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**RESEARCH ARTICLE**

# The nephroprotective effects of vardenafil against amikacin induced nephrotoxicity in rabbits

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## *Abstract*

**Background**

Nephrotoxicity is one of the most important side effects and therapeutical limitations of aminoglycoside antibiotics. Nephrotoxicity appears in 10–25% of therapeutic courses. Amikacin nephrotoxicity has been considered to result mainly from tubular damage. Vardenafil is a selective and potent inhibitor of specific phosphodiesterase 5 enzyme. It is an oral therapy for the treatment of erectile dysfunction.

**Aim of the study**

To study the nephroprotective effects of vardenafil against amikacin induced nephrotoxicity in rabbits.

**Materials and methods**

Twenty four domestic male rabbits were randomly divided into three groups , each of eight animals as follow:

Group1:(control group) treated with dimethylsulfoxide (DMSO) orally.

Group 2: (amikacin group) or (induction group) treated with daily dose of (100 mg/kg/IM ) for 21 days.

Group 3: (vardenafil group) which treated with daily oral dose of (2 mg/kg) and given amikacin (100mg/kg/ IM) at fourth day and both continued till day 24. Animals were sacrificed at day 25. Changes in serum levels of creatinine, urea, albumin, total protein, tissue malondialdehyde (MDA) and histopathological scores of all groups were measured.

**Results**

Serum levels of creatinine, urea, albumin, total protein, tissue MDA and histopathological scores analysis were done at day 25 after induction of nephrotoxicity, showed that vardenafil caused significant reduction of serum levels of creatinine, urea, tissue MDA and histopathological scores ($p<0.05$) in comparison to amikacin group.

Serum levels of albumin and total protein , vardenafil caused significant elevation of serum levels of albumin and total protein ($p<0.05$) in comparison to amikacin group .

**Conclusion**

Vardenafil was exerted nephroprotective effect through correction the investigation parameters of this study that had been changed due to amikacin.

## Introduction:

Like other aminoglycosides, amikacin has a very narrow therapeutic index, and the concentrations needed for optimal efficacy are close to those having a risk of toxicity. Amikacin is a concentration- dependent drug, with the rate of killing of microorganisms being proportional to the drug concentrations achieved in serum, especially peak concentrations.(1)

Aminoglycosides induced nephro and ototoxicity, which are the limiting factors for their clinical use, in which the oxygen free radicals have been involved. (2) Wojckch Lesniak *et al*., (2005) found that aminoglycosides, exert their adverse renal effect by generation of reactive oxygen species. (3) Additionally, it has been demonstrated that aminoglycoside form a complex with mitochondrial $Fe^{+2}$ to catalyze the formation of free radicals. (4)

Vardenafil is a potent and selective inhibitor of phosphodiesterase 5 enzyme (PDE5).(5,6) It differs from that of sildenafil and tadalafil reflecting differing pharmacological properties. Vardenafil, a potent and highly selective PDE-5 inhibitor may be restoring glomerular filtration rate (GFR) by enhancing nitrus oxide (NO) induced cyclic gaunosine monophosphate (cGMP) formation and accumulation. (7)

It has newly been recognized that PDE5 inhibitors are capable of protecting tissues against oxidative stress damage by inhibiting reactive oxygen species (ROS) formation, due to increasing cGMP level. (8) Increased cGMP level lead to increasing nitrus oxide (NO) bioavailability.(9)

## Methodology

### Experimental Animals

Twenty four healthy domestic male rabbits age between 8-10 months, weight 1250-1750 gram, bought from local market used in the experiment were randomly divided into three groups, each group of eight animals as follow :

Group1: Control group treated with 0.5 ml/kg body weight (BW) of dimethylsulfoxide (DMSO) orally once daily and continued till day 24, then animals were sacrificed at day 25.

Group2: Amikacin group treated with daily dose of (100 mg/kg/IM)(10) for 21 days pretreated for 3 days with 0.5ml/ kg BW/day DMSO orally and both continued till day 24, then animals were sacrificed at day 25.

Group3: Vardenafil group which treated with daily oral dose of (2 mg/kg)(11) for 24 days and given amikacin (100mg/kg/ IM) at fourth day and both continued till day 24. Animals were sacrificed at day 25.

### Preparation of serum samples

Blood samples were aspirated from heart of rabbits after 21 days of amikacin administration directly by intra-cardiac puncture. The clot was dispersed with glass rod and then centrifuged for 15-20 minutes at 3000 rpm and the supernatant was used for the estimation of serum levels of creatinine, urea, albumin and total protein.(12)

### Determination of serum creatinine level

Serum creatinine concentrations were determined according to Jaffe reaction using ready-made kit for this purpose. which was expressed in (mg/ml). (13)

### Determination of serum urea level

Serum urea levels were determined using urease-modified Barthelot reaction by a ready-made kit for this purpose. Which was expressed in (mg/ml). (14)

### Determination of serum albumin level

Principle of the method , albumin in the presence of bromcresol green at a slightly acid pH produces a color change of the indicator from yellow-green to green-blue. Which was expressed in (g/dl). (15)

### Determination of serum total protein

Serum total protein was determined using Biuret colorimetric method. Which was expressed in (g/dl). (16)

### Preparation of tissue homogenate

After the animals have been sacrificed by anesthetic ether, kidneys were quickly excised, placed in chilled phosphate buffer solution (PH 7.4) at $4^0$C, blotted with filter paper and weighed. One gram of organ was then taken to prepare 10% tissue homogenate using the same buffer solution utilizing tissue homogenizer at set 3 for 1minute at $4^0$C. All preparations were freshly prepared and kept frozen ($-70^0$C) unless worked immediately.(17)

### Measurement of tissue malondialdehyde (MDA)

The concentration of renal tissue MDA level was measured using ELISA technique , principle of the assay employs the competitive inhibition enzyme immunoassay technique. Which was expressed in (ng/ml). (18)

**Histopathological evaluation**

The kidneys of each animal were removed. Small pieces of fresh tissue were fixed in 10% neutral formalin and processed. Paraffin sections 5µm thick were stained with hematoxylin and eosin. The histopathological changes were evaluated in several sections from each group.

**Statistical analysis**

Student's-t test was used for the evaluation of statistical significance. Difference was considered significant at $P < 0.05$ level. All values were expressed as mean ± SEM .

## Results

**Effect of treatments on serum levels of creatinine and urea**

The effects of amikacin on the renal function showed significant increase ($p<0.05$) in the serum levels of both creatinine and urea of rabbits treated with 100 mg/kg/day of amikacin compared to the corresponding levels in the control group of animals, while there are a significant decrease ($p<0.05$) in the serum levels of both creatinine and urea of rabbits treated with 100 mg/kg/ day of amikacin + 2 mg/kg/day of vardenafil compared to the corresponding levels of rabbits treated with 100 mg/kg/ day of amikacin. Serum levels for creatinine were (1.243±0.15 , 0.652±0.019)(mg/ml) and that for urea were (118.981±12.883 , 40.450±0.835) (mg/ml) in groups of amikacin and vardenafil with amikacin respectively (table1).

**Table 1. Effect of amikacin and the combination of vardenafil with amikacin on the serum creatinine and urea levels .**

| **Group N= 8** | **Serum Creatinine level ( mean ± SEM) (mg/ml)** | **Serum Urea level ( mean ± SEM) (mg/ml)** |
|---|---|---|
| Control | 0.605±0.022 | 34.097±1.395 |
| Amikacin | 1.243±0.15 [a] | 118.981±12.883 [a] |
| Amikacin pretreated with vardenafil | 0.652±0.019 [b c] | 40.450±0.835 [b c] |

N = Number of animals , SEM = standard error of mean, a = significant change when being compared to that of control group ($p < 0.05$) , b = significant change when being compared to that of amikacin group ($p < 0.05$), c = non-significant change when being compared to that of control group.

**Effect of treatments on serum levels of albumin and total protein**

The effects of amikacin on the renal function showed significant decrease ($p<0.05$) in the serum levels of both albumin and total protein of rabbits treated with 100 mg/kg/ day of amikacin compared to the corresponding levels in the control group of animals , while there are a significant increase ($p<0.05$) in the serum levels of both albumin and total protein of rabbits treated with 100 mg/kg/ day of amikacin + 2 mg/kg/day of vardenafil compared to the corresponding levels of rabbits treated with 100 mg/kg/ day of amikacin. Serum levels for albumin were (2.357±0.042, 2.758±0.054) (g/dl) and that for total protein were (4.600±0.132 , 5.762±0.144) (g/dl) in group of amikacin and vardenafil with amikacin respectively (table 2) .

**Table 2. Effect of amikacin and the combination of vardenafil with amikacin on the serum albumin and total protein levels .**

| Group N= 8 | Serum Albumin level (mean ± SEM) (g/dl) | Serum Total protein level (mean ± SEM) (g/dl) |
|---|---|---|
| Control | 2.83±0.097 | 6.055±0.256 |
| Amikacin | 2.357±0.042 [a] | 4.600±0.132 [a] |
| Amikacin pretreated with vardenafil | 2.758±0.054 [b c] | 5.762±0.144 [b c] |

N = Number of animals , SEM = standard error of mean ,a = significant change when being compared to that of control group ($p < 0.05$) , b = significant change when being compared to that of amikacin group ($p < 0.05$), c = non-significant change when being compared to that of control group .

**Effect of treatments on tissue MDA level**

The effects of amikacin on the renal function showed significant increase ($p<0.05$) in the tissue MDA level of rabbits treated with 100 mg/kg/ day of amikacin compared to the corresponding level in the control group of animals, while there is a significant decrease ($p<0.05$) in the tissue MDA level of rabbits treated with 100 mg/kg/ day of amikacin + 2 mg/kg/day of vardenafil compared to the corresponding level of rabbits treated with 100 mg/kg/ day of amikacin. Tissue MDA levels were (145.390±1.105, 125.295±2.106) (ng/ml) in groups of amikacin and vardenafil with amikacin respectively (table 3) .

**Table 3. Effect of amikacin and the combination of vardenafil with amikacin on the tissue MDA level .**

| Group N= 8 | Tissue MDA level (mean ± SEM) (ng/ml) |
|---|---|
| Control | 113.443±1.672 |
| Amikacin | 145.390±1.105 [a] |
| Amikacin pretreated with vardenafil | 125.295±2.106 [a b] |

N = Number of animals , SEM = standard error of mean ,a = significant change when being compared to that of control group ($p < 0.05$) , b = significant change when being compared to that of amikacin group ($p < 0.05$).

**Effect of treatments on histopathological score**

The effects of amikacin on the renal function showed significant elevation ($p<0.05$) in the histopathological scores of renal tissue of rabbits treated with 100 mg/kg/ day of amikacin compared to the corresponding value in the control group of animals , while there are a significant decrease ($p<0.05$) in the histopathological scores of renal tissue of rabbits treated with 100 mg/kg/ day of amikacin + 2 mg/kg/day of vardenafil compared to the corresponding to rabbits treated with 100 mg/kg/ day of amikacin.

Histopathological scores were (3.75±0.068, 2.125±0.05) in groups of amikacin and vardenafil with amikacin respectively (table 4). Histopathological examination of the renal sections in different groups showed in figures 1, 2 and 3 .

**Table 4. Effect of amikacin and the combination of vardenafil with amikacin in the histopathological scores .**

| **Group N= 8** | **Histopathological scores (mean ±SEM)** |
|---|---|
| Control | 0 |
| Amikacin | 3.75±0.068 [a] |
| Amikacin pretreated with vardenafil | 2.125±0.05 [a b] |

N = Number of animals , SEM = standard error of mean, a = significant change when being compared to that of control group ($p < 0.05$), b = significant change when being compared to that of amikacin group ($p < 0.05$) .

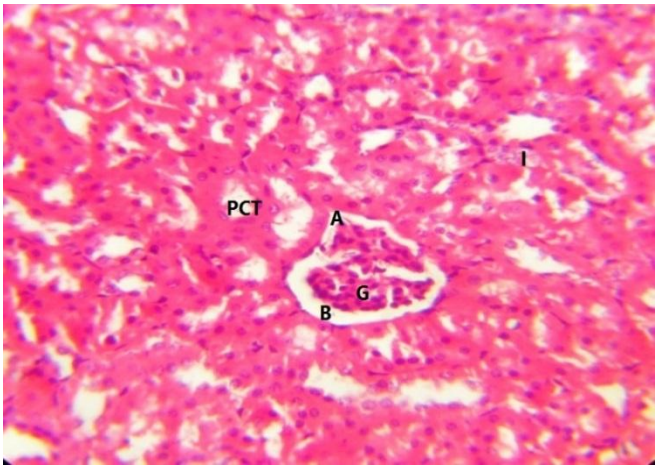


Figure 1. Light microscopic section of rabbit kidney tissue of control group (treated with DSMO 0.5ml/ kg/day) for 24 days showing normal kidney tissue. H&E (40X) , (G) Glomerulus ,(B) Bowman's capsule, (PCT) Proximal convoluted tubule, ( I) interstitial tissue , (A) Afferent arteriole .

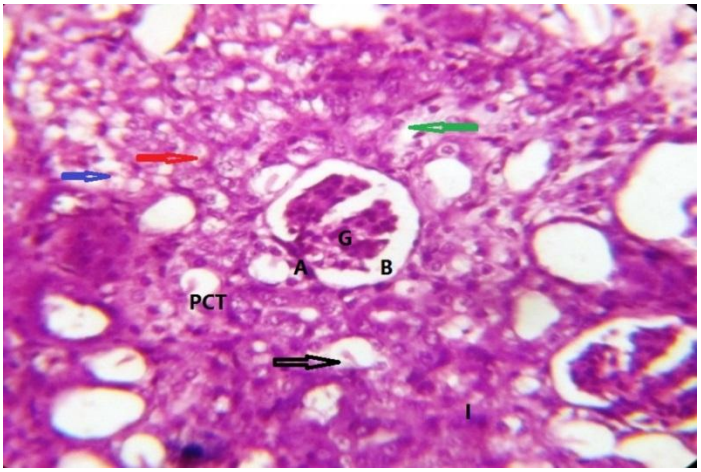


Figure 2. Light microscopic section of rabbit kidney tissue of amikacin group (treated with amikacin 100mg/ kg BW/day) for 21 days showing tubular epithelial cell swelling(red arrow), vacuolar degeneration (green arrow), necrosis (blue arrow) and desquamation (black arrow) involving 75% of cortical tubules. H&E (40X). (G)Glomerulus ,(B) Bowman's capsule,(PCT) Proximal convoluted tubule,(I) Interstitial tissue ,(A) Afferent arteriole.

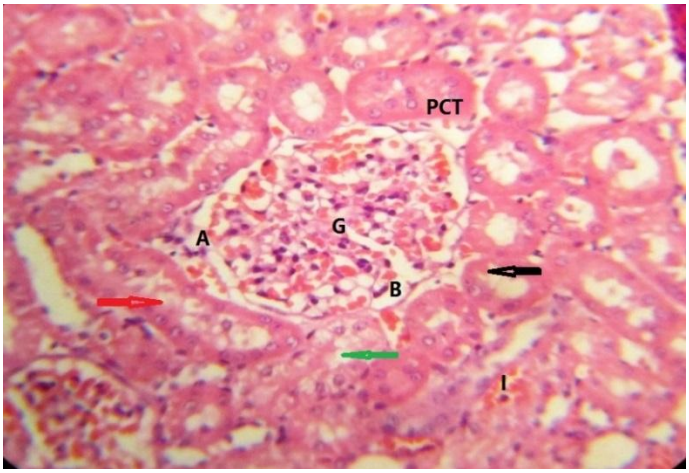


Figure 3. Light microscopic section of rabbit kidney tissue of vardenafil group ( vardenafil 2 mg/ kg /day given amikacin 100 mg/kg/day after 3 days) for 24 days showing tubular epithelial cell swelling ( red arrow), vacuolar degeneration (green arrow) and desquamation (black arrow) with absence of necrosis , involving 25% of cortical tubules .H&E (40X) . (G)glomerulus ,(B) Bowman's capsule,(PCT) Proximal convoluted tubule, (I) Interstitial tissue ,(A) Afferent arteriole .

## Discussion

### Amikacin-induced nephrotoxicity

Aminoglycosides nephrotoxicity, functional and morphological, is well established. Amikacin has proved to possess serious nephrotoxic side effects which may end in acute renal failure. [19]

It has been reported that amikacin may induce free radical production which involves a variety of pathological processes. [20,21]

In the present study ,amikacin had induced nephrotoxicity by causing significant elevation of renal function parameters including serum urea and creatinine levels in comparison with the control group (tables 1).The elevation in serum urea and creatinine levels may be attributed to create reactive oxygen species (ROS) that has central key role in the mechanisms that lead to tubular necrosis and decrease glomerular filtration rate ,so this will lead to reduced urea and creatinine clearance that is associated with elevation of serum urea and creatinine and this results are compatible with a study done by. [22]

In addition to the above mechanism, aminoglycosides directly disturb glomerular physiology, reducing glomerular filtration rate (GFR) by lowering the afferent glomerular arteriolar output. [23]

In the present study , amikacin had induced nephrotoxicity by causing significant reduction of serum albumin and total protein levels in comparison with the control group (table 2). The reduction in serum albumin and total protein levels can be due to glomerular changes ,which lead to loss of protein in urine with consequent reduction of serum protein. The same findings are also demonstrated by other study which showed that loss of glomerular filtration barrier selectivity, due to the neutralization of its negative charges contributes to proteinuria, especially under conditions in which tubular reabsorption is impaired such as in tubular necrosis. [24]

Other mechanism which explains the proteinuria is the competition of aminoglycosides with proteins for the megalin–cubilin endocytic complex (membrane transporter) in the proximal tubule, and thus impairs their reabsorption and increases their excretion. [25-28]

In the present study, amikacin had induced nephrotoxicity by causing significant elevation of tissue MDA level in comparison with the control group(table 3).The elevation in tissue MDA level was attributed to oxidative stress and free radical generation through lipid peroxidation .This is agreed with the results reported by. [29-31]

In the present study, amikacin had induced nephrotoxicity by causing significant elevation of the histopathological changes which clarified by raising the score index of renal tissue through changes like tubular epithelial cell swelling ,vacuolar degeneration, necrosis and desquamation involving 75% of cortical tubules in comparison with the control group .

It was shown that amikacin causes damage to the kidney tissue via inflammation and excessive oxidative stress. [32,33] As well as it caused elevation of tissue MDA which indicates severe kidney damage (table 4). Histopathological results of this study indicates that severe degenerations at the proximal tubular cells could be correlated with the harmful effects of amikacin parallel to high tissue MDA level. This is also recorded by. [34-36]

### Effects of vardenafil against amikacin induced nephrotoxicity

In the present study, vardenafil treatment results in an improvement of serum levels of creatinine, urea , albumin, total protein, tissue MDA and histopathological scores compared to amikacin group (tables 1,2,3 and 4)

Vardenafil, a potent and highly selective PDE-5 inhibitor causes restoration of GFR and reducing serum levels of urea and creatinine by enhancing NO-induced cGMP formation and accumulation. [37]

Vardenafil enhances renal blood flow by stimulating intra-cellular cGMP in ischemic acute renal failure in rats.[37] The results of this study is compatible with other studies. [38,39]

It improves serum levels of albumin and total protein through improving GFR and reduction of proteinuria ,by leading to reduced intracellular availability of $Ca^{+2}$ and increased NO bioavailability. [40] This work of the study is reported with other studies. [7,41]

Vardenafil reduces tissue MDA level , and recently PDE5 inhibitors are capable of protecting tissues against oxidative stress damage by inhibiting ROS formation, due to increasing cGMP levels. Our results are in agreement with other studies published. [42,43]

The results of present study indicate that vardenafil is effective in reducing the scores of histopathological changes, tubular epithelial cell swelling , vacuolar degeneration and desquamation with absence of necrosis involving 25% of cortical tubules in the present model and agreed with this studies . [44,45] It is concluded that vardenafil could be useful for reducing the nephrotoxic effects of amikacin. Further studies are needed to elucidate the mechanisms of protective effect of vardenafil.

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