

The Effects of Diclofenac on Renal Toxicity Disorders Induced by Gentamicin in Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Gentamicin, an antibiotic is used to treat infection with gram-negative bacteria. Due to side effects such as renal disorders, its use is limited. In this research the effect of diclofenac on gentamicin induced nephrotoxicity was examined.

METHODS: Male Wistar rats (n=32) weighting between 200-250 g were randomly divided into 4 groups (n=8 in each group): 1- control group received no drug, 2- treatment group with gentamicin (100 mg/kg/day i.p) for 8 days, 3- treatment group with diclofenac (0.5 mg/kg/day i.p) for 8 days, 4- treatment group with gentamicin (100 mg/kg/day i.p) and diclofenac (0.5 mg/kg/day i.p) for 8 days. In ninth day, blood pressure and renal artery blood flow and also level of urea, creatinine, magnesium, sodium, potassium, and osmolality were measured in urine and plasma samples. Finally, histological study was performed by using Hematoxylin and Eosin staining.

FINDINGS: Co-treatment with diclofenac significantly decreased fractional excretion of potassium (328 ± 17.6 ; $p < 0.001$), fractional excretion of sodium (0.885 ± 0.005 ; $p < 0.001$), excretion of urea (56.08 ± 5.56 ; $p < 0.001$), urine osmolality (439 ± 14.3 ; $p < 0.01$) and creatinine clearance ($p < 0.001$, 0.427 ± 0.01 ml/min/kg) but decreased renal blood flow (3.99 ± 0.2), which had previously been reduced by gentamicin.

CONCLUSION: The results of the study showed that diclofenac exacerbates kidney disorders caused by gentamicin.

KEY WORDS: Gentamicine, Nephrotoxicity, Diclofenac, Rat.

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Introduction

Gentamicin is an antibiotic of aminoglycoside which the side effects, like renal toxicity, have limited its use (1). The renal toxicity of gentamicin is closely related to the accumulation of gentamicin by megalin and cubilin in proximal tubular epithelial cells (2). Gentamicin induces renal toxicity by various mechanisms; gentamicin increases the mitochondrial pores after its entry into cytosol, releasing cytochrome C and producing reactive oxygen species (ROS) (3). Gentamicin enters the cell through endocytosis and accumulates in lysosomes, and by increasing the permeability of the lysosomal membranes by producing reactive oxygen species, it induces apoptosis in proximal tubule cells (4).

Gentamicin reduces the glomerular filtration factor (Kf) and glomerular filtration rate (GFR) (5, 6). Gentamicin causes inflammation in the interstitial tissue of the kidneys, extensive tubular necrosis and forms of protein membranes due to cellular pouring into the lumen (7). Glomerular basement membrane thickening, necrosis and vacuolation of close proximal tubular epithelial cells have been observed by gentamicin (8). Sodium diclofenac is a non-steroidal anti-inflammatory drug (NSAID) that is used to reduce fever, pain and for acute and chronic inflammation (9). Non-selective NSAIDs, such as diclofenac and indomethacin, inhibit the prostaglandin production enzymes, the enzymes (COX-1) and (COX-2) (10). The inhibition of prostaglandin production in the kidneys leads to a decrease in renal blood flow and glomerular filtration. These drugs cause inflammation and cell necrosis in kidney tubules (9). Considering the effects of diclofenac and the effect of gentamicin on acute renal failure, the aim of this study was to evaluate the effects of diclofenac alone and the use of diclofenac and gentamicin on kidney function.

Methods

This experimental study was carried out on 32 Wistar male rats weighing 200-250 grams after approval at the Ethics Committee of Arak Medical University 248.1394. The animals were kept in 12-hour of daylight and 12-hour of darkness alternately and at ambient temperature of $23 \pm 2^\circ\text{C}$ and free access to water and food.

Method for preparing diclofenac and gentamicin: Sodium diclofenac (Sigma-Aldrich) was dissolved in 0.125 mg/0.5 ml of normal saline and injected

intraperitoneally of 0.5 mg/kg dosage to each animal. 25 mg of gentamicin powder was dissolved in ml1 of normal saline and injected intraperitoneally of 100 mg/kg dosage to each animal (11, 12).

Studied groups:

Control group: No drugs received.

Gentamycin group: received intraperitoneal injection of gentamicin 100 mg/kg (Alborz Drug-Iran) for 8 consecutive days.

Diclofenac group: received intraperitoneal injection of diclofenac (0.5 mg/kg) (Sigma-American) for 8 consecutive days.

The diclofenac+gentamicin group: received intraperitoneal injection of gentamycin 100 mg/kg and diclofenac 0.5 mg/kg for 8 consecutive days. After the last injection, the animals were placed in a cage of metabolism, their urine was collected over a period of 12 hours, and then the urine volume was measured by gravimetry method. For this purpose, the volume of urine collected was weighed. Urine weight per ml indicates the amount of soluble materials in urine. The amount of systolic blood pressure was measured from tail artery with tail cuff and the help of ower labP device (Australia-AD Instruments) (12, 15).

The left kidney artery was removed and the kidney blood flow rate was measured by a flow meter (11). For this purpose, after fixation of blood flow, the mean blood flow of kidney artery was measured for 30 minutes. Blood samples were taken from abdominal aorta with cold heparin syringe. After separating the plasma, the level of creatinine and blood urea nitrogen (BUN) was measured using the Autoanalyzer (Netherlands-selectra-XL) and Sodium, Potassium, Magnesium were measured using Flame photometer (Italy-FP20SEAC) (16,17).

Osmolality of urine and plasma samples was determined using osmometric device (Germany -030 Osmomat gonotec) (18). After removing both kidneys and weighing them, they were put in 10% formalin. The following equations were used to calculate the Urinary Excretion of Sodium (UNaV) and Urinary Excretion of potassium (UKV[°]), Fractional excretion of sodium (FENa) and Fractional excretion of potassium (FEK), creatinine clearance (Ccr) and urine flow (V[°]) (Table 1) (11, 17).

Histopathologic studies: After fixing the left kidney, a paraffin mold was prepared. 5 micron sections were stained with two colors of hematoxylin and eosin. The pathology and tissue changes in the glomerular, tubular and vascular sections were compared among the

groups by pathologist. Increasing Bowman capsule space, creating protein templates within the lumen, tubular cell vacuolation, glomerular hyperemia, and tubular cell necrosis in the tubular section were studied. The damage caused was graded according to the calculation percentage. In the absence of damage, grade 0, 1-25% of damage grade 1, 25-50% of damage grade 2, 50-75% of damage grade 3, 100-75% of damage grade 4 were considered (11, 12, 19).

Table1. The formula for calculation of Urinary Excretion of Sodium ($\dot{A} \text{ UNaV}$) and Urinary Excretion of potassium (UKV°), Fractional excretion of sodium (FENa) and Fractional excretion of potassium (FEK), creatinine clearance (Ccr) from the following equations was used. (V° is the amount of urine flow)

Variable	Calculation formula
$V^\circ (\mu\text{l}/\text{min.gkw})$	$(1000 \times \text{UFR}) / (\text{KW} \times 720)$
$\text{CCr} (\text{ml}/\text{min.gkw})$	$(V^\circ / 1000 \times U_{\text{Cr}}) / P_{\text{Cr}}$
$\text{UNaV}^\circ (\mu\text{mol}/\text{min.gkw})$	$(V^\circ \times U_{\text{Na}}) / 1000$
$\text{UKV}^\circ (\mu\text{mol}/\text{min.g kw})$	$(V^\circ \times U_{\text{K}}) / 1000$
FENa	$(U_{\text{Na}} \times P_{\text{Cr}}) / (P_{\text{Na}} \times U_{\text{Cr}}) \times 100$
FEK	$(U_{\text{K}} \times P_{\text{Cr}}) / (P_{\text{K}} \times U_{\text{Cr}}) \times 100$

Statistical analysis: All data were analyzed using Graph pad prism software version 6 and one-way ANOVA and then Tukey test as well as nonparametric tests of kurskl wallis and Dunnett and $p < 0.05$ was considered significant.

Results

Effects of diclofenac on renal blood flow and systolic blood pressure: Systolic blood pressure did not change significantly between the two groups (Fig 1). The renal blood flow decreased significantly in the gentamicin group ($4.94 \pm 0.3 \text{ ml}/\text{min}$) and in the diclofenac group ($5.17 \pm 0.07 \text{ ml}/\text{min}$) compared to the control group ($7.86 \pm 0.33 \text{ ml}/\text{min}$) ($p < 0.001$). Reducing renal blood flow by administration of gentamycin and diclofenac simultaneously was not significant in comparison to gentamicin group (Fig 2). Effects of diclofenac on creatinine clearance (Ccr), **Urinary Excretion of Sodium ($\dot{A} \text{ UNaV}$) and Fractional excretion of sodium (FENa) and Urinary excretion of potassium (UKV°) and Fractional excretion of potassium (FEK):** creatinine clearance in the gentamicin group ($0.732 \pm 1.0 \text{ ml}/\text{min}/\text{kg}$) and in diclofenac group ($0.727 \pm 0.07 \text{ mg}/\text{min}/\text{kg}$) showed a significant decrease compared to the control group ($1.44 \pm 0.08 \text{ mg}/\text{min}/\text{kg}$).

Concurrent administration of gentamycin and diclofenac ($0.427 \pm 0.01 \text{ mg}/\text{min}/\text{kg}$) resulted in a significant reduction in creatinine clearance compared to gentamicin group ($p < 0.001$) (Table 2).

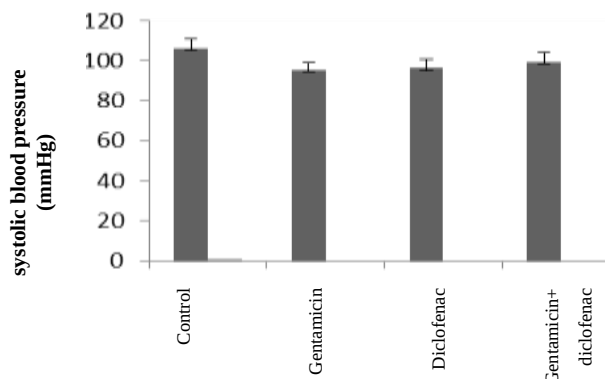


Figure 1. Comparison of systolic blood pressure changes in different groups

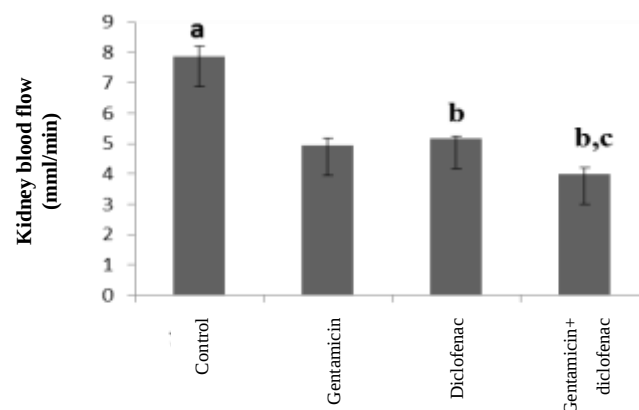


Figure 2. Comparison of blood flow changes in the kidney artery

Non-similar letters indicate a significant difference in blood flow reduction ($p < 0.001$), but the same letters do not show a significant difference. Reducing blood flow in the gentamicin and diclofenac simultaneously treatment group compared to the diclofenac group was significantly different ($p < 0.05$). Gentamycin and diclofenac simultaneously reduced blood flow compared to the gentamicin group, but this was not significant. The Fractional excretion of sodium in the gentamicin group (4.2 ± 0.3) was significantly increased compared to the control group (0.39 ± 0.002) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (0.888 ± 0.005) resulted in a significant reduction in Fractional excretion of sodium, compared to the gentamicin group ($p < 0.001$) (Table 2). The

Fractional excretion of potassium in the gentamicin group (458 ± 7.7) showed a significant increase compared to the control group (35.5 ± 5) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (328 ± 17.6) significantly reduced the Fractional excretion of potassium compared to gentamicin group ($p < 0.001$) (Table 2).

The Urinary Excretion of sodium in the gentamicin group (2.2 ± 0.6 mmol/min/kg) showed a significant increase compared to the control group (0.924 ± 0.01 mmol/min/kg) ($p < 0.001$) and in the diclofenac group (0.723 ± 0.01 mmol/min/kg), compared to the control group, decreased significantly ($p < 0.05$). Concurrent administration of gentamycin and diclofenac (1.1 ± 0.04 mmol/min/kg) resulted in a significant reduction in Urinary Excretion of sodium compared to the gentamicin group ($p < 0.001$). The Urinary Excretion of potassium did not change significantly among the groups (Table 2).

The effects of diclofenac on urinary excretion of sodium u [Na], potassium u [k], magnesium u [Mg], creatinine u [Cr], urea u [BUN] and Osmolu: urinary excretion of sodium in the gentamicin group (76.4 ± 2 μ mol/mL) was significantly higher than the control group (53.9 ± 1.3 μ mol/ml) ($p < 0.001$) and in the diclofenac group, (33.7 ± 1.1 μ mol/ml) showed a significant decrease compared to the control group ($p < 0.001$). Concurrent administration of diclofenac and gentamicin (56.9 ± 3.1 μ mol/mL) resulted in a significant reduction in urinary excretion of sodium compared to gentamicin group ($p < 0.001$) (Table 3). Urinary excretion of potassium in the gentamicin group (192.4 ± 17.5 μ mol/mL) showed a significant increase compared to the control group (120.1 ± 5.74 μ mol/ml) ($p < 0.001$), and in the diclofenac group (73.56 ± 5.61 μ mol/mL) showed a significant decrease compared to the control group ($p < 0.001$).

Concurrent administration of gentamicin and diclofenac (107.5 ± 11.06 μ mol/ml) significantly decreased potassium excretion compared to gentamicin group ($p < 0.001$) (Table 3). Magnesium urinary concentration in the gentamicin group (6.85 ± 0.5 μ mol/mL) showed a significant increase compared to the control group (2.75 ± 0.24 μ mol/ml) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (1.7 ± 0.1 μ g/L) resulted in an increase in magnesium urinary concentration in the gentamicin group, but this increase was not significant (Table 3). The urinary concentration of creatinine in the gentamicin group (23.1 ± 1.4 mg/dL) and in the

diclofenac group (23.1 ± 3.1 mg/dL) significantly decreased compared to the control group (0.7 ± 0.9 mg/dL)/50) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (15.1 ± 0.7 mg/dL) significantly decreased urinary creatinine concentration ($p < 0.001$) (Table 3).

Urinary urea concentration in the gentamicin group (81.4 ± 3.72 mg/dL) and diclofenac (82.18 ± 8.73 mg/dl) significantly decreased compared to the control group (5.82 ± 2.1155 mg/dl) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (56.08 ± 5.56 mg/dL) resulted in a significant decrease in urinary urea concentration compared to gentamicin group ($p < 0.05$) (Table 3). Urinary osmolality in the gentamicin group (709 ± 24 mOsm/kgH₂O) and diclofenac (702 ± 7.26 mOsm/kgH₂O) showed a significant decrease compared to the control group (1502 ± 1.54 mOsm/kgH₂O) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (439 ± 14.3 mOsm/kgH₂O) significantly decreased urinary osmolality compared to gentamicin ($p < 0.001$) (Table 3). **The effects of diclofenac on Plasma levels of sodium [Na](p), potassium [k](p), magnesium [Mg](p), creatinine [Cr](p), urea [BUN](p) and osmolality (osmolp):** plasma creatinine concentration in the gentamicin group (2.52 ± 0.2 mg/dL) and in the diclofenac group (1.4 ± 0.1 mg/dL), compared to the control group (0.25 ± 0.05 mg/dL), there was a significant increase ($p < 0.001$). The concurrent administration of gentamicin and diclofenac (2.9 ± 0.33 mg/dL) significantly increased plasma creatinine concentrations ($p < 0.001$) (Table 4).

Plasma urea concentration increased significantly in the gentamicin group (74.25 ± 4.9 mg/dL) and diclofenac group (57.41 ± 1.7 mg/dl) compared to the control group (21.3 ± 1.0 mg/dL) ($p < 0.001$). Concurrent administration of gentamicin and diclofenac (93.9 ± 2.7 mg/dL) significantly increased plasma urea concentration compared to gentamicin group (Table 4). Plasma magnesium concentration in the gentamicin group (μ mol/mL 0.07 1.7) showed a significant decrease compared to the control group (3.8 ± 0.2 μ mol/ml) ($p < 0.001$).

The concurrent administration of gentamicin and diclofenac (μ mol/mL, 0.15 ± 0.1) 1) decreased the concentration of magnesium in plasma compared to the gentamicin group, but this was not significant. The concentration of sodium, potassium and plasma osmolality in any of the groups did not show any significant change (Table 4).

Effects of diclofenac on tissue changes: In the gentamicin group, necrosis of the tubules (1-grade), increased Bowman capsule space (2nd grade), vacuolation (2nd grade) ($p<0.01$), and the formation of protein templates (2nd grade) and glomerular hyperemia (2grade) significantly increased compared to the control group (0 grade) ($p<0.001$). In the diclofenac group, necrosis (1grade), increased

Bowman capsule space (2nd grade) ($p<0.01$) and vacuolation (2nd grade) increased significantly compared to the control group ($p<0.05$). The concurrent administration of gentamicin and diclofenac in cases such as vacuolation (3rd grade) and increase in the Bowman capsule space (3rd grade) increased compared to the gentamicin group, but this increase was not significant (Table 5, Fig. 1).

Table 2. Comparison of creatinine clearance (Ccr), Urinary Excretion of Sodium (UNaV) and Fractional excretion of sodium (FENa) and Urinary excretion of potassium (UKV) and Fractional excretion of potassium (FEK) among the four studied groups

Parameters group	Control	Gentamicin	Diclofenac	Gentamicin + diclofenac	P-value
Fractional excretion of potassium FEK%	^a 43.9±3.23	^b 458±39.7	^c 29.6±3.01	^d 328±17.6	<0.001
Fractional excretion of sodium FENa%	^a 0.39±0.002	^b 2.4±0.3	^c 0.231±0.004	^d 0.885±0.005	<0.001
Urinary excretion of potassium UKV ^o (mmol/min/kg)	^a 2.23±0.1	^a 2.2±0.4	^a 2.15±0.3	^a 2.14±0.07	>0.05
Urinary Excretion of Sodium UNaV ^o (mmol/min/kg)	^a 0.924±0.01	^b 2.2±0.6	^c 0.723±0.01	^d 1.1±0.04	<0.001
Clearance of creatinine (ml/min/kg)	^a 1.44±0.08	^b 0.732±0.01	^b 0.727±0.007	^c 0.427±0.01	<0.001

Groups are marked with Latin letters. Non-identical letters indicate a significant difference in the fractional excretion of potassium and fractional excretion of sodium, Urinary Excretion of Sodium and creatinine clearance, but the identical letters do not show a significant difference. Urinary Excretion of potassium did not change significantly among the groups. The results were expressed as mean±standardized error of mean (SEM) for 8 rats in each group using statistical analysis (One-way ANOVA-TUKEY).

Table 3. Comparison of urinary excretion of sodium (Nau), potassium ([ku]), magnesium ([Mgu]), creatinine ([Crul]), urea (BUNu) and osmolality between the four studied groups

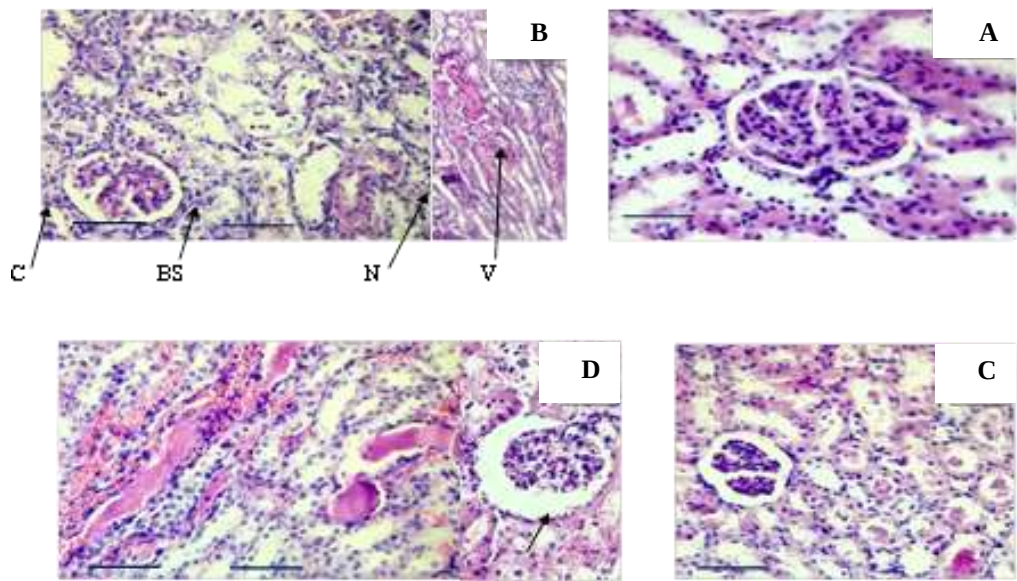
Parameters	Control	Gentamicin	Diclofenac	Gentamicin+diclofenac	P-value
[Na] _u (μmol/mL)	^a 53.9±1.38	^b 76.4±2	^c 33.7±1.1	^a 56.9±3.1	<0.001
[K] _u (μmol/mL)	^a 120.1±5.74	^b 192.4±17.5	^c 73.56±5.6	^a 107.5±11.06	<0.001
[Mg] _u (μmol/mL)	^a 2.75±0.4	^b 6.85±0.5	^c 3.18±0.3	^b 7.1±0.1	<0.001
[Cr] _u (mg/dL)	^a 50.7±1.9	^b 23.1±1.4	^b 23.1±1.3	^c 15.1±0.7	<0.001
[BUN] _u (mg/dL)	^a 576±29.1	^b 407±18.6	^b 410.9±18.65	^c 280.4±27.78	<0.001
Osmol _u (mOsm/kgH ₂ O)	^a 1502±54.1	^b 709±24	^b 702±26.7	^c 439±14.3	<0.001

Table 4. Comparison of plasma levels of sodium ([Na]_p), potassium ([k]_p), magnesium ([Mg]_p), creatinine ([Cr]_p), urea ([BUN]_p), osmolp) between four studied group

Parameters	Control	Gentamicin	Diclofenac	Gentamicin + diclofenac	P-value
[Na] _p (μmol/mL)	^a 144±5.8	^a 141±5.4	^a 143±8	^a 145±9	>0.05
[K] _p (μmol/mL)	^a 4.12±0.3	^a 4.18±0.3	^a 3.9±0.6	^a 4.1±0.2	>0.05
(μmol/mL) [Mg] _p	^a 3.8±0.2	^b 1.7±0.07	^c 2.9±0.1	^b 1.5±0.1	<0.001
[Cr] _p (mg/dL)	^a 0.52±0.05	^b 2.25±0.2	^c 1.4±0.1	^d 2.9±0.03	<0.001
[BUN] _p (mg/dL)	^a 21.3±1.06	^b 74.25±4.9	^c 57.4±1.7	^d 93.9±2.7	<0.001
Osmol _p (mOsm/kgH ₂ O)	^a 362±27.4	^a 323±7.1	^a 323±28.1	^a 321±9.79	>0.05

Table5. Comparison of necrosis, vacuolation, increase of Bowman capsule space, formation of casts and glomerular hyperemia between the four studied groups

Group	Control	Gentamicin	Diclofenac	Gentamicin + diclofenac	P-value
Prameters					
Necrosis	^a 0±0	^b 1.25±0.164	^b 1.25±0.164	^b 1.38±0.183	<0.01
Vacuolation	^a 0±0	^b 2.75±0.164	^b 2.63±0.183	^c 3.75±0.164	<0.01
Increase of Bowman capsule space	^a 0±0	^b 2±0	^b 2±0	^c 3±0	<0.001
Formation of protein templates	^a 0±0	^b 2.25±0.164	^c 1.25±0.164	^b 2.38±0.183	<0.001
Glomerular hyperemia	^a 0±0	^b 2.05±0.14	^c 1.2±0.16	^b 2±0.13	<0.001



C=intratubular cast, BS=Bowman's space, N=necrosis, V=vacuolization

Figure1. Comparison of tissue changes between different groups. A. control group with normal. glomerular and tubular structure. B. Gentamicin group with tubular cell necrosis, formation of protein templates, increase of b Bowman capsule space and vacuolation. C. Diclofenac group A slight increase in Bowman capsule space and the formation of protein templates; D-diclofenac+gentamicin; formation of intense protein templates; severe increase in Bowman capsule space; severe glomerular hyperemia (staining of hematoxylin and Eosin with a magnification of 400X and a scale bar of 100 µm)

Discussion

The results of this study showed that gentamicin and diclofenac separately and simultaneously increase the plasma concentration of creatinine and urea, decrease creatinine clearance, urea and also reduce their urinary excretion. Changes in these parameters indicate a higher renal impairment and toxicity. Changes in renal function can be due to decreased glomerular filtration (Kf) or necrosis of tubular cells, followed by a decrease in the number of functional nephrons, resulting in a decrease in GFR (20, 21). Administration of diclofenac reduced the creatinine clearance and significantly increased its plasma concentration compared to the control group, which are consistent with previous studies (22). Concomitant

treatment with gentamicin and diclofenac significantly increased plasma creatinine and significantly reduced creatinine clearance compared to gentamicin group. Diclofenac affects renal function by degrading epithelial cells, increasing kidney necrosis and fibrosis, as well as tubular and glomerular atrophy (9). Similar to previous studies (23), gentamicin increased urinary excretion of sodium and potassium as a result of increased FENa and FEK. Gentamicin can cause oxidative inhibition of Na +/K + ATPase and sodium channels causing cellular swelling, loss of membrane integrity and necrosis. The fractional excretion of sodium in the diclofenac group decreased compared to the control group, but this was not significant. Concurrent treatment with gentamicin and diclofenac

resulted in a significant reduction in fractional excretion of sodium compared to the gentamicin group. Previous studies have shown that cyclooxygenase enzyme inhibitors such as diclofenac and indomethacin have an increased effect on the expression of the NKCC co-transporter in a thick branch of ascending limb of loop of Henle, which increases the rate of NaCl reabsorption, and also diclofenac has an increased effect on the expression of NHE exchangers (25).

Therefore, the diclofenac reduction effect on fractional excretion of sodium may be due to imbalance tubular absorption of sodium. The results showed that plasma osmolality did not change significantly between groups. However, urinary osmolality in the gentamicin group was significantly lower than the control group. Studies have shown that the reduction of urinary osmolality by gentamicin is due to its effect on reducing the expression of AQP2 (26). Urinary osmolality decreased in the diclofenac group as compared to the control group, which may be due to non-oliguric renal failure in the diclofenac group (22). The simultaneous treatment of gentamicin and diclofenac significantly reduced osmolality compared to gentamicin, due to the effect of these two agents on the kidney through different mechanisms. In this study, blood pressure in different groups did not show a significant difference. The reason for not changing blood pressure was the interference of short-term and middle-term mechanisms of blood pressure regulation (27).

In this study, gentamicin reduced arterial blood flow in the kidney, which, according to previous studies, can be associated with an increase in renal vascular resistance (28). Increased kidney resistance is due to the activation of tubular glomerular feedback (TGF) (1). This increased resistance is due to increased production of vasoconstriction mediators such as Platelet Activating Factor (PAF) and endothelin-1 in the kidney vessels and mesangial organs, as well as increased resistance under the direct effect of

gentamicin on the vascular cells (29, 30). In the diclofenac group, the renal blood flow decreased compared to the control group, which may be due to the effect of diclofenac inhibitor on COX-1 and COX-2 enzymes, inhibiting these enzymes preventing the production of prostaglandins.

One of the most important prostaglandins, prostaglandin 2E and prostaglandin 2I, plays an important role in renal vasodilation and increased blood flow (10). Also, inhibition of cyclooxygenase 1 and 2 enzymes diverts arachidonic acid pathway to the production of contractile leukotrienes, which in addition to reducing the blood flow of the kidneys, can increase the permeability of the capillary and tubular disorders (31).

Histologic studies showed that gentamicin increased necrosis, vacuolation, increased bowman capsule space, and increased formation of protein templates compared to the control group. In the diclofenac group, necrosis, vacuolation, increase in Bowman capsule space, formation of protein templates and glomerular hyperemia were increased in comparison with the control group (32, 33). The simultaneous treatment of gentamicin and diclofenac resulted in a greater increase in vacuolation and a greater increase in bowman space and glomerular hyperemia than in the gentamicin group, but this increase was not significant.

The results showed that the use of diclofenac simultaneously with gentamicin exacerbated and worsened hemodynamic changes, disorder of soluble excretion and tissue changes caused by gentamicin, which could be due to the effect of diclofenac in inhibiting the production of prostaglandins and thus reducing the flow Kidney blood.

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References

1. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney Int.* 2011;79(1):33-45.
2. Schmitz C, Hilpert J, Jacobsen C, Boensch C, Christensen EI, Luft FC, et al. Megalin deficiency offers protection from renal aminoglycoside accumulation. *J Biol Chem.* 2002;277(1):618-22.
3. Morales AI, Demaille D, Prieto M, Puente A, Briones E, Arévalo M, et al. Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. *Kidney Int.* 2010;77(10):861-9.
4. Denamur S, Tyteca D, Marchand-Brynaert J, Van Bambeke F, Tulkens PM, Courtoy PJ, et al. Role of oxidative stress in lysosomal membrane permeabilization and apoptosis induced by gentamicin, an aminoglycoside antibiotic. *Free Radic Biol Med.* 2011;51(9):1656-65.
5. Morales AI, Rodríguez-Barbero A, Vicente-Sánchez C, Mayoral P, López-Novoa JM, Pérez-Barriocanal F. Resveratrol inhibits gentamicin-induced mesangial cell contraction. *Life Sci.* 2006;78(20):2373-7.
6. Martínez-Salgado C, Eleno N, Tavares P, Rodríguez-Barbero A, García-Criado J, Bolaños JP, et al. Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. *Kidney Int.* 2002;62(5):1682-92.
7. Derakhshanfar A, Bidadkosh A, Kazeminia S. Vitamin E protection against gentamicin-induced nephrotoxicity in rats: a biochemical and histopathologic study. *Iran J Vet Res.* 2007;8(3):231-8.
8. Stojiljković N, Veljković S, Mihailović D, Stojiljković M, Radovanović D, Randelović P. The effect of calcium channel blocker verapamil on gentamicin nephrotoxicity in rats. *Bosni J Basic Med Sci.* 2008;8(2):170-6.
9. Aydın G, Gökçimen A, Öncü M, Çicek E, Karahan N, Gökalp O. Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. *Turk J Veterin Animal Sci.* 2003;27(5):1131-40.
10. Lomas AL, Grauer GF. The renal effects of NSAIDs in dogs. *J Am Animal Hos Associat.* 2015;51(3):197-203.
11. Hajhashemi S, Hamidzad Z, Rahbari A, Ghanbari F, Motealeghi ZA. Effects of cobalamin (vitamin b12) on gentamicin induced nephrotoxicity in rat. *Drug Res (Stuttg).* 2017;67(12):710-718.
12. Ahmadi M, Hajhashemi S, Chehrei A, Hosseini N. Therapeutic effects of *Urtica dioica* methanolic extract on gentamicin induced nephrotoxicity in rats. *Koomesh.* 2014;15(2):220-31. [In Persian].
13. Patil CR, Jadhav RB, Singh PK, Mundada S, Patil PR. Protective effect of oleanolic acid on gentamicin induced nephrotoxicity in rats. *Phytother Res.* 2010;24(1):33-7.
14. Tonussi CR, Ferreira SH. Mechanism of diclofenac analgesia: direct blockade of inflammatory sensitization. *Eur J Pharmacol.* 1994;251(2-3):173-9.
15. Whitesall SE, Hoff JB, Vollmer AP, D'Alecy LG. Comparison of simultaneous measurement of mouse systolic arterial blood pressure by radiotelemetry and tail-cuff methods. *Am J Physiol Heart Circ Physiol.* 2004;286(6):2408-15.
16. Ashtiyani SC, Zohrabi M, Hassanpoor A, Hosseini N, Hajhashemi S. Oral administration of the aqueous extract of *Rosmarinus officinalis* in rats before renal reperfusion injury. *Iran J Kidney Dis.* 2013 Sep;7(5):367-75.
17. Hajhashemi S, Jafarian T, Ahmadi M, Rahbari A, Ghanbari F. Ameliorative effects of *zataria multiflora* hydro-alcoholic extract on gentamicin induced nephrotoxicity in rats. *Drug Res.* 2018.
18. Laustsen C, Østergaard JA, Lauritzen MH, Nørregaard R, Bowen S, Søgaard LV, et al. Assessment of early diabetic renal changes with hyperpolarized [1-¹³C] pyruvate. *Diabet/Metabol Res Rev.* 2013;29(2):125-9.
19. Al-Shabanah OA, Aleisa AM, Al-Yahya AA, Al-Rejaie SS, Bakheet SA, Fatani AG, et al. Increased urinary losses of carnitine and decreased intramitochondrial coenzyme A in gentamicin-induced acute renal failure in rats. *Nephrol Dial Transplant.* 2010;25(1):69-76.
20. Savin V, Karniski L, Cuppage F, Hodges G, Chonko A. Effect of gentamicin on isolated glomeruli and proximal tubules of the rabbit. *J Technic Method Pathol.* 1985;52(1):93-102.
21. Martínez-Salgado C, Rodríguez-Barbero A, Tavares P, Eleno N, Lida e, López-Novoa J, et al. Role of calcium in gentamicin-induced mesangial cell activation. *Cell Physiol Biochem.* 2000;10(1-2):65-72.
22. Besen A, Kose F, Paydas S, Gonlusen G, Inal T, Dogan A, et al. The effects of the nonsteroidal anti-inflammatory drug diclofenac sodium on the rat kidney, and alteration by furosemide. *Int Urolog Nephrol.* 2009;41(4):919-26.

23. Gowrisri M, Kotagiri S, Vrushabendra Swamy B, Archana Swamy P, Vishwanath K. Anti-oxidant and nephroprotective activities of *Cassia occidentalis* leaf extract against gentamicin induced nephrotoxicity in rats. *Res J Pharm Biol Chem Sci*. 2012;3:684-94.
24. Cuzzocrea S, Thiemermann C, Salvemini D. Potential therapeutic effect of antioxidant therapy in shock and inflammation. *Curr Med Chem*. 2004;11(9):1147-62.
25. Fernández-Llama P, Ecelbarger CA, Ware JA, Andrews P, Lee AJ, Turner R, et al. Cyclooxygenase inhibitors increase Na-K-2Cl cotransporter abundance in thick ascending limb of Henle's loop. *Am J Physiol-Ren Physiol*. 1999;277(2):219-26.
26. Sohn EJ, Kang DG, Lee HS. Protective effects of glycyrrhizin on gentamicin-induced acute renal failure in rats. *Pharmacol Toxicol*. 2003;93(3):116-22.
27. Wu X, Kentner R, Stezoski J, Kochanek PM, Jackson EK, Carlos TM, et al. Intraperitoneal, but not enteric, adenosine administration improves survival after volume-controlled hemorrhagic shock in rats. *Crit Care Med*. 2001;29(9):1767-73.
28. Morales AI, Buitrago JM, Santiago JM, Fernández-Tagarro M, López-Novoa JM, Pérez-Barriocanal F. Protective effect of trans-resveratrol on gentamicin-induced nephrotoxicity. *Antioxidant Redox Signal*. 2002;4(6):893-8.
29. Valdivielso JM, Rivas-Cabañero L, Morales AI, Arévalo M, López-Novoa JM, Pérez-Barriocanal F. Increased renal glomerular endothelin-1 release in gentamicin-induced nephrotoxicity. *Int J Experiment Pathol*. 1999;80(5):265.
30. Shahbazi F, Dashti-Khavidaki S, Khalili H, Lessan-Pezeshki M. Potential renoprotective effects of silymarin against nephrotoxic drugs: a review of literature. *J Pharm Pharmaceut Sci*. 2012;15(1):112-23.
31. Gulbins E, Parekh N, Rauterberg E, Schlottmann K, Steinhausen M. Cysteinyl leukotriene actions on the microcirculation of the normal and split hydronephrotic rat kidney. *Eur J Clin Investigat*. 1991;21(2):184-96.
32. Gökçimen A, Aydin G, Isen u, Karaöz E, Malas MA, Öncü M. Effect of diclofenac sodium administration during pregnancy in the postnatal period. *Fet Diagnos Thera*. 2001;16(6):417-22.
33. Gokcimen A, Akdogan G, Karaoz E. Structural and biochemical changes in liver and renal tissues induced by an acute high dose of diclofenac sodium in rats. *Biomed Res*. 2000;11(3):293-302.