimental study.

**Place and Duration of Study:** This study was carried out in the Department of Anatomy Baqai Medical University and Muhammad Medical College, Mirpurkhas from June 2011 to November 2011.

**Materials and Methods:** For this study, 30 adult albino rats were taken. They were divided into three groups ; A, B and C. The animals in group-A given normal saline 10 ml/kg/day intraperitoneal for 2 weeks. Group-B received gentamicin 100 mg/kg/day intraperitoneal for 2 weeks and group-C receives gentamicin 100mg/kg/day intraperitoneal with vitamin-E 2 mg/kg/day orally for 2 weeks. On day 15 all animals were sacrificed with deep ether anesthesia. Their kidneys were removed, fixed in 10 % formalin. Representative blocks were taken and embedded in liquid paraffin. For routine histological examination 5  $\mu$ m thick section cut by microtome and stained with H&E, PAS and silver methenamine. Renal histology was done under light microscope to see the proximal and distal tubular diameter and count

**Results:** No significant ( $P>0.05$ ) changes were observed in the histopathology of kidney tissues of the groups A and C rats. The group B significantly ( $P<0.001$ ) affected the histopathology of kidney.

**Conclusion:** It may be concluded that gentamicin produces changes in kidney, which may be attributed to ischaemia resulting in tubular necrosis in adult albino rats simultaneous administration of vitamin-E partially protect the morphological and histological changes induced by gentamicin.

**Key Words:** Gentamicin, Vitamin-E, Adult albino rats, Kidneys.

## INTRODUCTION

Gentamicin is an aminoglycoside bactericidal antibiotic that works by binding the 30S subunit of bacterial ribosome, interrupting protein synthesis, used to treat many types of bacterial infection, particularly those caused by gram negative organism.<sup>1</sup>

Gentamicin cause deleterious effects on kidney function, especially with respect to solute homeostasis, maintenance of renal perfusion and glomerular filtration. Renal toxicity can be a result of hemodynamic changes, direct injury to cells and tissue.<sup>2</sup> Studies that evaluated episodes of acute tubular necrosis (ATN) or Acute interstitial nephritis (AIN) due to antibiotics (e.g. aminoglycosides) has been reported to be upto 36%.<sup>3,4</sup> Most episodes of drug-induced renal dysfunction are reversible, with function returning to baseline when the medication is discontinued. Chronic renal injury can however, be induced by some medications, leading to chronic tubulointerstitial inflammation, papillary necrosis.<sup>5,6</sup> Heightened physician awareness is necessary if renal injury and associated morbidity from renal failure are not to be prevented.

Vitamin E is the collective name for a set of 4 related  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and the corresponding four

tocotrienols  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - which are fat-soluble vitamins with antioxidant properties<sup>7,8</sup>. The major sources of Vitamin-E are avocado, nuts, such as almonds or hazelnuts, red palm oil, seeds, spinach, green leafy vegetables, vegetable oils (canola), corn, sunflower, soybean, cottonseed, olive oil, wheat germ, wholegrain foods, milk and asparagus<sup>9</sup>. The administration of vitamin E (antioxidant) has been shown to be beneficial in prevention and attenuation of renal scarring in numerous animal models of kidney diseases<sup>10</sup>. Antioxidative (tocopherol) therapies have been shown to prevent acute decrease in renal function induced by ischemia, contrast media and drugs like diclofenac sodium (NSAID)<sup>11</sup>.

## MATERIALS AND METHODS

This study was carried out during the period from June 2011 to November 2011, in the Department of Anatomy Baqai Medical University and Muhammad Medical College, Mirpurkhas. For this experimental study 30 adult albino rats aged 12 weeks, weighing ranging from 180gm to 200gm were used. They were originally obtained from Charles River breeding laboratories, Brooklyn, Massachusetts, USA, and were cross bred at the animal house of Muhammad Medical College, Mirpurkhas. The animals were kept in the

animal house on a balanced diet. They were put under observation for one week prior to the experimental procedure for assessment of their state of health on basis of weight gain or loss.

The animals used in this study were divided into 3 groups: A, B and C. the animals in each group were kept in a separate cage and labeled. Each animal was weighed period to treatment.

**Group-A (10 Animals):** In this group each animal received normal saline 10 ml/kg/day intraperitoneal once daily for 2 weeks.

**Group-B (10 Animals):** In this group each animal received Gentamicin 100 mg/kg/day intraperitoneal once daily for 2 weeks.

**Group-C (10 Animals):** In this group each animal received Gentamicin 100 mg/kg/day intraperitoneal and vitamin-E ( $\alpha$ -tocopherol acetate) 2 mg/kg/day dissolved in olive oil given orally by feeding tube once daily for 2 weeks.

On day 15 the animals were sacrificed kidneys were removed, bisected in two halves, one half fixed in 10% formalin and second in alcoholic formalin. The tissues were sectioned and mounted on slides. They were stained by Haematoxylin & Eosin, silver methamine and periodic acid Schiff stain.

The morphological changes in renal parenchyma were observed under light microscope. Five observations for each parameter were recorded in each animal. Proximal and distal tubular counts were made under 8x ocular and 40x objective with counting reticule in randomly selected five fields in the cortex of the kidney and proximal and distal tubular diameter was measured with the help of ocular micrometer. The data was subjected to statistical analysis Student 't' test was employed to see the significance of the results.

## RESULTS

**Observations in Group-A (Control):** In H&E stained sections the histological structure in the cortical and

medullary portion appeared absolutely normal without any change in either glomeruli or tubules as shown in Figure 1.

In PAS stained sections the brush border on the apical surface of proximal tubular epithelial cells stained magenta in colour and almost filled the tubule. The glycogen content of the cytoplasm of proximal tubular cells was quite normal. The basement membrane of proximal and distal tubules also stained magenta, which was distinct and regular.

Silver methenamine stained sections revealed basement membrane of glomeruli, Bowman's capsule and proximal and distal tubules which was faint in outline, and unmeasurable by light microscopy.

The mean values of number of proximal convoluted tubules per unit area as noted in group-A was  $24.0 \pm 0.49$ . when group-A compared with group-B highly significant increase ( $P<0.001$ ) was noted in group-A, however, when group-A compared with group-C statistically non-significant difference ( $P>0.05$ ) was observed.

The mean values of diameter of proximal tubules measured in unit area in group-A was  $50.9 \pm 0.74 \mu\text{m}$ , which when compared with group-B, statistically significant decrease ( $P<0.05$ ) was noted in group-A, however, when compared with group-C, no significant difference ( $P>0.05$ ) was observed.

The mean values of number of distal tubules per unit area, as observed in Group-A was  $22.7 \pm 0.56$ , which when compared with that in group-B, a highly significant increase ( $P<0.001$ ) was observed in group-A, however, when compared with group-C, no significant change was noticed.

The mean values of diameter of distal tubules per unit area in group-A was  $38.4 \pm 0.37 \mu\text{m}$ , which when compared with group-B, a highly significant decrease ( $P<0.001$ ) was noted in group-A, however, when compared with group-C, no significant change occurred.

**Table No.1: Comparison of Proximal and distal tubular counts and diameters per unit area between groups A and B.**

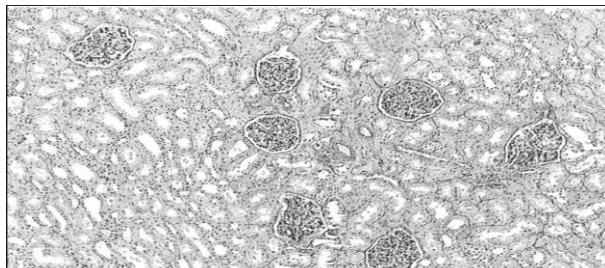
	Group A Controls (n=10)	Group B Gentamicin (n=10)	P-value
	Mean $\pm$ S.D $\pm$ SEM	Mean $\pm$ S.D $\pm$ SEM	
Proximal Tubular Count per unit area (Under Reticule)	$24.0 \pm 1.56 \pm 0.49$	$16.1 \pm 2.08 \pm 0.66^{**}$	0.001
Mean Diameter of Proximal Tubules (Under Ocular Micrometer)	$50.9 \pm 2.33 \pm 0.74$	$54.3 \pm 3.07 \pm 0.97^{*}$	0.030
Mean Distal Tubular Count (Under Reticule)	$22.7 \pm 1.77 \pm 0.56$	$14.5 \pm 1.08 \pm 0.34^{**}$	0.001
Mean Diameter of Distal Tubules (under Ocular Micrometer)	$38.4 \pm 1.16 \pm 0.37$	$54.5 \pm 1.85 \pm 0.59^{**}$	0.001

**Table No.2: Comparison of Proximal and distal tubular counts and diameters per unit area between groups A and C.**

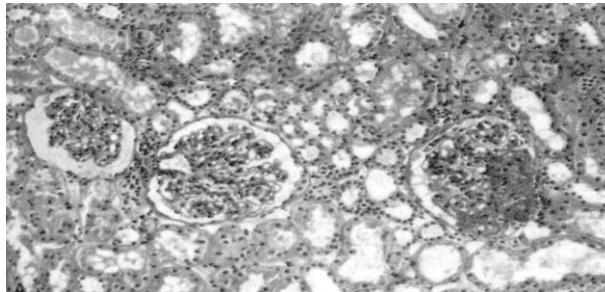
	Group A Controls (n=10)	Group C Gentamicin with Vitamin E (n=10)	P-value
	Mean $\pm$ S.D $\pm$ SEM	Mean $\pm$ S.D $\pm$ SEM	
Proximal Tubular Count per unit area (Under Reticule)	24.0 $\pm$ 1.56 $\pm$ 0.49	22.9 $\pm$ 2.08 $\pm$ 0.66	0.419
Mean Diameter of Proximal Tubules (Under Ocular Micrometer)	50.9 $\pm$ 2.33 $\pm$ 0.74	51.6 $\pm$ 2.85 $\pm$ 0.90	0.856
Mean Distal Tubular Count (Under Reticule)	22.7 $\pm$ 1.77 $\pm$ 0.56	20.7 $\pm$ 2.11 $\pm$ 0.67	0.067
Mean Diameter of Distal Tubules (under Ocular Micrometer)	38.4 $\pm$ 1.16 $\pm$ 0.37	39.8 $\pm$ 1.01 $\pm$ 0.32	0.079

**Table No.3: Comparison of Proximal and distal tubular counts and diameters per unit area between groups B and C.**

	Group B Gentamicin (n=10)	Group C Gentamicin with Vitamin E (n=10)	P-value
	Mean $\pm$ S.D $\pm$ SEM	Mean $\pm$ S.D $\pm$ SEM	
Proximal Tubular Count per unit area (Under Reticule)	16.1 $\pm$ 2.08 $\pm$ 0.66 **	22.9 $\pm$ 2.08 $\pm$ 0.66	0.001
Mean Diameter of Proximal Tubules (Under Ocular Micrometer)	54.3 $\pm$ 3.07 $\pm$ 0.97 *	51.6 $\pm$ 2.85 $\pm$ 0.90	0.01
Mean Distal Tubular Count (Under Reticule)	14.5 $\pm$ 1.08 $\pm$ 0.34 **	20.7 $\pm$ 2.11 $\pm$ 0.67	0.001
Mean Diameter of Distal Tubules (under Ocular Micrometer)	54.5 $\pm$ 1.85 $\pm$ 0.59 **	39.8 $\pm$ 1.01 $\pm$ 0.32	0.001



**Figure No.1:** Photomicrograph of 5  $\mu$ m thick H&E stained paraffin section of rat kidney from group-A (control), showing normal architecture of renal cortex under low magnification. x101

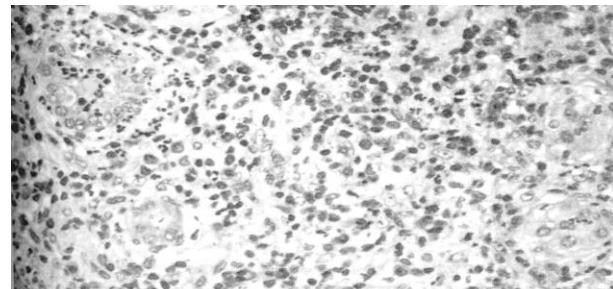


**Figure No.2:** Photomicrograph of 5  $\mu$ m thick H&E stained paraffin section of rat kidney from group-B treated with gentamicin, showing dilated blood vessels with marked infiltration of inflammatory cells and damaged tubules. x205.

**Observations in Group-B:** In H&E stained sections the interstitium of renal cortical area was sparse with few inflammatory cells but no marked oedema, many

dilated and congested blood vessels were observed as shown in Figure 2.

In PAS stained sections the brush border at the luminal surface appeared scanty and indistinct and at some places it was completely absent. The intracellular glycogen content of the proximal as well as distal tubules was moderately depleted. However, the basement membrane of proximal and intact.



**Figure No.3:** Photomicrograph of 5  $\mu$ m thick H&E stained paraffin section of rat kidney from group-C treated with gentamicin, and vitamin-E, showing almost normal proximal convoluted tubules (PT) and distal convoluted tubules (DT) x416.

In silver methenamine stained sections the basement membrane was visible as intensely stained black line around proximal and distal tubules which was quite thickened in some tubules but still not measurable by light microscopy.

The mean values of number of proximal convoluted tubule per unit area observed in group-B was 16.1  $\pm$  0.66, which when compared with that in group-C, a

highly significant decrease ( $P<0.001$ ) was noted in group-B.

The mean values of diameter of proximal tubules per unit area in group-B was  $54.3 \pm 0.97 \mu\text{m}$ , which when compared with group-C, highly significant increase ( $P<0.001$ ) occurred in group-B.

Mean values of distal tubular count per unit area as observed under high magnification in group-B was  $14.5 \pm 0.34$ , which when compared with group-C, highly significant decrease ( $P<0.001$ ) was observed in group-B.

Mean values of diameter of distal tubules per unit area in group-B was  $54.5 \pm 0.59 \mu\text{m}$ , which when compared with that in group-C, highly significant increase ( $P<0.001$ ) was noticed in group-B.

**Observation in Group-C:** In H&E stained sections the histological structure in the cortical and medullary portion appeared absolutely normal without any change in either glomeruli or tubules as shown in Figure 3.

In PAS stained sections showing normal brush borders at the apical surface of proximal tubules cells. It was well defined and almost filled the lumen of proximal tubules. The intracellular cytoplasm had normal glycogen content, basement membrane also appeared a regular outline.

The basement membrane of proximal and distal tubules was observed in silver methenamine stained sections. These sections showed uniformly continuous black stained basement membrane in both tubules.

The mean values of the number of proximal convoluted tubules per unit area as observed under high magnification in group-C was  $22.9 \pm 0.66$ , which when compared with that in group-A, no significant change was observed. However, when compared with group-B statistically highly significant increase ( $P<0.001$ ) was noted in group-C.

The mean values of diameter of proximal tubules per unit area in group-C was  $51.6 \pm 0.90 \mu\text{m}$ , which when compared with that in group-A, statistically no change was noticed. However when compared with group-B statistically significant decrease ( $P<0.01$ ) was noted in Group-C.

The mean values of the number of distal tubules per unit area as observed under high magnification in group-C was  $20.7 \pm 0.67$ , which when compared with that in group-A, no significant change was observed. However when compared with group-B statistically highly significant increase ( $P<0.001$ ) was noted in Group-C.

The mean values of diameter of distal tubules per unit area in group-C was  $39.8 \pm 0.32 \mu\text{m}$ , which when compared with that in group-A, statistically no change was noticed. However when compared with group-B statistically highly significant decrease ( $P<0.001$ ) was noted in Group-C.

## DISCUSSION

Nephrotoxicity induced by Gentamicin has been found even in therapeutic doses.

Vitamin-E, an antioxidant is known to be a potent scavenger of free radicals which have been implicated in over hundred conditions in humans including ischaemia of many organs<sup>12</sup>.

Studies on the Gentamicin have shown that prolonged administration of this drug should be considered as a risk for nephrotoxicity<sup>13</sup>. In the present study three groups of animals were used group-A acted as control, group-B received Gentamicin while group-C received gentamicin and vitamin-E.

The effect of both these drugs were observed including number and diameter of proximal and distal convoluted tubules.

The proximal tubular count was not changed significantly in group-C, when compared with control group-A, whereas a significant decrease in number of tubules per unit area in group-B occurred which may be attributed to damage to the tubular epithelial cells by ischaemia<sup>14</sup>.

The highly significant increase observed in the diameter of proximal tubules in group-B as compared to groups A and C, may be attributed to degeneration of cells in proximal tubules resulting in apparent increase in their diameter<sup>15</sup>.

The total number of distal tubules in group-B was significantly lower when compared with group A and C. The decrease in number of tubules may be attributed to focal ischaemic necrosis of some of the tubules resulting in their numbers<sup>16</sup>.

The diameter of distal tubules in group-B showed highly significant increase as compared to that in groups A and C, which may be attributed to vacuolar degeneration of cells which fill the lumen of damaged tubules resulting in increase in diameter<sup>16</sup>.

## CONCLUSION

It may be concluded that gentamicin produces changes in kidney, which may be attributed to ischaemia resulting in tubular necrosis in albino rats and simultaneous administration of vitamin E partially protect the morphological and histological changes induced by gentamicin.

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