J Appl Biotechnol Rep. 2024 June;11(2):1334-1343

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2024.426639.1691

Original Article

The Protective Effects of Alpha-Tocopherol Against Gentamicin -Induced Nephrotoxicity: The Potential Role of the Nrf2/NQO1 Pathway

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Received November 24, 2023; **Accepted** February 27, 2024; **Online Published** June 20, 2024

Abstract

Introduction: Aminoglycosides like gentamicin can cause nephrotoxicity by increasing reactive oxygen species (ROS) and reducing antioxidants. The transcription factor Nrf2 regulates antioxidant genes like NQO1 to combat oxidative stress. This study evaluated Nrf2/NQO1 involvement in gentamicin renal toxicity and vitamin E protection.

Materials and Methods: 24 rats were divided into control, gentamicin, vitamin E, and gentamicin plus vitamin E groups. Gentamicin (100 mg/kg) and vitamin E (250 mg/kg) were given intraperitoneally for 8 days. Kidney function, oxidative stress, Nrf2/NQO1 expression, and histology were analyzed.

Results: Gentamicin significantly increased serum creatinine by 1.98-fold (1.43 ± 0.49 vs 0.72 ± 0.16 mg/dl, p <0.01) and BUN by 5.58-fold (252.3 ± 78.13 vs 45.18 ± 7.26 mg/dl, p <0.0001) compared to control. Gentamicin also markedly suppressed renal Nrf2 mRNA expression by 83% and NQO1 by 79% versus control (p <0.0001). Vitamin E partially alleviated the functional impairment and downregulation of Nrf2 and NQO1 caused by gentamicin. The vitamin E group displayed the highest Nrf2 (2.8-fold vs control) and NQO1 (1.6-fold vs control) expression among all groups (p <0.0001).

Conclusions: Gentamicin appears to cause nephrotoxicity partly by suppressing Nrf2/NQO1 antioxidant defense. Vitamin E provided renoprotection by scavenging ROS and potentially reactivating Nrf2/NQO1. The study suggests oxidative stress is an important mechanism in aminoglycoside kidney toxicity that may be mitigated by appropriate antioxidants. Evaluating Nrf2/NQO1 modulation provides insights into gentamicin nephrotoxicity and related kidney injuries.

Keywords: Acute kidney Injury, Gentamicin, α-Tocopherol, Nrf2, NQO1

Citation: Ashrafi S, Heidari R, Ashrafi MR, Chamanara M, Dadpay M, Ebrahimi M. The Protective Effects of Alpha-Tocopherol Against Gentamicin-Induced Nephrotoxicity: The Potential Role of the Nrf2/NQO1 Pathwa. J Appl Biotechnol Rep. 2024;11(2):1334-1343. doi:10.30491/JABR.2024.426639.1691

Introduction

The kidneys play a crucial role in maintaining body fluid homeostasis by filtering out metabolites and minerals from the blood.[1] In addition to their capacity to metabolize, concentrate, extract, and secrete poisonous compounds, the kidneys are particularly susceptible to various types of toxins. There is acute renal failure (ARF) or acute kidney injury (AKI) due to glomerular lesions, degenerative changes, and necrosis of the tubular epithelium.[2,3] Symptoms of renal disappointment include a fast decline in glomerular filtration rate (GFR), disturbances of extracellular liquid electrolyte volume and balance, corrosive base homeostasis, and the maintenance of nitrogenous side effects caused by protein catabolism, such as blood urea nitrogen (BUN) and creatinine.[4,5] The relationship between oxidative stress and AKI is becoming more evident.[6] The majority of AKI cases acquired in hospitals are caused by nephrotoxicity. Drug-induced nephrotoxicity is approximately 14% to 26% among adults and Drug-induced AKI accounts for 19-26% of hospitalized cases.[7,8]

Nephrotoxicity is one of the most serious side effects of therapeutic doses of gentamicin, an aminoglycoside antibiotic used to treat infections.[9] Gentamicin, an aminoglycoside antibiotic used to treat infections, is nephrotoxic.[10] Increased plasma creatinine and urea levels and severe necrosis of the

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proximal renal tubules are signs of gentamicin-induced nephrotoxicity, which is followed by deterioration and renal failure.[11,12] Gentamicin decreases renal antioxidant enzyme activity and increases kidney reactive oxygen species (ROS).[13] Oxidative pressure plays a significant role in drug-induced nephrotoxicity. Therefore, antioxidants such as vitamin E are used to prevent drug-induced nephrotoxicity.[14]

Antioxidants such as vitamin E control free radical production and regulate peroxidation. One of the antioxidants that protects against kidney toxicity is vitamin E called alpha-tocopherol. α-Tocopherol induces phase II enzymes in the cells by amplifying the antioxidant system. The Keap1/Nrf2 pathway is activated by pretreatment with α-tocopherol by promoting Nrf2 translocation to the nucleus and increasing Nuclear factor erythroid-2-related factor-2 (Nrf2) expression. Consequently, phase II enzyme expression rises: Quinone oxidoreductase 1 (NQO1), Glutamate cysteine ligase (GCL), Superoxide dismutase (SOD), Glutathione-S-transferase, Heme oxygenase 1, and total antioxidant capacity. Consequently, cells are protected from gentamicin-induced cell death by strengthening their antioxidant defenses.[15]

Previous research showed that olive leaf and vitamin E significantly prevented gentamicin sulfate-induced kidney toxicity. In this research, the levels of antioxidant enzymes increased significantly and the levels of malondialdehyde (MDA), serum creatinine, and urea decreased in all treatment groups.[16] Nrf2 is a cellular oxidative stress sensor that protects cells from harmful effects caused by toxic damage and elevated reactive oxygen species.[17,18] Under normal conditions, Nrf2 transcription is inhibited by a Kelch-like ECH-associated protein 1 (Keap 1) inhibitor (Figure 1). Induction of renal failure by gentamicin leads to significant changes in internal antioxidant substances and Nrf2 expression.[19] Lee et al, showed that genetic defect in Nrf2 increases susceptibility to ischemic and nephrotoxic AKI in mice. These findings showed that after ischemia-reperfusion (I/R) injury, the expression levels of several genes encoding proteins and antioxidant enzymes are increased in healthy mice, but not in Nrf2-deficient mice.

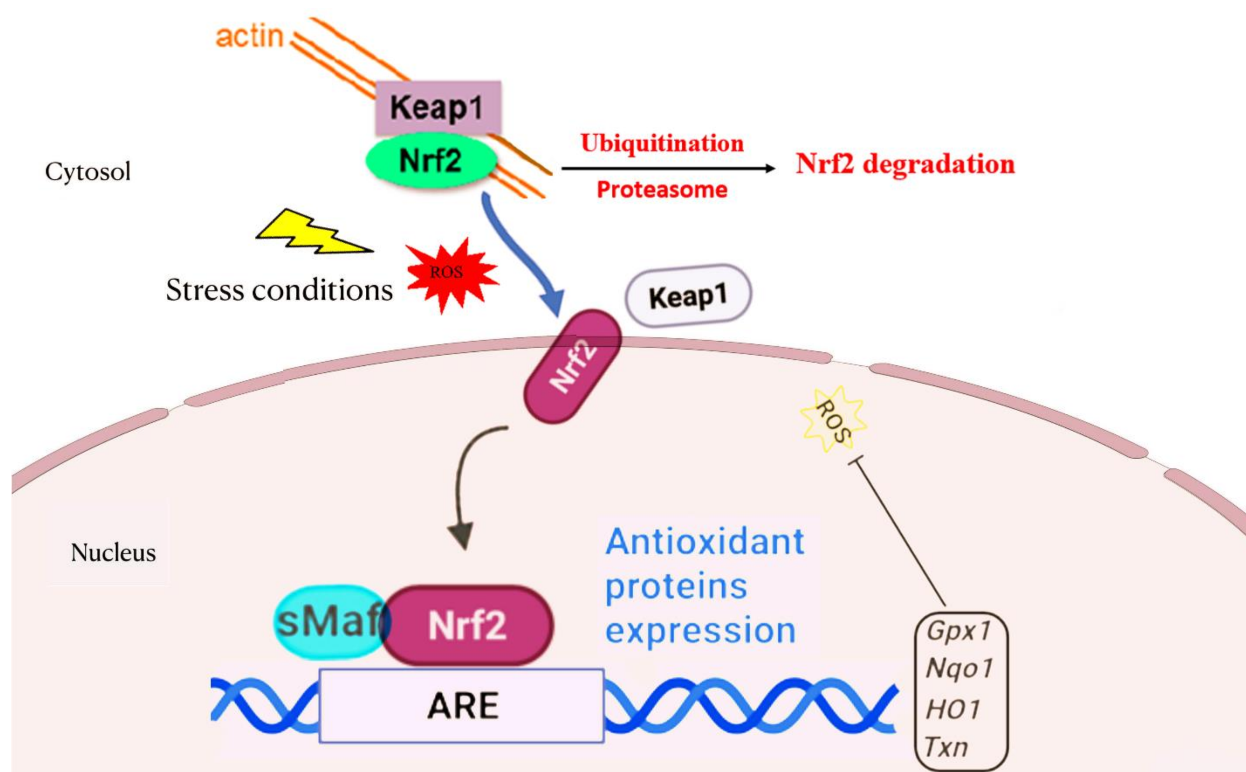

Figure 1. The Relationship between Nrf2 Transcription Factor Activation and Cellular Antioxidant Systems.

Nrf2 controls the expression of subsequent genes like GST, HO-1, NOQ1, and SOD-1. These genes encode detoxification and antioxidant enzymes alongside related proteins. In response to oxidants, antioxidants, xenobiotics, UV light, heavy metals, and other cellular protective factors, NQO1, NQO2, and other genes are frequently expressed and induced.[20] It was demonstrated in the human NQO1 experiment that this enzyme is active toward α-tocopherol-quinone and reduces it to α-tocopherol hydroquinone, protecting the cell membrane from damage.[21]

Numerous pieces of evidence demonstrate the protective function of Nrf2 and NQO1 in oxidative stress-related diseases, including AKI. Additionally, this investigation aims to elucidate the molecular mechanisms underlying

gentamicin-induced renal toxicity, paving the way for future studies to identify effective therapeutic interventions for such damage. The novel aspect of the current study is the focus on elucidating the role of the Nrf2/NQO1 pathway in gentamicin-induced nephrotoxicity and the potential protective effects of vitamin E. While previous studies have evaluated oxidative stress and antioxidant enzymes in aminoglycoside kidney toxicity, the specific involvement of Nrf2/NQO1 signaling has not been thoroughly characterized. This study aims to provide new insights into the molecular mechanisms of gentamicin renal damage by assessing temporal alterations in Nrf2 and NQO1 expression. The results can reveal whether suppression of this key cellular antioxidant pathway contributes to the nephrotoxic effects of gentamicin. In addition, the ability of vitamin E to potentially restore Nrf2/NQO1 expression may explain its amelioration of functional and histological impairment. Overall, further investigation of Nrf2/NQO1 modulation will establish a stronger foundation for prevention and treatment of acute kidney injury. Elucidating the role of this pathway in gentamicin cytotoxicity and vitamin E protection represents a novel contribution to existing knowledge in this field.

Materials and Methods

Drugs and Chemicals

Gentamicin was purchased from the Caspian Company (Tehran, Iran). vitamin E (α-Tocopherol) was purchased from the Caspian Company (Tehran, Iran).

Animals

Male Wistar rats (8 weeks old) weighing 210 + 40 g were obtained from the Royan mouse breeding center. The animals were randomly divided into groups of 6 and 3 or 4 animals were placed in each cage. The animals were maintained in the animal laboratory of Tehran university of medical sciences. They were maintained under standard conditions with a 12-hour light-dark cycle, at 22-24 °C, and 40-60% humidity. Animals were given ad libitum access to food and water.

Experimental Protocol

A total of 24 adult male wistar were obtained for this study. The rats were divided randomly into 4 groups of 6 animals each using a computer-generated randomization chart. The treatment groups were as follows:

Control group (C): Received intraperitoneal injections of normal saline at a dose of 1 cc/kg body weight once daily. Gentamicin group (G): Received intraperitoneal injections of gentamicin sulfate at a dose of 100 mg/kg body weight once daily. Vitamin E group (E): Received intraperitoneal injections of α-tocopherol (vitamin E) at a dose of 250 mg/kg body weight once daily. Combination group (T): Received intraperitoneal injections of α-tocopherol at 250 mg/kg followed by gentamicin sulfate at 100 mg/kg after a gap of 30 minutes, once daily. The treatments were administered for a duration of 8 consecutive days. The dose and duration of gentamicin and vitamin E were selected based on prior studies demonstrating nephrotoxicity with gentamicin and protection with vitamin E.

Samples Collection and Preparation

At the end of the treatment period, rats were anesthetized with ketamine (60 mg/kg) and xylazine (8 mg/kg) IP injection. 5 cc of blood was collected by cardiac puncture, and centrifuged to collect serum. Renal blood urea nitrogen (BUN) and creatinine (Cr) levels were measured. After blood sampling, both kidneys were removed, and one-half of the right kidney was fixed in 10% neutral buffered formalin for histopathological examination. The left kidney was divided into small pieces immediately snap frozen in liquid nitrogen and stored at −80 °C for molecular evaluation.

Assessment of BUN and Cr Plasma Levels

Measuring BUN and Cr levels is a measure to check kidney damage and function. The concentrations of these enzymes were quantified through colorimetric assays using enzymatic methods and expressed in milligrams per deciliter (mg/dl), as described in previous study.[22]

Assessment of Nrf2 and NQO1 Genes Expression Changes by Real-time PCR

Total RNA was extracted by an RNA extraction kit (Favorgen, Taiwan) from kidney tissues. The cDNA was synthesized using cDNA Synthesis Kit (Favorgen, Taiwan). SYBR Green fluorescence real-time PCR was performed on Nrf2 and NQO1 genes with a Rotor-GeneQ instrument (Qiagen, Hilden, Germany), as described in previous study.[23] Primers were designed using Premier Biosoft's AlleleID primer design software version 7.5), as described in previous study (Table 1).[24] A 20μl was used for each Real Time PCR reaction, containing SYBR Green (RealQ plus Master Mix Green without ROX, Ampliqon, Denmark), specific primers, and cDNA. As part of the amplification program, 95 °C was first denaturated for 15 minutes, followed by 40 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s. All sample reactions were performed in triplicate, and melting analysis was carried out following each reaction to verify the specificity of the products and the absence of primer dimers.

Histopathological Evaluation

Formalin-fixed kidney samples were processed and embedded in paraffin blocks. Sections of 5 μm thickness were cut using a rotary microtome and stained with hematoxylin and eosin (H&E) for microscopic examination. An expert histopathologist blinded to group assignments

performed qualitative evaluation of the kidney sections. Multiple high-power fields (400x magnification) were examined in the cortex and medulla of each section. The presence and severity of histological parameters were semi-quantitatively scored on a scale of 0 to 3+, with 0 indicating no abnormal changes and 3+ indicating severe changes. The parameters assessed included: vascular congestion, tubular dilation, tubular casts, cytoplasmic vacuolization of tubular epithelial cells, tubular necrosis, interstitial inflammation, and glomerular changes. Photomicrographs were captured using an Olympus BX43 light microscope equipped with a DP21 imaging system. ImageJ software was utilized to quantify tubular dilation, cast formation, and percent necrosis in representative fields.

Table 1. Nrf2, NQO1, and GAPDH Primers Utilized for RT-PCR Analyses

Genes	Forward (5´-3´)	Reverse (5´-3´)
NQO1	GCATCCAATCCTCCACCCA	ACAAGTTAGTCCCTCAGCCATT
Nrf2	GCCATTAGTCAGTCGCTCT	GTGCCTTCAGTGTGCTTC
GAPDH	CAACGGCACAGTCAAGGC	CTCAGCACCAGCATCACC

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.3.0 software. Normality of data distribution was evaluated by the Shapiro-Wilk test. For normally distributed data, comparisons between multiple groups were made by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For non-normally distributed data, the Kruskal-Wallis test followed by Dunn's post hoc test was utilized for comparisons between multiple groups. Histopathological scoring was analyzed using the non-parametric Mann-Whitney U test. p values less than 0.05 were considered statistically significant.

Results

Renal Histological Parameters

The grading of all histopathological parameters is summarized in Table 2. The findings from the histopathological evaluations show the absence of damage in groups C and E. The size and number of glomeruli in group G are normal. The presence of vascular congestion and mild interstitial inflammation was detectable in this group, while it was not statistically different from groups C and E (p >0.05). Dilation of tubules with epithelial necrosis, cell shedding with intratubular protein casts, and cytoplasmic vacuolation of proximal tubule cells were observed with relatively high and significant intensity in group G compared to groups E and C (p <0.001).

In group T, we have dilated tubules, shedding of epithelial cells, and necrosis with moderate intensity compared to group G, but there is no statistically significant difference (p >0.05). Dilation of tubules along with epithelial necrosis and shedding of cells in group T is visible compared to groups C and E with moderate and significant damage (p <0.05). Vacuolarization of the cytoplasm of proximal tubule cells was observed with relatively moderate intensity, but it was not statistically different from group G (p >0.05). Protein casts with milder intensity than group G can be seen in the lumen of tubules, but no difference was observed in the statistical analysis (p >0.05). In group T, there were intratubular casts with moderate and significant intensity compared to groups E and C (p <0.05) (Table 3, Figure 2).

Table 2. The Grading of all Histopathological Parameters

Parameters / Grade	Vascular congestion	Tubular dilatation	Caste	Vacuolization of epithelial cells	Tubular necrosis	Interstitial inflammation	Glomerular changes
0	No changes						
1+	Mild changes						
2+	Moderate changes						
3+	Severe changes						

Table 3. Histopathological Changes in Renal Tissues

Histopathological Parameters	C	E	G	T
Glomerular damage	-	-	+	-
Vascular congestion	-	-	+	+
Tubular epithelial cell necrosis	-	-	+++	++
Tubular casts	-	-	++	+
Inflammation	-	-	+	-
Tubular dilation	-	-	++	+
Tubular vacuolation	-	-	++	+

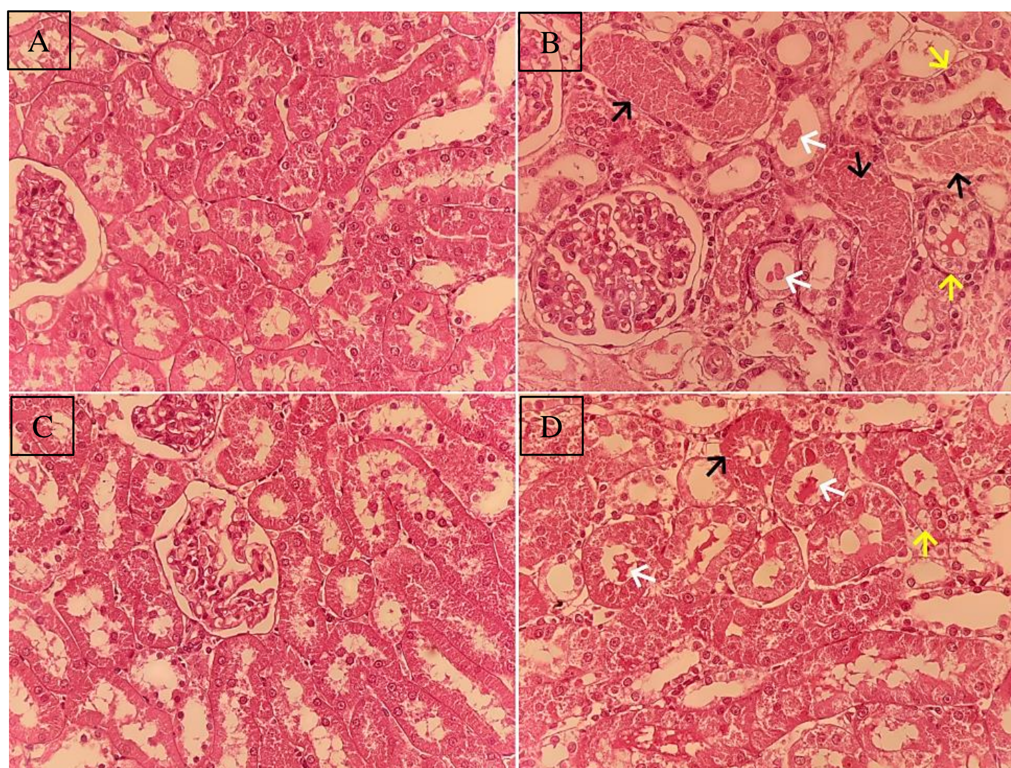

Figure 2. Histopathology of Renal Tissue. A) cross-sections of glomeruli in the (C) group; B) cross-sections of glomeruli in the (G) group; C) cross-sections of glomeruli in the (E) group; D) cross-sections of glomeruli in the (T) group A and C kidney cortexes from the (C) and (E) groups, respectively, show normal kidney histology. (G) group showing marked damage to renal tubules. Extensive vacuolization of epithelial cells, separation of epithelial cells from the basement membrane, kidney epithelial degeneration (yellow arrows), necrosis (black arrows), and intratubular protein casts (white arrows) in the (G) and (T) groups are seen. The (T) group was associated with a significant reduction in the amount of tubular damage. (Hematoxylin and eosin; original magnification × 400).

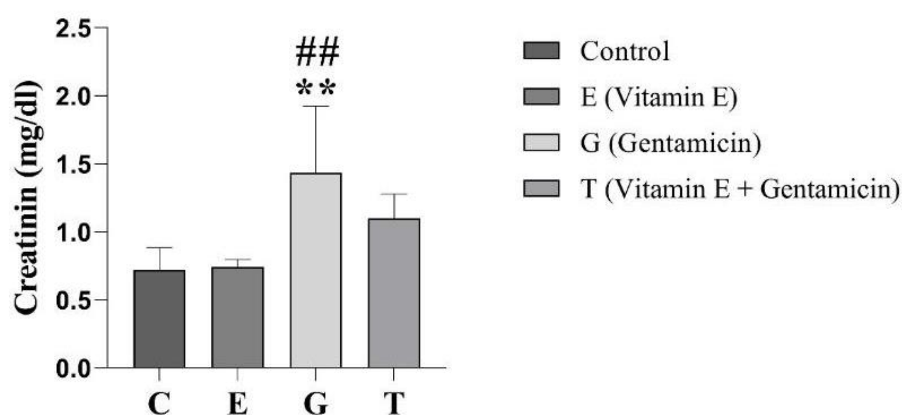

Figure 3. Comparison of the Mean Plasma Level of Cr in Groups C, E, G, and T in Rats. Values are presented as mean ± SD for 6 rats in each group. $^{**}p$ <0.01 compared to the C group and $^{##}p$ <0.01 compared to the E group.

Serum Cr and BUN Levels

Assessment of blood urea nitrogen (BUN) and creatinine (Cr) levels provides an indication of kidney function and damage. In the present study, intraperitoneal administration of gentamicin sulfate at 100 mg/kg body weight daily for 8 consecutive days resulted in markedly elevated serum BUN and Cr levels in the gentamicin (G) group compared to the control (C) group (Figures 3 and 4, Table 4). Specifically, the mean serum Cr concentration showed an increase of 1.98-fold in the G group relative to the C group (1.43 ± 0.49 vs 0.72 ± 0.16 mg/dl, p <0.01). Similarly, the average serum BUN level displayed a 5.58-fold elevation in the G group compared to the C group (252.3 ± 78.13 vs 45.18 ± 7.26 mg/dl, p <0.0001). These findings are consistent with renal dysfunction and structural damage induced by gentamicin, as reported extensively in previous studies.

The increases in BUN and Cr likely reflect impaired glomerular filtration and tubular dysfunction associated with

gentamicin nephrotoxicity. Gentamicin accumulation in proximal tubule cells leads to increased permeability, necrosis, and shedding of cells into the tubule lumen. This disrupts normal reabsorptive capacity and leaks proteins and functional elements into urine. Elevated BUN signifies increased urea nitrogen retention due to reduced glomerular filtration. Elevated Cr results from decreased tubular secretion and increased tubular reabsorption. Therefore, monitoring BUN and Cr levels serves as a reliable indicator of kidney injury in this model of gentamicin nephrotoxicity, validating the detrimental effects of gentamicin on renal structure and function.

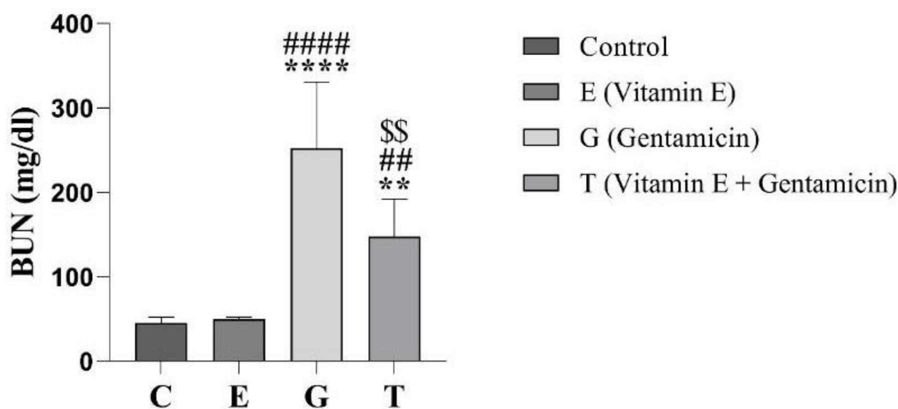

Figure 4. Comparison of Mean Plasma Level of BUN in Groups C, E, G, and T in Rats. The values are presented as the mean ± SD for 6 rats in each group. $^{**}p<0.01$ and $^{****}p<0.0001$ compared to the C group, $^{\#\#}p<0.01$ and $^{\#\#\#\#}p<0.0001$ compared to the E group, and $^{\$\$}p<0.01$ compared to the G group.

Table 4. Biochemical Data in the Four Experimental Groups

	C	E	G	T
Cr	0.72 ± 0.16	0.74 ± 0.05	1.43 ± 0.49^{bd}	1.10 ± 0.17
BUN	45.18 ± 7.26	49.55 ± 2.93	252.3 ± 78.13^{ac}	147.4 ± 44.43^{bde}

Values are presented as mean ± SD for 6 rats in each group. Compared to the C group: $^{a}p<0.0001$, $^{b}p<0.01$, compared to the E group: $^{c}p<0.0001$, $^{d}p<0.01$, compared to the G group: $^{e}p<0.01$.

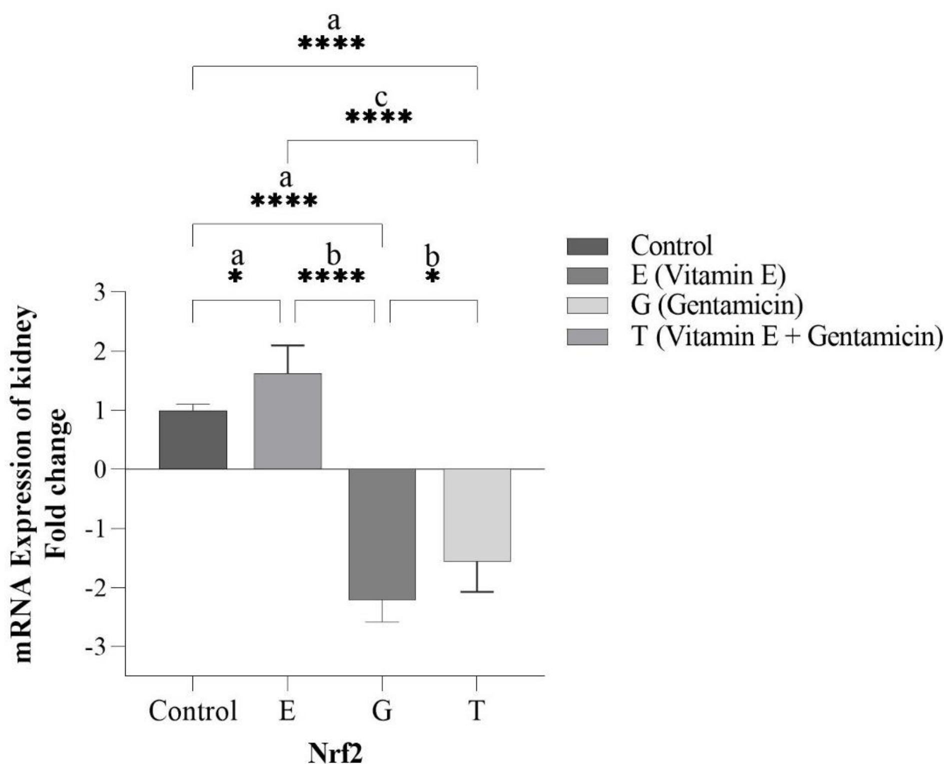

Figure 5. Comparison of the Average Expression of the Nrf2 Gene in Groups C, E, G, and T in Rats (n = 6). Values are presented as fold change. $^{*}p<0.05$ and $^{****}p<0.0001$. a) compared to group C; b) compared to group G; and c) compared to group T.

Effect on Nrf2 and NQO1 Gene Expression

As shown in Figure 5, the expression level of the Nrf2 gene in group E had a relatively significant increase in expression compared to the C group ($p < 0.05$). Also, in group E, compared to G and T groups, we saw a more intense and significant expression ($p < 0.0001$). The level of Nrf2 gene expression in the G and T groups has decreased significantly compared to the C group ($p < 0.0001$). Also, in the inter-group comparison, we saw a more severe and significant decrease in expression in the G and T groups compared to the E group ($p < 0.0001$). The G group has shown a greater decrease in expression than group T, which indicates the destructive effects of gentamicin in this group ($p < 0.05$). Group T has shown a lower expression decrease than the G group, which indicates the therapeutic effects of vitamin E in this group ($p < 0.05$) (Table 5).

Table 5. Expression Levels of Nrf2 and NQO1 Genes in Rats (n = 6)

Gene expression / Group	C	E	G	T
NQO1	–	↑[d]	[bc]↓	[bce]↓
Nrf2	–	↑[ad]	[bc]↓	[bcf]↓

Compared to the C group: [a]$p < 0.05$, [b]$p < 0.0001$, compared to the E group: [c]$p < 0.0001$, compared to the G group: [d]$p < 0.0001$, [e]$p < 0.01$, [f]$p < 0.05$.

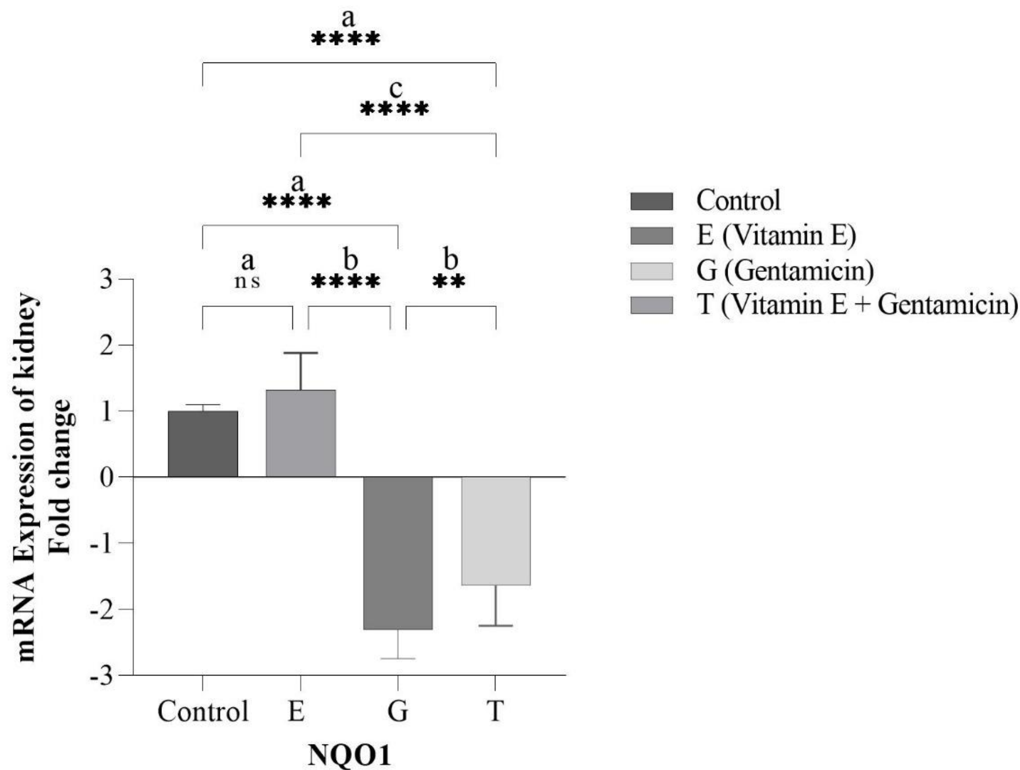

Figure 6. Comparison of the Average Expression of the NQO1 Gene in Groups C, E, G, and T in Rats (n = 6). Values are presented as fold change. $^{**}p < 0.01$ and $^{****}p < 0.0001$. a) compared to group C; b) compared to group G; and c) compared to group T.

As shown in Figure 6, the expression level of the NQO1 gene in group E increased compared to the C group, but this difference with the C group was not statistically significant ($p = 0.0520$). Also, in group E compared to the G and T groups, we saw a stronger and significant increase in expression ($p < 0.0001$). The level of NQO1 gene expression in the G and T groups shows a relatively strong and significant decrease compared to the C group ($p < 0.0001$). Also, in the inter-group comparison, we saw a more intense and significant decrease in the expression in groups G and T compared to group E ($p < 0.0001$). Group G has shown a greater decrease in expression than the T group, which indicates the destructive effects of gentamicin in this group ($p < 0.01$). Group T showed a lower expression reduction (protective effect) than the G group, which indicates the therapeutic effects of vitamin E in this group ($p < 0.01$) (Table 5).

Discussion

The goal of the present study was to evaluate the temporal pattern of changes in expression of the Nrf2 upstream target gene and NQO1 downstream gene involved in intracellular antioxidant pathways during separate and combined

administration of gentamicin and vitamin E. Aminoglycoside antibiotics like gentamicin can cause nephrotoxic effects through toxicity of renal tubules, reduced glomerular filtration, and decreased renal blood flow.[25] Gentamicin-induced nephrotoxicity limits its clinical usage as an antibiotic for severe gram-negative infections due to dose-dependent toxicity. At low levels, reactive oxygen species (ROS) play important roles in normal cell signaling and function. However, elevated ROS overwhelm endogenous antioxidant defenses causing damage to DNA, proteins, lipids and other cellular components.[25] Chronic inflammation ensues when cellular damage is not completely repaired, with release of free radicals, prostaglandins, adhesion molecules, proinflammatory cytokines and other detrimental factors.[26] Aminoglycosides exert their nephrotoxic effects by generation of ROS. Antioxidants such as vitamin E have demonstrated protective effects against gentamicin toxicity in some studies.[12,27] Cellular resistance to oxidative stress relies heavily on the Nrf2/ARE pathway. Numerous downstream genes like NAD(P)H quinone oxidoreductase-1 (NQO1) are also regulated by Nrf2. These regulatory mechanisms can potentially be exploited as therapeutic antioxidant strategies.

Microscopic evaluation showed gentamicin caused pathological changes in renal tubules, glomeruli and interstitium including degeneration and necrosis of proximal tubule cells, tubular dilation, intratubular protein casts and interstitial inflammation. Vacuolization, detachment and necrosis of epithelial cells was evident, mostly in the S3 segment of proximal tubule and thick ascending limb.[28-30] Desquamation of tubule cells was observed, indicating cell damage. Our findings agree with previous studies using varying gentamicin doses and treatment durations.[28,31] Gentamicin is excreted unchanged in urine by glomerular filtration and partially reabsorbed by proximal tubule cells, leading to accumulation in lysosomes.[32,33] Other study reported tubular necrosis, parenchymal destruction and cell sloughing in rats after 5 days of gentamicin 100 mg/kg.[31] Yildirim et al. showed vascular congestion, tubule necrosis and casts in rats following 8 days of gentamicin 80 mg/kg.[28] Al-Majed et al. demonstrated glomerular atrophy, apoptosis, tubular necrosis and interstitial nephritis after 8 days of gentamicin 80 mg/kg.[29]

Tubular casts were frequently observed, mainly containing Tamm-Horsfall protein from damaged thick ascending limbs.[34] Glomerular dysfunction and defective tubular reabsorption increases urinary protein excretion, forming casts.[35] Gentamicin accumulates in proximal tubule cells via endocytosis, concentrating in lysosomes, Golgi and endoplasmic reticulum where it disrupts cell signaling.[36] Reduced ATP, increased cell volume, ROS generation and caspase/cathepsin activation mediate cytotoxicity.[37] Cytoplasmic gentamicin affects mitochondria, inducing apoptosis by activating caspases and inhibiting ATP production. By increasing superoxide and hydroxyl radicals, gentamicin causes vasoconstriction, hypoxia and oxidative injury.[38,39] It forms iron complexes that catalyze free radical formation.[38] Decreased renal blood flow, ischemia and oxygen deprivation promote tubular apoptosis and necrosis.[37]

Vitamin E ameliorated gentamicin-induced nephrotoxicity, likely by scavenging ROS. Yang et al. reported tubular mitochondria produce abundant ROS during gentamicin accumulation.[40] As an antioxidant, vitamin E quenches superoxide, perhydroxyl and hydroxyl radicals.[41] It inhibited lipid peroxidation and enhanced antioxidant activity in kidney toxicity models.[42] Vitamin E also attenuated functional impairment and histological damage, facilitating structural and functional preservation.[43]

Gentamicin markedly increased serum creatinine and BUN compared to control and vitamin E groups, indicating compromised glomerular filtration and tubular dysfunction.[44,45] Vitamin E attenuated the increases, demonstrating renal protection. Bashan et al. showed increased creatinine and BUN after 8 days of gentamicin 100 mg/kg, which was mitigated by antioxidants.[44] Previous study demonstrated significant BUN and creatinine elevation following gentamicin 80 mg/kg for 5-7 days.[45] Vitamin E decreased BUN and creatinine levels compared to gentamicin alone, although some increase persisted versus control.[46] Other antioxidants like N-acetylcysteine have also alleviated functional impairment from gentamicin and cisplatin.[47]

Gentamicin administration resulted in markedly suppressed gene expression of both Nrf2 and its downstream target NQO1 compared to control groups. In contrast, the vitamin E treated group displayed significantly higher expression of these genes compared to gentamicin and control groups. The NQO1 enzyme plays an important role in cellular antioxidant defense by stimulating production of antioxidants like vitamin E through its ability to catalyze reduction of quinones, thus limiting reactive oxygen species generation.[47]

The increased expression of Nrf2 and its downstream target NQO1 observed with vitamin E treatment may be attributable to activation of the Nrf2/ARE cytoprotective signaling pathway. As mentioned previously, under conditions of oxidative stress, Nrf2 is able to dissociate from its endogenous inhibitor protein Keap1 in the cytoplasm.[48] This allows Nrf2 to translocate into the nucleus, where it can bind to antioxidant response elements (AREs) in the regulatory regions of target genes like NQO1.

Binding of Nrf2 to AREs induces the transcription of these cytoprotective genes involved in the antioxidant response. Several previous studies have demonstrated that vitamin E treatment can activate this Nrf2/ARE pathway by facilitating Nrf2 nuclear translocation and upregulating the expression of NQO1 and other antioxidant enzymes.[49,50]

Therefore, it appears vitamin E may preserve Nrf2/NQO1 expression in the face of gentamicin-induced oxidative kidney damage by acting as an Nrf2 activator. In contrast,

gentamicin suppresses this key cellular antioxidative pathway. Further exploration is still needed to fully elucidate the specific molecular mechanisms by which vitamin E enhances Nrf2/ARE signaling and NQO1 expression as an indirect antioxidant. Nonetheless, this ability likely contributes to its amelioration of oxidative renal injury caused by gentamicin.

Conclusion

considering the role of oxidative stress as a primary or secondary harmful factor in gentamicin-induced renal failure, as well as the treatment of this damage by vitamin E, the findings of the present study showed that the change in Nrf2 gene expression has a dual role in this study, it can play a role in gentamicin-induced nephrotoxicity and also act as an upstream control gene that directly affects other key regulatory elements associated with the production and/or activation of downstream cellular antioxidant systems which are mediated by Nrf2. Nrf2 provides the possibility to fight against such pathological conditions of AKI through increasing antioxidant capacities such as NQO1 gene expression. Investigation of the expression changes of these genes can provide a clue for the next step in investigating the therapeutic role of NQO1 activation and/or inhibition in different time periods of vitamin E and gentamicin administration.

Authors' Contributions

Conceptualization: ME, RH, SA; Methodology: RH, ME, MC, MD; Formal analysis: RH, SA, MRA; Writing – review & editing: SA, RH, MC, MRA, MD, ME.

Ethical Approval

The present study protocol was conducted after receiving the Code of Ethics from the National Committee of Ethics in Biomedical Research (Approval ID: IR.AJAUMS.REC. 1401.022). All methods were carried out in accordance with relevant guidelines and regulations. All experiments were conducted under the Aja University of Medical Sciences. University Guidelines for the humane treatment of experimental animals. All methods are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org) for the reporting of animal experiments.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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