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ORIGINAL ARTICLE

Adenosine Improved Indices of Gentamicin-induced Nephrotoxicity in Rats

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ABSTRACT

Background: Gentamicin is an aminoglycoside antibiotic used in the treatment of gram-negative bacterial infections; however, due to its adverse effects such as nephrotoxicity, its application has been curtailed. Thus, this study aimed to evaluate the therapeutic effects of adenosine on gentamicin-induced nephrotoxicity.

Materials and Methods: In this experimental study, thirty-five male Wistar rats were divided into five groups of control, gentamicin, adenosine, concurrent gentamicin and adenosine, and post-gentamicin adenosine treatment groups (n=7 for each group). Afterwards, systolic blood pressure, renal blood flow (RBF), as well as urea, creatinine, sodium, potassium, and osmolality levels were quantified. Malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP) biochemical analyses were also performed on renal tissue.

Results: Concurrent adenosine treatment could significantly inhibit the enhanced levels of sodium and MDA excretion and compensate for attenuated RBF and FRAP levels caused by gentamicin. In the post-treatment adenosine group, compared to the gentamicin group, elevated relative excretions of sodium, potassium, and MDA, induced by gentamicin treatment, were significantly reduced and urinary excretion of urea was enhanced.

Conclusion: Adenosine could diminish gentamicin-induced nephrotoxicity through anti-inflammatory effects, vasodilation, and attenuation of oxidative stress.

Key Words: Adenosine, Gentamicin, Nephrotoxicity, Oxidative stress

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INTRODUCTION

Gentamicin is an aminoglycoside antibiotic administered in the treatment of gram-negative bacterial infections; however, due to its adverse effects such as nephrotoxicity, its application has been limited. Gentamicin-induced nephrotoxicity is closely linked with drug accumulation in tubular cells, which can lead to a number of morphological and biochemical alterations [1-3]. This effect is attributed to endocytic receptors called megalin and cubilin [4].

Several mechanisms are involved in the gentamicin-induced nephrotoxicity including: 1) Impact on mitochondrion: after entering the cytosol, gentamicin affects mitochondrion and temporarily increases mitochondrial permeability by transitional pore, releases cytochrome C, and causes respiratory chain dysfunctions. This mechanism enhances production of reactive oxygen species (ROS) of electron transport chain [5-8]; 2) Lysosomal accumulation: after entering the cell through endocytosis, gentamicin accumulates in the lysosome and increases permeability of lysosomal membrane by producing ROS, which results in apoptosis in tubular cells [9]; and 3) Impact on mesangial cells: in the glomerular area, gentamicin induces contraction in glomerular mesangial cells and consequently, reduces glomerular filtration coefficient (Kf) and glomerular filtration rate (GFR). Enhanced production of superoxide anions plays an important role in the induction of contraction in mesangial cells [9, 10].

Gentamicin treatment leads to inflammation in the renal interstitial tissue, extensive tubular necrosis, and formation of protein casts caused by scattering of tubular cells into the tubule lumen [9]. Regarding this type of damage

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mechanism, oxidative stress and inflammation play a major role in gentamicin-induced nephrotoxicity [11]. Despite these side effects, gentamicin is administered on a large scale due to its cost efficiency, low resistance, and expedient and broad anti-bacterial effects [12].

Endogenous nucleoside adenosine is composed of adenine base and ribose monosaccharide. Adenosine is present in all tissues and operates as a vital component of the energy production system. Adenosine is generated by all mammalian cells and plays a pivotal role in adaptation to hypoxia [13, 14]. Adenosine is an androgenic compound found in the interior and exterior of the cells. Extracellular adenosine mainly acts as a signaling molecule. Adenosine level increases during negative energy balance, when the rate of adenosine 5′-triphosphate (ATP) hydrolysis is higher than its synthesis.

In pathological conditions, adenosine accumulates in the kidneys; therefore, elevated consumption of ATP in the kidney, impaired renal perfusion, hypoxia, and inflammation precipitate production of adenosine. Adenosine in the kidney regulates renin release, GFR, and renal vascular tonus. Adenosine is also a critical regulator of glomerular-tubular feedback (TGF) [14]. Adenosine receptors (A1, A2A, A2B, and A3) belong to the large family of G-protein coupled receptors [13-15]. Adenosine receptor expression alters under ischemic, hypoxic, or inflammatory conditions. Production of renal adenosine and use of its receptors can relieve acute renal injury [14].

Due to anti-inflammatory, antioxidant, and vasodilator properties of adenosine and tissue damage mechanisms of gentamicin-induced nephrotoxicity, in the present study, we aimed to study the effects of concurrent and post-treatment administrations of adenosine on renal problems caused by gentamicin prescription.

MATERIALS AND METHODS

Study design

This experimental study was conducted on 35 male Wistar rats, weighing between 200 and 250 g. The animals were kept in cages under similar controlled conditions in terms of light (12 h light-12 h dark intermittently), room temperature (23±2˚C), and free access to food and water.

The studied groups were: 1) control group: they received no medication; 2) gentamicin group: they were administered intraperitoneal (IP) gentamicin 100 mg/kg (Alborz Darou Co., Iran) during eight consecutive days [16]; 3) adenosine group: they received IP adenosine 10 mg/kg (A9251 Sigma-Aldrich) for eight consecutive days [17]; 4) gentamicin and adenosine concurrent treatment group: this group received IP gentamicin 100 mg/kg together with IP adenosine 10 mg/kg for eight consecutive days; and 5) gentamicin and post-treatment adenosine group: the animals received IP gentamicin 100 mg/kg for eight consecutive days and then after the ninth day IP adenosine 10 mg/kg was administered for another eight days.

The study interventions

On the ninth day of the concurrent protocol and the seventeenth day of the post-treatment protocol, after receiving the last dose of the drugs, the animals were placed in metabolic cages for 12 h and their urine volume was measured using gravimetric analysis. The animals were weighed after leaving the metabolism cage. After injection of thiopental sodium (50-60 mg/kg; Sandoz, GmbH, Estonia), systolic blood pressure was measured using tail cuff and PowerLab device (AD Instruments, Australia) [18].

Afterwards, a longitudinal incision was made on the abdominal surface by a cutter. Both kidneys were observed by opening the abdomen. The left renal artery and vein were separated and renal blood flow (RBF) was quantified using Flowmeter device with a special probe (T402, America) for 30 min, and then blood flow was recorded as a graph [19].

Blood samples were drawn from the abdominal aorta by dint of a cold heparinized syringe. After extraction of plasma, creatinine (Cr), and blood urea nitrogen (BUN) levels were measured using AutoAnalyzer (Selectra-XL, Netherlands) [20]. Sodium and potassium levels were assessed by means of flame photometer (SEAC-20Fp, Italy) [21]. Moreover, osmolality was evaluated by osmometer (Gonotec Osmomat-030, Germany) [22].

Thereafter, creatinine clearance level and absolute and relative excretions of potassium and sodium were determined based on the following formula:

CCr(µl/min/gkw)=(V°/1000×UCr)/PCr; absolute sodium excretion UNaV° (µmol/min/gkw)=(V°×UNa)/1000; absolute excretion of potassium UKV° (µmol/min/gkw)=(V°× UK)/1000; relative sodium excretion based on percentile FENa=(UNa×PCr)/(PNa×UCr)×100; and relative potassium excretion base on percentile FEK=(Uk×PCr)/(Pk×UCr)×100.

After removal of both kidneys and weighing them, to measure FRAP and MDA resulting from lipid peroxidation by ROS, the right kidney was kept in liquid nitrogen and then was quickly transferred to a freezer at -20˚C. For measuring MDA of renal tissue, Ohkawa method [23] was used. In addition, to determine FRAP we applied

Benzie and Strain method [24].

After removing the capsule, the left kidney was placed in 10% buffered formalin, and after regular tissue passage and fixation, a paraffin mold was made from it and after preparing 5-micron slices, it was stained with hematoxylin and eosin acid. Tissue analysis was performed by a pathologist after provision of a proper slide.

Afterwards, Bowman's space, reduction of the number of red blood cells (RBC), percentage of glomerular injury, scattering of tubular epithelial cells into the tubule lumen, production of protein casts inside the tubule lumen, as well as vacuolation and necrosis of tubular cells were examined.

The resulting damage was graded based on computational percentage. The outcomes were graded as "grade 0" (no damage), "grade 1" (1-25% damage), "grade 2" (25-50% damage), "grade 3" (50-75% damage), and "grade 4" (75-100% damage) [25].

Statistical analysis

Finally, to analyze the data, One-way ANOVA, Tukey's test, Kruskal-Wallis, and Dunnett's test were run using SPSS, version 20. P-values equal to or less than 0.05 were considered statistically significant.

Ethical considerations

All the ethical codes established by Committee of Monitoring Laboratory Animals of Arak University of Medical Sciences were considered for all the experiments on animals.

RESULTS

The effects of adenosine on RBF and systolic blood pressure: compared to the control group, RBF significantly decreased in the gentamicin and adenosine groups (5.4±0.3 ml/min and 6.2±0.3 ml/min vs. 7.8±0.2 ml/min; P<0.001). Concurrent treatment with adenosine for eight consecutive days (7.02±0.2 ml/min) enhanced RBF compared to the gentamicin group (P<0.001). RBF in the post-treatment adenosine group (6.04±0.2 ml/min) was higher than the gentamicin group; however, this difference was not significant. Systolic blood pressure was not significantly different among the groups (Figure 1).

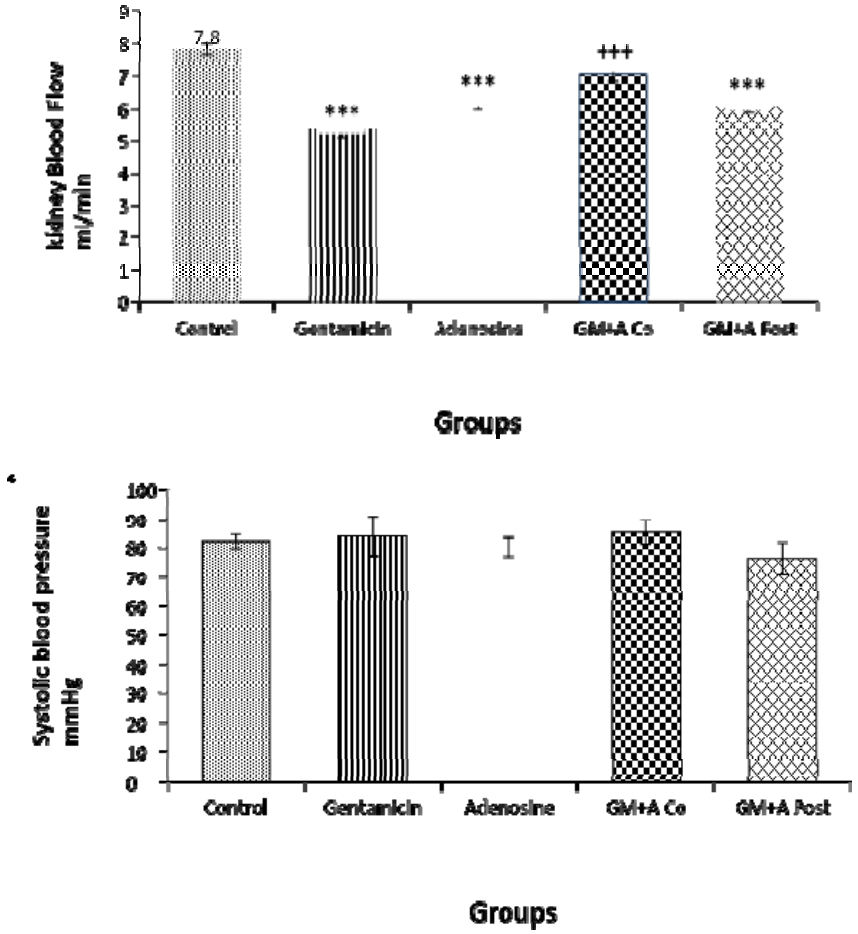

Figure 1. Comparison of A) systolic blood pressure and B) renal blood flow among the groups
Compared to the control group: ***P<0.001, **P<0.01, *P<0.05; Compared to the gentamicin group: +++P<0.001, ++P<0.01, +P<0.05; Results are expressed as mean± standard error of the mean for seven rats in each group.

Effects of adenosine on creatinine clearance (C_{Cr}), absolute ($U_{Na}V_o$) and relative (FE_{Na}) excretions of sodium, and absolute (U_kV_o) and relative (FE_k) excretions of potassium: the outcomes showed that as compared to the control group (0.26±0.1 ml/min/kg), creatinine clearance was significantly lower in the gentamicin group (0.009±0.002 ml/min/kg; P<0.05). No significant differences were observed between the adenosine (0.21±0.1 ml/min/kg) and control groups.

Compared to the gentamicin group, there was an insignificant increase in creatinine clearance in the concurrent and post-treatment adenosine groups (0.07±0.03 ml/min/kg and 0.05±0.02 ml/min/kg, respectively). Relative excretion of sodium in the gentamicin group significantly increased compared to the control group (30.5±5.7% vs. 0.88±0.05%; P<0.001).

Relative excretion of sodium in the adenosine group (0.90±0.20%) was not significantly different from the control group. The concurrent (11.4±1.8%) and post-treatment (2.4±0.6%) adenosine groups manifested a significant reduction in relative excretion of sodium compared to the gentamicin group (P<0.001). Relative excretion of potassium in the gentamicin group was significantly higher than the control group (756.9±66.9% vs. 64.4±5.4%; P<0.001).

With respect to relative excretion of potassium, the adenosine group (52.05±7.8%) was not significantly different from the control group. Relative excretion of potassium in the concurrent adenosine group (457.2±160.4%) was not significantly different from the gentamicin group. However, elative excretion of potassium in the post-treatment adenosine group (185.03±34.8%) was significantly lower than the gentamicin group (P<0.01). The groups were not significantly different in terms of absolute excretion of sodium and potassium (Table 1).

Effects of adenosine on urinary levels of sodium ($[Na]_u$), potassium ($[K]_u$), creatinine ($[Cr]_u$), urea ($[BUN]_u$), and osmolality ($Osmolu_u$): urinary sodium concentration in the gentamicin group was significantly higher than the control group (219.3±45.5 µmol/mL vs. 98.7±14.9 µmol/mL; P<0.01). Concentration of urinary sodium in the adenosine group (56.8±13.7 µmol/mL) was not significantly different from the control group. Urinary concentration of sodium in the concurrent adenosine group (77.3±10.2 µmol/mL) was significantly lower than the gentamicin group (P<0.001).

Urinary concentration of sodium in the post-treatment adenosine group (176.7±22.1 µmol/mL) was not significantly different from the gentamicin group. Urinary concentrations of creatinine in both gentamicin (7.6±0.9 mg/dL; P<0.001) and adenosine (30±4.7 mg/dL) groups were significantly lower than the control group (68.3±14 mg/dL; P<0.05). In both concurrent (14.4±4.7 mg/dL) and post-treatment (30.2±5.8 mg/dL) adenosine groups, urinary concentration of creatinine was insignificantly higher than the gentamicin group.

Urinary concentration of urea was significantly lower in the gentamicin group compared to the control group (1166.7±192.6 mg/dL vs. 2033.3±297.4 mg/dL; P<0.05). In addition, there was an insignificant difference between the adenosine (1500±163.3 mg/dL) and control groups in terms of urinary urea concentration. Urinary concentration of urea was significantly higher in the post-treatment adenosine group (2380±120.5 mg/dL) compared to the gentamicin group (P<0.001)

Urine osmolality in the gentamicin group was insignificantly higher than the control group (1773.1±270.1 mOsmkgH_2O vs. 1200.6±141.5 mOsmkgH_2O). Urine osmolality in the adenosine group (1453±241.9 mOsmkgH_2O) was not significantly different from the control group

Table 1. Comparison of creatinine clearance (C_{Cr}), absolute ($U_{Na}V_o$) and relative (FE_{Na}) excretions of sodium, and absolute (U_kV_o) and relative (FE_k) excretions of potassium

Groups	Parameters				
	FE_k%	FE_{Na}%	U_kV^o (mmol/min/kg)	$U_{Na}V^o$ (mmol/min/kg)	C_{cr} (ml/min/kg)
Control	64.4±5.4	0.88±0.05	0.57±0.15	0.2±0.05	0.26±0.1
Gentamicin	*** 756.9±66.9	*** 30.5±5.73	0.38±0.06	0.3±0.05	* 0.009±0.002
Adenosine	52.05±7.8	0.90±0.2	0.44±0.16	0.14±0.07	0.11±0.05
Gentamicin + adenosine (concurrent)	* 457.2±160.4	** +++ 11.4±1.8	0.58±0.09	0.3±0.06	0.07±0.03
Gentamicin + adenosine (post-treatment)	++ 185.03±34.8	+++ 2.4±0.6	0.51±0.20	0.2±0.09	0.05±0.02

Compared to control group: ***P<0.001, **P<0.01, *P<0.05; Compared to the gentamicin group: +++P<0.001, ++P<0.01, +P<0.05; Results were expressed as mean±standard error of the mean for seven rats in each group.

Table 2. Comparison of urinary concentrations of sodium ($[Na]_u$), potassium ($[K]_u$), creatinine ($[Cr]_u$), urea ($[BUN]_u$), and osmolality ($Osmol_u$) among the groups

Groups	Parameters				
	$[Na]_u$ (µmol/mL)	$[K]_u$ (µmol/mL)	$[Cr]_u$ (mg/dL)	$[BUN]_u$ (mg/dL)	$Osmol_u$ (mOsm/kgH_2O)
Control	98.7±14.9	219.7±32.1	68.3±14	2033.3±297.4	1200.6±141.5
Gentamicin	** 219.3±45.5	254±39.6	*** 7.6±0.9	* 1166.7±192.6	1773.1±270.1
Adenosine	56.8±13.6	191.2±28.1	* 30±4.7	1500±163.3	1453±241.9
Gentamicin + Adenosine (concurrent)	+++ 77.3±10.2	+ 109.1±13.2	*** 14.4±4.7	* 914.3±107.9	+ 941.9±73.2
Gentamicin + Adenosine (post treatment)	176.7±22.1	274.7±25.8	* 30.2±5.8	+++ 2380±120.5	1925.1±179.4

Compared to control group: ***P<0.001, **P<0.01, *P<0.05; Compared to the gentamicin group: +++P<0.001, ++P<0.01, +P<0.05; Results were expressed as mean±standard error of the mean for seven rats in each group.

There was a significant reduction in urine osmolality in the concurrent adenosine group (941.9±73.2 mOsmkgH_2O) compared to the gentamicin group (P<0.05; Table 2)

Effects of adenosine on plasma concentrations of sodium ($[Na]_p$), potassium ($[K]_p$), creatinine ($[Cr]_p$), urea ($[BUN]_p$), and osmolality ($Osmol_p$): the results revealed that administration of gentamicin (1.1±0.1 mg/dL) enhanced plasma creatinine concentration in comparison to the control group (0.8±0.08 mg/dL), but the difference was not significant. The adenosine group (0.6±0.05 mg/dL) was not significantly different from the control group in terms of plasma concentration of creatinine. Adenosine treatment (0.7±0.05 mg/dL) insignificantly reduced plasma concentration of creatinine compared to gentamicin administration. There was a significant increase in plasma concentration of urea in the gentamicin group compared to the control group (73.6±21.2 mg/dL vs. 25.3±2.6 mg/dL; P<0.05).

An insignificant difference in plasma concentration of urea was observed between the adenosine (27.3±2.7 mg/dL) and control groups. Plasma concentration of urea was insignificantly lower in the post-treatment adenosine group (27.3±1.7 mg/dL) compared to the gentamicin group. Plasma osmolality and plasma concentrations of sodium and potassium were not significantly different between the groups (Table 3).

The effects of adenosine on the MDA and FRAP levels in renal tissue: the results of this study manifested a significant increase in MDA level in the gentamicin group compared to the control group (18.5±0.6 µmol/gkw vs. 12.9±1.5 µmol.gkw; P<0.05). The adenosine group (6.1±0.8 µmol/gkw) also showed a significant reduction of MDA level compared to the control group (P<0.01). Moreover, there was a significant reduction of MDA in the concurrent (10.8±1.5 µmol/gkw) and post-treatment (8.2±1.2 µmol/gkw) adenosine groups compared to the gentamicin group (P<0.001).

FRAP level in renal tissue was significantly lower in the gentamicin group compared to the control group (9.8±0.6 mmol/gkw vs. 17.1±2.1 mmol/gkw; P<0.01). On the contrary, the adenosine group (22.1±1.1 mmol/kgw) showed no significant differences from the control group in terms of FRAP. There was also a significant

Table 3. Comparison of plasma concentrations of sodium ($[Na]_p$), potassium ($[K]_p$), creatinine ($[Cr]_p$), urea ($[BUN]_p$), and osmolality ($Osmol_p$)

Groups	Parameters				
	$[Na]_p$ (µmol/mL)	$[K]_p$ (µmol/mL)	$[Cr]_p$ (mg/dL)	$[BUN]_p$ (mg/dL)	$Osmol_p$ (mOsm/kgH_2O)
Control	142.7±2.6	4.7±0.2	0.8±0.08	25.3±2.6	300.7±3.8
Gentamicin	142.6±2.4	5.2±0.2	1.1±0.1	* 73.6±21.2	303.3±8.5
Adenosine	147.9±1.2	5±0.2	0.6±0.05	27.3±2.7	295.1±1.9
Gentamicin + adenosine (concurrent)	140.7±1.9	4.7±0.2	*** 1.9±0.4	* 80.4±19.8	223.3±8.2
Gentamicin + adenosine (post-treatment)	146.3±1.2	4.8±0.2	0.7±0.05	27.3±1.7	284.4±2.5

Compared to the control group: ***P<0.001, **P<0.01, *P<0.05; Compared to the gentamicin group: +++P<0.001, ++P<0.01, +P<0.05; Results were expressed as mean±standard error of the mean for seven rats in each group.

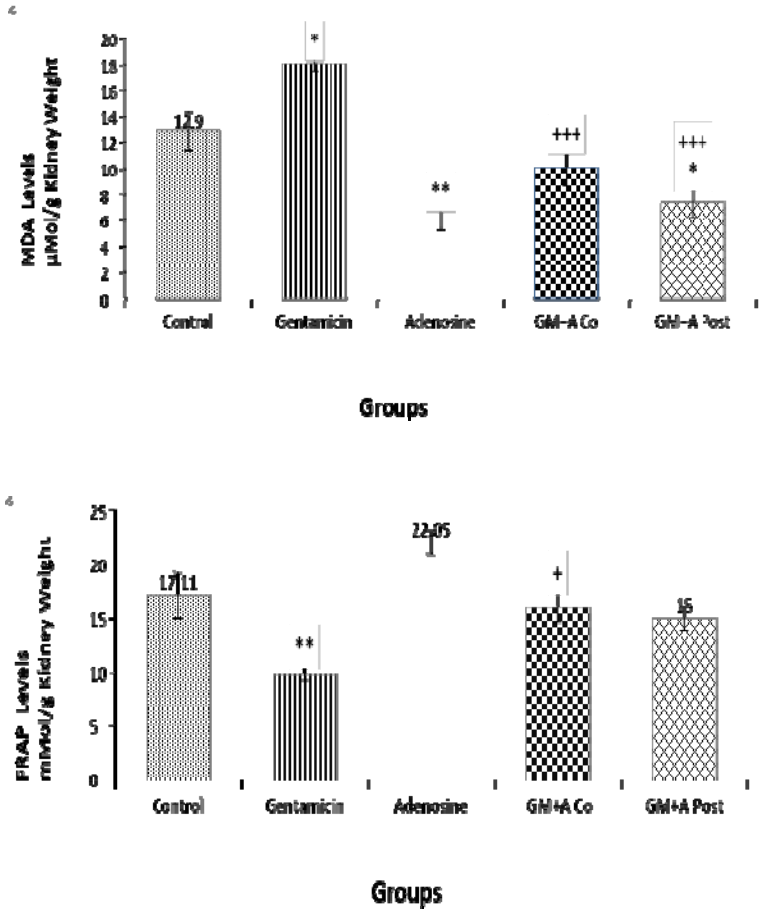

Figure 2. Comparison of MDA and FRAP levels between the groups; Compared to the control group: ***P<0.001, **P<0.01, *P<0.05; Compared to the gentamicin group: +++P<0.001, ++P<0.01, +P<0.05; Results are expressed as mean± standard error of the mean for seven rats in each group.

raise in FRAP level in the concurrent adenosine treatment group (16.1±1.4 mmol/gkw) in comparison to the gentamicin group (P<0.05). Finally, an insignificant increase of FRAP level was observed in the post-treatment adenosine group (15±1.1 mmol/gkw) compared to the gentamicin group (Figure 2).

The effects of adenosine on histological changes (Figure 3): based on the results, increased Bowman's space (grade 4), reduced number of RBCs of the glomeruli (grade 3), necrosis of tubular cells (grade 3), formation of protein casts within the tubule lumen (grade 3), vacuolation of tubular cells (grade 3; P<0.001), and scattering of cells into the tubule lumen (grade 3; P<0.01) in the gentamicin group were not significantly different from the control group (grade 0).

In addition, the adenosine group (grade 0) did not show any significant differences from the control group (Figure 3).

Compared to the gentamicin group, the concurrent adenosine treatment for eight consecutive days resulted in a significant decrease of cellular necrosis (grade 2), reduction of the Bowman's space (grade 3), formation of protein casts within the tubule lumen (grade 2), scattering of cells into the tubule lumen (grade 1; P<0.05), and vacuolation of tubular cells (grade 1; P<0.01), as well as expansion in the number of RBCs in the glomeruli (grade 1; P<0.05).

In the post-treatment adenosine group, compared to the gentamicin group, there was a significant decrease in cellular necrosis (grade 2), cell scattering (grade 2; P<0.05), Bowman's space (grade 2), formation of protein casts (grade 1), and cellular vacuolation (grade 1; P<0.01), as well as an increase in the number of RBCs in the glomeruli (grade 1; P<0.05; Table 4).

DISCUSSION

The results of this study revealed that gentamicin leads to nephrotoxicity, which is associated with elevated plasma concentrations of urea and creatinine, as well as reduced creatinine and urea clearance and urinary

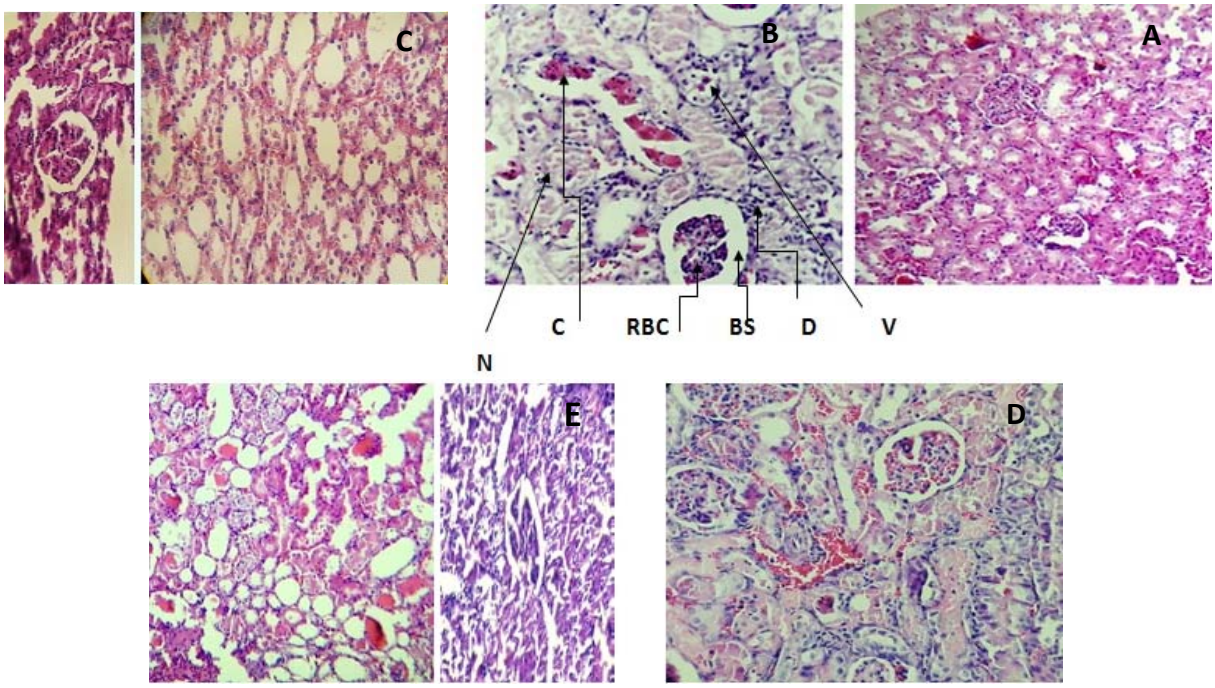

Figure 3. Comparison of renal histological between different groups
A- Control group with glomerular and normal tubular structure (× 40); B- Gentamicin group with tubular cell necrosis, formation of protein casts inside the tubule lumen, cells scattering into the tubule lumen, vacuolation of tubular cells, increased Bowman's space, and reduced number of red blood cells in glomerulus(× 40); C- Adenosine group with normal glomerular and tubular structures (× 40); D- Concurrent treatment with adenosine with reduced tubular cell necrosis, formation of protein casts inside the tubule lumen, cell scattering (× 40), vacuolation of tubular cells, increased Bowman's space, and enhanced number of red blood cells in glomerulus (× 40); E- Post-treatment with adenosine group with reduced tubular cell necrosis, formation of protein casts inside tubule lumen, cell scattering, vacuolation of tubular cells, increased Bowman's space, and elevated number of red blood cells in glomerulus.
RBC: Red blood cells, BS: Bowman's space, N: Necrosis, C: Intratubular cast, D: Downfall, V: Vacuolization

Table 4. Comparison of necrosis level, protein casts, cell scattering, vacuolation, the reduced number of red blood cells, increased Bowman's space, and total glomerular injury

Groups	Parameters						
	Necrosis	Formation of protein casts	Cell scattering	Vacuolation	Reduced number of red blood cells	Increased Bowman's space	Total glomerular injury
Control	0	0	0	0	0	0	0
Gentamicin	*** 3	*** 3	** 3	*** 3	*** 3	*** 4	*** 3
Adenosine	0	0	0	0	0	0	0
Gentamicin + adenosine (concurrent)	*+ 2	*+ 2	*+ 1	++ 1	+ 1	**+ 3	**+ 2
Gentamicin + adenosine (post-treatment)	*+ 2	++ 1	*+ 2	*++ 1	*+ 1	*++ 2	*+++ 1

Compared to the control group: ***P<0.001, **P<0.01, *P<0.05; Compared to the gentamicin group: +++P<0.001, ++P<0.01, +P<0.05; Results are expressed as mean±standard error of the mean for seven rats in each group

excretion. These factors, which might be induced by decreased Kf or necrosis of tubular cells with subsequent reduction in the number of functional nephrons, and in turn, GFR decline, are indicators of nephrotoxicity [26, 27].

Creatinine clearance and plasma concentration levels were not significantly different between the adenosine and control groups. In the concurrent and post-treatment adenosine groups, compared to the gentamicin group, there was an insignificant enhancement in creatinine clearance. Previous studies demonstrated that adenosine could dramatically improve renal function, GFR, creatinine clearance, and renal histology using its A_1 and A_{2B} receptors in the kidneys [13].

Quite in line with other studies, in this study, gentamicin increased urinary excretion of sodium and potassium, which in turn, resulted in higher FE_{Na} and FE_K [28]. Gentamicin had an inhibitory effect on Na^+/K^+_{ATPase} [3]. Na^+/K^+_{ATPase} provides the required gradient force for transferring sodium and potassium ions, and its inhibition leads to accumulation of

sodium and water in the cell, cell swelling, and ultimately, cellular necrosis and excretion of sodium and potassium ions [29].

Relative and urinary excretions of sodium ions were not considerably different between the adenosine and control groups. The concurrent and post-treatment adenosine significantly reduced relative sodium excretion in comparison to the gentamicin group. In the proximal tubule, Na^+/H^+ (NHE3) transmitter is mainly responsible for reabsorption of Na+; therefore, by activating A_1 adenosine receptor, it reactivates NHE3 and increases sodium reabsorption [30].

Plasma osmolality did not significantly vary among the groups. The gentamicin group exhibited a slight increase of urinary osmolality compared to the control group, which was probably due to increased urinary excretion of ions. Aminoglycoside-induced nephrotoxicity lowered urine osmolality and increased urine volume, which result from decreased expression of aquaporin-2 water channel and aquaporin-1, which are vital for urinary concentration [31, 32].

In the current study, urine osmolality and volume were not significantly different in the gentamicin group. Urinary osmolality in the concurrent adenosine treatment group reduced significantly compared to the gentamicin group. Activation of adenosine A_1 receptor in renal tubule stimulates NHE3, Na/P_i, and transmission of Na/glucose, and as a result, increases sodium reabsorption and diminishes its urinary excretion [30].

Gentamicin treatment significantly increased MDA and reduced FRAP levels in renal tissue. Gentamicin treatment impaired antioxidant defense system, undermined levels of superoxide dismutase, glutathione peroxidase, and catalase, and enhanced lipid peroxidation [33]. In the adenosine group, compared to the control group, there was not a significant reduction in MDA; moreover, FRAP level in the adenosine group was not significantly different from the control group.

Compared to the gentamicin group, in the concurrent adenosine treatment group, a significant reduction of MDA level was observed. FRAP level also increased significantly in the concurrent adenosine treatment group compared to the gentamicin group. The post-treatment adenosine group showed a significant reduction in MDA and a slight increase in FRAP level compared to the gentamicin group. Through A_{2A} receptor, adenosine reduces myeloperoxidase activity and release of ROS by enhancing cAMP activity and activation of kinase A protein in neutrophils [14].

In the present study, there was not a significant difference between the groups in terms of blood pressure. Given the short- and long-term blood pressure regulation mechanisms, stability of blood pressure might be due to involvement of short- and medium-term mechanisms.

The kidneys are long-term regulators of blood pressure, and patients with end-stage renal failure face hypertension. In addition, our study showed that mean arterial pressure and heart rate in intraperitoneal or intra-intestinal adenosine administration groups were similar to the control group during hemorrhagic shock and resuscitation [34].

Gentamicin treatment decreased RBF compared to the control group. Gentamicin reduced RBF through boosting renal vascular resistance. Reduced RBF plays an important role in reducing GFR, as well [35, 36]. Increased renal vascular resistance is primarily due to activation of TGF. Gentamicin impairs function of membrane transporters, which leads to loss of tubular reabsorption and activation of TGF. This feedback is a protective mechanism to prevent water and electrolytes loss after tubular damage, but it will eventually mitigate within 1-24 hours [9]. Additionally, gentamicin promotes resistance through increased production of vasoconstrictor mediators such as platelet-activating factor (PAF), endothelin-1, and arachidonic acid metabolites (mainly thromboxane A_2) in the renal vascular system and mesangial cells. This vascular resistance results from direct effects of gentamicin on vascular cells [37, 38].

RBF reduced in the adenosine group in comparison to the control group. Studies suggested that endogenous and exogenous A_1 adenosine receptors reduced GFR and cortex blood flow due to contraction of renal arteries and TGF mechanism [14]. The reduced blood flow might be due to higher activation of A_1 receptors under this physiological condition. A_1 receptors are broadly expressed in the kidneys, especially in afferent arteriole, mesonephric cells, juxta-glumerular cells, direct vessels, juxta-tubule, thin branches of Henle's loop, thick ascending branch of Henle's loop, central collecting ducts, and epithelium cells of the papilla [15].

Compared to the gentamicin group, concurrent treatment with adenosine significantly elevated RBF. In addition, there was an insignificant increase in RBF in the post-treatment adenosine group. Adenosine production increases and the expression of its receptor alter in the course of pathological conditions [14]. Increased adenosine

in the kidney leads to activation of low-affinity receptors such as A_{2B}. A_{2B} and A_{2A} are two adenosine receptors mainly located in the renal artery, which cause deep vasodilatation of the cortex and increased blood flow in the medulla [14].

The potent vasodilator property of adenosine is because of A_{2B} receptor activation, which can neutralize vasoconstriction through A_1 receptor [13, 39]. A_{2A} receptors could diminish TGF and consequently, increase RBF by stimulating endothelial inducible nitric oxide synthase (iNOS) enzymes in the afferent arteriole [40].

Histological analysis presented that unlike the control group, the gentamicin group manifested tubular cell necrosis, formation of protein casts, reduction of RBCs, as well as vacuolation of tubular cells and cells in the tubule lumen. Renal tissue in the adenosine group was not significantly different from the control group. Both concurrent and post-treatment adenosine administrations had protective effects on renal tissue and improved tubular and glomerular structures.

Adenosine had anti-inflammatory properties, and by A_1 receptor through $G_{i,}$ it activates ERK MAPK and PIK3, which in turn, causes HSP27 phosphorylation and attenuates apoptosis, inflammation, and necrosis [14].

By reducing the release of pre-inflammatory cytokines, derived from macrophages such as TNF-α, IL-6, and IL-8, adenosine reduced inflammation in glomerulonephritis through A_{2A} receptors. Furthermore, A_{2B} adenosine receptor decreases inflammation in renal tubular endothelial and epithelial cells through G_s and cAMP signaling pathways [14]. Additionally, inflammatory markers such as activation of NF-κB extensively reduce accumulation of granulocytes and myeloperoxidase [13]. Thus, considering the anti-inflammatory properties of adenosine, its concurrent and post-treatment administrations improve glomerular and tubular structures. Another study indicated that cisplatin could boost expression of A_1 receptor in the kidneys by production of ROS and activation of nuclear factor-kappa and can protect cells against cisplatin-induced nephrotoxicity [41].

CONCLUSION

Concurrent IP injections of adenosine and gentamicin for eight consecutive days had a protective effect on renal tissue and improved hemodynamic parameters and antioxidant defense conditions. Post-treatment with adenosine also improved excretion of ions and enhanced antioxidant defense systems.

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CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Javaid R, Aslam M, Javaid R, Nizami Q, Javed K, Azhar MU. Extract of ferula foetida regel reverses gentamicin-induced nephrotoxicity in rats. EXCLI J. 2012; 11:760-6.
2. Ajami M, Eghtesadi S, Pazoki-Toroudi H, Habibey R, Ebrahimi SA. Effect of crocus sativus on gentamicin induced nephrotoxicity. Biol Res. 2010; 43(1):83-90.
3. Banday AA, Farooq N, Priyamvada S, Yusufi AN, Khan F. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sci. 2008; 82(9-10):450-9.
4. Dagil R, O'shea C, Nykjær A, Bonvin AM, Kragelund BB. Gentamicin binds to the megalin receptor as a competitive inhibitor using the common ligand binding motif of complement type repeats: insight from the nmr structure of the 10th complement type repeat domain alone and in complex with gentamicin. J Biol Chem. 2013; 288(6):4424-35.
5. Morales AI, Detaille 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.
6. Zorov DB. Amelioration of aminoglycoside nephrotoxicity requires protection of renal mitochondria. Kidney Int. 2010; 77(10):841-3.
7. Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Di Paola R, Britti D, et al. A role for superoxide in gentamicin-mediated nephropathy in rats. Eur J Pharmacol. 2002; 450(1):67-76.
8. Walker PD, Barri Y, Shah SV. Oxidant mechanisms in gentamicin nephrotoxicity. Renal Failure. 1999; 21(3-4):433-42.
9. 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.
10. Martinez-Salgado C, Lopez-Hernandez FJ, Lopez-Novoa JM. Glomerular nephrotoxicity of aminoglycosides. Toxicol Appl Pharmacol. 2007; 223(1):86-98.
11. Huang SS, Zheng RL. Rosmarinic acid inhibits angiogenesis and its mechanism of action in vitro. Cancer Lett. 2006; 239(2):271-80.
12. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother. 1999; 43(5):1003-12.
13. Bauerle JD, Grenz A, Kim JH, Lee HT, Eltzschig HK. Adenosine generation and signaling during acute kidney injury. J Am Soc Nephrol. 2011; 22(1):14-20.
14. Yap SC, Lee HT. Adenosine and protection from acute kidney injury. Curr Opin Nephrol Hypertens. 2012; 21(1):24-32.
15. Vallon V, Mühlbauer B, Osswald H. Adenosine and kidney function. Physiol Rev. 2006; 86(3):901-40.
16. 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.
17. Kaster MP, Rosa AO, Rosso MM, Goulart EC, Santos AR, Rodrigues AL. Adenosine administration produces an antidepressant-like effect in mice: evidence for the involvement of A1 and A2A receptors. Neurosci Lett. 2004; 355(1):21-4.
18. 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):H2408-15.
19. Hashimoto S, Huang YG, Castrop H, Hansen PB, Mizel D, Briggs J, et al. Effect of carbonic anhydrase inhibition on GFR and renal hemodynamics in adenosine-1 receptor-deficient mice. Pflügers Arch. 2004; 448(6):621-8.
20. Ameen NM, Altubaigy F, Jahangir T, Mahday IA, Mohammed EA, Musa OA. Effect of Nigella sativa and bee honey on pulmonary, hepatic and renal function in Sudanese in Khartoum state. J Med Plant Res. 2011; 5(31):6857-63.
21. Vallon V, Rose M, Gerasimova M, Satriano J, Platt KA, Koepsell H, et al. Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. Am J Physiol Renal Physiol. 2013; 304(2):F156-67.
22. Laustsen C, Østergaard JA, Lauritzen MH, Nørregaard R, Bowen S, Søgaard LV, et al. Assessment of early diabetic renal changes with hyperpolarized [1-(13)C] pyruvate. Diabetes Metab Res Rev. 2013; 29(2):125-9.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-8.
24. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996; 239(1):70-6.
25. 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. 2009; 25(1):69-76.
26. Savin V, Karniski L, Cuppage F, Hodges G, Chonko A. Effect of gentamicin on isolated glomeruli and proximal tubules of the rabbit. Lab Invest. 1985; 52(1):93-102.
27. Martnez-Salgado C, Rodrguez-Barbero A, Tavares P, Eleno N, lida E, Lpez-Novoa J, et al. Role of calcium in gentamicin-induced mesangial cell activation. Cell Physiol Biochem. 2000; 10(1-2):65-72.
28. Gowrisri M, Kotagiri S, Vrushabendra Swamy BM, Archana Swamy P, Vishwanath KM. Anti-oxidant and nephroprotective activities of cassia occidentalis leaf extract against gentamicin induced nephrotoxicity in rats. Res J Pharm Biol Chem Sci. 2012; 3(3):684-94.
29. Xiao AY, Wei L, Xia S, Rothman S, Yu SP. Ionic mechanism of ouabain-induced concurrent apoptosis and necrosis in individual cultured cortical neurons. J Neurosci. 2002; 22(4):1350-62.
30. Osswald H, Schnermann J. Methylxanthines and the kidney. Handb Exp Pharmacol. 2011; 200:391-412.
31. Guo X, Nzerue C. How to prevent, recognize, and treat drug-induced nephrotoxicity. Cleveland Clin J Med. 2002; 69(4):289-90.
32. Bae WK, Lee J, Park JW, Bae EH, Ma SK, Kim SH, et al. Decreased expression of Na/K-ATPase, NHE3, NBC1, AQP1 and OAT in gentamicin-induced nephropathy. Korean J Physiol Pharmacol. 2008; 12(6):331-6.
33. Thounaojam MC, Jadeja RN, Devkar RV, Ramachandran AV. Sida rhomboidea. Roxb leaf extract ameliorates gentamicin induced nephrotoxicity and renal dysfunction in rats. J Ethnopharmacol. 2010; 132(1):365-7.
34. 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.
35. Morales AI, Buitrago JM, Santiago JM, Fernández-Tagarro M, López-Novoa JM, Pérez-Barriocanal F. Protective effect of trans-resveratrol on gentamicin-induced nephrotoxicity. Antioxid Redox Signal. 2002; 4(6):893-8.
36. Klotman PE, Yarger WE. Reduction of renal blood flow and proximal bicarbonate reabsorption in rats by gentamicin. Kidney Int. 1983; 24:638-43.
37. 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 Exp Pathol. 1999; 80(5):265-70.
38. Dashti-Khavidaki S, Shahbazi F, Khalili H, Lessan-Pezeshki M. Potential renoprotective effects of silymarin against nephrotoxic drugs: a review of literature. J Pharm Pharm Sci. 2012; 15(1):112-23.
39. Feng MG, Navar LG. Afferent arteriolar vasodilator effect of adenosine predominantly involves adenosine A2B receptor activation. Am J Physiol Renal Physiol. 2010; 299(2):F310-5.
40. Carlström M, Wilcox CS, Welch WJ. Adenosine A2A

receptor activation attenuates tubuloglomerular feedback responses by stimulation of endothelial nitric oxide synthase. Am J Physiol Renal Physiol. 2011; 300(2):F457-64.

41. Bhat SG, Mishra S, Mei Y, Nie Z, Whitworth CA, Rybak LP, et al. Cisplatin up-regulates the adenosine A (1) receptor in the rat kidney. Eur J Pharmacol. 2002; 442(3):251-64.