J Renal Inj Prev. 2018; 7(2): 73-77.

http://journalrip.com DOI: 10.15171/jrip.2018.18

Journal of Renal Injury Prevention

# Ameliorative effect of ferulic acid on gentamicin-induced nephrotoxicity in a rat model; role of antioxidant effects

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**ARTICLE INFO**

*Article Type:*
Original

*Article History:*
Received: 20 August 2017
Accepted: 7 December 2017
Published online: 14 January 2018

*Keywords:*
Gentamicin
Nephrotoxicity
Ferulic acid
Free radicals
Acute renal failure

**ABSTRACT**

**Introduction:** The nephrotoxicity of gentamicin is thought to be a dangerous side effect in the use of this drug caused by the formation of oxidative stress and the production of reactive oxygen species (ROS) and nitrogen species. Antioxidant agents play important roles in reducing oxidative stress.
**Objectives:** In this study, the antioxidant role of ferulic acid has been studied in nephrotoxicity caused by gentamicin.
**Materials and Methods:** In this experimental study, 50 Spraque-Dawley rats were randomly divided into 5 groups. The first group was considered as the control group and other groups received different doses of ferulic acid + 80 mg/kg gentamycin for 8 days by intraperitoneally injection. After the treatment, blood samples were prepared from animals. Then, the right and left kidneys were removed, paraffin blocks prepared, and the rest of the tissues were lysed. Three samples were used to evaluate serum creatinine and urea, as well as urine creatinine, histopathologic status and oxidative stress factors levels, respectively. To compare the results of different groups, Mann-Whitney U test was used.
**Results:** Ferulic acid treatment decreased tissue malondialdehyde (MDA), serum urea and creatinine, urine albumin/creatinine ratio and neutrophil gelatinase-associated lipocalin (NGAL), and also increased superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Ferulic acid reduced tubular necrosis and eosinophilic casts in gentamycin group induced nephrotoxicity.
**Conclusion:** Ferulic acid can be effective in inhibiting gentamicin-induced nephrotoxicity.

*Implication for health policy/practice/research/medical education:*
The most common symptoms of nephrotoxicity include decreased glomerular filtration, increased creatinine, blood urea nitrogen, uric acid, alkaline phosphatase, and electrolyte changes. Ferulic acid is a natural compound with a phenolic group which is found mainly in citrus, rice and coffee. In numerous studies, many roles have been identified for this substance, including anticancer, nephroprotective, hepatoprotective and anti-inflammatory activities.

*Please cite this paper as:* Hasanvand A, Mir S, Mohammadrezaei Khorramabadi R, Darabi S. Ameliorative effect of ferulic acid on gentamicin-induced nephrotoxicity in a rat model; role of antioxidant effects. J Renal Inj Prev. 2018;7(2):73-77. DOI: 10.15171/jrip.2018.18.

## Introduction

Nephrotoxicity is the most important side effect associated with the consumption (for more than 7 days) of aminoglycoside antibiotics such as gentamicin. The prevalence of this complication is over 30%. It brings about the incidence of 10%-20% of acute renal failure (1-3). However, different prescribing options can have a variety of implications (4). The most common symptoms of nephrotoxicity include decreased glomerular filtration, increased creatinine, blood urea nitrogen (BUN), uric acid, alkaline phosphatase, and electrolyte changes (5,6). The main mechanism for the generation of renal toxicity

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with gentamicin is the production of reactive oxygen species (ROS) and NOS (reactive nitrogen species) in renal cells, which can lead to inflammation and apoptosis of mesangial cells (2,5,7). Several studies have emphasized the role of antioxidant agents such as vitamins C and E, garlic and garden cress water in the reduction of functional damage caused by the effects of ROS and NOS on renal cells (2,7-9). Ferulic acid is a member of the family of phenolic acids. It has a strong antioxidant activity in scavenging free radicals and is able to reduce oxidative stress by up-regulating the cellular protection systems such as homoxygenase and reducing the expression of cytotoxic enzymes like inducible nitric oxide synthase (iNOS) (10,11).

## Objectives

Despite the nephrotoxic effects of gentamicin, this drug is still used as the first or second choice for a wide range of clinical conditions due to its sustainability, high bactericidal effect and low cost. Therefore, the antioxidant role of ferulic acid has been studied in reducing the nephrotoxic effects of gentamicin in this study.

## Materials and Methods

### *Study design*

This experimental study was performed on 50 Spraque-Dawley rats weighing 290-270 g. At the time of the study, all animals were kept in a standard condition of 12 hours of light and 12 hours of darkness at 23°C, with adequate air conditioning and water and food supplied free of charge.
The rats used in this experiment were randomized divided into five groups: Group 1; the control group, which did not receive any medication.
Group 2; nephrotoxicity group (induced with intraperitoneal injection of gentamycin (80 mg/kg) for 8 days).
Group 3; nephrotoxicity group (induced with intraperitoneal injection of gentamicin [80 mg/kg]) and treatment with ferulic acid (25 mg/kg for 8 days).
Group 4; nephrotoxicity group (induced with intraperitoneal injection of gentamicin [80 mg/kg]) and treatment with ferulic acid (50 mg/kg for 8 days).
Group 5; nephrotoxicity group (induced with intraperitoneal injection of gentamicin [80 mg/kg]) and treatment with Ferulic acid (100 mg/kg for 8 days).
At the end of the eighth day, all animals were anesthetized with pentobarbital sodium (60 mg/kg) and their blood samples were prepared. Blood samples were centrifuged at 4000 r/min and serum was isolated and kept at -20°C for the evaluation of BUN, creatinine, neutrophil gelatinase-associated lipocalin (NGAL) and kidney enzymes.

### *Evaluation of kidney structure by tests*

Creatinine, urea, the ratio of albumin to urine creatinine and NGAL were used to evaluate the renal structure. To evaluate these factors, serum samples and related kits (prepared by Diasis Diagnostic System, Istanbul, Turkey) were used (12).

### *Assessment of biochemical tests*

After animal anesthesia and blood collection, to evaluate the level of oxidative stress factors in the kidney (malondialdehyde [MDA], glutathione peroxidase [GPx], catalase [CAT] and superoxide dismutase [SOD]) on the eighth day, the right and left kidneys were separated. Then, kidney tissues were lysed with buffer (Tris HCl /pH = 7.5) and eventually its supernatant was separated. For evaluation of the level of oxidative stress factors, we used the relevant ELISA kits prepared by Sigma Aldrich (12).

### *Kidney histopathology*

Briefly, paraffin blocks were prepared from kidney samples and were cut into a thickness of 3 µm with microtome. The samples staining was performed using hematoxylin and eosin (H&E), and then the severity of tissue necrosis and degeneration of epithelial cells were evaluated (12).

### *Ethical issues*

The research followed the tenets of the Declaration of Helsinki. This project was approved by Ethics Committee of Lorestan University of Medical Sciences. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Lorestan University of Medical Sciences.

### *Data analysis*

Kruskal-Wallis test was used for statistical significance, followed by the Mann-Withney U test as a post hoc test. All statistics are shown as means ± standard deviation (SD). Data were calculated significant for $P$ value <0.05.

## Results

### *The Effect of ferulic acid on kidney structural kidney*

### *Assessment of the levels of serum urea*

Gentamicin significantly increased serum urea in group 2 (GIN) as compared to the control group ($P$ <0.001). However, Administration of ferulic acid (25, 50 and 100 mg/kg), in groups III, IV and V compared to nephrotoxicity group resulted in a significant reduction in serum urea (Figure 1A).

### *Assessment of the levels of serum creatinine*

Serum creatinine had a significant increase in nephrotoxicity group (GIN) in comparison with the control group ($P<0.001$). As shown, the levels of serum creatinine decreased in rats after administration of ferulic acid in groups 3 (25 mg/kg), 4 (50 mg/kg) and 5 (100 mg/kg) depending on the dose ($P<0.05$) (Figure 1B).

### *The ratio of albumin to urine creatinine*

As shown in Figure 1C the level of albumin/urine creatinine significantly increased in nephrotoxicity group (GIN) rats compared to the healthy groups on days 8 after nephrotoxicity induction. Moreover, our results demonstrated that administration of ferulic acid (at doses 50 and 100 mg/kg) in day 8 after nephrotoxicity induction

depressed the albumin/urine creatinine levels compared to GIN groups.

### *Effect of ferulic acid on the quantity of NGAL*

Figure 1D shows the findings of NGAL measurement. As shown, the level of NGAL significantly higher in the nephrotoxicity group than in the control group after induction of nephrotoxicity in rats. Findings indicated that administration of ferulic acid (depending on the dose) decreased the concentration of NGAL in the nephrotoxicity rats.

### *The effect of ferulic acid on oxidative stress indices*

Gentamicin significantly increased the amount of MDA in group 2 (GIN) and also remarkably decreased the amount of SOD, GPx and CAT as compared to the control group ($P$ <0.05). Administration of ferulic acid in groups 3 (25 mg/kg), 4 (50 mg/kg) and 5 (100 mg/kg) significantly decreased MDA and noticeably increased SOD, GPx and CAT depending on the dose ($P$ < 0.05) (Figure 1E-H).

### *The effect of ferulic acid on tubular necrosis and eosinophilic casts*

Administration of gentamicin alone induced tubular necrosis and eosinophilic casts in kidney tissue when compared with control groups. Ferulic acid (50 and 100 mg/kg) decreased tubular necrosis in kidney tissue induced by gentamicin. However, ferulic acid 100 mg/kg alone reduced eosinophilic casts in renal tissue.

## Discussion

Nephrotoxicity caused by the consumption of gentamicin is the most important limitation of the therapeutic use of this antibiotic. Despite the control and follow up of patients treated with gentamicin, it remains an important side effect (5,13). The main aspect of this disorder is tubular damage that occurs after stimulating the production of ROS and oxidative stress by gentamicin (14-16). Various studies have indicated that gentamicin is able to disturb the redox balance via reducing the antioxidant activity of the body, such as SOD, GPx and CAT. Moreover, it results

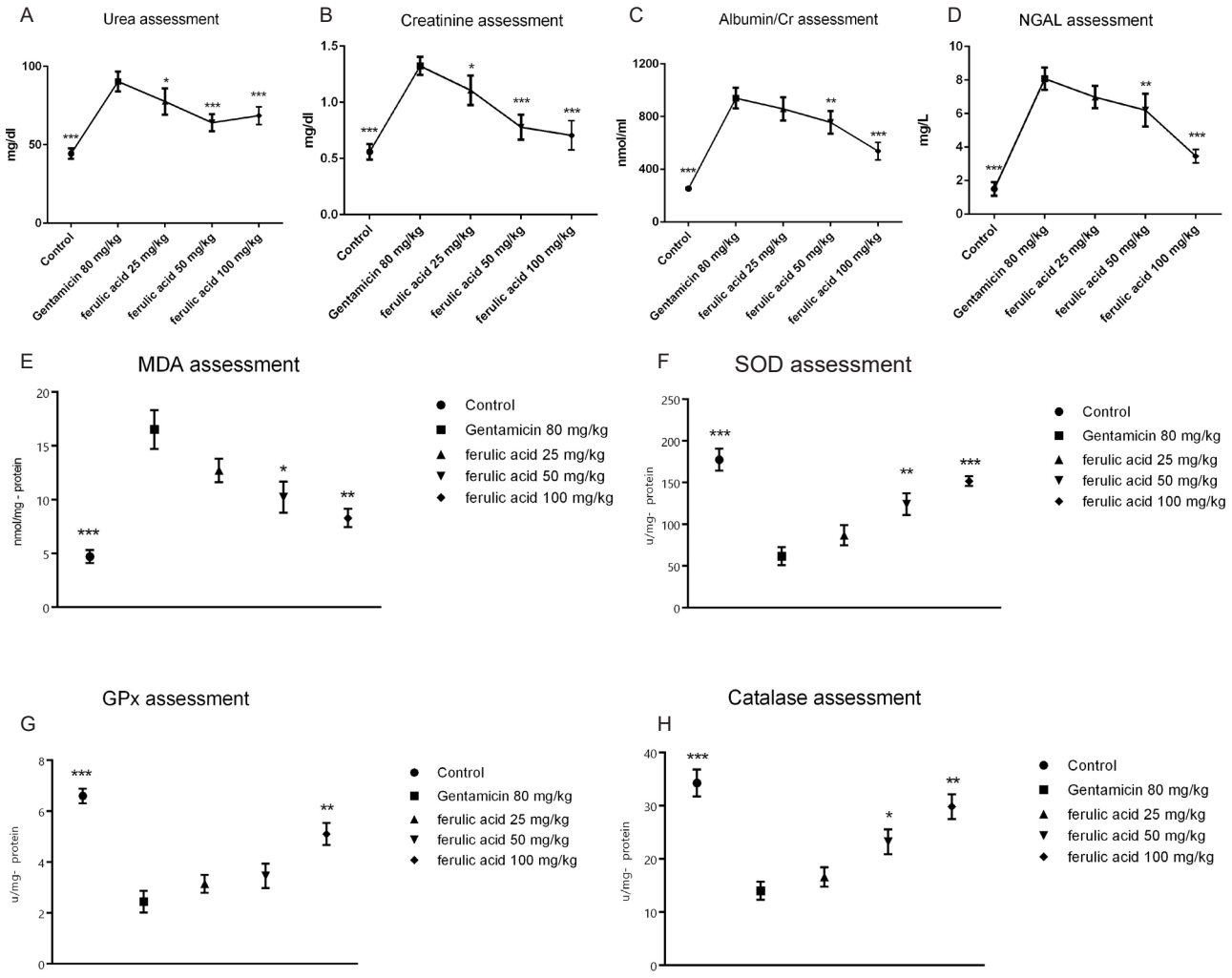


**Figure 1.** Effects of ferulic acid administration on (A) serum urea level, (B) serum creatinine level, (C) albumin/urine creatinine ratio, (D) NGAL level, (E) MDA level, (F) SOD level, (G) GPx level, and (H) CAT level (n = 8 rats per each group).
* $P$ < 0.5, ** $P$ < 0.05, ** $P$ < 0.01, *** $P$ < 0.001 vs Gentamicin group.

in high levels of free radicals and eventually multiple complications such as lipid peroxidation and MDA production (15,17-19). We also achieved a similar result by measuring the serum levels of these enzymes as well as the MDA level, and showed that following the injection of gentamicin during a specific period, the level of antioxidant activity was reduced and, as a result, increased MDA levels. Several studies demonstrated incremental changes in serum or urine levels of various compounds such as urea and creatinine, albumin / creatinine ratio (16-20), due to decreased glomerular filtration rate, as well as NGAL (as a sensitive and specific marker for early detection of renal injury) (21-23) in renal injury. This finding has also been proved in the present study.

The roles of antioxidant agents such as Tetramethylpyrazine (8), hydroxytyrosol (20), vitamin C (24) and garlic extract (2) have been indicated in several studies to improve nephrotoxicity caused by the use of gentamicin and to reduce the symptoms of the disorder. In our study, we also investigated the role of ferulic acid as an antioxidant agent in improving nephrotoxicity caused by gentamicin. Ferulic acid is a natural compound with a phenolic group which is found mainly in citrus, rice, coffee, etc. In numerous studies, many roles have been identified for this substance, including anticancer, nephroprotective, hepatoprotective and anti-inflammatory activity (25). Sompong et al demonstrated the inhibitory effect of ferulic acid in glycation and oxidative damage of proteins in 2016 (26). Manikandan et al have confirmed the positive role of ferulic acid in the improvement of glycerol-induced renal damage which occurs following the activation of nuclear factor kappa (NF-βB) in 2014 (27).

Accordingly, Gerin et al found that ferulic acid could play an inhibitory role in liver damage due to oxidative stress induced by formaldehyde. Having hydroxyl and phenoxyl groups in its structure, ferulic acid can neutralize free radicals (25). Furthermore, it responds to cellular stress and oxidative damage by increasing the level of antioxidant systems such as SOD, GPx and CAT enzymes and decreasing the expression of cytotoxic enzymes (11).

## Conclusion

Our results showed that co-administration of gentamicin and ferulic acid dose dependently could increase the antioxidant markers and scavenging of free radicals which lead in turn to the reduction of the lipid peroxidation marker (MDA) and gentamicin toxicity. Renal function that improves following the alleviation of poisoning and the reduction of biochemical and structural signs confirmed this. It is suggested that the effects of ferulic acid on other aspects of nephrotoxicity and intracellular signaling pathways will be investigated in the future.

## Acknowledgments

The authors are especially grateful to Lorestan University of Medical Sciences, Khorramabad, Iran. Also, the authors thank the head and staff of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences.

## Authors' contribution

SD designed the study. AH, SM, RMK and SD generated the data collection sheet, performed the data collection, and wrote the discussion. SM and RMK conducted the literature review and wrote the introduction. AH wrote the methods. SD conducted the statistical analysis. SM and RMK wrote the results. All authors read, revised, and approved the final manuscript.

## Conflict of interests

The authors declared no conflict of interests.

## Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

## Funding/Support

This study (As the complementary work) was supported by Lorestan University of Medical Sciences with (Grant # A-10-1758-2).

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