# Prophylaxis With Diosmin Mitigates Kidney Damage Induced by Gentamicin in Rats

**Seyed Sajad Hossieni Geshnigani**
Ahvaz Jundishapur University of Medical Sciences
**Masoud Mahdavinia**
Ahvaz Jundishapur University of Medical Sciences
**Mojtaba Kalantar**
Ahvaz Jundishapur University of Medical Sciences
**Mehdi Goudarzi**
Ahvaz Jundishapur University of Medical Sciences
**Layasadat Khorsandi**
Ahvaz Jundishapur University of Medical Sciences
**Hadi Kalantar** ( kalantar-h@ajums.ac.ir )
Ahvaz Jundishapur University of Medical Sciences

**Research Article**

**Keywords:** Gentamicin, Nephrotoxicity, Oxidative stress, Inflammation, Diosmin

**Posted Date:** July 21st, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-571486/v1

# Abstract

## Background

Gentamicin is a crucial aminoglycoside antibiotic but it is used only to treat severe bacterial infections, because of its high nephrotoxicity among patients. We evaluated the preventive effects of diosmin (as a natural ingredient) on gentamicin-related renal damage in rats.

## Methods

In this research, 28 male Wistar rats were assigned to 4 groups: control, gentamicin (100 mg/kg, (i.p.), daily for 1 week), and gentamicin plus diosmin (50mg/kg, p.o., daily for two weeks), diosmin (50mg/kg/day, p.o. for two weeks). After, the final gavage, blood specimens were gathered for determining serum blood urea nitrogen (BUN) and creatinine. kidneys used for biochemical, inflammation and histological test.

## Results

Creatinine, BUN, nitric oxide, malondialdehyde, TNF-α and *IL*-1β concentrations significantly increased and glutathione, catalase, glutathione peroxidase, and superoxide dismutase activities decreased after gentamicin treatment. Creatinine, BUN, nitric oxide, malondialdehyde, tumour necrosis factor α (TNF-α), interleukin 1 beta (IL-1β) concentrations significantly reduced and glutathione level, catalase and glutathione peroxidase activities significantly increased via co-administration with diosmin.

## Conclusion

Diosmin had ameliorative impacts against gentamicin-related kidney injury that can be owing to its antioxidant, and anti-inflammatory activities.

# Background

Aminoglycosides antibiotics including gentamicin (GEN) are agents extensively applied to treat severe gram-negative infections[1]. Gentamicin is a strong antibacterial drug, it is associated with nephrotoxic adverse effects. Gentamicin causes 10%-30% of acute renal failures[2, 3]. The commonest symptoms are enhanced serum blood urea nitrogen (BUN), level creatinine (Cr), reduced glomerular filtration (GF), vast tubular edema (TE) and serious proximal tubule injury (PTI)[4–6]. Renal toxicity by GEN is caused by generating reactive nitrogen species (RNS) and reactive oxygen species (ROS) in cells, resulting in oxidative stress, inflammatory pathways and apoptosis activation. Also, GEN can deplete protective antioxidants, like glutathione content (GSH) and reduce the activity of different enzymes, like glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD)[2].

Accordingly, agents with promising anti-inflamatory, anti-oxidative and nephroprotective effects have been examined thorough the past years[7]. Flavonoids are natural compounds characterized by

antioxidant and anti-inflammatory effects available in vegetables, fruits etc. Diosmin (DIO), is a natural flavonoid and obtained from the hesperidin and different plants. Pharmacokinetic (PK) features have revealed that DIO is rapidly changed to diosmetin (DIM). Its $T_{1/2}$ (half-life) is between 26–43 hours[8, 9]. Diosmin has many pharmacological properties, such as anti-inflammatory, antioxidant, anti-diabetic, anti-atherosclerotic, anti-hyperlipidaemia, anti-peptic ulcer, anti-apoptotic, antimutagenic effects[8, 10–15]. However, the impacts of DIO on GEN-related nephrotoxicity have not been surveyed in prior researchs. Now we examined the impacts of DIO on GEN-related renal injury in an animal model.

# Methods

### Chemicals

Diosmin (CAS Number: 520-27-4), Gentamicin (CAS Number: 1405-41-0) and other chemicals and reagents prepared from Sigma-Aldrich (Germany).

### Animals

Twenty-eight male Wistar albino rats (250–300g) were obtained from the animal house of Ahvaz Jundishapur University of Medical Sciences (AJUMS). Then, for seven days the rats were placed in a 12:12 h sleed-wake cycle in ideal states (normal moisture, 65±5%; temperature, 23±3°C).

### Experimental Protocol

The animals were allocated to 4 groups of 7 rats at random.

Group I received normal saline (10ml/kg of DIO vehicle) for fourteen days, and 0.9% normal saline (GEN vehicle, i.p.) from the eighth day to the fourteenth day.

Group II was used as GEN group and received 100mg/kg gentamicin for 7 continuous days, from day 8 to day 14[16].

Group III was used as GEN + DIO group and received DIO (50mg/kg, p.o.) [8] for 14 continuous days, from 7 consecutive days before GEN (100mg/kg, i.p.) administration as well as 7 days together with GEN administration.

Group IV was used as DIO group, rats received DIO (50mg/kg, p.o.) alone for 14 continuous days.

After 24h from final dose administration, all the rats were anesthetized by xylazine-ketamine (8/80 mg/kg). Blood sampling was taken from the heart and the serum samples was isolated by centrifuging (15 min / 1500 rpm / +4 °C) and kept at −20 °C to determine serum kidney markers, Cr and BUN. The rats were sacrificed with cervical dislocation and their kidneys were removed. For histopathological evaluation, the right kidney removed and fixed in formalin (10%), whereas the left kidney was promptly

and homogenized (1/10 w/v) on cool Tris buffer (pH 7.4) and served at –20 °C regarding inflammation and biochemical tests.

### Determination of serum Cr and BUN levels

Serum Cr concentration was measured by creatinine colorimetric kit (BioMérieux, France)[17]. Accordingly, Cr in alkaline solution is reacted with picrate for forming a colored complex, whose color was assessed at 492 nm. Blood urea nitrogen concentration was determined by urea enzymatic colorimetric kit (Linear Chemicals, S.L., Spain). Accordingly urea is hydrolyzed with water and urease for producing ammonia and carbon dioxide. The ammonia ions are reacted by hypochlorite and salicylate for forming green dye (2, 2 dicarboxyl-indophenol), whose color was assessed at 580 nm.

### Bradford assay

The total protein content was calculated considering the Bradford's approach[18].

### Renal malondialdehyde (MDA) assay

The tissue MDA concentration was measured based on previous reports[19, 20]. In brief, tissue homogenate (0.5 ml) was added to trichloroacetic acid (TCA, 10%, w/v; 1.5 ml), followed by centrifugation (5000 rpm / 12 min), and transferring 1.5 ml of each specimen supernatant into a test tube including thiobarbituric acid (TBA) solution (0.67% w/v; 2 ml). We then centrifuged (4000 rpm / 15 min) and its absorbance was read by the microplate-reader (at 532 nm). The standard curve was built in the concentration of 1 to 10 µM of tetraethoxypropane.

### Renal nitric oxide (NO) assay

Griess assay was used to identify NO level, so that 100 µl of the sample was mixed with 100 µl acidic Griess reagent and the absorbance was determined by an ELISA reader (RayBiotech, Canada) at 540 nm[21].

### Renal GSH content assay

The GSH concentration of renal tissue was assessed based on Ellman's method[22]. In summary, supernatant 1 mL was blended with 1 mL of 4% sulfosalicylic acid, then it was centrifuged at 1200 rpm for a quarter-hour at 4 °C. Then, 2.7 mL of 0.1 M phosphate buffer (pH 7.4) and 0.2 mL of 5,5-dithiobis 2-nitrobenzoic acid (DTNB) (40 mg/10 mL of 0.1 M phosphate buffer, pH 7.4) were mixed. Afterward, the yellow color was investigated instantly at 412 nm by an ELISA reader (RayBiotech, Canada).

### Renal CAT, SOD and GPx assay

CAT, SOD and GPx activities were assessed using a commercial kit designed for rat, based on the producer's guideline (ZellBio GmbH, Germany).

### Assessment of pro-inflammatory cytokines

Pro-inflammatory cytokines of the kidney tissues were assessed through ELISA and commercial kits. Tumour necrosis factor α (TNF-α) and interleukin 1 beta (IL-1β) concentrations were measured by ELISA kits (IBL International Co.).

### Histopathological evaluations

Following blood sampling, the kidneys were isolated immediately followed by fixation in formalin (10%) and dehydrating in graded alcohol concentrations and, embedding in paraffin. The obtained sections (4 to 6μm) were stained by hematoxylin and eosin (H&E) and 6 microscopy slides per rat were assessed for histological alterations, like red blood cells (RBCs) congestion, inflammatory cell infiltration, glomeruli and proximal tubule cells injury (degeneration, cell swelling). To assess proximal tubule injuries, the mean rate of injured tubules were calculated via dividing the count of tubules in a random microscopic field by the overall count of tubules in the similar field and the obtained value multiplied by 100. Accumulating inflammatory cells and RBCs was categorized into four classes: normal (0), mild (1), medium (2) or severe (3) and the mean values were regarded. Regarding each slide, the average of 6 field was determined. We read slides in a "blind" manner and examining under light microscope with 400× magnification.

### Statistical analysis

Values are provided as mean ± S.D. The one way ANOVA was applied for comparing the results of the groups, Tukey post-hock test was employed to compare the findings between groups. P values of $p<0.05$ were regarded as significant.

## Results

### Impact of GEN and DIO on serum Cr and BUN Levels

Gentamicin administrated for seven days led to a significant increase in serum Cr and BUN concentrations *(P <0.001)* than normal saline group (Figure 1A, B). Nonetheless, an elevation in the Cr and BUN levels were significantly *(P<0.05 and P<0.01* respectively*)* reduced by DIO pretreatments compared to GEN group. Diosmin alone caused no change in the renal function examinations than the normal saline group (Figure 1A, B).

### Impact of GEN and DIO on renal oxidative stress biomarkers

Tissue MDA and NO concentrations significantly increaseed in the GEN-administrated renal injury group than the normal saline group *(P< 0.001)*. An increase due to GEN were significantly reduced after

pretreatment with DIO (*P<0.01)*. Diosmin treatment alone showed no significant effect on MDA and NO contents of the normal saline group (Figure 2A, B).

### Effect of GEN and DIO on renal antioxidants factors

In GEN group, the renal GSH content, SOD, GPx, and CAT activities indicated a significantly *(P< 0.001)* reduced than those in the normal saline rats. In the DIO + GEN group, the renal amount of GSH, GPx and CAT activities showed a significant increase than the GEN-intoxicated rats *(P<0.05, P<0.01, P<0.05*, respectively*)*. Diosmin treatment alone caused no significant effect on GSH level, GPx, SOD and CAT activities of the normal saline group (Figure 3A-D).

### Effect of GEN and DIO on renal inflammatory cytokines

Regarding the normal saline group, GEN treatment significantly elevated the tumor necrosis factor -α and interleukin-1β concentrations in rats *(P<0.001)*. However, pretreatment of rats with DIO caused a significant decrease in TNF-α and IL-1β concentrations than GEN-intoxicated group *(P<0.01 and P<0.001* respectively*)*. DIO alone caused no a significant impact on TNF-α and IL-1β levels (Figure 4A, B).

### Histopathological Analysis

Figure 5 and Table 1 summarize the histopathological alterations in kidneys of all groups. Light microscopic assessment of kidneys in the rats treated with NS and DIO caused no structural Changes in kidney tissues. Changes in the appearance of glomeruli and the loss of tissue organization were observed in of kidneys in rats treated with GEN. Also, swelling and degeneration of cells and proximal renal tubules, infiltration of inflammatory cells and accumulating RBCs were detected in the kidney sections in this group. Kidney samples of animals administrated with DIO and GEN showed a significant improve in glomeruli and renal tubules, than the GEN-administrated rats.

Tabale 1. Effects of DIO and GEN on damage scores in renal tissues

| Groups | Proximal cell degeneration | Proximal cell swelling (%) | Infiltration of inflammatory cells | Accumulation of RBCs |
|---|---|---|---|---|
| NS | - | 0.32±0.03 | 0.08±0.02 | 0.09±0.02 |
| DIO | - | 0.21±0.04 | 0.07±0.01 | 0.09±0.03 |
| GEN | 8.53±1.82*** | 18.3%±3.1*** | 2.1±0.8*** | 1.82 ± 0.4*** |
| GEN+DIO | 1.96±0.22***## | 3.3%±0.73**## | 0.6±0.4*# | 0.53 ± 0.2*# |

*Significant with NS group, **p < 0.05*, ***p < 0.01*, ****p < 0.001*.

#Significant with GEN Group. *#p <0.05*, *##p < 0.01*.

NS: Normal saline, DIO: Diosmin, GEN: Gentamicin.

# Discussion

Our findings indicated that DIO protected kidneys to GEN-related nephrotoxicity in rats. Creatinine and BUN levels were considered as indices of damage to kidney. Reduced glomerular filtration causes an elevation in serum Cr and BUN concentrations[23, 24]. We found that rats receiving GEN for 7 continuous days showed nephrotoxicity, because of an increase in serum Cr and BUN concentrations that is consistent with other reports[7, 25]. In our study, there are a correlation between such changes in biochemical parameters and the renal histological characteristics. On the other hand, the elevated BUN and creatinine concentrations in rats receiving GEN were restored by DIO administration, which can be linked to antioxidant effect of DIO[26, 27]. Oxidative stress and ROS due to GEN causes the structural and functional deterioration of the kidney. In an organism, GEN is metabolized and activates free radicals generation that affect proteins, membrane phospholipids, and nucleic acids, leading to alterations in the function and structure of these molecules resulting in tissue injury[28–31]. Malondialdehyde can disrupt enzyme activities, change the intracellular ion balance via influencing ion exchange in the membranes, increase the cell membrane permeability and cause base modifications in DNA structure[28]. Similar to other reports, we found that GEN could increase MDA concentration[32, 33], and it reduced significantly after DIO administration.

Nitric oxide can be produced with nitric oxide synthase (NOS) and affects many patho-physiological conditions, like inflammation and oxidative injury[28, 34, 35]. Nitric oxide plays a role to regulate the renal hemodynamics and renal tubular function[35]. Reduced glomerular damage (GD) and glomerulosclerosis (GS) were observed by decreasing nitric oxide production[36]. GEN-related oxidative damage causes NO generation and its reaction with superoxide radicals produces very toxic ROS, like per-oxynitrite, leading to renal damge[5, 37]. The result of this study showed that GEN treatment could increase the NO concentrations in renal tissue of rats that may be due to oxidative stress. Pretreatment with DIO attenuated the NO concentrations and ameliorated the renal dysfunction because of the GEN-related oxidative damage.

Antioxidants show a direct and indirect protective effect against the damages of oxidative stress[38]. Nonetheless, GSH as a non-enzymatic anti-oxidant is a ROS scavenger protecting against the harmful effect of free radicals. Superoxide dismutase can catalyze the dismutation of superoxide radical to hydrogen peroxide ($H_2O_2$), CAT can break down and inhibit $H_2O_2$ change to free radical, whereas GPx can utilize GSH for preventing hydroperoxide generation[38–40].

Our findings showed that GEN induces GSH concentration depletion because of extra production of ROS or elevated use in the formation of proteins with –SH group and reduced SOD, CAT, and GPx antioxidant enzymes activity linked to overproduction of ROS[28, 41, 42]. Diosmin treatment restores the GSH concentrations, GPx and CAT activities in kidney tissue of GEN-related toxicity groups by its ROS scavenging and/or enhancing anti-oxidant effect. Diosmin showed no significant difference in SOD activity. Previous studies have shown that DIO could exert its anti-oxidant activity directly via ROS scavenging as well as indirectly via upregulation of anti-oxidant enzymes[10, 14, 15].

Gentamicin-related renal tubular necrosis may induces/stimulates inflammatory responses and promote migration of monocytes and macrophages tissue injury area[43]. The nuclear factor kappa B (NF-κB) activation against GEN-related oxidative stress and other disorders is an important transcription marker in the renal inflammatory actions via regulation of many gene expressions of cytokines, like IL-1β and TNF-α[44–46]. The outcomes of this report showed that GEN administration could upregulate the inflammatory reaction evidenced by increased IL-1β and TNF-α concentration in kidney. Flavonoids inhibit the NF-κB pathway effective to regulate inflammatory markers, including IL-6, IL-1β and TNF-α[47, 48]. Our findings indicated that DIO prevented ranal damage by inhibition of IL-1β and TNF-α. So, IL-1β and TNF-α inhibition is a cornerstone to understand anti-inflammatory mechanism of DIO. Previous reports have revealed that the antiinflammatory impacts of DIO could mediate its protection to hepatotoxicity[49], gastric injury[10], ulcerative colitis[50], cognitive impairment[51] and brain oxidative damage[27].

# Conclusion

Diosmin is effective against development of GEN-induced nephrotoxicity. The DIO effectiveness to treat GEN-related nephrotoxicity is associated with its antioxidant and antiinflammatory effects. Thus, DIO may be an appropriate strategy to prevent progression of nephrotoxicity and may be a drug employed for kidney damage.

# Abbreviations

**GEN**: Gentamicin; **BUN**: Blood urea nitrogen; **Cr**: Creatinine; **GF**: Glomerular filtration; **TE**: Tubular edema; **PTI**: Proximal tubule injur; **RNS**: Reactive nitrogen species; **ROS**: Reactive oxygen species; **GSH**: Glutathione content; **GPx**: Glutathione peroxidase; **CAT**: Catalase; **SOD**: Superoxide dismutase; **DIO**: Diosmin; **PK**: Pharmacokinetic; **DIM**: Diosmetin; **$T_{1/2}$**: Half-life; **AJUMS**: Ahvaz Jundishapur University of Medical Sciences; **MDA**: Malondialdehyde; **TCA**: Trichloroacetic acid; **TBA**: Thiobarbituric acid; **NO**: Nitric oxide; **DTNB**: 5,5-dithiobis 2-nitrobenzoic acid; **TNF-α**: Tumor necrosis factor-α; **IL-1β**: Interleukin IL-1β; **H&E**: Hematoxylin and eosin; **RBCs**: Red blood cells; **NS**: Normal saline; **PD**: Proximal tubule degeneration; **PS**: Proximal cell swelling; **NOS**: nitric oxide synthase; **GD**: Glomerular damage; **GS**: Glomerulosclerosis; **NF-κB**: Nuclear factor kappa B.

# Declarations

### Author's contributions

**HK:** Supervision, Funding acquisition, Conceptualization, Writing - Original Draft, **MM:** Writing - Review & Editing, Conceptualization, **SSHG:** Investigation, Project administration, **MK:** Formal analysis, Conceptualization, **MG:** Formal analysis, Methodology, **LK**: Formal analysis, Methodology.

### Funding

This study is delivered from a Pharm. D. Thesis of Seyed Sajad Hossieni Geshnigani and was financially supported by Toxicology Research Center (Grant number: TRC-9718), funded by the Vice Chancellor of Research, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

### Acknowledgments

The authors would like to thank Vice Chancellor of Research, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

### Ethicas approval

All experiments were done following the principles for the care and use of laboratory animals in research and confirmed by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. (**Ethics code:** IR.AJUMS.ABHC.REC.1397.062).

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

### Competing interests

The authors declare that they have no competing interests.

### Author details

[1]Toxicology Research Center, Medical Basic Sciences Research Institute, Department of Toxicology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [2]Department of Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [3]Shoushtar Faculty of Medical Sciences, Shoushtar, Iran. [4]Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [5]Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Department of Toxicology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

## References

1. Trevor AJ, Katzung BG, Masters SB, Kruidering-Hall M: *Pharmacology examination & board review*. McGraw-Hill Medical New York, NY, USA:; 2010.
2. Hasanvand A, Kharazmkia A, Mir S, Khorramabadi RM, Darabi S: Ameliorative effect of ferulic acid on gentamicininduced nephrotoxicity in a rat model; role of antioxidant effects. *Journal of Renal Injury Prevention* 2018, 7:73–77.

3. SUNDIN DP, SANDOVAL R, MOLITORIS BA: Gentamicin inhibits renal protein and phospholipid metabolism in rats: implications involving intracellular trafficking. *Journal of the American Society of Nephrology* 2001, 12:114–123.
4. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ: New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney international* 2011, 79:33–45.
5. Christo JS, Rodrigues AM, Mouro MG, Cenedeze MA, de Jesus Simões M, Schor N, Higa EMS: Nitric oxide (NO) is associated with gentamicin (GENTA) nephrotoxicity and the renal function recovery after suspension of GENTA treatment in rats. *Nitric Oxide* 2011, 24:77–83.
6. Kopple JD, Ding H, Letoha A, Ivanyi B, Qing DPY, Dux L, Wang HY, Sonkodi S: l-carnitine ameliorates gentamicin-induced renal injury in rats. *Nephrology Dialysis Transplantation* 2002, 17:2122–2131.
7. Karataş Y, Seçilmiş MA, Karayaylalı İ, Doran F, Büyükafşar K, Şingirik E, Saglıker Y, Dikmen A: Effect of tempol (4-hydroxy tempo) on gentamicin-induced nephrotoxicity in rats. *Fundamental & clinical pharmacology* 2004, 18:79–83.
8. Jain D, Bansal MK, Dalvi R, Upganlawar A, Somani R: Protective effect of diosmin against diabetic neuropathy in experimental rats. *Journal of integrative medicine* 2014, 12:35–41.
9. Senthamizhselvan O, Manivannan J, Silambarasan T, Raja B: Diosmin pretreatment improves cardiac function and suppresses oxidative stress in rat heart after ischemia/reperfusion. *European Journal of Pharmacology* 2014, 736:131–137.
10. Arab HH, Salama SA, Omar HA, Arafa E-SA, Maghrabi IA: Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. *PloS one* 2015, 10:e0122417.
11. Kuntz S, Wenzel U, Daniel H: Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. *European journal of nutrition* 1999, 38:133–142.
12. Queenthy SS, John B: Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats. *European journal of pharmacology* 2013, 718:213–218.
13. Srinivasan S, Pari L: Ameliorative effect of diosmin, a citrus flavonoid against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. *Chemico-Biological Interactions* 2012, 195:43–51.
14. Tahir M, Rehman MU, Lateef A, Khan AQ, Khan R, Qamar W, O'Hamiza O, Ali F, Hasan SK, Sultana S: Diosmin abrogates chemically induced hepatocarcinogenesis via alleviation of oxidative stress, hyperproliferative and inflammatory markers in murine model. *Toxicology Letters* 2013, 220:205–218.
15. Yasım A, Özbağ D, Kılınç M, Çıralık H, Toru İ: The effect of diosmin-hesperidin combination treatment on the lipid profile and oxidativeantioxidative system in high-cholesterol diet-fed rats. *Türk Göğüs Kalp Damar Cerrahisi Dergisi* 2011, 1:55–61.
16. Abdelrahman R: Protective effect of apocynin against gentamicin-induced nephrotoxicity in rats. *Human & experimental toxicology* 2018, 37:27–37.

17. Henry RJ: Clinical chemistry, principles and technics. 1964.
18. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 1976, 72:248–254.
19. Buege JA, Aust SD: [30] Microsomal lipid peroxidation. In *Methods in enzymology. Volume* 52: Elsevier; 1978: 302–310
20. Kalantar H, Sabetkasaei M, Shahriari A, Haj Molla Hoseini M, Mansouri S, Kalantar M, Kalantari A, Khazaei Poul Y, Labibi F, Moini-Zanjani T: The effect of rapamycin on oxidative stress in MCF-7 and MDA MB-231 human breast cancer cell lines. *Jundishapur Journal of Natural Pharmaceutical Products* 2016, 11.
21. Tracey WR, Linden J, Peach MJ, Johns RA: Comparison of spectrophotometric and biological assays for nitric oxide (NO) and endothelium-derived relaxing factor (EDRF): nonspecificity of the diazotization reaction for NO and failure to detect EDRF. *Journal of Pharmacology and Experimental Therapeutics* 1990, 252:922–928.
22. Ellman GL: Tissue sulfhydryl groups. *Archives of biochemistry and biophysics* 1959, 82:70–77.
23. Kalantar M, Houshmand G, Kalantar H, Asadi M, Goudarzi M: Protective effect of hydroalcoholic extract of Lavandula officinalis L. on gentamicin induced nephrotoxicity in rats. *Journal of Babol University of Medical Sciences* 2016, 18:62–67.
24. Ural M, Özgüner M, Şenal D, Sütçü R, Delibaş N: Effects of Vitamin C, Vitamin E and verapamil on cyclosporin induced nephrotoxicity in rats: a light microscobic study. *Medical Journal of Süleyman Demirel University Faculty of Medicine* 2004, 12:28–35.
25. Ghaznavi R, Faghihi M, Kadkhodaee M, Shams S, Khastar H: Effects of nitric oxide on gentamicin toxicity in isolated perfused rat kidneys. *Journal of Nephrology* 2005, 18:548.
26. Abdel-Daim MM, Khalifa HA, Abushouk AI, Dkhil MA, Al-Quraishy SA: Diosmin attenuates methotrexate-induced hepatic, renal, and cardiac injury: a biochemical and histopathological study in mice. *Oxidative medicine and cellular longevity* 2017, 2017.
27. Elhelaly AE, AlBasher G, Alfarraj S, Almeer R, Bahbah EI, Fouda MM, Bungău SG, Aleya L, Abdel-Daim MM: Protective effects of hesperidin and diosmin against acrylamide-induced liver, kidney, and brain oxidative damage in rats. *Environmental Science and Pollution Research* 2019, 26:35151–35162.
28. Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS, Bodhankar SL: Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. *Renal failure* 2016, 38:996–1006.
29. Ateşşahin A, Karahan I, Yilmaz S, Çeribaşi A, Princci I: The effect of manganese chloride on gentamicin-induced nephrotoxicity in rats. *Pharmacological research* 2003, 48:637–642.
30. Karahan İ, Ateşşahin A, Yılmaz S, Çeribaşı A, Sakin F: Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. *Toxicology* 2005, 215:198–204.
31. Özcan O, Erdal H, Çakırca G, Yönden Z: Oxidative stress and its impacts on intracellular lipids, proteins and DNA. *J Clin Exp Invest* 2015, 6:331–336.

32. Edeogu C, Kalu ME, Famurewa AC, Asogwa NT, Onyeji GN, Ikpemo KO: Nephroprotective effect of Moringa oleifera seed oil on gentamicin-induced nephrotoxicity in rats: biochemical evaluation of antioxidant, anti-inflammatory, and antiapoptotic pathways. *Journal of the American college of nutrition* 2020, 39:307–315.
33. Samarghandian S, Azimi-Nezhad M, Mehrad-Majd H, Mirhafez SR: Thymoquinone ameliorates acute renal failure in gentamicin-treated adult male rats. *Pharmacology* 2015, 96:112–117.
34. Raygude KS, Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL: Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. *Inflammopharmacology* 2012, 20:331–341.
35. Sarkar A, Sengupta A, Mukhrjee A, Guru A, Patil A, Kandhare AD, Bodhankar SL: Antiulcer potential of morin in acetic acid-induced gastric ulcer via modulation of endogenous biomarkers in laboratory animals. *Pharmacologia* 2015, 6:273–281.
36. Narita I, Border WA, Ketteler M, Noble NA: Nitric oxide mediates immunologic injury to kidney mesangium in experimental glomerulonephritis. *Laboratory investigation; a journal of technical methods and pathology* 1995, 72:17–24.
37. Ali BH, Al Za'abi M, Blunden G, Nemmar A: Experimental gentamicin nephrotoxicity and agents that modify it: a mini-review of recent research. *Basic & clinical pharmacology & toxicology* 2011, 109:225–232.
38. Bami E, Ozakpınar OB, Ozdemir-Kumral ZN, Köroglu K, Ercan F, Cirakli Z, Sekerler T, Izzettin FV, Sancar M, Okuyan B: Protective effect of ferulic acid on cisplatin induced nephrotoxicity in rats. *Environmental toxicology and pharmacology* 2017, 54:105–111.
39. Erseçkin V, Mert H, İrak K, Yildirim S, Mert N: Nephroprotective effect of ferulic acid on gentamicin-induced nephrotoxicity in female rats. *Drug and chemical toxicology* 2020:1–7.
40. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J: Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology* 2007, 39:44–84.
41. Cao L, Zhi D, Han J, Kumar Sah S, Xie Y: Combinational effect of curcumin and metformin against gentamicin-induced nephrotoxicity: Involvement of antioxidative, anti-inflammatory and antiapoptotic pathway. *Journal of food biochemistry* 2019, 43:e12836.
42. Derakhshanfar A, Bidadkosh A, Kazeminia S: Vitamin E protection against gentamicin-induced nephrotoxicity in rats: a biochemical and histopathologic study. 2007.
43. Balakumar P, Rohilla A, Thangathirupathi A: Gentamicin-induced nephrotoxicity: do we have a promising therapeutic approach to blunt it? *Pharmacological research* 2010, 62:179–186.
44. Bae EH, Kim IJ, Joo SY, Kim EY, Choi JS, Kim CS, Ma SK, Lee J, Kim SW: Renoprotective effects of the direct renin inhibitor aliskiren on gentamicin-induced nephrotoxicity in rats. *Journal of the Renin-Angiotensin-Aldosterone System* 2014, 15:348–361.
45. Handa O, Naito Y, Takagi T, Shimozawa M, Kokura S, Yoshida N, Matsui H, Cepinskas G, Kvietys PR, Yoshikawa T: Tumor necrosis factor-α-induced cytokine-induced neutrophil chemoattractant-1 (CINC-

1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-κB. *Journal of Pharmacology and Experimental Therapeutics* 2004, 309:670–676.

46. Zhu B, Wu G, Wang C, Xiao Y, Jin J, Wang K, Jiang Y, Sun Y, Ben D, Xia Z: Soluble cluster of differentiation 74 regulates lung inflammation through the nuclear factor-κB signaling pathway. *Immunobiology* 2020, 225:152007.
47. Kang SR, Park KI, Park HS, Lee DH, Kim JA, Nagappan A, Kim EH, Lee WS, Shin SC, Park MK: Anti-inflammatory effect of flavonoids isolated from Korea Citrus aurantium L. on lipopolysaccharide-induced mouse macrophage RAW 264.7 cells by blocking of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathways. *Food Chemistry* 2011, 129:1721–1728.
48. Michalski J, Deinzer A, Stich L, Zinser E, Steinkasserer A, Knippertz I: Quercetin induces an immunoregulatory phenotype in maturing human dendritic cells. *Immunobiology* 2020, 225:151929.
49. Tahir M, Rehman MU, Lateef A, Khan R, Khan AQ, Qamar W, Ali F, O'Hamiza O, Sultana S: Diosmin protects against ethanol-induced hepatic injury via alleviation of inflammation and regulation of TNF-α and NF-κB activation. *Alcohol* 2013, 47:131–139.
50. Shalkami A, Hassan M, Bakr A: Anti-inflammatory, antioxidant and anti-apoptotic activity of diosmin in acetic acid-induced ulcerative colitis. *Human & experimental toxicology* 2018, 37:78–86.
51. Shabani S, Mirshekar MA: Diosmin is neuroprotective in a rat model of scopolamine-induced cognitive impairment. *Biomedicine & Pharmacotherapy* 2018, 108:1376–1383.

# Figures

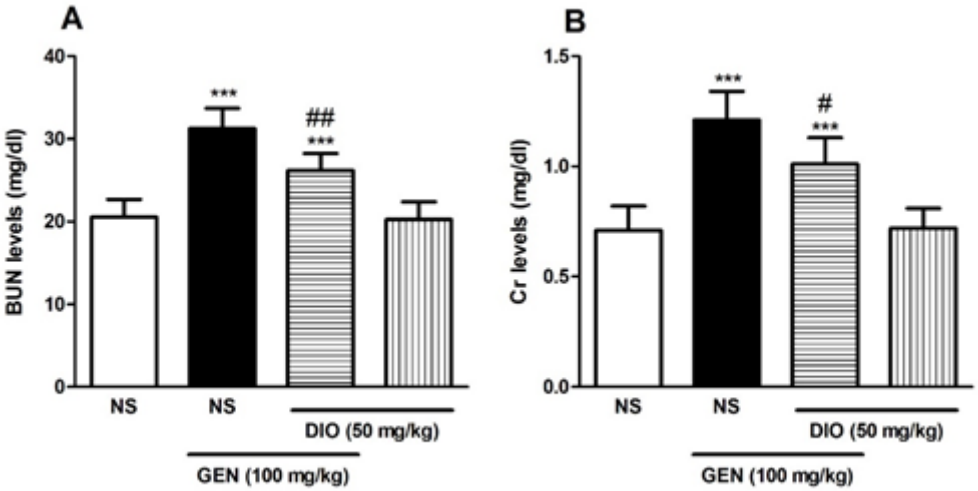
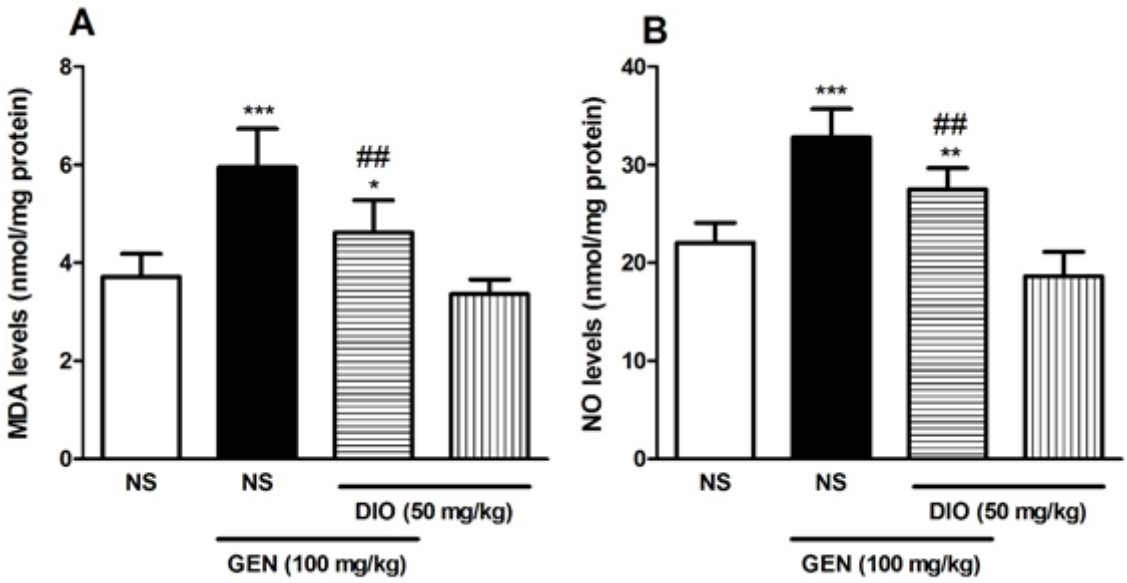


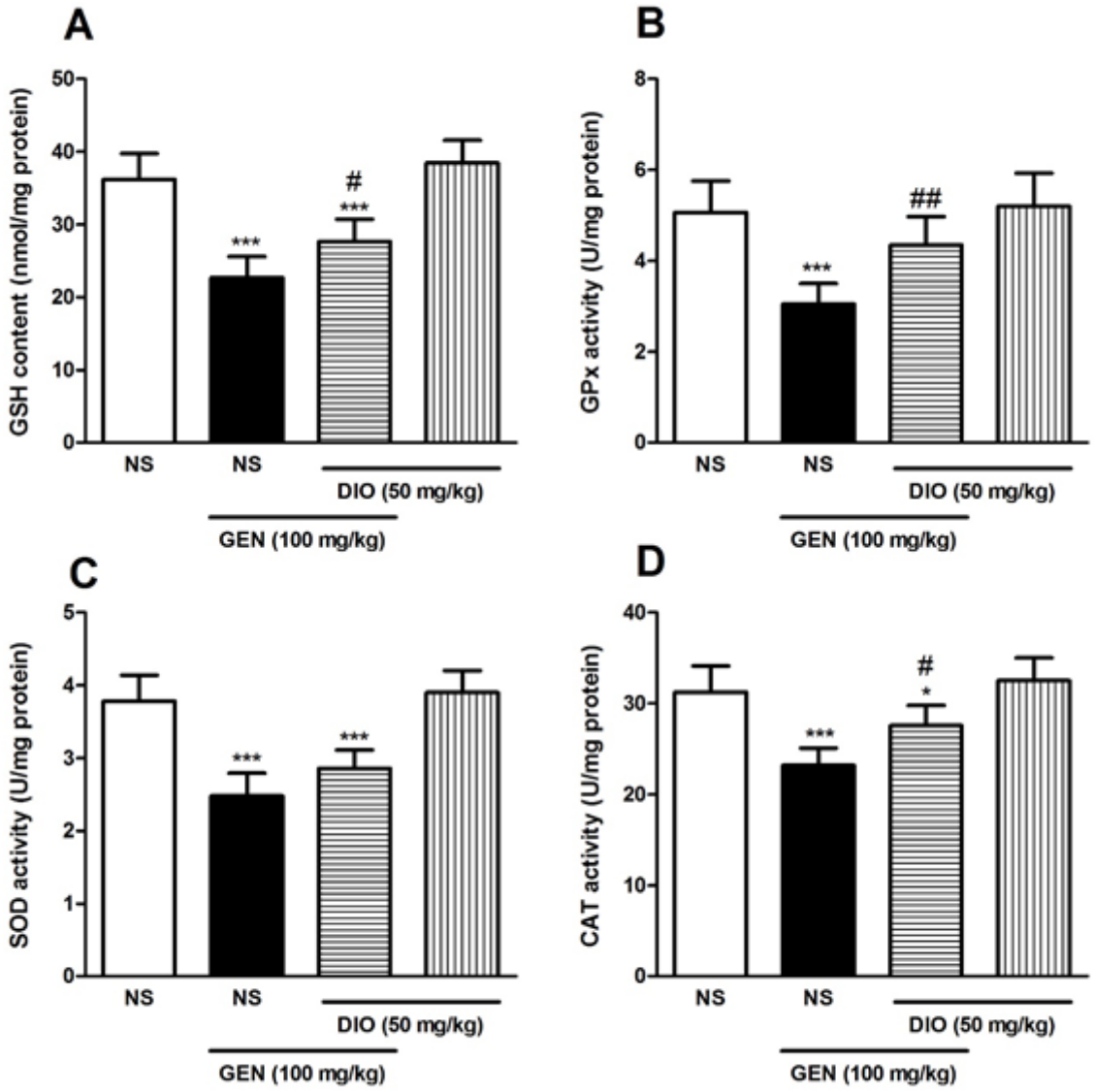

**Figure 1**

Impacts of DIO and GEN on plasma BUN and Cr levels. *Significant difference in comparison with the NS group (***P<0.001). #Significant difference in comparison with the GEN group (# P<0.05, ## P < 0.01). DIO: Diosmin, GEN: Gentamicin, NS: Normal saline. BUN: Blood urea nitrogen, Cr: Creatinine,



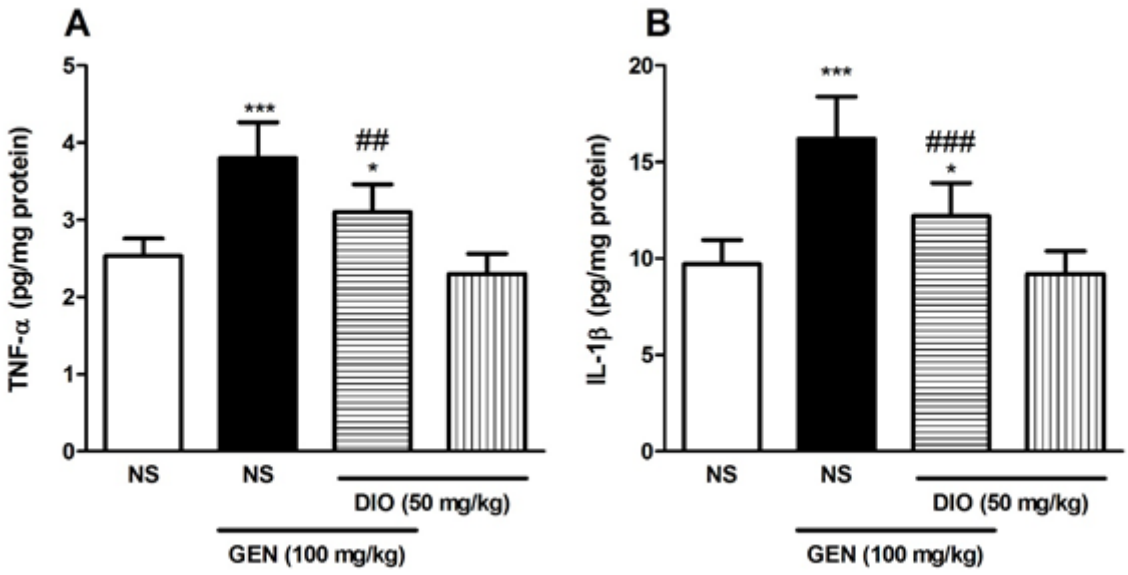

## Figure 2

Impacts of DIO and GEN on tissue MDA and NO levels. *Significant difference in comparison with the NS group (***P<0.001). #Significant difference in comparison with the GEN group (## P < 0.01). MDA: Malondialdehyde, NO: Nitric oxide, DIO: Diosmin, GEN: Gentamicin, NS: Normal saline.



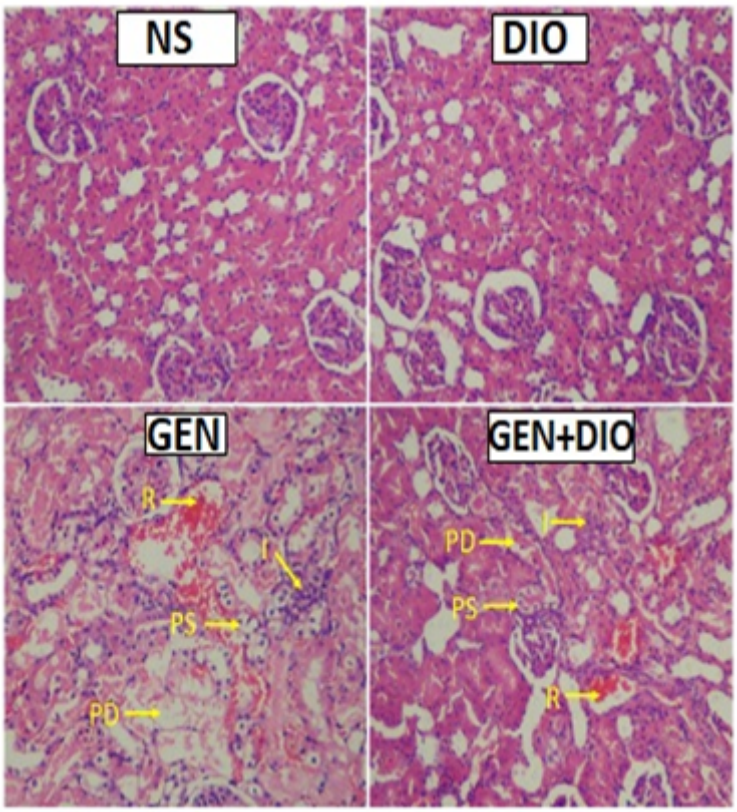

## Figure 3

Impacts of DIO and GEN on tissue GSH levels, GPx, SOD and CAT activities. *Significant difference in comparison with the NS group ((*P<0.05, ***P<0.001). #Significant difference in comparison with the CP group (# P<0.05, ## P < 0.01). NS: Normal saline, GEN: Gentamicin, DIO: Diosmin, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase.




## Figure 4

Impacts of DIO and GEN on tissue TNF-α and IL-1β levels. *Significant difference in comparison with the NS group (***P<0.001). #Significant difference in comparison with the CP group (## P < 0.01, ### P < 0.001). NS: Normal saline, GEN: Gentamicin, DIO: Diosmin, TNF-α: Tumor necrosis factor-α, IL-1β: Interleukin 1 beta.




## Figure 5

Histological changes (kidney sections stained by H&E, X 400) showing impacts of DIO on GEN-related renal toxicity. NS: Normal saline, GEN: Gentamicin, DIO: Diosmin, PD: Proximal tubule degeneration, PS: Proximal cell swelling, R: Accumulation of RBCs, I: Infiltration of inflammatory cells.