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RESEARCH ARTICLE

Comparison of the effect of vitamin C and selenium nanoparticles on gentamicin-induced renal impairment in male rats: a biochemical, molecular and histological study

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ABSTRACT

Renal failure caused by gentamicin is mainly mediated through oxidative damage, inflammation, and apoptosis. Hence, vitamin C and selenium, which have antioxidant, anti-inflammatory, and anti-apoptotic properties, and their nanoparticle forms, which have recently received attention, may reduce gentamicin-induced side effects. Therefore, the aim of this study was to investigate the therapeutic effects of vitamin C and selenium, and their nanoparticles on gentamicin-induced renal damage in male rats. 128 adult male Wistar rats were randomly divided into equal sixteen controlled and treated groups. Serum levels of uric acid, blood urea nitrogen, urea, and creatinine were measured. Renal levels of oxidative parameters such as MDA, SOD, and CAT and inflammatory parameters including IL-1β, and TNF-α were measured. Renal expression of Nrf2, NF-κB, Bcl-2, caspase-3, BAX and mTORc1 was also evaluated. The results showed that gentamicin causes oxidative damage, inflammation, apoptosis and disruption of autophagy in kidney tissue in a dose-dependent manner. However, treatment with vitamin C, selenium and their nanoparticles could significantly improve these effects. Also, the results showed that the inflammatory and oxidative parameters and the expression of genes involved in them and apoptosis in the gentamicin groups treated with vitamin C nanoparticles and selenium nanoparticles reduced significantly compared to those treated with vitamin C and selenium. It can be concluded that vitamin C, selenium and their nanoparticles can improve gentamicin-induced kidney damage by inhibiting oxidative damage, inflammation and apoptosis-induced by autophagy, and can be a good option for kidney damage caused by gentamicin or as an adjunctive treatment to reduce its side effects.

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Gentamicin; vitamin C nanoparticle; selenium nanoparticle; nephrotoxicity

Introduction

Gentamicin is an aminoglycoside antibiotic used to treat several types of bacterial infections. It is also combined with β-lactam antibiotics to produce a synergistic effect. Due to its high clinical efficacy, low drug resistance, and low cost, gentamicin is used as a first-line antibiotic for many serious and life-threatening infectious diseases (Krause et al. 2016). However, its use is limited due to nephrotoxicity and ototoxicity. Nephrotoxicity is due to damage to the renal cortex, especially in the proximal tubule due to the destruction of cellular organs including mitochondria and cell necrosis. This phenomenon causes proteinuria and increased serum levels of urea and creatinine, similar to what is seen in acute kidney injury (AKI) (Fuchs et al. 2016). It is an unpleasant clinical condition that causes kidney dysfunction due to structural damage. Recent studies have shown that high levels of free radicals and inflammatory mediators (Basile et al. 2012) and apoptosis in glomerular cells and tubular cells are involved in the pathogenesis of renal failure induced by gentamicin (Makris and Spanou 2016). Gentamicin increases the production of reactive oxygen species (ROS) and leads to oxidative damage through the oxidation of cellular compounds such as lipids, proteins, and nucleic acids (Cui et al. 2015; Ratliff et al. 2016). Moreover, oxidative damage induced by various factors including ethanol and gentamicin may associate with apoptosis, inflammation, and autophagy (Cui et al. 2019; Fathi et al. 2021). Autophagy is a cellular mechanism responsible for the removal of damaged organs, deformed proteins, and dysfunctional proteins (Ahmed Mustafa et al. 2021). Furthermore, the kidneys are prone to damage due to constant exposure to chemicals and the clearance of drug metabolites and other excretory metabolites. It is also known well that oxidative stress accelerates the degeneration process in the structure and function of many organs, and promotes chronic diseases, cancer, diabetes, and drug-related

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degeneration (Akbari 2013; Akbari et al. 2016; Liguori et al. 2018; Nimrouzi et al. 2020). Therefore, it seems that modifying these pathophysiological pathways by supplements and nutrients such as vitamins and essential elements that have antioxidant, anti-inflammatory and anti-apoptotic effects may be an effective therapeutic strategy.

Vitamin C and selenium are powerful and essential natural antioxidants (Zoidis et al. 2018) that are directly responsible for removing free radicals and strengthening antioxidant systems inside and outside the cell (Akbari et al. 2016). In addition to antioxidant activity, these two natural compounds strengthen the immune system and have strong antibacterial activity (Hoffmann and Berry 2008; Carr and Maggini 2017). Vitamin C is water-soluble and acts as a non-enzymatic and chain-breaking antioxidant by donating electrons and direct removal of free radicals (Akbari et al. 2014b) and can prevent oxidative damage to DNA, mutations, cell membranes, and other macromolecules (Lee et al. 2004). Selenium is an essential microelement that has antioxidant activity by scavenging free radicals and as a cofactor of antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase (Zoidis et al. 2018). In addition, there are other selenoproteins that act as anti-inflammatory and anti-apoptotic agents (Tinggi 2008). Protective effects of vitamin C and selenium on the kidney against lead-induced damage, radio waves, and intoxication with drugs such as gentamicin have been reported (Akbari et al. 2014c, 2014a; Raeeszadeh et al. 2021). However, their use is limited due to dose-dependent responses, short half-life due to low stability (Akbari et al. 2016), the narrow safe dose range, and uncontrolled use (Filipović et al. 2021). Such factors have limited the clinical use of selenium and vitamin C in the prevention and treatment of many diseases as a treatment or adjunctive therapy. However, today the use of some new technologies such as nanotechnology to solve these problems is very welcome.

Nanotechnology has made it possible to use many materials at the nanoscale for industrial, food, and pharmaceutical applications (Filipović et al. 2021; Raeeszadeh et al. 2021). In recent years, nanotechnology has been able to make significant progress in various fields of life sciences and medicine in order to diagnose, treat and prevent diseases. Changes in properties such as stability, half-life, bioavailability and availability are some of the most important pharmacokinetic properties that nanotechnology has facilitated the pharmaceutical application of many compounds (Jeevanandam et al. 2018). Vitamin C nanoparticles and selenium nanoparticles have been considered by many scientists and researchers due to their high bioavailability, low toxicity, extensive biological activity, and antioxidant properties (Hosnedlova et al. 2018; Raeeszadeh et al. 2021). In this regard, the healing effects of selenium nanoparticles and vitamin C nanoparticles against cancer, bacterial and viral infections, neurological diseases, diabetes, as well as the toxicity of some drugs and some other toxins such as ethanol, cadmium and lead have been well reported (Ferro et al. 2021; Filipović et al. 2021; Lin et al. 2021; Raeeszadeh et al. 2021). It seems that vitamin C nanoparticles and selenium nanoparticles can significantly improve gentamicin-induced kidney damage. Therefore, this study was performed to elucidate the potential protective properties of vitamin C and selenium nanoparticles on gentamicin-induced kidney damage compared to treatment with selenium or vitamin C alone by evaluating oxidative stress, inflammation, apoptosis and necroptosis signaling pathways.

Materials and methods

Animals

All stages of this study were in accordance with the guidelines of the Helsinki Declaration (Helsinki Declaration, revised, 2013) and were also approved by the animal ethical committee of Hawler Medical University and the research ethics committee affiliated with the scientific and research group affiliated with Dr. Abolfazl Akbari with approved no. SRA-0017/12-2021. In this study, 128 adults male Wistar rats (200–220, 8 weeks old) were kept in a controlled breeding-house in temperature (22 ± 2 °C), brightness (12 h bright: 12 h dark), and humidity (30%). Animals had free access to water and chow.

Study design

In this study, 128 male Wistar rats were randomly divided into sixteen groups ($n = 8$). Groups included control, gentamicin (intraperitoneal injection (IP), 100 mg/kg, for 6 consecutive days), gentamicin (IP, 200 mg/kg, for 6 consecutive days), gentamicin (IP, 300 mg/kg, for 6 consecutive days), gentamicin 100 + vitamin C (200 mg/kg, for 28 consecutive days), gentamicin100 + selenium (2 mg/kg, for 28 consecutive days), gentamicin 100+ vitamin C nanoparticles (200 mg/kg), gentamicin 100 + selenium nanoparticles (2 mg/kg), gentamicin200-selenium, gentamicin200-vitamin C, gentamicin200-nanovitamin C, gentamicin 200-nanoselenium, gentamicin300-selenium, gentamicin300-vitamin C, gentamicin300-nanovitamin C, gentamicin300-nanoselenium. Vitamin C, its nanoparticles and sodium selenite as a supplement containing selenium (2 mg/kg) and its nanoparticles (US research nanomaterials, Inc, USA) were taken daily by gavage.

Sampling

At the end of the study period, the animals were anesthetized after fasting overnight with thiopental (IP, 40 mg/kg). After ensuring the anesthesia of the animals, blood pressure and heart rate were measured in the controlled and treated groups. After this stage, blood samples were taken by cardiac puncture. The blood samples were incubated (37 °C for 10 min) and then centrifuged (15 min at 2000 rpm). The supernatant was finally separated and stored at −80 °C for biochemical assessment latter. In the final stage, sacrificed animals and kidney sampling were performed for molecular and histological evaluation.

Measurement of biochemical parameters

The serum levels of acid uric, blood nitrogen, urea and creatinine were measured by spectrophotometric methods to evaluate kidney function. Renal levels of malondialdehyde

(MDA) (Ohkawa et al. 1979), superoxide dismutase (SOD) (Sun and Zigman 1978), catalase (CAT) (Aebi 1984) were measured. Interleukin (IL)-1β and tumor necrosis factor (TNF) -α were measured in kidney tissue using the ELISA kits (Shanghai Crystal Day Biotech Co., Shanghai, China).

Molecular measurements

The renal expression of NF-κB, HMGB1, TLR4, Bcl-2, Caspase-3, BAX, Nfr2 and mTORc1 was measured in the controlled and treated rats. Total RNA was extracted from the kidney tissue of rats using TRIZol® LS reagent according to the manufacturer's instructions (Berda, Netherlands). Then samples were treated with DNase I enzyme to avoid genomic DNA amplification. RNA concentration was determined using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Reverse transcription was performed to synthesize cDNA using Ipsogen RT Kit (Qiagen, GmbH, Hilden, Germany). PCR primers for NF-κB, HMGB1, TLR4, Bcl-2, Caspase-3, BAX, Nfr2 and mTORc1 genes were obtained from previous studies (Table 1) and specific primers to amplify GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) as an internal control (housekeeping) gene. The reaction of Real-Time PCR was performed using Real Q Plus 2x Master Mix Green (Amplicon, Denmark) by Rotor gene Corbett 6000 instrument (Corbett Research, Australia). The fluorescent quantitative PCR reaction in volume 20 µl included of 10 µl RT 2x QuantiNova SYBR Green RTPCR Master Mix (Qiagen, Germany), 0.4 µl forward primer (10 µM), 0.4 µl reverse primer (10 µM), 3 µl cDNA (50 ng) and 6.2 µl RNAse/DNAse free water. The reaction conditions were as follows: 95 °C for 5 min followed by 40 cycles including of 95 °C for 15 sec and 59 °C for GAPDH and 59–60 °C for target genes for 35 sec and extension in 72 °C for 20 sec. Cycle threshold (Ct) values were obtained for housekeeping gene and each specific PCR and ΔCt values were calculated. The expression of target genes was normalized to the expression of GAPDH. Quantification of expression of target genes was performed using 2-ΔΔCt method (Livak and Schmittgen 2001).

Histological examination

Considering that the renal cortex, especially the proximal tubule, is the target site of gentamicin (Parsons et al. 2000). In this study, some features such as tubular dilation, tubular atrophy in the cortex, hemorrhage, degeneration, and severe necrosis were evaluated in the cortical part of the kidney tissue. For this purpose, the kidney tissue was fixed in a 10% formalin solution for 48 h. it was then replaced with a 4% formalin solution. All samples were embedded in block paraffin. Sections with 5 µm were stained with hematoxylin and eosin (H&E). histopathological assessment was performed by A histologist using a light microscope (x40).

Statistical analysis

All data were analyzed using SPSS 16.0 software and one-way analysis of variance (ANOVA). Post hoc multiple comparisons Tukey 'test was used to compare the mean between groups. $P < 0.05$ was considered as the least statistically significant level. The results were expressed as mean ± standard error of the mean (mean ± SEM).

Results

The results of biochemical parameters

The results showed that different doses of gentamicin could significantly alter the serum blood urea nitrogen, urea, creatinine and uric acid compared to the control group. The highest level of blood urea nitrogen, urea, creatinine, and uric acid was observed in the gentamicin 3 group compared to other groups receiving gentamicin. The lowest level of these parameters was observed in the gentamicin 1 group receiving vitamin C nanoparticles and selenium nanoparticles. The results showed that the vitamin C and selenium and their nanoparticles in the groups receiving gentamicin could significantly improve kidney function (Table 2).

Table 1. Sequence of primers used in this study.

Gene	Sequence	Size product	Accession no.
HMGB-1	Forward 5′-GTAATTTTCCGCGCTTTTGT-3′ Reverse 5′-TCATCCAGGACTCATGTTCAGT-3′	114	NM_012963.2
TLR-4	Forward 5′-AGGATGATGCCAGGATGATGTC-3′ Reverse 5′-TCAGGTCCAGGTTCTTGGTTGAG-3′	195	NM_019178.2
NF-κB	Forward 5′-GCACCAAGACCGAAGCAAT-3′ Reverse 5′-CGTAACCGCGTAGTCGAAGA-3′	143	NM_001276711.1
mTORc1	Forward 5′- GGTGGACGAGCTCTTTGTCA −3′ Reverse 5′- AGGAGCCCTAACACTCGGAT −3′	225	NM_019906.2
Nrf2	Forward 5′-AAAGACAAACATTCAAGCCGATTAG-3′ Reverse 5′-TTGCTCCTTGGACATCATTTCAT-3′	141	NM_031789.2
Bcl-2	Forward 5′-GCTACGAGTGGGATACTGGAGATGA-3′ Reverse 5′-ACAGCGGGCGTTCGGTTG-3′	103	NM_016993.2
BAX	Forward 5′-AGGGTGGCTGGGAAGGC-3′ Reverse 5′- TGAGCGAGGCGGTGAGG- 3′	93	XM_039087751.1
Caspase-3	Forward 5′-GCAGCAGCCTCAAATTGTTGAC-3′ R 5′-TGCTCCGGCTCAAACCATC-3′	144	NM_012922.2
GAPDH	Forward 5′-ATGACTCTACCCACGGCAAG-3′ Reverse 5′-TACTCAGCACCAGCATCACC-3′	136	NM_017008.4

Table 2. The mean ± SEM levels of kidney function evaluation parameters in controlled and treated groups.

Group/parameters	BUN (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	13.25 ± 2.64^{a}	21.36 ± 3.64^{a}	0.664 ± 0.02^{a}	1.26 ± 0.31^{a}
Gentamicin 1	29.31 ± 3.69^{b}	36.26 ± 5.69^{b}	1.23 ± 0.01^{b}	2.67 ± 0.12^{b}
Gentamicin 2	37.54 ± 6.54^{c}	48.89 ± 6.47^{c}	1.87 ± 0.21^{c}	3.74 ± 0.22^{c}
Gentamicin 3	43.58 ± 4.39^{c}	54.67 ± 6.57^{d}	2.96 ± 0.35^{d}	5.64 ± 0.12^{d}
Gentamicin 1-Vitamin C	13.15 ± 3.5^{a}	19.15 ± 3.64^{a}	0.654 ± 0.012^{a}	1.23 ± 0.13^{a}
Gentamicin 1-Vitamin C nanoparticles	13.64 ± 3.6^{a}	18.43 ± 3.6^{a}	0.644 ± 0.011^{a}	1.23 ± 0.9^{a}
Gentamicin 1-Selenium	13.57 ± 3.16^{a}	18.23 ± 5.47^{a}	0.596 ± 0.011^{a}	1.32 ± 0.21^{a}
Gentamicin 1-Selenium nanoparticles	13.64 ± 1.4^{a}	17.87 ± 3.4^{a}	0.575 ± 0.011^{a}	1.28 ± 0.12^{a}
Gentamicin 2-vitamin C	18.64 ± 3.64^{d}	36.15 ± 6.14^{b}	0.801 ± 0.012^{a}	1.78 ± 0.18^{e}
Gentamicin 2-nano-vitamin C	12.36 ± 3.94^{a}	23.3 ± 4.5^{a}	0.684 ± 0.014^{a}	1.29 ± 0.34^{a}
Gentamicin 2-selenium	16.58 ± 3.48^{d}	37.87 ± 5.94^{b}	0.792 ± 0.012^{a}	2.23 ± 0.21^{b}
Gentamicin 2-nano-selenium	12.64 ± 4.47^{a}	23.61 ± 4.8^{a}	0.714 ± 0.012^{a}	1.58 ± 0.26^{e}
Gentamicin 3-vitamin C	23.21 ± 2.58^{e}	39.41 ± 6.14^{b}	1.011 ± 0.034^{e}	2.17 ± 0.18^{b}
Gentamicin 3-nano-vitamin C	14.25 ± 2.47^{a}	24.45 ± 6.4^{e}	0.764 ± 0.014^{a}	1.69 ± 0.34^{e}
Gentamicin 3-selenium	19.47 ± 5.47^{d}	41.29 ± 6.61^{f}	0.989 ± 0.012^{e}	2.48 ± 0.34^{b}
Gentamicin3-nanoselenium	13.44 ± 3.47^{a}	27.14 ± 7.48^{g}	0.647 ± 0.025^{a}	1.78 ± 0.16^{e}

Mismatched lowercase letters indicate a significant difference among the controlled and treated groups ($n = 8$, $p < 0.05$). BUN: Blood urea nitrogen.

Table 3. The mean ± SEM levels of oxidative parameters in kidney tissue in the controlled and treated groups.

Group/parameters	SOD (U/mgProtein)	CAT (U/mgProtein)	MDA (U/mgProtein)
Control	33.15 ± 6.47^{a}	21.57 ± 3.54^{a}	0.502 ± 0.012^{a}
Gentamicin 1	19.24 ± 5.69^{b}	11.36 ± 4.15^{b}	1.341 ± 0.021^{b}
Gentamicin 2	14.26 ± 3.14^{c}	8.06 ± 2.45^{c}	1.891 ± 0.041^{c}
Gentamicin 3	9.14 ± 2.19^{d}	6.16 ± 1.31^{d}	2.441 ± 0.031^{d}
Gentamicin1-Vitamin C	34.24 ± 3.64^{a}	21.54 ± 4.73^{a}	0.617 ± 0.01^{a}
Gentamicin 1-Vitamin C nanoparticles	34.14 ± 7.14^{a}	22.24 ± 5.36^{a}	0.512 ± 0.01^{a}
Gentamicin 1-Selenium	34.45 ± 6.47^{a}	20.14 ± 4.37^{a}	0.626 ± 0.01^{a}
Gentamicin 1-Selenium nanoparticles	33.34 ± 3.4^{a}	22.68 ± 5.14^{a}	0.521 ± 0.01^{a}
Gentamicin2- Vitamin C	25.35 ± 6.48^{e}	16.24 ± 3.14^{e}	0.826 ± 0.014^{e}
Gentamicin2- nano-vitamin C	29.14 ± 8.14^{f}	19.34 ± 3.54^{a}	0.601 ± 0.001^{a}
Gentamicin2- Selenium	26.45 ± 5.47^{e}	15.89 ± 3.15^{e}	0.842 ± 0.024^{e}
Gentamicin2-nano-selenium	33.14 ± 6.57^{a}	20.15 ± 3.64^{a}	0.597 ± 0.004^{a}
Gentamicin3- Vitamin C	28.35 ± 6.04^{f}	13.12 ± 2.65^{b}	1.426 ± 0.14^{b}
Gentamicin3- nano-vitamin C	33.14 ± 6.34^{a}	18.74 ± 4.84^{a}	0.971 ± 0.01^{e}
Gentamicin3- Selenium	24.75 ± 5.07^{e}	14.89 ± 3.15^{e}	$1.372 \pm 0.\ 24^{b}$
Gentamicin3- nano-selenium	32.34 ± 6.27^{a}	18.15 ± 3.64^{a}	0.879 ± 0.14^{e}

Mismatched lowercase letters indicate a significant difference among the controlled and treated groups ($n = 8$, $p < 0.05$).

Selenium and vitamin C and their nanoparticles could improve gentamicin-induced oxidative damage in kidney tissue

In this study, the renal levels of the SOD, CAT, and MDA and changes in the renal expression of Nrf2 were assessed to evaluate the status of oxidative damage induced by gentamicin. The highest amount of MDA and the lowest of SOD and CAT levels were observed in the gentamicin-3 group compared to the control group. Changes in these parameters were observed in a dose-dependent manner in the gentamicin-receiving groups (Table 3). Moreover, the renal expression of Nrf2 significantly decreased by gentamicin in a dose-dependent manner compared to the control group (Figure 1). However, treatment with vitamin C and selenium and their nanoparticles could significantly improve its expression in the gentamicin groups (Figure 1).

Selenium and vitamin C and their nanoparticles could improve gentamicin-induced inflammation in kidney tissue

The renal levels of IL-1β and TNF-α and changes in the renal expression of NF-κB, TLR-4 and HMGB1 were measured to evaluate gentamicin-induced inflammation. The results showed that the expression of NF-κB, TLR-4, and HMGB1 and the levels of IL-1β and TNF-α in kidney tissue significantly increased by gentamicin in a dose-dependent manner compared to the control group. However, treatment with vitamin C and selenium and their nanoparticles in the gentamicin-receiving groups could significantly decrease the expression of these genes compared to the gentamicin-receiving groups (Table 4 and Figure 2).

Selenium and vitamin C and their nanoparticles could improve gentamicin-induced apoptosis in kidney tissue

In this study, the renal expression of Bcl-2, BAX, and caspase-3 was evaluated as genes involved in apoptosis induced by gentamicin. The results showed that the expression of BAX and caspase-3 significantly increased and the expression of Bcl-2 decreased in the gentamicin-receiving groups in a dose-dependent manner compared to the control group. The lowest expression of Bcl-2 and the highest expression of BAX, and caspase-3 were observed in the gentamicin 3 group compared to other groups. However, treatment with vitamin C and selenium and their nanoparticles in the gentamicin-receiving groups could significantly improve the expression of these genes compared to the gentamicin groups (Figure 3).

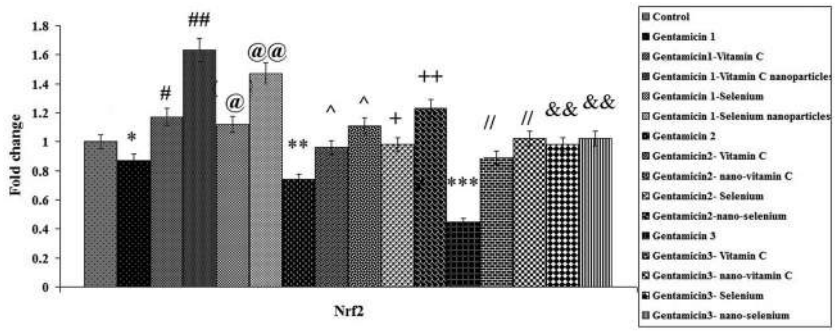

Figure 1. The expression of Nrf2 in kidney tissue of controlled and treated groups. *, **, and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively compare to the control group. # and ## represent $p < 0.05$ and $p < 0.01$ respectively compare to the G1 group. @ and @@ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G1 group. ^, + and ++ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G2 group. // and && represent $p < 0.01$ compare to the G3 group.

Table 4. The mean ± SEM levels of inflammatory parameters in kidney tissue in the controlled and treated groups.

Group/parameters	IL-1β (ng/mgProtein)	TNF-α (pg/mgProtein)
Control	0.742 ± 0.012^{a}	1.14 ± 0.12^{a}
Gentamicin 1	1.721 ± 0.021^{b}	3.62 ± 0.11^{b}
Gentamicin 2	2.381 ± 0.041^{c}	5.68 ± 0.33^{c}
Gentamicin 3	2.841 ± 0.031^{d}	6.48 ± 0.38^{d}
Gentamicin1-Vitamin C	0.627 ± 0.01^{a}	1.78 ± 0.36^{a}
Gentamicin 1-Vitamin C nanoparticles	0.522 ± 0.01^{e}	1.23 ± 0.09^{a}
Gentamicin 1-Selenium	0.636 ± 0.01^{a}	1.22 ± 0.01^{a}
Gentamicin 1-Selenium nanoparticles	0.511 ± 0.01^{e}	1.08 ± 0.02^{a}
Gentamicin2- Vitamin C	0.846 ± 0.014^{d}	2.23 ± 0.21^{e}
Gentamicin2- nano-vitamin C	0.651 ± 0.01^{a}	1.34 ± 0.24^{a}
Gentamicin2- Selenium	0.882 ± 0.024^{d}	2.47 ± 0.43^{e}
Gentamicin2-nano-selenium	0.627 ± 0.04^{a}	1.33 ± 0.34^{a}
Gentamicin3- Vitamin C	1.226 ± 0.14^{f}	3.19 ± 0.47^{b}
Gentamicin3- nano-vitamin C	1.041 ± 0.01^{g}	1.58 ± 0.36^{a}
Gentamicin3- Selenium	$1.72 \pm 0.\ 24^{b}$	3.41 ± 0.51^{b}
Gentamicin3- nano-selenium	1.019 ± 0.14^{g}	1.63 ± 0.74^{a}

Mismatched lowercase letters indicate a significant difference among the controlled and treated groups ($n = 8$, $p < 0.05$).

Selenium and vitamin C and their nanoparticles could improve mTORc1 expression in kidney tissue in the controlled and treated groups

Mammalian/mechanistic target of rapamycin complex 1 (mTORc1) as a protein complex act as a nutrient/energy/redox sensor. The expression mTORc1 was evaluated in kidney tissue in the controlled and treated groups. The renal expression of mTORc1 significantly decreased by gentamicin in a dose-dependent manner compared to the control group. However, treatment with vitamin C and selenium and their nanoparticles could significantly improve mTORc1 expression in the gentamicin-receiving groups compared to the gentamicin-receiving groups (Figure 4).

Histological results

Gentamicin is prone to damage to the renal cortex, especially the proximal tubule. Microscopic examination showed that gentamicin-induced glomerular damage occurs with increased glomerular degeneration and dilatation of the proximal tubule. The severity of these lesions increased with increasing gentamicin dose. Cortical interstitial edema with expansion and congestion of blood capillaries was widely seen in the interstitial spaces of the kidney tissue of all rats treated with gentamicin. The most damage was observed in the gentamicin 3 group compared to the control group. However, vitamin C and selenium and their nanoparticles could significantly improve the severity of damage caused by different doses of gentamicin. The greatest improvement was in the gentamicin groups receiving selenium nanoparticles and vitamin C nanoparticles. (Figure 5).

Discussion

Acute kidney damage occurs in 10–25% of people receiving gentamicin and is known to be one of the most nephrotoxic drugs in the aminoglycoside class (Krause et al. 2016; Randjelovic et al. 2017). This effect can be affected by the dose, frequency, duration of treatment, and concomitant use of some drugs such as NSAIDs (Alarifi et al. 2012; Krause et al. 2016; Randjelovic et al. 2017). In addition, increasing age, pregnancy, hypothyroidism, renal and hepatic insufficiency, decreased effective volume, and metabolic acidosis also increases the risk of toxicity of aminoglycosides, especially gentamicin (Basile et al. 2012). Consistent with these findings, our results showed that gentamicin in a dose-dependent manner significantly causes oxidative damage, inflammation, and apoptosis in kidney tissue. Our results also showed that serum levels of blood nitrogen, urea, creatinine and uric acid were significantly increased in a dose-dependent manner in the gentamicin-receiving groups without treatment compared with the control group. Previous studies have shown that gentamicin induces renal toxicity due to the production of oxygen free radicals such as H2O2 and

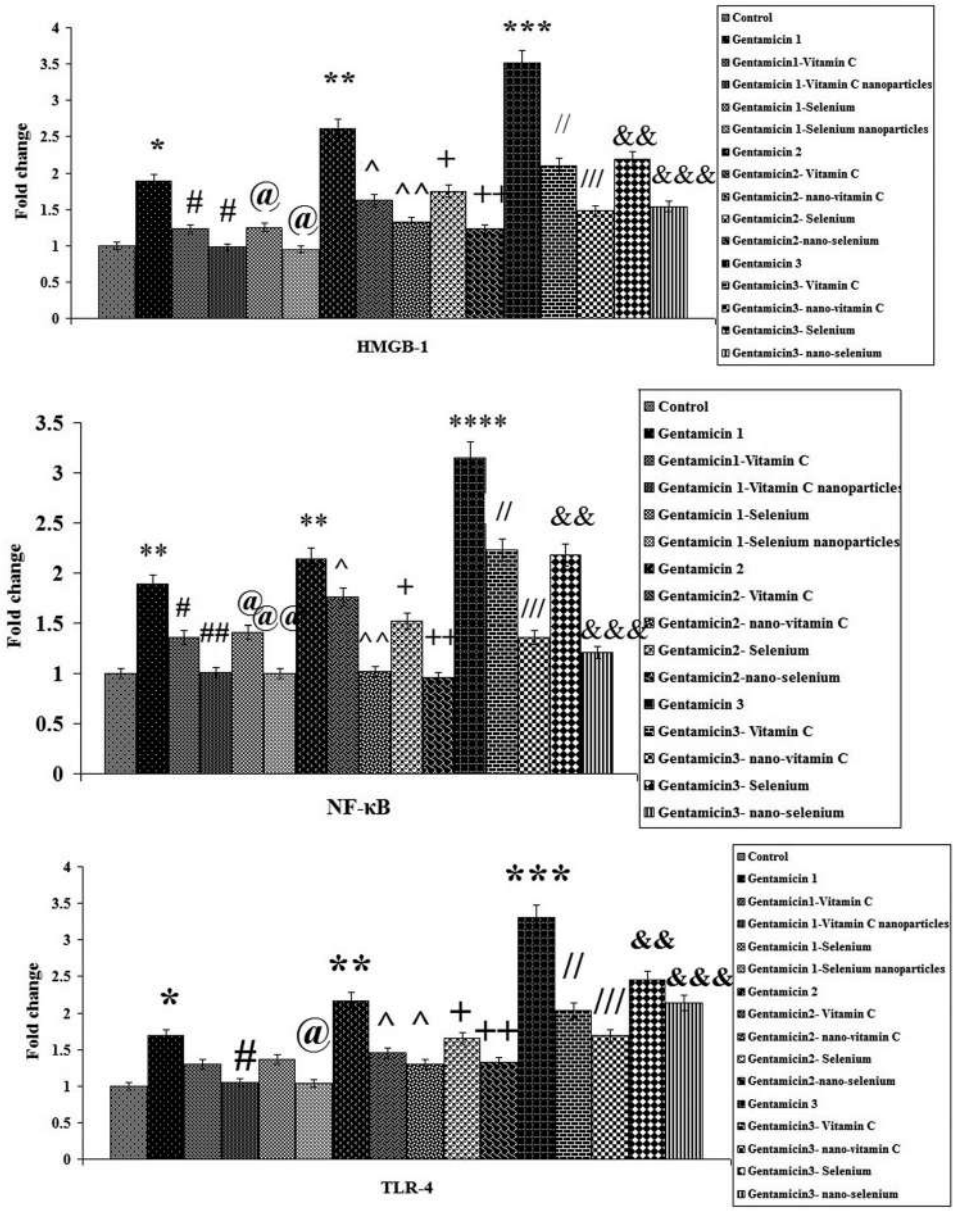

Figure 2. The expression of HMGB1, TLR-4 and NF-κB in kidney tissue of the controlled and treated groups. *, **, and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively compare to the control group. # and ## represent $p < 0.05$ and $p < 0.01$ respectively compare to the G1 group. @ and @@ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G1 group. ^ and ^^ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G2 group. + and ++ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G2 group. // or && and /// or &&& represent $p < 0.01$ and $p < 0.001$ respectively compare to the G3 group.

O2−. These free radicals cause increase membrane lipid peroxidation and contraction of mesangial cells (Martínez-Salgado et al. 2002). In addition, free radicals destroy collagen and other components of the glomerular basement membrane, so can disrupt the function of the glomerulus and tubule (Savin et al. 1985). These events lead to a decrease in the glomerular filtration rate (GFR), ultrafiltration coefficient, urinary concentration, and proteinuria (Vallon and Komers 2011). In addition, the production of circulating vasoactive mediators including nitric oxide is reduced leading to a reduction in glomerular hydrostatic pressure and GFR (Carlström 2021). Therefore, increasing serum levels of creatinine, urea, uric acid, and BUN in our study seems reasonable due to the reduced filtration rate.

The results of SPSS analysis showed that Nrf2 expression and tissue levels of antioxidant enzymes decreased in the gentamicin-receiving groups in a dose-dependent manner compared with the control group. The fact that gentamicin can cause oxidative damage by increasing free radicals and damage caused by mitochondrial activity was well demonstrated by Cui et al. (2015). However, we have shown that in addition to increasing ROS levels, decreasing levels of antioxidant enzymes and impairing the expression of their controlling gene, Nrf2, can cause oxidative damage to kidney tissue. Our results also showed that the levels of inflammatory parameters (TNF-α and IL-1β) and genes expression of NF-κB, TLR-4 and HMGB1 involved in inflammation and expression of Bcl-2, BAX and caspase-3 genes involved in apoptosis in kidney tissue increased in the gentamicin-receiving groups. Inflammation, oxidative damage, and apoptosis are the main mechanisms of tissue damage and organ dysfunction (Mittal et al. 2014; Nasiri et al. 2021), which are well

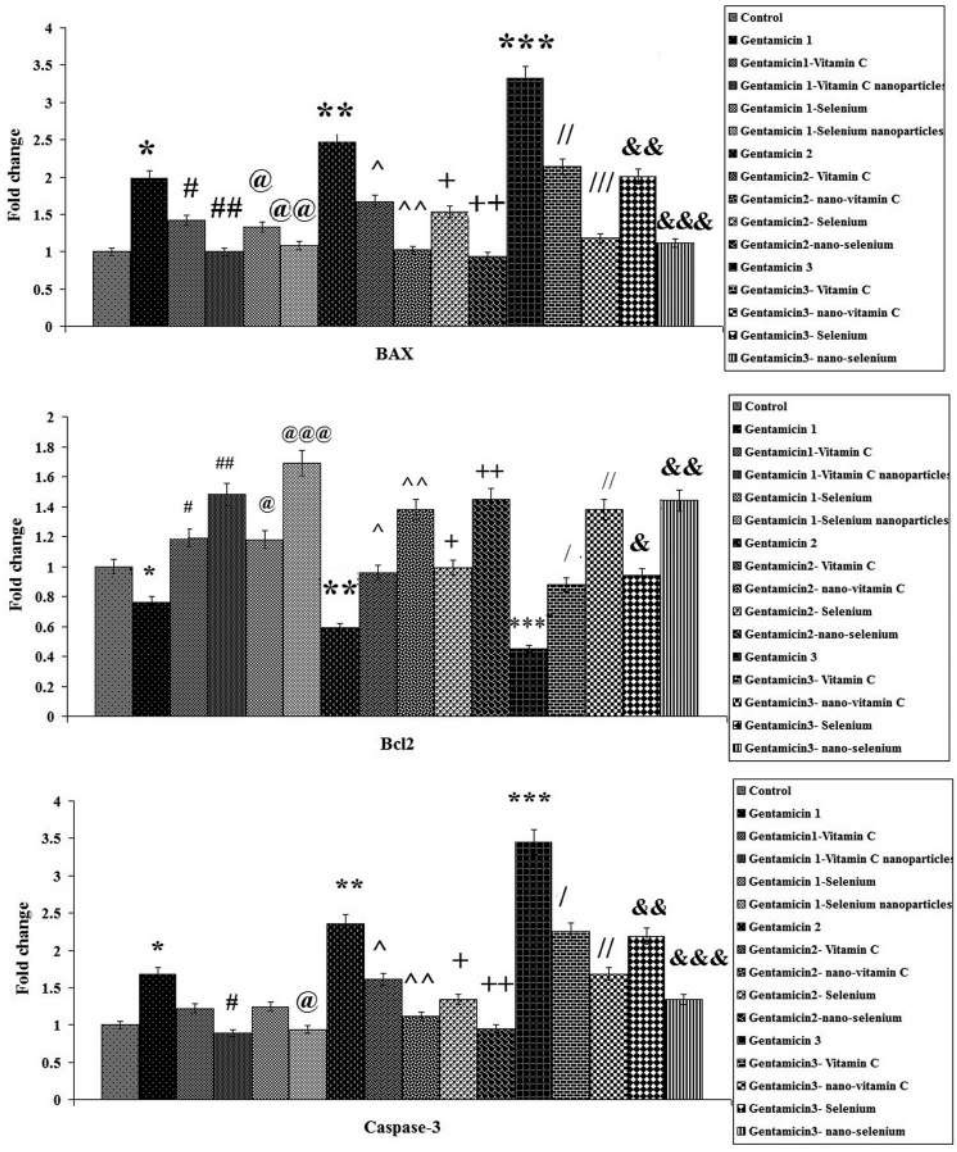

Figure 3. The expression of Bcl-2, BAX and caspase-3 in kidney tissue of the controlled and treated groups. *, **, and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively compare to the control group. # and ## represent $p < 0.05$ and $p < 0.01$ respectively compare to the G1 group. @, @@ and @@@ represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively compare to the G1 group. ^ and ^^ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G2 group. + and ++ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G2 group. // or && and /// or &&& represent $p < 0.01$ and $p < 0.001$ respectively compare to the G3 group.

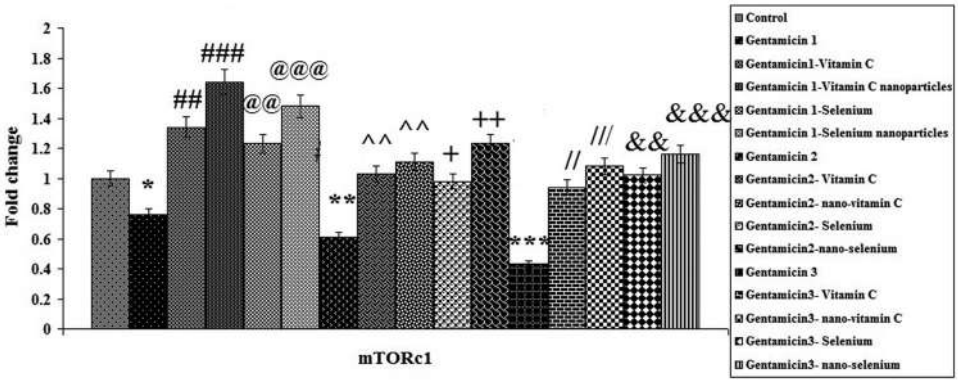

Figure 4. The expression of mTORc1 in kidney tissue of the controlled and treated groups. *, **, and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively compare to the control group. ## and ### represent $p < 0.01$ and $p < 0.001$ respectively compare to the G1 group. @@ and @@@ represent $p < 0.01$ and $p < 0.001$ respectively compare to the G1 group. ^^ represent $p < 0.01$ compare to the G2 group. + and ++ represent $p < 0.05$ and $p < 0.01$ respectively compare to the G2 group. // or && and /// or &&& represent $p < 0.01$ and $p < 0.001$ respectively compare to the G3 group.

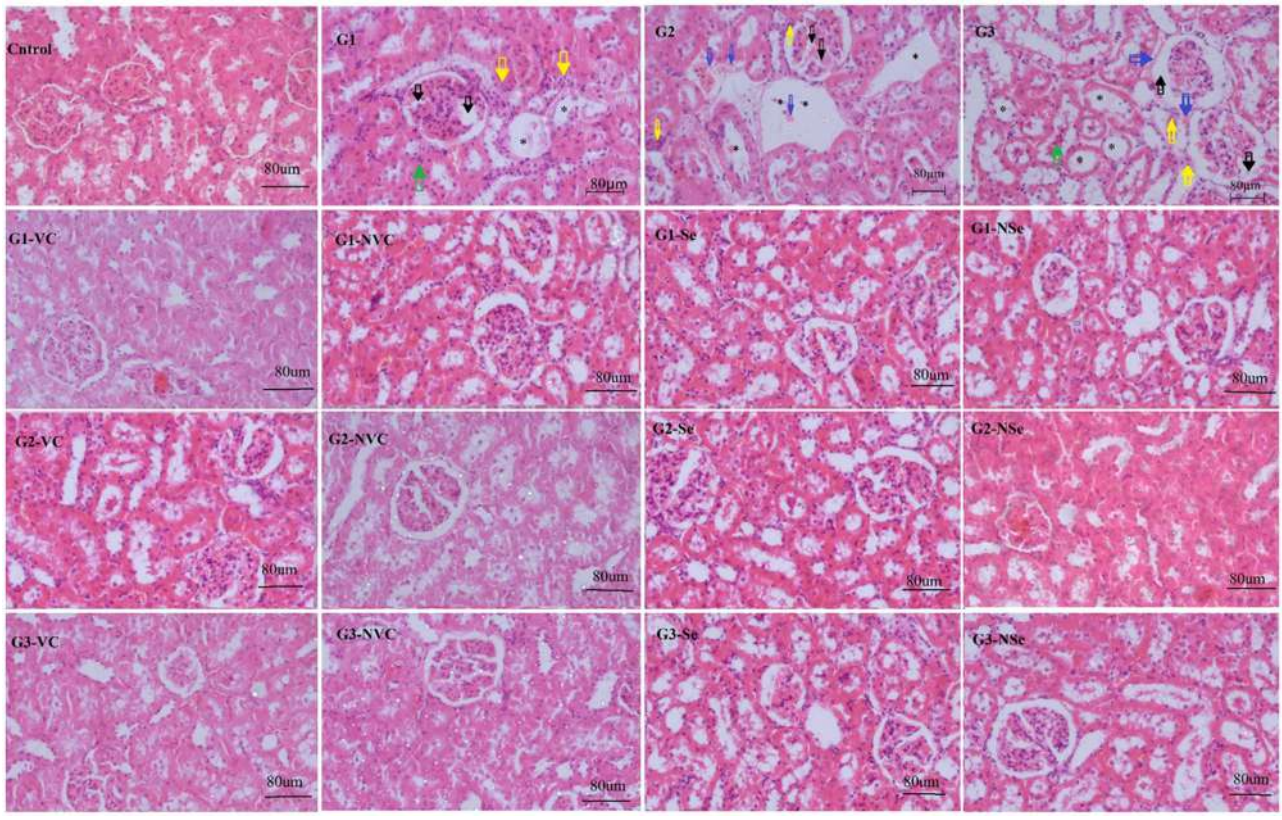

Figure 5. Cortical sections, especially proximal tubules, and glomeruli stained with hematoxylin and eosin in different groups. The results showed dilated tubes and tubular atrophy in the cortex of all gentamicin-receiving groups compared to the control group. The severity of tubal dilatation and tubular atrophy was increased by increasing the dose of gentamicin. In addition, severe degeneration and necrosis were seen in most of the complex proximal tubules and to some extent the distal tubules. These changes were improved in the groups receiving vitamin C and selenium and their nanoparticles compared to their control groups. The greatest improvement was in the groups of 100 mg gentamicin treated with vitamin C nanoparticles and selenium nanoparticles. Most damages were seen in the gentamicin-3 group, which received vitamin C and selenium. Glomerular Degeneration (Green Arrow), Mononuclear Cells Infiltration (Yellow Arrow), Hemorrhage (Blue Arrow), Renal Tubular Atrophy (Black Arrow) and Renal Tubular Dilatation (Asterisk). The scale is 80 μm.

supported by our results. The NF-κB/TNF-α/IL-1β, HMGB1/TLR-4/IL-1β/NF-κB, and HMGB1/TNF-α pathways play an effective role in organ dysfunction by inducing cellular abnormalities including apoptosis and oxidative damage (Lucas and Maes 2013; Mittal et al. 2014; Nimrouzi et al. 2020; Rasoulinejad et al. 2021). Therefore, inhibiting or strengthening genes involved in these pathways can play an effective role in improving gentamicin-induced nephrotoxicity. In line with this proposal, our results showed that the use of vitamin C and selenium and their nanoparticles could improve oxidative damage, inflammation, and apoptosis induced by the different doses of gentamicin in kidney tissue. Our results showed that vitamin C and selenium and their nanoparticles could increase the activity of the antioxidant enzymes and the expression of Nrf2 in kidney tissue in the gentamicin-receiving groups. Inhibition of oxidative damage in kidney tissue by treatment with vitamin C can be due to the reduction of ROS production, increase in the activity of antioxidant enzymes, and expression of Nrf2 genes. Previous studies have shown that vitamin C and selenium play an effective role in improving the activity of enzymatic such as thioredoxin reductase, glutathione s-transferases, and glutathione peroxidase and non-enzymatic antioxidants such as glutathione (D'Aniello et al. 2017; Demirci et al. 2017; Hosnedlova et al. 2018; Yang et al. 2018; Esmaeilizadeh et al. 2020). Studies have shown that reducing the levels of free radicals and increasing the expression of Nrf2 is directly related to reducing the expression of NF-κB and the levels of TNF-α and IL-1β, and can improve the state of inflammation (Ahmed Mustafa et al. 2021; Nasiri et al. 2021). On the other hand, increasing BAX and caspase-3 expression and decreasing Bcl-2 expression play an effective role in inhibiting apoptosis (Rex et al. 2019). In support of this evidence, our results well demonstrated that vitamin C and selenium and their nanoparticles could inhibit apoptosis in kidney tissue by down-regulating Bax and caspase-3 expression, up-regulating Bcl-2 expression and inhibiting the renal levels of TNF-α and IL-1β, and the mTORc1, NF-κB, HMGB1/TLR4 pathways in the gentamicin-receiving groups. These results indicated a pleasant condition that not only improves kidney tissue but also reduces the serum biochemical parameters associated with kidney function. Consistent with these results, previous studies have shown well that vitamin C and selenium play an effective role in improving AKT/mTOR, inflammation, oxidative stress, and apoptosis (Akbari et al. 2016; Demirci et al. 2017). Yang et al. (2018) showed that ascorbic acid inhibits D-galactose-induced senescence by inhibiting the production of free radicals and activating AKT/mTOR signaling in mesenchymal stem cells (Yang et al. 2018). Son et al. (2004) showed that vitamin C in a dose-dependent manner could attenuate inflammation by inhibiting the NF-κB activation (Son et al. 2004). Liu et al. (2021) showed that selenium

could attenuate inflammation-induced by LPS via scavenging intracellular ROS and activating PI3K/Akt/mTOR signaling pathway (Liu et al. 2021). mTORC1 acts as a sensor of nutrient and cell redox status. It also controls autophagy, which is involved in the removal of damaged or aging macromolecules and biological organs from the cytoplasm (Cui et al. 2015). Cui et al. (2015) showed that rapamycin could improve gentamicin-induced toxicity in proximal tubules by increasing autophagy and decreasing the interstitial infiltration of inflammatory cells (Cui et al. 2015). In addition, the AKT/mTOR pathway is associated with the ROS pathways, and the interaction of the two can affect cellular signaling. Evidences have shown that inhibition of AKT/mTOR pathway increased level of ROS by down-regulating MnSOD (Yang et al. 2018). Therefore, the modulation of this pathway by vitamin C and selenium and their nanoparticles not only improves autophagy in the kidney, but also reduces the damage caused by gentamicin by inhibiting inflammation, oxidative stress and apoptosis. A comparison of the results of the gentamicin groups receiving vitamin C and selenium showed that despite the changes in the gentamicin groups receiving vitamin C, there was no significant difference between them. Given these results, it is possible that both vitamin C and selenium have been able to improve gentamicin-induced damage by similar pathways. However, the gentamicin groups receiving vitamin C nanoparticles and selenium nanoparticles had better improvement effects compared to vitamin C and selenium. These results can be due to several reasons, including changes in the physical and chemical nature of vitamin C and selenium, or due to involvement in other cellular pathways responsible for the rapid elimination of gentamicin or tissue repair (Ferro et al. 2021; Raeeszadeh et al. 2021; 2021).

On the other hand, previous studies have shown that gentamicin causes renal damage mainly in the renal cortex especially the proximal tube through tubular necrosis (Randjelovic et al. 2017). Moreover, approximately one-fifth of the total blood volume enters the renal cortex, especially in areas where glomeruli are densely packed, and during each treatment, large amounts of vitamin C and selenium are exposed to cells in these areas. On the other hand, the use of nanoparticle technology has improved bioavailability and access to cells by changing the physicochemical nature (Jeevanandam et al. 2018). Nanoparticles with high bioavailability and low toxicity may be more efficient and safer (Chenthamara et al. 2019). Therefore, a more significant improvement for the gentamicin groups receiving vitamin C nanoparticle and selenium nanoparticle compared to gentamicin groups receiving vitamin C and selenium are reasonable and justifiable. Inhibition of oxidative damage due to exposure of vitamin C and selenium to mitochondria and cellular respiratory system improves the function of mitochondria and membrane organs including endoplasmic reticulum and inhibition of enzymes such as xanthine oxidase and NADPH oxidase (Apostolova and Victor 2015).

In addition, although we did not measure the glomerular filtration rate in this study, vitamin C, selenium, and their nanoparticles could reduce the serum levels of BUN, urea, acid uric and creatinine by improving renal function (Esmaeilizadeh et al. 2020). The role of free radicals and inflammatory mediators in decreasing GFR and tissue damage is previously described. Therefore, the role of vitamin C, selenium and their nanoparticles to improve renal structure and function especially GFR by eliminating free radicals and inhibiting inflammatory reactions and apoptosis is obvious. It has been well shown that vitamin C and its nanoparticles play an important role in improving the intercellular matrix, including collagen, by modulating the TGF-β pathway (D'Aniello et al. 2017). In addition, it seems that selenium and vitamin C and their nanoparticles through antioxidant properties and removal of free radicals, especially superoxide anion, can improve the renal damage caused by gentamicin by modulating the activity of the renin-angiotensin- aldosterone system (RAAS) (Rehman et al. 2012).

Our results also showed that some mediators and genes in the gentamicin group receiving vitamin C nanoparticles had better results than in the gentamicin group receiving selenium nanoparticles. In fact, this may be due to the extensive biological roles of vitamin C and its structural nature than selenium. Vitamin C has antimicrobial, antibacterial, antiviral, antiparasitic, and antifungal properties (Mousavi et al. 2019; Mumtaz et al. 2021). It is a powerful antioxidant and pro-oxidant due to its low reducing potential, which has a significant role in clearing free radicals and improving the antioxidant status of cells and tissues in the body (Akbari et al. 2019). The antioxidant activity of vitamin C comes from 2, 3-enediol (Akbari et al. 2016). Vitamin C is also a strong modulator of the immune system and enhances innate and consistent immune functions (Carr and Maggini 2017). Selenium-related studies in line with our study have also shown that selenium and its nanoparticles have antioxidant property (Filipović et al. 2021). Selenium acts as a cofactor for the reduction of antioxidant enzymes, such as glutathione peroxidases and certain forms of thioredoxin reductase. The glutathione peroxidase family of enzymes catalyzes certain reactions that remove reactive oxygen species such as hydrogen peroxide and organic hydroperoxides (Penglase et al. 2014). It seems that selenium and its nanoparticles could improve the damage caused by gentamicin only by increasing the clearance of free radicals and strengthening the antioxidant system (Tapiero et al. 2003; Kondaparthi et al. 2019). In fact, the inhibition of oxidative damage can inhibit damage induced by gentamicin.

In the end, before drawing conclusions, it is necessary to address some limitations of this study. In this study, we tried to show that the use of vitamin C and selenium and their nanoparticles can be useful as an adjunctive treatment with gentamicin to control its side effects, especially kidney toxicity. However, although in this study, the animals in the patient groups received the same dose of gentamicin, but the severity of the disease in some of them was different, so that sometimes it confuses us. Furthermore, previous studies have limited the use of high doses of selenium and its nanoparticles due to their toxic effects (Yang and Xia 1995; Filipović et al. 2021). Therefore, a dose-dependent study is recommended to determine the lethal dose (LD50) of acute and chronic toxicity. In addition, due to the many biological functions of vitamin C and

the physicochemical changes it acquires due to being nanoscale, it seems that vitamin C nanoparticles have higher therapeutic effects than vitamin C and selenium or its nanoparticles. Therefore, it seems that due to the limitation of selenium intake, vitamin C nanoparticles intake should be prioritized.

Conclusions

It can be concluded that vitamin C and selenium and their nanoparticles could significantly improve gentamicin-induced kidney damage. Moreover, vitamin C nanoparticles and selenium nanoparticles had better healing effects than vitamin C and selenium. In addition, vitamin C intake should be prioritized due to the limitation of selenium intake. Therefore, the use of selenium and vitamin C and their nanoparticles can be used as a treatment option for acute kidney damage or as adjunctive therapy with gentamicin.

Author contributions

Su Zheng: Data curation; Formal analysis; Resources; Software; Writing-original draft; Afrah Hameed Sultan: Data curation; Formal analysis; Investigation; Methodology; Resources; Writing-original draft; Prshng Tofiq Kurtas: Conceptualization; Resources; Validation; Writing-review & editing. Layla Abdulsattar Kareem: Formal analysis; Investigation; Methodology; Resources; Software; Validation; Writing-original draft. A. Akbari: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

Disclosure statement

The authors declare that no conflict of interest exists.

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