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Protective effects of Adenosine on Gentamicin-Induced Nephrotoxicity in rat

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ABSTRACT

Introduction: Gentamicin, an aminoglycoside antibiotic widely used for treating gram-negative bacterial infections, is limited in clinical practice due to its nephrotoxic effects. This study was designed to test the hypothesis that adenosine attenuates gentamicin-induced nephrotoxicity by mitigating oxidative stress and inflammation.

Materials and Methods: Thirty-five male Wistar rats were randomly allocated into five groups (n = 7 each): control, gentamicin, adenosine, gentamicin plus adenosine (concurrent treatment), and adenosine post-treatment. Systolic blood pressure, renal blood flow (RBF), and serum levels of urea, creatinine, sodium, potassium, and osmolality were measured. In addition, renal tissue was analyzed for malondialdehyde (MDA) content and ferric reducing antioxidant power (FRAP).

Results: Concurrent adenosine administration significantly prevented the gentamicin-induced increase in sodium excretion and renal MDA levels, while restoring reduced RBF and FRAP values ($p < 0.05$). In the post-treatment group, adenosine significantly attenuated the gentamicin-induced increases in urinary sodium, potassium, and MDA level, while promoting a higher urinary urea output compared with the gentamicin group ($p < 0.05$).

Conclusion: Adenosine attenuates gentamicin-induced nephrotoxicity, likely through vasodilatory, anti-inflammatory, and antioxidant mechanisms.

Keywords: Adenosine, Gentamicin, Nephrotoxicity, Oxidative stress.

Introduction

Gentamicin is an aminoglycoside antibiotic widely used in the management of gram-negative bacterial infections; however, its clinical application is substantially restricted due to adverse effects such as nephrotoxicity. Gentamicin-induced nephrotoxicity is primarily associated with drug accumulation in renal tubular epithelial cells, leading to a series of morphological and biochemical alterations (1–3). This accumulation is mediated through endocytic receptors, notably megalin and cubilin (4).

Multiple mechanisms contribute to gentamicin-induced nephrotoxicity:1. Mitochondrial dysfunction: Upon entering the cytosol, gentamicin disrupts mitochondrial integrity by increasing the permeability transition pore, releasing cytochrome C, and impairing the respiratory chain. These alterations result in excessive generation of reactive oxygen species (ROS) from the electron transport chain (5–8). 2. Lysosomal accumulation: Internalized gentamicin undergoes endocytosis and accumulates in lysosomes, where it promotes lysosomal membrane permeabilization through ROS production, ultimately triggering apoptosis in tubular epithelial cells (9). 3. Effects on mesangial cells: Within the glomerulus, gentamicin induces contraction of mesangial cells, thereby reducing the glomerular ultrafiltration coefficient (Kf) and glomerular filtration rate (GFR).

Gentamicin administration induces renal interstitial inflammation, extensive tubular necrosis, and protein cast formation, which result from the detachment and sloughing of tubular epithelial cells into the tubular lumen. Enhanced generation of superoxide anions plays a critical role in triggering mesangial cell contraction (9,10). In this context, oxidative stress and

inflammation represent key mechanisms underlying gentamicin-induced nephrotoxicity (11). Nevertheless, despite these adverse effects, gentamicin remains widely prescribed due to its affordability, low resistance profile, and potent, broad-spectrum antibacterial activity (12).

The endogenous nucleoside adenosine, composed of an adenine base and a ribose sugar, is ubiquitously present in mammalian tissues and functions as a fundamental component of cellular energy metabolism. Produced by virtually all mammalian cells, adenosine plays a pivotal role in adaptation to hypoxic stress (13,14). Adenosine is an endogenous purine nucleoside that exists both intracellularly and extracellularly; the latter primarily serves as a signaling molecule. Extracellular adenosine levels rise during states of negative energy balance, when the rate of adenosine 5′-triphosphate (ATP) hydrolysis exceeds its synthesis.

Under pathological conditions, adenosine tends to accumulate in the kidney, where excessive ATP consumption, impaired renal perfusion, hypoxia, and inflammation drive its overproduction. In renal physiology, adenosine regulates renin secretion, glomerular filtration rate (GFR), and renal vascular tone, and also serves as a central mediator of tubuloglomerular feedback (TGF) (14). Adenosine receptors (A1, A2A, A2B, and A3) are members of the G-protein–coupled receptor (GPCR) superfamily (13–15). Notably, the expression of these receptors is dynamically modulated under ischemic, hypoxic, and inflammatory conditions. Activation of renal adenosine signaling pathways through its receptors has been shown to attenuate acute kidney injury (14).

Given the anti-inflammatory, antioxidant, and vasodilatory properties of adenosine, and considering the tissue injury mechanisms underlying gentamicin-induced nephrotoxicity, the present study aimed to investigate the effects of adenosine administered either concurrently with, or following, gentamicin treatment on renal dysfunction and injury.

Materials and Method

The study was carried out on 35 adults male Wistar rats (200–250 g). The animals were housed in standard cages under controlled environmental conditions, including a 12-h light/12-h dark cycle, constant room temperature (23 ± 2 °C), and free access to food and water. All experimental procedures were performed in accordance with the ethical guidelines of the Committee for the Care and Use of Laboratory Animals at Arak University of Medical Sciences.

The animals were randomly assigned to five experimental groups:

1. Control group: received no medication;
2. Gentamicin group: administered intraperitoneal (IP) gentamicin (100 mg/kg; Alborz Darou Co., Iran) for eight consecutive days (16);
3. Adenosine group: administered IP adenosine (10 mg/kg; A9251, Sigma-Aldrich) for eight consecutive days (17);
4. Concurrent gentamicin + adenosine group: received IP gentamicin (100 mg/kg) and IP adenosine (10 mg/kg) concurrently for eight consecutive days;
5. Post-treatment adenosine group: received IP gentamicin (100 mg/kg) for eight consecutive days, followed by IP adenosine (10 mg/kg) from day 9 to day 16.

On day 9 (for the concurrent treatment group) and day 17 (for the post-treatment group), after administration of the final dose, animals were placed in metabolic cages for 12 h, and urine output was determined gravimetrically. Body weight was recorded upon removal from the metabolic cages. Subsequently, systolic blood pressure was measured under thiopental sodium anesthesia (50–60 mg/kg; Sandoz GmbH, Estonia) using a tail-cuff method with a PowerLab system (AD Instruments, Australia) (18).A midline abdominal incision was then performed to expose the kidneys. The left renal artery and vein were carefully isolated, and renal blood flow (RBF) was assessed for 30 min using a flowmeter with a specific probe (T402, USA), and values were recorded graphically (19). Blood samples were collected from the abdominal aorta using a chilled heparinized syringe. Plasma was separated, and levels of creatinine (Cr) and blood urea nitrogen (BUN) were determined using an AutoAnalyzer (Selectra-XL, Netherlands) (20). Plasma sodium ((Na+)) and potassium ((K+)) concentrations were measured by flame photometry (SEAC-20Fp, Italy) (21), and plasma osmolality was assessed using an osmometer (Gonotec Osmomat-030, Germany) (22).

Creatinine clearance (CCr) as well as absolute and fractional excretions of sodium and

potassium were calculated using the following equation:

$$CCr(\mu l/min/gkw) = (V°/1000 \times UCr)/PCr;$$

Absolute sodium excretion (UNaV°, µmol/min/gkw) was calculated as:

$$UNaV^{\circ} (\mu mol/min/gkw) = (V° \times UNa)/1000$$

Absolute potassium excretion (UKV°, µmol/min/gkw) was calculated as:

$$UKV^{\circ}(\mu mol/min/gkw) = (V^{\circ} \times UK)/1000$$

Relative sodium excretion based on percentile $FENa=(UNa \times PCr)/(PNa \times UCr) \times 100$

Fractional sodium excretion (FENa, %) was determined using the formula:

$$FENa=(UNa \times PCr)/(PNa \times UCr) \times 100;$$

Relative potassium excretion base on percentile $FEK=(Uk \times PCr)/(Pk \times UCr) \times 100$.

Following kidney excision and weighing, the right kidney was snap-frozen in liquid nitrogen and subsequently stored at –20 °C for biochemical analyses of ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA), an indicator of lipid peroxidation by reactive oxygen species (ROS). Renal tissue MDA levels were measured using the method described by Ohkawa et al. (23), while FRAP was assessed using the Benzie and Strain protocol (24). The left kidney, after capsule removal, was fixed in 10% buffered formalin. Following standard histological processing and paraffin embedding, 5-µm tissue sections were prepared and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed by a blinded pathologist using well-prepared slides. The following parameters were assessed: Size of Bowman's space, reduction in the number of red blood cells (RBCs), percentage of glomerular injury, desquamation of tubular epithelial cells into the lumen, intraluminal protein cast formation, and evidence of tubular cell vacuolation and necrosis.

Histopathological damage was graded semi-quantitatively based on the percentage of affected tissue as follows: grade 0, no damage; grade 1, 1–25% damage; grade 2, 26–50% damage; grade 3, 51–75% damage; and grade 4, 76–100% damage (25).For statistical analysis, one-way ANOVA followed by Tukey's post hoc test, as well as Kruskal–Wallis and Dunnett's tests, were performed using SPSS software (version 20.0, IBM Corp., Armonk, NY, USA). A p-value ≤ 0.05 was considered indicative of statistical significance.

Results

Effects of Adenosine on Renal Blood Flow and Systolic Blood Pressure

Renal blood flow (RBF) was significantly reduced in both the gentamicin and adenosine groups compared to controls (5.4 ± 0.3 and 6.2 ± 0.3 vs. 7.8 ± 0.2 ml/min, respectively; $P < 0.001$). Concurrent administration of adenosine for eight consecutive days significantly improved RBF relative to the gentamicin group (7.02 ± 0.2 ml/min; $P < 0.001$). In the post-treatment adenosine group, RBF (6.04 ± 0.2 ml/min) was higher than in the gentamicin group, although this difference did not reach statistical significance (Figure 1). No significant differences in systolic blood pressure were observed among the groups.

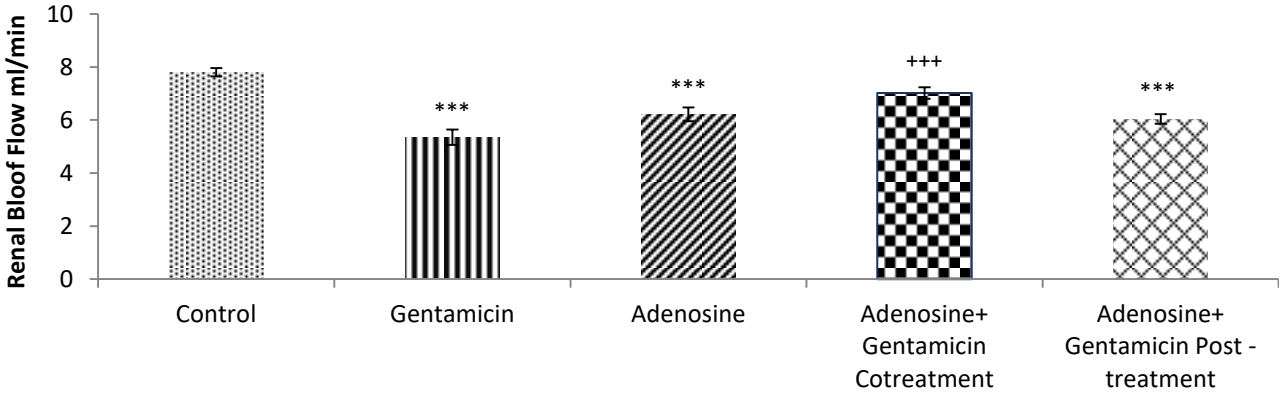

Figure 1. Comparison of renal blood flow among the groups
Compared to the control group: $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$
Compared to the gentamicin group: $^{+++}P<0.001$, $^{++}P<0.01$, $^{+}P<0.05$
Results are expressed as mean± standard error of the mean for seven rats in each group.

Effects of Adenosine on Creatinine Clearance and Electrolyte Excretion

Creatinine clearance (CCr) was significantly reduced in the gentamicin group compared with controls (0.009 ± 0.002 vs. 0.26 ± 0.1 ml/min/kg; P < 0.05). In contrast, no significant difference was observed between the adenosine group (0.21 ± 0.1 ml/min/kg) and the control group. Compared with the gentamicin group, creatinine clearance showed a nonsignificant increase in both the concurrent (0.07 ± 0.03 ml/min/kg) and post-treatment (0.05 ± 0.02 ml/min/kg) adenosine groups. Relative sodium excretion (FENa) was markedly elevated in the gentamicin group compared to controls (30.5 ± 5.7% vs. 0.88 ± 0.05%; P < 0.001). In contrast, the adenosine group (0.90 ± 0.20%) did not differ significantly from controls. Both concurrent (11.4 ± 1.8%) and post-treatment (2.4 ± 0.6%) adenosine administration significantly reduced FENa compared with the gentamicin group (P < 0.001).

Relative potassium excretion (FEK) was also significantly higher in the gentamicin group than in controls (756.9 ± 66.9% vs. 64.4 ± 5.4%; P < 0.001). FEK in the adenosine group (52.05 ± 7.8%) did not differ significantly from controls. In the concurrent adenosine group (457.2 ± 160.4%), FEK was not significantly different from the gentamicin group. However, post-treatment adenosine administration significantly reduced FEK compared with gentamicin (185.03 ± 34.8% vs. 756.9 ± 66.9%; P < 0.01).

Absolute excretions of sodium and potassium (UNaV°, UKV°) did not differ significantly among the groups (Table 1).

Effects of Adenosine on Urinary Electrolytes, Creatinine, Urea, and Osmolality

Urinary sodium concentration ([Na]u) was significantly elevated in the gentamicin group compared with controls (219.3 ± 45.5 vs. 98.7 ± 14.9 μmol/mL; P < 0.01). In the adenosine group (56.8 ± 13.7 μmol/mL), [Na]u did not differ significantly from controls. Concurrent adenosine administration significantly reduced [Na]u compared with the gentamicin group (77.3 ± 10.2 μmol/mL; P < 0.001), whereas post-treatment adenosine administration (176.7 ± 22.1 μmol/mL) showed no significant difference relative to gentamicin. Urinary creatinine concentration ([Cr]u) was significantly lower in both gentamicin (7.6 ± 0.9 mg/dL; P < 0.001) and adenosine (30.0 ± 4.7 mg/dL; P < 0.05) groups compared with controls (68.3 ± 14.0 mg/dL). In the concurrent (14.4 ± 4.7 mg/dL) and post-treatment (30.2 ± 5.8 mg/dL) adenosine groups, [Cr]u was higher than in the gentamicin group, though this difference did not reach statistical significance. Urinary urea concentration ([BUN]u) was significantly decreased in the gentamicin group compared with controls (1166.7 ± 192.6 vs. 2033.3 ± 297.4 mg/dL; P < 0.05). The adenosine group (1500 ± 163.3 mg/dL) did not differ significantly from controls. Post-treatment adenosine administration significantly increased [BUN]u relative to gentamicin (2380 ± 120.5 mg/dL; P < 0.001). Urine osmolality was higher in the gentamicin group than in controls (1773.1 ± 270.1 vs. 1200.6 ± 141.5 mOsm/kg H_2O), although this difference was not statistically significant. Osmolality in the adenosine group (1453 ± 241.9 mOsm/kg H_2O) was comparable to controls. In contrast, concurrent adenosine administration significantly reduced urine osmolality compared with the gentamicin group (941.9 ± 73.2 mOsm/kg H_2O; P < 0.05; Table 2).

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.

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.

Plasma creatinine concentration ([Cr]p) was elevated in the gentamicin group (1.1 ± 0.1 mg/dL) compared with controls (0.8 ± 0.08 mg/dL), although the difference did not reach statistical significance. In the adenosine group (0.6 ± 0.05 mg/dL), [Cr]p was comparable to controls, while post-treatment adenosine administration (0.7 ± 0.05 mg/dL) showed a nonsignificant reduction relative to gentamicin. Plasma urea concentration ([BUN]p) was significantly increased in the gentamicin group compared with controls (73.6 ± 21.2 vs. 25.3 ± 2.6 mg/dL; P < 0.05). No significant difference was observed between the adenosine (27.3 ± 2.7 mg/dL) and control groups. In the post-treatment adenosine group (27.3 ± 1.7 mg/dL), [BUN]p was lower than in the gentamicin group, though the difference was not significant. Plasma osmolality, as well as plasma sodium ([Na]p) and potassium ([K]p) concentrations, did not differ significantly among the experimental groups (Table 3).

Effects of Adenosine on Renal Tissue MDA and FRAP Levels

Renal malondialdehyde (MDA) content was significantly increased in the gentamicin group compared with the control group (18.5 ± 0.6 vs. 12.9 ± 1.5 µmol/gkw; *P* < 0.05). In contrast, the adenosine group (6.1 ± 0.8 µmol/gkw) demonstrated a significant reduction relative to controls (*P* < 0.01). Both concurrent (10.8 ± 1.5 µmol/gkw) and post-treatment (8.2 ± 1.2 µmol/gkw) adenosine administration resulted in markedly lower MDA levels compared with gentamicin alone (*P* < 0.001). Ferric reducing antioxidant power (FRAP) was significantly reduced in the gentamicin group relative to controls (9.8 ± 0.6 vs. 17.1 ± 2.1 mmol/gkw; *P* < 0.01). Adenosine treatment alone (22.1 ± 1.1 mmol/gkw) did not differ significantly from controls. Concurrent adenosine administration (16.1 ± 1.4 mmol/gkw) significantly increased FRAP compared with the gentamicin group (*P* < 0.05), whereas post-treatment adenosine (15 ± 1.1 mmol/gkw) induced only a nonsignificant elevation (Figure 2).

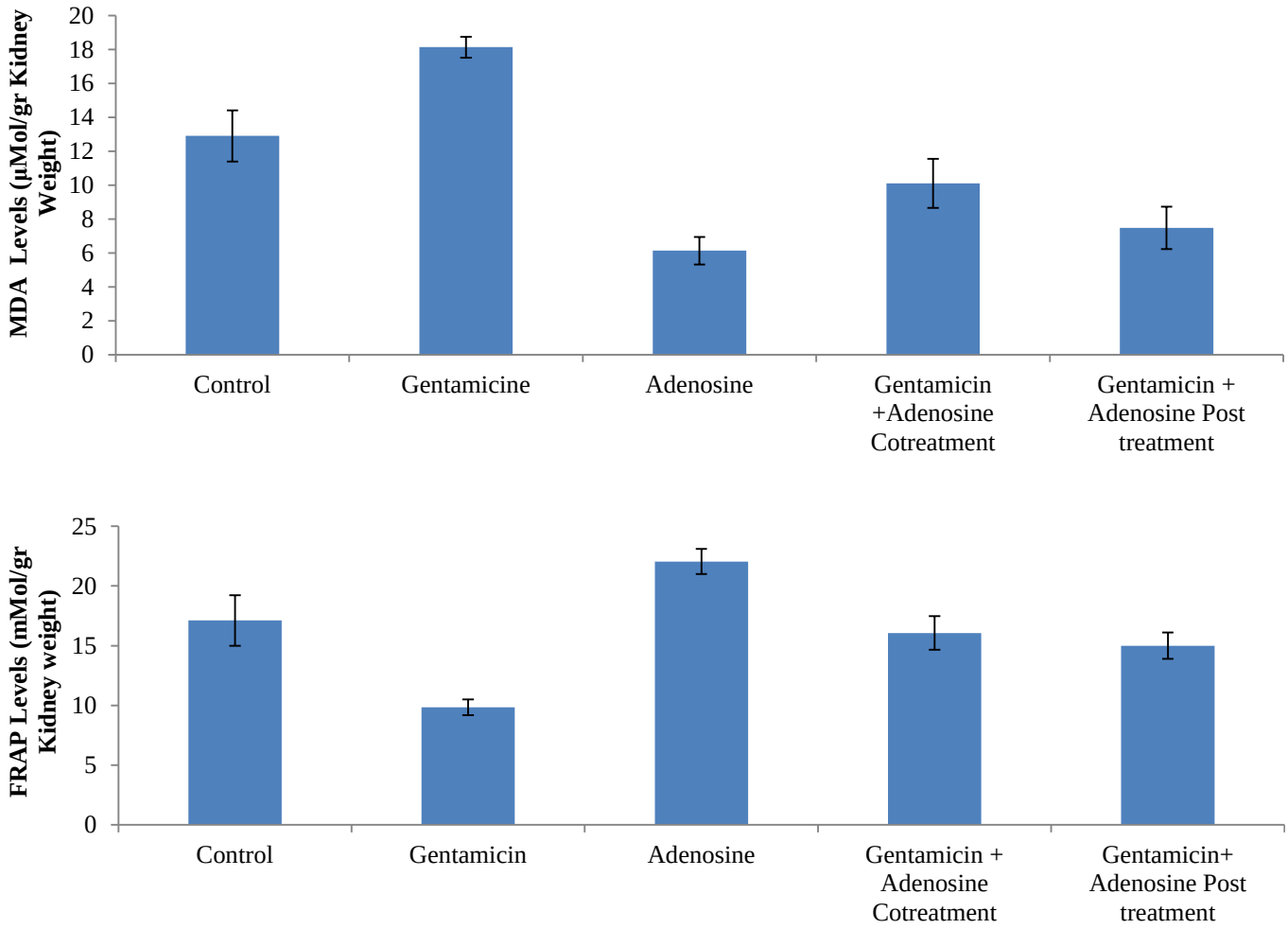

Figure 2. Comparison of MDA and FRAP levels between the groups
Compared to the control group: $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$
Compared to the gentamicin group: $^{+++}P<0.001$, $^{++}P<0.01$, $^{+}P<0.05$
Results are expressed as mean± standard error of the mean for seven rats in each group.

Effects of Adenosine on Histological Alterations (Figure 3)

Histological analysis revealed marked renal damage in the gentamicin group, characterized by widened Bowman's space (grade 4), reduced glomerular RBC count (grade 3), tubular cell necrosis (grade 3), intraluminal protein cast formation (grade 3), tubular cell vacuolation (grade 3; *P* < 0.001), and shedding of tubular cells into the lumen (grade 3; *P* < 0.01), compared with the control group (grade 0). In contrast, the adenosine group (grade 0) exhibited no significant alterations relative to controls (Figure 3).

Histological Effects of Adenosine in Concurrent and Post-Treatment Protocols

Compared with the gentamicin group, concurrent administration of adenosine for eight consecutive days markedly attenuated renal injury, as evidenced by reduced tubular necrosis (grade 2), narrowing of Bowman's space (grade 3), diminished intraluminal protein cast formation (grade 2), decreased cell shedding into the tubular lumen (grade 1; *P* < 0.05), and attenuated tubular cell vacuolation (grade 1; *P* < 0.01). Furthermore, a significant increase in the number of glomerular RBCs was observed (grade 1; *P* < 0.05).

Similarly, in the post-treatment adenosine group, histological improvements were also evident compared to the gentamicin group, including reduced tubular necrosis (grade 2), decreased cell shedding (grade 2; *P* < 0.05), narrowing of Bowman's space (grade 2), reduced protein cast formation (grade 1), and decreased tubular cell vacuolation (grade 1; *P* < 0.01). This group also demonstrated a significant increase in glomerular RBCs (grade 1; *P* < 0.05) (Table 4).

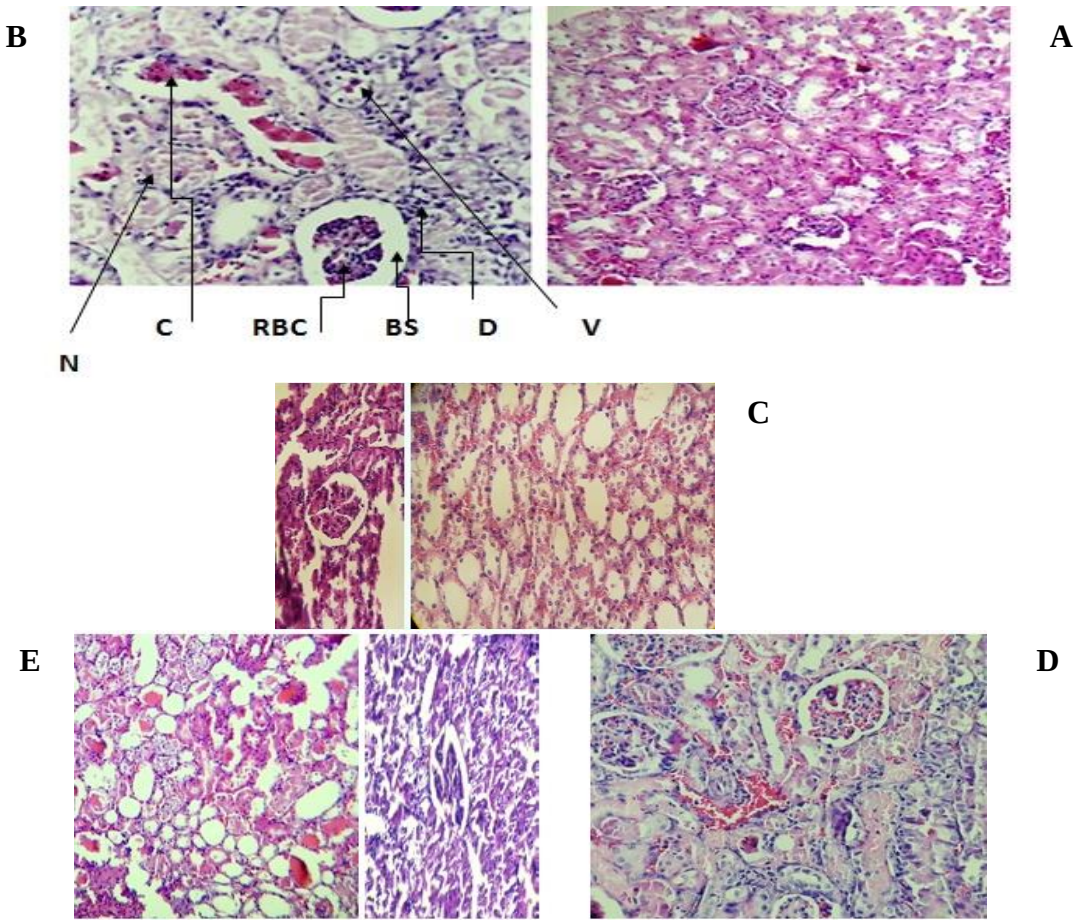

Figure 3. Comparison of renal histological between different groups

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

Parameters / Groups	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.

Discussion

The present study demonstrated that gentamicin induces nephrotoxicity, as evidenced by elevated plasma urea and creatinine concentrations, decreased creatinine and urea clearance, and impaired urinary excretion. These alterations, likely resulting from reduced filtration coefficient (Kf) or tubular cell necrosis with subsequent nephron loss, ultimately contribute to glomerular filtration rate (GFR) decline, which is a hallmark of nephrotoxicity [26, 27]. Creatinine clearance and plasma levels were not significantly altered between the adenosine and control groups. Interestingly, concurrent and post-treatment administration of adenosine yielded a modest, though not statistically significant, improvement in creatinine clearance compared with gentamicin. Consistent with previous reports, adenosine has been shown to enhance renal function, GFR, creatinine clearance, and histological integrity via activation of A1 and A2B receptors in the kidney [13]. In agreement with earlier studies, gentamicin administration increased urinary sodium and potassium excretion, leading to elevated FENa and FEK [28]. This effect can be attributed to inhibition of Na^+/K^+-ATPase activity [3], an enzyme essential for generating the sodium–potassium gradient. Inhibition of this transporter disrupts ionic homeostasis, resulting in sodium and water accumulation, cellular swelling, necrosis, and subsequent ion excretion [29]. In contrast, sodium excretion in the adenosine group did not differ from controls, while concurrent and post-treatment administration significantly reduced sodium loss compared with gentamicin. This effect may be mediated by A1 receptor–induced reactivation of the Na^+/H^+ exchanger (NHE3), thereby enhancing proximal sodium reabsorption [30]. Although plasma osmolality did not differ significantly across groups, gentamicin treatment was associated with a mild increase in urinary osmolality, likely due to excessive ion excretion. Aminoglycoside nephrotoxicity is typically characterized by impaired urinary concentrating ability, mediated by reduced aquaporin-1 and aquaporin-2 expression [31, 32]. In this study, urinary osmolality was markedly reduced following concurrent adenosine treatment, consistent with enhanced sodium reabsorption through A1 receptor activation of NHE3, Na/Pi, and Na/glucose transporters [30].

Oxidative stress also played a central role in gentamicin-induced renal injury, as reflected by increased malondialdehyde (MDA) levels and decreased ferric reducing antioxidant power (FRAP). Gentamicin impairs the antioxidant defense system by reducing superoxide dismutase, glutathione peroxidase, and catalase activities, while simultaneously enhancing lipid peroxidation [33]. Although adenosine alone did not significantly alter oxidative stress markers, concurrent administration effectively reduced MDA and increased FRAP compared with gentamicin. Post-treatment administration also reduced MDA and slightly increased FRAP. These findings may be explained by adenosine-mediated suppression of myeloperoxidase activity and reduced reactive oxygen species (ROS) release through A2A receptor–dependent activation of cAMP and protein kinase A in neutrophils [14]. Hemodynamic parameters, including blood pressure, remained unchanged across groups, possibly due to compensatory short- and medium-term mechanisms. While the kidneys act as long-term regulators of blood pressure, no significant alterations were detected within the timeframe of this study. This is consistent with previous findings reporting stable mean arterial pressure and heart rate following intraperitoneal or intraintestinal adenosine administration during hemorrhagic shock and resuscitation [34].

Gentamicin also reduced renal blood flow (RBF), largely by increasing vascular resistance through tubuloglomerular feedback (TGF) activation and stimulation of vasoconstrictor mediators such as platelet-activating factor, endothelin-1, and thromboxane A2 [35–38]. Conversely, concurrent adenosine administration significantly improved RBF, while post-treatment led to a modest, nonsignificant increase. These vasodilatory effects are likely mediated by activation of low-affinity A2A and A2B receptors, predominantly expressed in renal vasculature, which counteract A1-mediated vasoconstriction and enhance cortical and medullary blood flow [13, 14, 39, 40]. Histological evaluation revealed extensive tubular necrosis, protein cast formation, and cellular vacuolation in the gentamicin group, whereas adenosine alone showed no significant alterations

compared with controls. Both concurrent and post-treatment administration of adenosine markedly improved renal architecture, reducing tubular injury and restoring glomerular integrity. The protective effects of adenosine can be attributed to its anti-inflammatory actions, including suppression of pro-inflammatory cytokines (TNF-α, IL-6, IL-8), inhibition of NF-κB activation, and attenuation of neutrophil infiltration via A2A and A2B receptor signaling [13, 14]. Additionally, adenosine-mediated activation of ERK MAPK and PI3K pathways through A1 receptors promotes HSP27 phosphorylation, thereby reducing apoptosis and necrosis [14]. Similar mechanisms have been observed in cisplatin-induced nephrotoxicity, where adenosine receptor upregulation protects against ROS-mediated injury [41].

Conclusion

Concurrent intraperitoneal administration of adenosine alongside gentamicin for eight consecutive days exerted significant renoprotective effects by improving renal hemodynamics, restoring antioxidant defenses, and preserving histological structures. Post-treatment with adenosine also conferred partial protection, particularly by reducing oxidative stress and improving ion handling. These findings suggest that adenosine, through activation of both A1 and A2 receptor subtypes, may represent a promising therapeutic approach to mitigate gentamicin-induced nephrotoxicity.

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Conflicts of Interest

The authors declare no conflicts of interest.

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