IJKD KIDNEY DISEASES

Role of Farnesoid Receptors and Nrf2-mediated Genes in Gentamicin-induced Nephrotoxicity in Rat: A Time-course Study

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Original Paper

Introduction. Farnesoid-X-activated receptor (FXR) is considered as an upstream controller which could influence the other key regulatory genes encoding cellular antioxidant defense system.
Methods. Thirty-five male Wistar rats (240 ± 20 g) were randomly allocated into five groups: 1) control, 2) received gentamicin (100 mg/kg/d) for three days (GM-3d), 3) seven days (GM-7d), 4) 10 days (GM-10d), and 5) 14 consecutive days (GM-14d). Biochemical measurements of BUN and serum creatinine (SCr), histological assessment of renal samples as well as molecular analysis using real-time qRT-PCR were used to investigate the pattern of changes in different levels.
Results. Administration of gentamicin was associated with a significant increase in the BUN and SCr until the 10th day, which then suddenly dropped at the day 14. Meantime, the maximum histological distortion was also seen on the 10th day but in a similar pattern, 14th day was associated with clear improvement. Compared to the control value, the maximum reduction in the mRNA expression of Farnesoid X-activated receptor (FXR), nuclear factor erythroid 2–related factor 2 (Nrf2) and Glutathione cysteine ligase-modulatory subunit (GCLM), occurred at the 3rd and 7th days, respectively. Compared to the control, the mRNA expression of the mentioned genes significantly increased up to day 14. Apart from the 3rd day, the mRNA expression of alpha-glutathione S-transferase (α-GST) and superoxide dismutase (SOD) showed a similar descending and ascending pattern at 7th and 10th days, respectively.
Conclusion. The expression of FXR, as an upstream controller gene and its downstream pathways mediated by Nrf2, could play a role in gentamicin-induced nephrotoxicity but the pattern of expression was rather biphasic at the acute phase or the subacute ones.

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INTRODUCTION

Gentamicin, an antibiotic agent belonging to the aminoglycoside family, is used to treat severe bacterial infections. Despite the potential therapeutic effects, nephrotoxicity has limited its clinical use. Active tubular accumulation of gentamicin in proximal convoluted tubules leads to epithelial cell damage and therefore development of various

degree of renal failure.[1] Several molecular aspects of gentamicin nephrotoxicity have been obtained from both *in vivo* and *in vitro* experimental findings. Binding to membrane phospholipids and changing the metabolism,[2,3] disruption of mitochondrial ATP synthesis and overproduction of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals[4] are examples of the mechanism of gentamicin-induced renal toxicity.

Farnesoid X-activated receptor (FXR) is a member of nuclear receptor superfamily, which is extensively expressed in the liver and intestine[5] as well as other tissues such as heart, kidneys, adrenal glands, and blood vessels.[6,7] These types of nuclear receptors also play an important role in regulating cellular redox status by controlling several upstream and downstream signaling pathways such as the nuclear factor erythroid 2–related factor 2 (Nrf2)-mediated genes involved in the cellular defense system. Glutathione cysteine ligase (GCL), superoxide dismutase (SOD) and alpha-glutathione S-transferase (α-GST) are some target genes which could mediate by such controlling pathways.[8-11]

Considering the important role of cellular defense system against direct nephrotoxic agents such as gentamicin, the present study was designed to find out the effects of gentamicin on time-course pattern of alteration in the FXR and its targeted genes involved in the cell antioxidant process during a 14-day interval. Such a time-course study might be helpful for understanding the best time to perform a therapeutic intervention to overcome against gentamicin-induced renal impairment.

MATERIALS AND METHODS

Animals

Thirty-five Wistar male rats, eight weeks old, were obtained from Royan Animal Breeding Center, Karaj, Iran. Animals were kept under standard conditions (12 hours light/dark cycle at 20 to 24 °C and 50 ± 5% relative humidity). They had free access to food and water during the study. The animal care, experimentation and procedures were performed according to the national guidelines and protocols approved by the Research Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1399.152) in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No.85-23, revised 1996).

Experimental Design and Protocol

Thirty-five male Wistar rats (240 ± 20 g) were randomly allocated into five groups (n = 7) including: 1) control group; and the other four groups received daily intraperitoneal injection of 100 mg/kg gentamicin for 3, 7, 10, and 14 consecutive days including: 2) three days (GM-3d), 3) seven days (GM-7d), 4) 10 days (GM-10d), and 5) 14 days (GM-14d). At the end of each respective times, animals were deeply anesthetized with intraperitoneal injections of the Ketamine (60 mg/kg) and Xylazine (8 mg/kg).

Biochemical Parameters

Blood samples were gently collected from the right ventricle to determine the levels of BUN and SCr by using biochemical kits (Pars Azmun Co, INC, Karaj, Iran), according to the manufacturer guidelines.

Histological Assessments

For histological assessment, after scarifying the rats, the left kidney was removed and immediately fixed in 10% formalin solution. Histological assessment has been performed according to the previous study protocol.[12] Tissue staining with Hematoxylin and Eosin (H&E), Masson's trichrome and Periodic acid shift (PAS) were performed for detecting any pathological signs of damages, fibrotic scars, or brush border loss.

Real Time-qRT PCR Assessment

Quantitative measurements of the renal FXR, Nrf2, GCLM, α-GST and SOD were performed using real time RT-PCR according to the previous protocol.[12,13] The exact nucleotide sequences of the genes and GAPDH primers were shown in Table 1.

Statistical Analysis

Between-group analysis was conducted using One-way analysis of variance (ANOVA) and in the case of any significant difference, it was followed by the Tukey as the post hoc test. All data was presented as mean ± SEM and a *P* value of less than .05 was considered statistically significant. Graphs were drawn using Graphpad Prism 8 (8.0.2) software.

RESULTS

Body Weights

The values of initial and final body weights

Table 1. Nucleotide Sequences of Primers

Genes	Primer Sequences (5′-3′)	
	Forward	Reverse
FXR	TGGGAATGTTGGCTGAATG	CCTGTGGCATTCTCTGTTTG
Nrf2	AGTGGATCTGTCAGCTACTC	GGGAATGTCTCTGCCAAAAG
α-GST	GTGGAGATTGATGGGATGAAG	GGGAATGTAAGGGTAATGGAG
GCLM	GACAAAACACAGTTGGAGCAG	GGTCATTGTGAGTCAGTAGCTG
SOD	TGTGTCCATTGAAGATCGTG	TCCAGCATTTCCAGTCTTTG
GAPDH	GCCTTCTCTTGTGACAAAGTG	CTTCCCATTCTCAGCCTT

as well as the mean difference between the two weights are summarized in Table 2. Compared to the control group, three days administration of gentamicin did not affect the final body weights so that the difference in the mean body weights were unchanged. Whereas, compared to the control group, the body weight difference was significantly decreased in 7th ($P < .05$), 10th ($P < .01$), and 14th ($P < .001$) days. The reduction in the mean body weight difference on the 14th day was negative because the final body weights in this group were lower than the initial ones (Table 2). Both final body weight ($P < .05$) and difference in the mean body weight ($P < .001$) of the 14th day was significantly lower than the 3rd day weights. Difference in the mean body weight of the 14th day was also lower compared to the 7th day ($P < .05$).

Biochemical Results

Levels of BUN and SCr have been shown in Figure 1. Compared with the control group, three days administration of the gentamicin was not associated with any significant changes in the levels of either BUN or SCr. However, in comparison with the control group, the levels of BUN ($P < .01$, $P < .001$) and SCr ($P < .001$, $P < .001$) were significantly higher

Table 2. The Effects of the Gentamicin Administration on the Animal's Body Weights in a 14-day Interval

Groups	Average initial weight (g)	Average final weight (g)	Average weight difference (g)
Control	234.7 ± 8.31	276.5 ± 13.69	41.83 ± 6.72
GM-3d	242.6 ± 3.03	270.7 ± 3.10	28.14 ± 2.46
GM-7d	239.5 ± 3.84	252.5 ± 7.70	13 ± 5.11*
GM-10d	254.2 ± 6.33	260.8 ± 8.81	6.67 ± 10.42**
GM-14d	242.1 ± 7.99	228.9 ± 10.43**$	-13.25 ± 25.20***$$$#

Data were presented as the mean ± SEM in the five groups (n = 7) including a control group and 4 gentamicin (100 mg/kg) treatment experimental groups which were treated for 3 (GM-3d), 7 (GM-7d), 10 (GM-10d) and 14 (GM-14d) days separately.
*$P < .05$, **$P < .01$, ***$P < .001$ vs. Control group; $^{\$}P < .05$, $^{\$\$\$}P < .001$ vs. GM-3d group; $^{\#}P < .05$ vs. GM-7d group

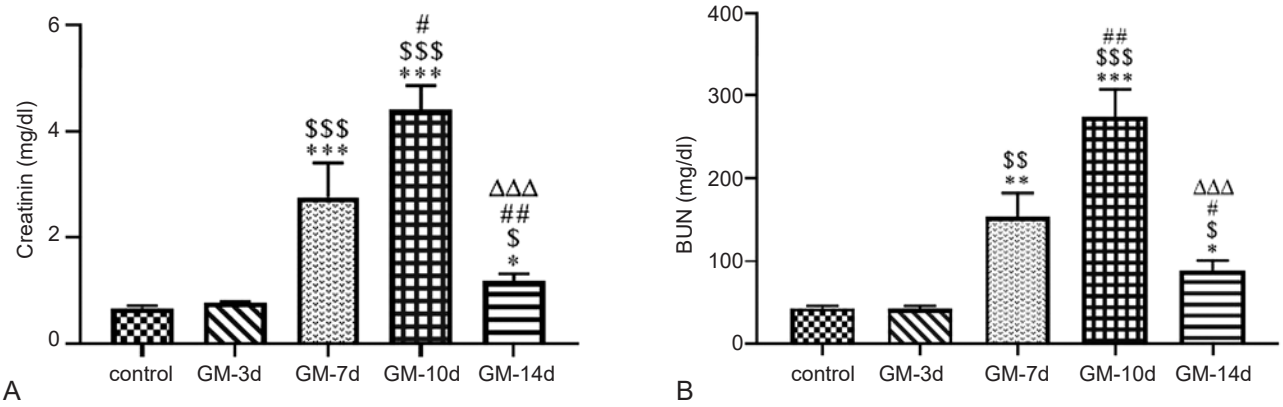

Figure 1. The effects of gentamicin (100 mg/kg/d) administration on the serum levels of (A) Creatinine (Cr) and (B) blood urea nitrogen (BUN) in a 14-day interval. Data were presented as mean ± SEM in the groups (n = 7) including a control group and 4 gentamicin treatment experimental groups were treated for 3 (GM-3d), 7 (GM-7d), 10 (GM-10d), and 14 (GM-14d) days; separately.
*$P < .05$, **$P < .01$, ***$P < .001$ vs. control group; $^{\$}P < .05$, $^{\$\$}P < .01$, $^{\$\$\$}P < .001$ vs. GM-3d group; $^{\#}P < .05$, $^{\#\#}P < .01$ vs. GM-7d group; $^{\Delta\Delta\Delta}P < .001$ vs. GM-10d group

on the 7th and 10th days, respectively. Levels of BUN ($P < .01$, $P < .001$) and SCr ($P < .001$, $P < .001$) were also significantly higher on the aforementioned days than that of the 3rd day values. The values of either BUN ($P < .01$) or SCr ($P < .05$) on the 10th day was significantly higher when compared with the 7th day values. Surprisingly, in comparison to the control group, we found a significant reduction in the levels of both BUN ($P < .05$) and SCr ($P < .05$) of day 14. Compared to the values of 7th and 10th days, the levels of BUN ($P < .05$, $P < .001$) and SCr ($P < .01$, $P < .001$) were significantly lower on the 14th day, respectively. Despite marked reduction in levels of BUN and SCr at 14 day, the values were significantly higher than that of the 3rd day ones ($P < .05$).

Histological Results

Changes in the cortical and outer medullary sections have been shown in Table 3 and Figures 2 to 6. As shown in the H&E staining of cortical parts (Figure 2), the 10th day samples were associated with the highest degree of alterations including increased glomerular size, decreased bowman's space, increased number of capillaries as well as the presence of RBCs in capillary lumens. In contrast, a marked improvement of glomerular size and bowman's space as well as number of capillaries occurred on the day 14. Moreover, compared to the control group, during the 14-day interval of gentamicin administration, cortical tubular damages were also detected on the 10th day and, to a lesser extent, on the 7th day (Figure 3). The later damages were characterized by development of leukocyte infiltration, cell desquamation, cytoplasmic vacuolization, apoptosis, pyknosis and necrosis. Surprisingly, 14th day was associated with a typical signs of

Table 3. The Effects of Gentamicin Administration on the Renal Histology of Five Groups (n = 7) Including a Control Group and 4 Gentamicin (100 mg/kg) Treatment Experimental Groups Which Were Treated for 3 (GM-3d), 7 (GM-7d), 10 (GM-10d), and 14 (GM-14d) Days Separately

H&E staining		Groups				
		Control	GM-3d	GM-7d	GM-10d	GM-14d
Cortex: Glomerulus						
1	Size of glomeruli	0	↑1	↑1.5*	↑2**	$0^{\#\#\Delta\Delta}$
2	Bowman's space size	0	0	0	$\downarrow 2^{***\$\$\$\#\#\#}$	$0^{\Delta\Delta\Delta}$
3	No. of capillaries	0	0	0	$\uparrow 3^{***\$\$\$\#\#\#}$	$0^{\Delta\Delta\Delta}$
4	No. of RBC in capillary lumen	0	1	2*	$3.5^{***\$\$}$	2**
Cortex: Tubules						
PT, TAL, DT, CCD						
1	Leukocyte infiltration	0	1	$3^{***\$}$	$3^{***\$}$	1
2	Desquamation	0	0	2**	$2^{**\$}$	1
3	Epithelial cytoplasmic cell vacuolization	0	1	$3.5^{***\$}$	2.5**	$1^{\#}$
4	Cortical apoptotic bodies	0	0	$2^{*\$\$}$	$2^{**\$\$}$	$0^{\#\#\Delta\Delta}$
5	Pyknotic nuclei	0	0	$2^{*\$\$}$	$2.5^{**\$\$}$	$0^{\#\Delta\Delta}$
6	tubular epithelial cell necrosis	0	0	$2^{*\$}$	$2.5^{**\$\$\$}$	$0^{\#\#\Delta\Delta\Delta}$
7	Tubular regeneration	0	0	1	$2^{**\$\$}$	$2^{***\$\$\$}$
Outer medulla						
PST (S3), TDL, TAL, MCD						
1	Vascular congestion	0	0	2*	$3.5^{***\$\$}$	2**
2	Intratubular proteinaceous casts	0	0	$3.5^{**\$\$}$	$3.5^{**\$\$}$	2
3	Desquamation	0	0	0	$0.5^{*\$\#}$	0^{Δ}
4	Epithelial cytoplasmic cell vacuolization	0	0	0	$0.5^{*\$\#}$	0^{Δ}
5	Apoptotic bodies	0	0	0	0	0
6	Tubular epithelial cell necrosis	0	0	0	$0.5^{*\$\#}$	0^{Δ}
PAS staining	Brush border loss	0	0	$3^{**\$\$}$	2	$2^{**\$\$}$
Mason'trichrome staining	Fibrotic bundle	0	1	2**	1*	1

note: 0: no abnormality detected; 1: damage/active changes up to < 20%; (Mild) 2: damage/active changes up to 20 to 40% (Moderate); 3: damage/active changes up to 40 to 60%; 4: damage/active changes up to 60 to 80%; 5: damage/active changes up to > 80%. Abbreviations: PT, proximal tubule; TAL, thick ascending limb; DT, distal tubule; CCD, cortical collecting duct; PST, proximal straight tubule (S3); TDL, thin descending limb; TAL, thick ascending limb; MCD, medullary collecting duct.
*$P < .05$, **$P < .01$, ***$P < .001$ vs. control group; $^{\$}P < .05$, $^{\$\$}P < .01$, $^{\$\$\$}P < .001$ vs. GM-3d group; $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs. GM-7d group and $^{\Delta}P < .05$, $^{\Delta\Delta}P < .01$, $^{\Delta\Delta\Delta}P < .001$ vs. GM-10d group.

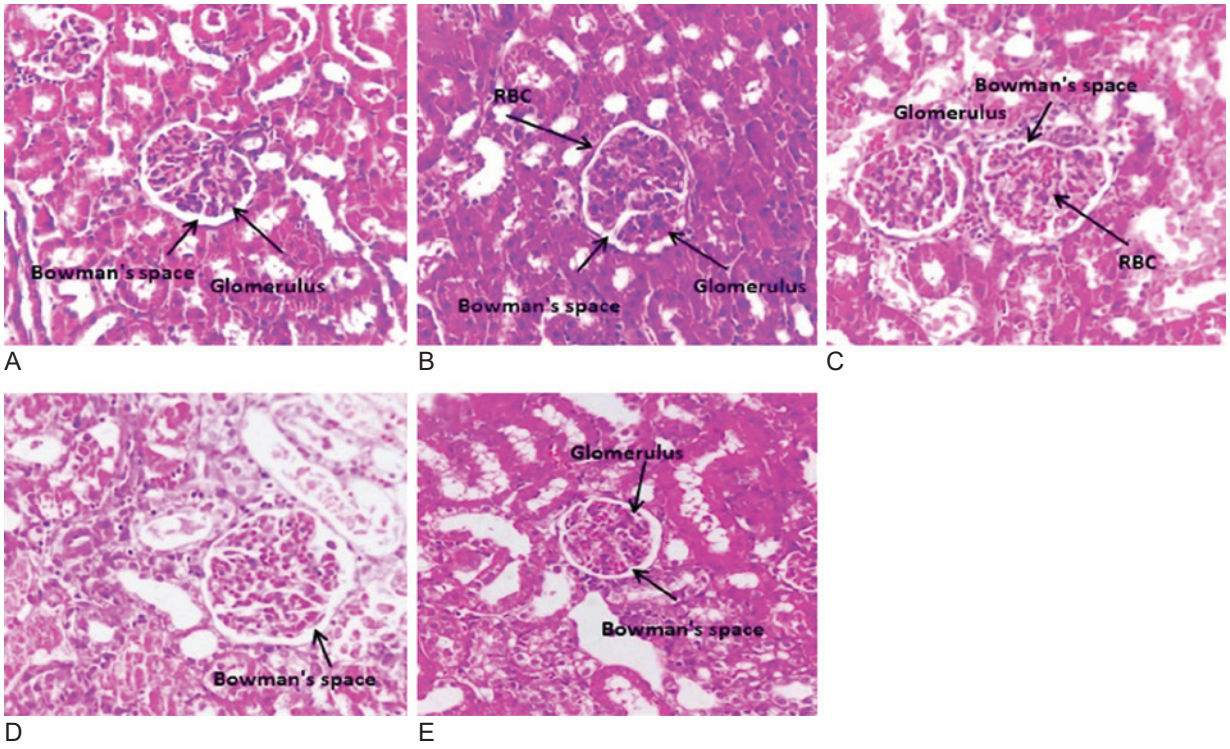

Figure 2. Histological changes of the rat left kidney following gentamicin (100 mg/kg/d) administration. Light Photomicrographs of the histological sections of the cortex focused on glomeruli parts (H&E staining; magnification ×400) from control group (A) and 4 gentamicin treatment experimental groups were treated for (B) 3 (GM-3d), (C) 7 (GM-7d), (D) 10 (GM-10d), and (E) 14 (GM-14d) days; separately.

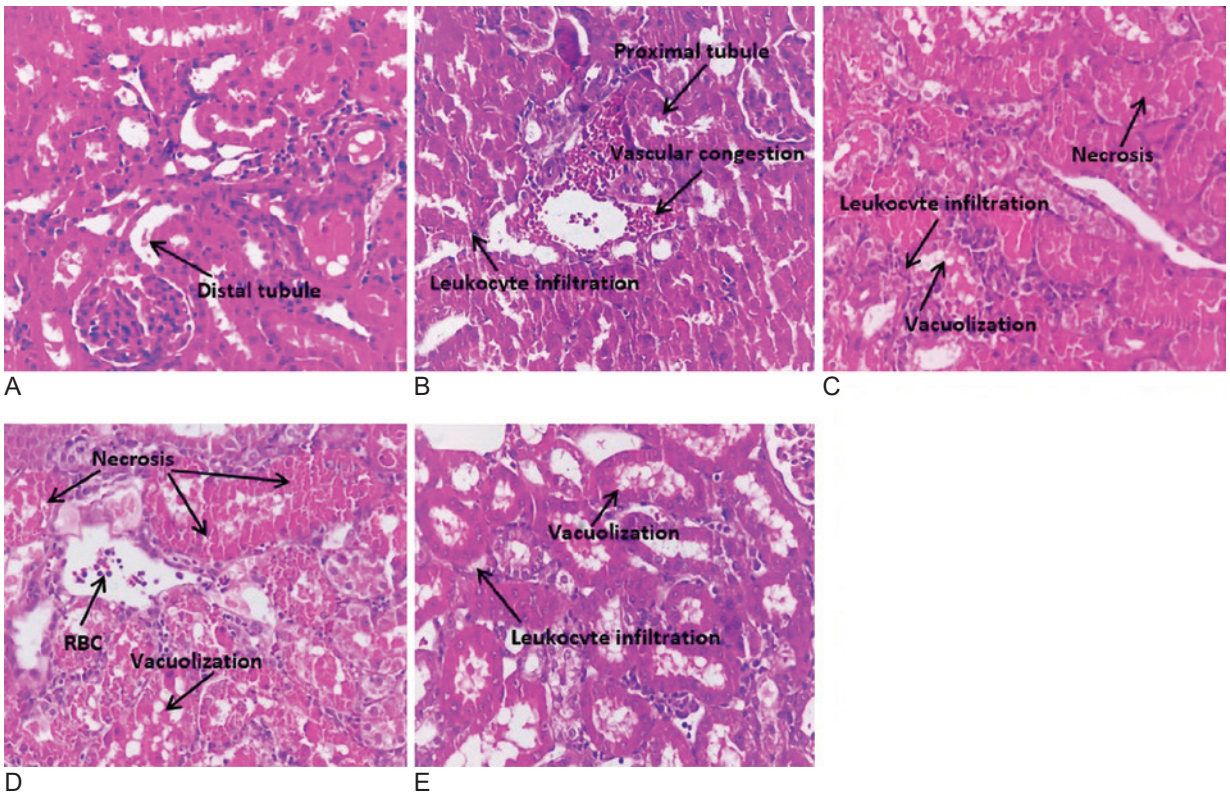

Figure 3. Histological changes of the rat left kidney following gentamicin (100 mg/kg/d) administration. Light Photomicrographs of the histological sections of the cortex focused on tubular parts (H&E staining; magnification ×400) from control group (A) and 4 gentamicin treatment experimental groups were treated for (B) 3 (GM-3d), (C) 7 (GM-7d), (D) 10 (GM-10d), and (E) 14 (GM-14d) days; separately.

tissue regeneration and recovery of the proximal tubules. As shown in Figure 4, compared to the control group, loss of the proximal tubule brush border did not occur at 3rd day but significant loss was detected on the 7th and 14th days. Although the brush border loss on the 10th day was clearly observed it was not significant compared to the control group. Histological assessment of the outer medullary sections showed a significant vascular congestion on the 7th, 10th, and 14th days, cast formation on the 7th and 10th days as well as cell desquamation, cytoplasmic vacuolization, and necrosis on the 10th day (Figure 5). In Mason's trichrome staining, thickening of collagen bundles (fibrotic bundles) was also observed on the 7th and 10th days, which was significant compared to the control group. Change in the collagen bundles was not statistically significant in the 3 and 14-day groups (Figure 6).

Molecular Findings

As shown in Figure 7, the gentamicin-induced change in the targeted genes expression are greatly variable during three to 14 days of the experiment. Compared to the control group, expression of FXR on the 3rd ($P < .01$) and 7th ($P < .001$) days, Nrf2 and GCLM on the 3rd ($P < .01$, $P < .01$), 7th ($P < .001$, $P < .05$), and 10th ($P < .05$, $P < .05$) days were significantly diminished (Figures 7 A, B, and C). Moreover, compared to the control value, the expression of FXR on day 14 was significantly lower ($P < .05$), however, mRNA expression of Nrf2 and GCLM at the same time were comparable to that of the control ones (Figures 7 B and C). The comparison between groups also showed a significant rise in mRNA expression of FXR ($P < .001$), Nrf2 ($P < .05$) and GCLM ($P < .01$) on the 10th day compared to the respective value on the 7th day. When compared to that of the 3rd day, Nrf2 expression at the 14th day ($P < .05$) was associated with a significant up-regulation. The similar 14-day pattern was also seen for GCLM ($P < .001$, $P < .01$, and $P < .01$) expression when compared to that of the 3rd, 7th and 10th days of

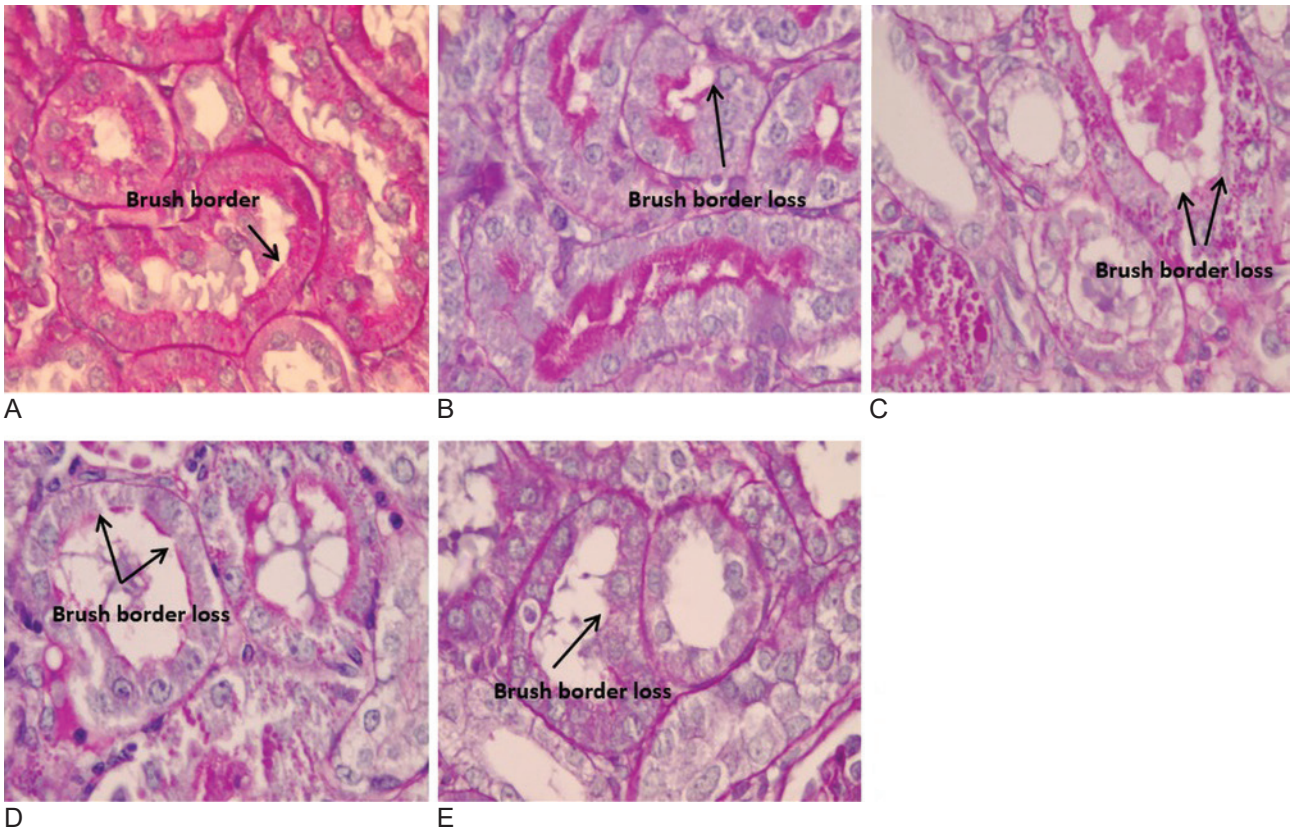

Figure 4. Histological changes of the rat left kidney following gentamicin (100 mg/kg/d) administration. Light Photomicrographs of the histological sections of the cortex focused on proximal tubule brush borders (PAS staining; magnification ×1000) from control group (A) and 4 gentamicin treatment experimental groups were treated for (B) 3 (GM-3d), (C) 7 (GM-7d), (D) 10 (GM-10d), and (E) 14 (GM-14d) days; separately.

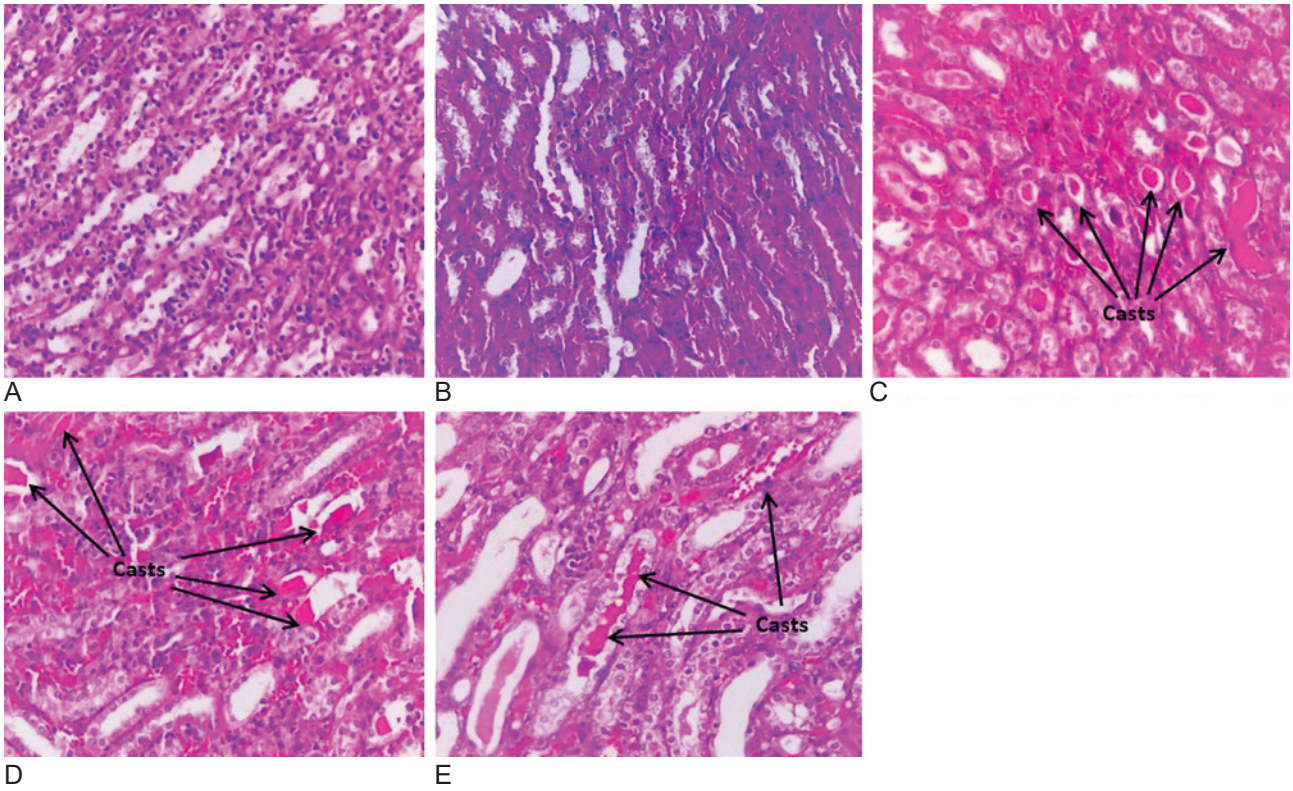

Figure 5. Histological changes of the rat left kidney (n = 7) following gentamicin (100 mg/kg/d) administration. Light Photomicrographs of histological sections of the outer medulla (H&E staining; magnification ×400) from control group (A) and 4 gentamicin treatment experimental groups were treated for (B) 3 (GM-3d), (C) 7 (GM-7d), (D) 10 (GM-10d), and (E) 14 (GM-14d) days; separately.

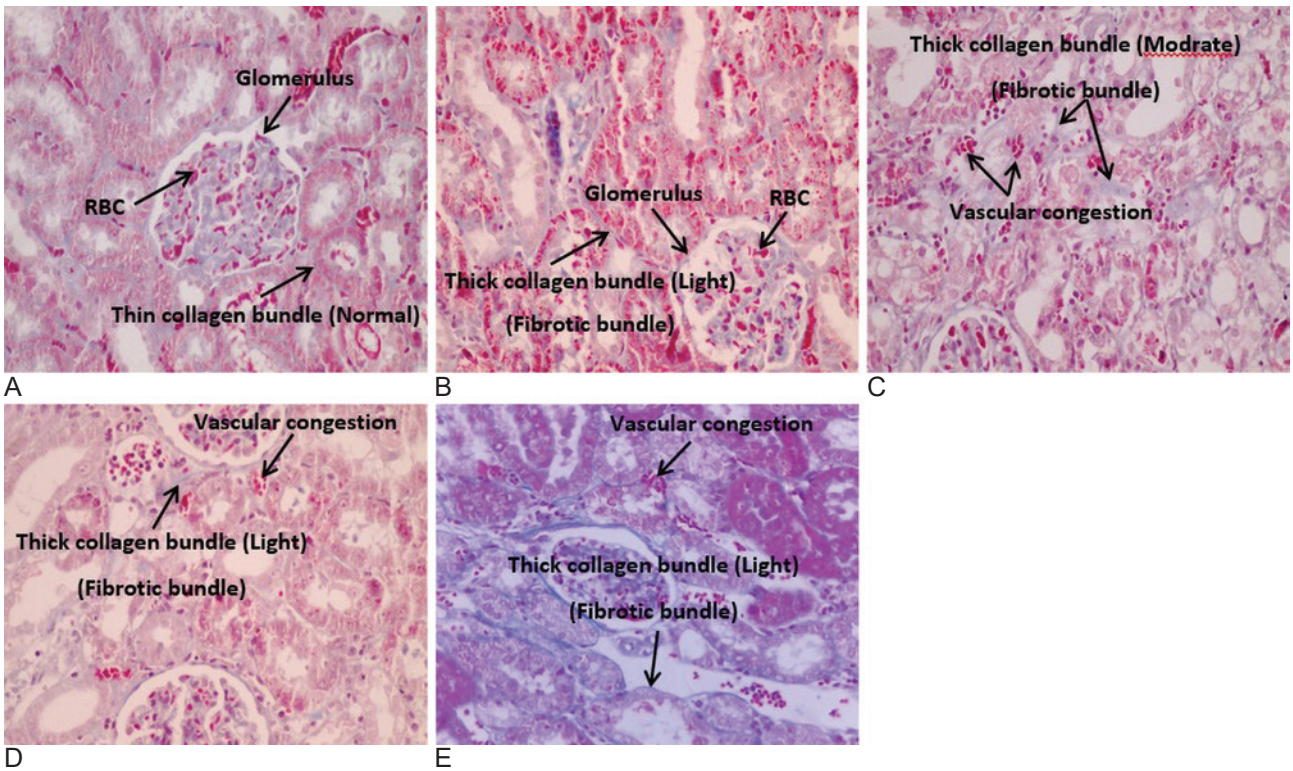

Figure 6. Histological changes of the rat left kidney (n = 7) following gentamicin (100 mg/kg/d) administration. Light Photomicrographs of histological sections of the cortex (Masson's trichrome staining; magnification ×400) from control group (A) and 4 gentamicin treatment experimental groups were treated for (B) 3 (GM-3d), (C) 7 (GM-7d), (D) 10 (GM-10d), and (E) 14 (GM-14d) days; separately.

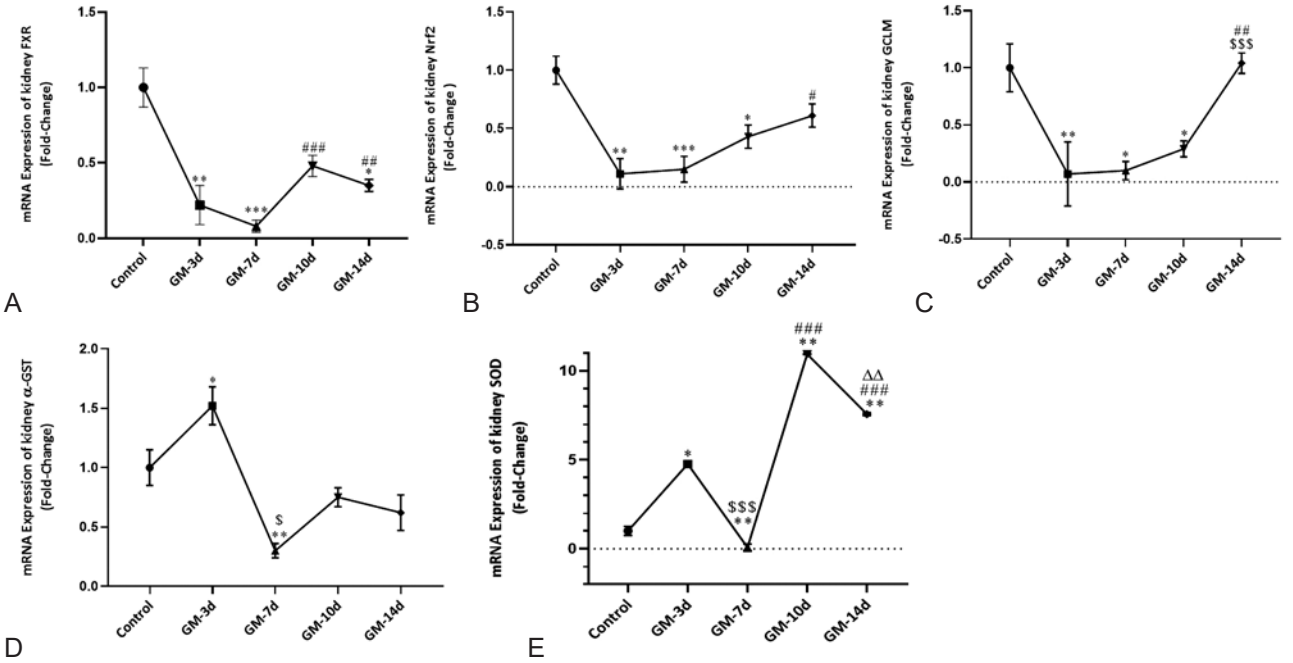

Figure 7. The effect of gentamicin (100 mg/kg/d) administration on the renal expression of targeted genes in a 14-day interval. Data were presented as mean ± SEM in the groups (n = 7) including (A) control group and 4 gentamicin treatment experimental groups were treated for (B) 3 (GM-3d), (C) 7 (GM-7d), (D) 10 (GM-10d), and (E) 14 (GM-14d) days; separately.
*$P < .05$, **$P < .01$, ***$P < .001$ vs. control group; $^{\$}P < .05$, $^{\$\$}P < .01$, $^{\$\$\$}P < .001$ vs. GM-3d group; $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs. GM-7d group; $^{\Delta\Delta}P < .01$, $^{\Delta\Delta\Delta}P < .001$ vs. GM-10d group

the control group (Figure 7 C).

On the other hand, the expression pattern of α-GST and SOD was not the same as the former genes. As shown in Figure 7 D, the expression of α-GST mRNA showed a significant reduction on day 7 so that the value at that time was significantly lower compared to the control ($P < .01$) and the 3rd day ($P < .05$). The SOD gene expression; however, was associated with more fluctuations. As shown in Figure 7 E, in comparison with the control, 3 days administration of gentamicin was associated with a significant increase in the expression of SOD mRNA ($P < .05$). Compared to the control or the 3rd day values, the expression dropped suddenly on the 7th day ($P < .01$, $P < .001$), which was as similar as the α-GST behavior at the same day. The second time up-regulation of SOD ($P < .01$) was seen on the 10th day when compared to the control group. The expression of SOD on the 14th day was still significantly higher ($P < .01$) than that of the control and the 7th day ($P < .001$) groups, although it was lower ($P < .01$) in comparison to the 10th day.

DISCUSSION

The present study was designed to find out the time-course pattern of changes in the expression of some upstream and downstream target genes involved in the intracellular antioxidant process concomitant with gentamicin administration. According to the previous investigations, almost all of the studies on gentamicin effects were designed for a fixed time in particular six ,[14] seven,[15-18] eight,[19-22] ten,[23] 14[24], or 15[1,25] consecutive days. There are just a few studies which have been published on time-dependent effects of gentamicin-induced nephrotoxicity.[26-28]

According to several *in vivo*[19,25,29] and *in vitro*[30-32] investigations, tubular cytotoxicity, particularly at the proximal section, is the main issue of gentamicin-induced nephrotoxicity which is characterized by massive apoptosis and necrosis of tubular epithelial cells. Direct drug toxicity comes from accumulation of the drug in the tubular epithelial cells especially in the proximal tubules which then is followed by a condition known as phospholipidosis.[33] The present study showed that similar to the previous studies,[25,34,35] administration of gentamicin led to development of drug-induced renal damage but the observed manifestations of this renal damage were rather unexpected during 14-day interval. Elevation

of the BUN and SCr started at the 7th day and continued up to the 14th day. Although the levels of BUN and SCr were significantly higher between days 7 to 10, surprisingly a significant reduction in these values occurred on the 14th day. While the later results seem rather in contrast with the reports of the studies, which had stated a time-dependent elevation of either BUN or Cr,[27,36] it was consistent with our parallel histological and/or molecular findings. In this regard, our histological assessment showed typical signs of tubular and, to a lesser extent, glomerular damages. Based on our histologic findings, the 7th and 10th days groups were associated with moderate to severe degrees of acute tubular necrosis (ATN), which was characterized by vacuolization, brush border loss, desquamation, apoptosis, and necrosis. Unexpectedly, again we encountered a marked improvement in almost all tubular damages indices on the 14th day, a phenomenon which was consistent with, and partially explained the marked reduction of the levels of BUN and SCr on the respective day. In consistence with our results, Chen *et al.* findings also exhibited a similar pattern.[28] In the Chen's study, the maximal tubular damage induced by gentamicin was seen on the 5th day and then it gradually recovered and reached the normal level on day 29.[28] The other time-course study on gentamicin nephrotoxicity by Cui *et al.*, revealed that during a 10-day interval, maximum rate of tubular damage occurred on the 10th day characterized by necrosis, apoptosis, oxidative and inflammatory damages.[27] Sudden drop in BUN and SCr on the 14th day was an unexpected finding that might be explained by marked increment in the proximal tubule regeneration along with a decrease in apoptosis and necrosis of the tubular cells. The reduced levels of BUN and Cr on day 14 may be due to the replacement of necrotic cells with newer cells, but this explanation cannot be determined based on the current findings and requires further research.

Overproduction of reactive oxygen species (ROS) could subsequently stimulate inflammatory responses by triggering production of several pro-inflammatory cytokines.[25,27,37] The regulatory role of numerous upstream pathways that mediate the activation of intracellular antioxidant systems is essential in this regard. Farnesoid receptors, one of the nuclear receptor superfamily members, play a role in the metabolism of lipids, cholesterol, and glucose in addition to bile acid. In the kidney, they are expressed mainly in the tubular and, to a lesser extent, in the glomerular sites. [38] In addition to their metabolic role, FXR is considered as upstream controller of the cellular redox state. This regulatory role is performed via connecting to several downstream signaling pathways such as Nrf2 which contributes to formation of glutathione and SOD.[39] Nrf2 plays a pivotal role in the regulation of the genes involved in the glutathione synthesis such as GCL. It also manages the xenobiotic detoxification by regulating the expression of genes involved in this process such as α-GST. [40] This study is the first to investigate the impact of time on the expression of the FXR and its downstream target genes during gentamicin administration, recognizing the significant function of these upstream controllers. According to the results and apart from some minor differences, two main time-course alterations in the gene expression have been observed. The first one was a similar trend of changes in the expression of FXR, Nrf2 and GCLM and the second one was for SOD and α-GST. For the first category, the maximum level of mRNA downregulation occurred during 3 to 7 days which was then followed by an ascending phase. So, the acute phase (up to 7 days) of gentamicin treatment has been associated with a clear downregulation in the FXR, Nrf2 and GCLM expression. One of the best researches which has precisely demonstrated the logical association between FXR signaling pathway and Nrf2-mediated induction of the antioxidant genes, was performed by the Gai *et al*.[39] As they showed in detail, FXR expression following hypoxia-induced renal damage was decreased in either acute or chronic phase, and administration of a selective FXR agonist either as a prophylactic or therapeutic agent, could strongly prevent the oxidative damage and inflammatory responses on proximal tubular cells.[39] As they precisely demonstrated, the mentioned protective effect of FXR activation mediated via the Nrf2, since the Nrf2 silencing could block FXR protective effects.[39] Consistent with Gai's findings, we also noticed a strong correlation between the fluctuations in the expression of the FXR and Nrf2 genes over a 14-day period of gentamicin

administration. The GCL is a rate-limiting enzyme in the glutathione synthesis which is induced by oxidative stress responses.[39] The GCLM or the modulatory subunit of the GCL is an indicator for glutathione synthesis.[11] The present study showed a positive correlation between the Nrf2 and GCLM mRNA expression.

Both SOD and α-GST mRNA expression showed a sudden drop on the 7th day. The second occurrence of an increase in altitude was observed between days 7 and 14. So, apart from the expression pattern of the 3rd day, in the other days the pattern was similar to those of FXR and Nrf2 in respective days. However, our findings were not consistent with the Gai's results regarding the correlation between the pattern of SOD and Nrf2 mRNA expressions on the 3rd day. According to the descending trend of the FXR and Nrf2 on the 3rd day, similar reduction in the SOD was expected. It might be suggested that such an elevation in SOD mRNA expression probably had been a compensatory response against the Xenobiotic-induced renal damage, which was mediated via other signaling pathways rather than Nrf2-mediated ones.

CONCLUSION

In conclusion, it appears that the downregulation of FXR and its subsequent regulator, Nrf2, during the initial stage of gentamicin treatment may contribute to the progression of renal damage caused by reactive oxygen species (ROS) in a pathological manner. Nonetheless, the pattern of expression was rather biphasic at the acute and sub-acute stages. Significant reduction in the expression of the cellular defense related genes including GCLM, SOD and α-GST, could be responsible for such structural and functional injuries. However, for certain interpretation of the present results, Western blot analysis is needed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

G. Bayat, S. Changizi-Ashtiyani and A. Khalili conceived and designed the study. MR. Ashrafi, G. Bayat, A. Khalili, SA. Hashemi performed the experimental animal procedure. A. Khalili, G. Bayat, S. Changizi-Ashtiyani and R. Mazloom, analyzed data and wrote the manuscript. All the authors contributed to conducting different experiments, read and approved the manuscript.

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