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Original Article

Anti-inflammatory and antioxidative properties of date pollen in the gentamicin-induced renal toxicity

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ABSTRACT

Introduction: Aminoglycoside antibiotics including gentamicin are used for treatment of gram-negative bacteria-induced infections; however, gentamicin has severe side effects such as nephrotoxicity. Date palm pollen (DPP), as a herbal medicine is believed to have some anti-inflammatory and antioxidative stress effects. In this study the protective effect of DPP extract were evaluated after its phytochemical analysis on gentamicin-induced nephrotoxicity.

Methods: Wistar rats allocated into five groups including control, sham, gentamicin and two groups that received gentamicin along with DPP extract (200 or 400 mg/kg). Plasma urea and creatinine concentrations were measured and oxidative stress was assessed by evaluating MDA, FRAP, CAT and SOD. NF-κB, TNF-α and ICAM-1 gene expression levels along with the leukocyte infiltration were measured for evaluating inflammation. Histopathological damages were also measured by studying H&E-stained tissue sections.

Results: The gentamicin receiving group had increased plasma urea and creatinine, increased MDA, and decreased FRAP, CAT and SOD activities in the kidney. The gentamicin administration also increased the TNF-α, NF-κB and ICAM-1 gene expression, infiltration of leukocytes and tissue damages in the kidney. DPP extract caused a partial or complete recovery of all these damages.

Conclusion: In conclusion, DPP extract protects the kidney against the side effects of gentamicin and improves its function and histopathological damages. The underlying mechanism is likely to decrease the NF-κB gene expression and consequently reducing pro-inflammatory cytokine genes expression, infiltration of leukocytes and oxidative stress. The DPP extract also increased the cellular antioxidant reserves.

Keywords:
Gentamicin
Nephrotoxicity
Date palm pollen
Inflammation
Oxidative stress
Herbal medicine

Introduction

Aminoglycoside antibiotics have a capability to cause nephrotoxicity and among them, gentamicin (GM) possess the highest renal toxicity (Balakumar et al., 2010). GM was discovered in the 1940s and is used to treat gram-negative and some gram-positive bacteria (Moreira Galdino et al., 2017). Despite this advantage, GM application is not eligible due to its side effects on vari-

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ous organs, including the kidneys. Several mechanisms have been considered for the harmful effects of GM on the kidney, one of which is to increase reactive oxygen species (ROS) and reduce the antioxidant enzymes (Moreira et al., 2014; Com et al., 2012; Khan et al., 2009). Also, GM leads to increased nuclear factor kappa B (NF-κB) expression by reducing NF-κB inhibitory protein, resulting in leukocyte infiltration and the stimulation of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) (Ansari et al., 2016; Sahu et al., 2013). Moreover, GM reduces glomerular filtration and thereby increases the concentration of nitrogenous wastes in the blood by reducing the ultrafiltration coefficient (Baylis et al., 1977; de-Barros-e-Silva et al., 1992). These mechanisms have been confirmed by studies showing that compounds with anti-inflammatory and antioxidant properties, including *Malva sylvestris*, Crocin, *Pimpinella anisum*, kiwifruit, atorvastatin and synaptic acid reduce GM-induced renal damages (Mohamadi yarijani et al., 2019; Mahmoud, 2017; Ashtiyani et al., 2017; Jaikumkao et al., 2016; Mohamadi Yarijani et al., 2016).

Medicinal plants have been used to treat many ailments since ancient times. One of them is date palm (*Phoenix dactylifera L.*), which has been commonly cultivated in the Middle East since 6,000 BC (Miller et al., 2003). In traditional medicine, dates and other palm tree products have been used to treat various diseases, of which is the date palm pollen (DPP). The DPP has been used as a dietary supplement, aphrodisiac, as well as for enhancement of fertility in men and women (Mokhtar and Samar, 2012; Tahvilzadeh et al., 2016). Numerous studies have reported the DPP constituents, including amino acids, minerals, vitamins (A, B, C and E), fatty acids (Mokhtar and Samar, 2012; Hassan, 2011; Basuny et al., 2013), estradiol, estriol, estrone, flavonoids, saponins (Abbas and Abdel-Monem, 2011) as well as many others depending on the species.

Recent studies have shown that DPP extracts have antioxidative (Abbas and Abdel-Monem, 2011; El-Neweshy et al., 2012), anti-inflammatory (Elberry et al., 2011; Metwaly et al., 2014), anti-toxicant (Eraslan et al., 2008) and liver protection effects (Uzbekova et al., 2003). As previously mentioned, inflammation and oxidative stress have been suggested to contribute to gentamicin-induced renal toxicity. Therefore, this study aimed to assess the alleviative effect of DPP hydroalcoholic extract, after its qualitative phytochemical analysis, on gentamicin-induced renal disturbances through measuring histopathological damages, oxidative stress, inflammation and functional disturbances, which has not been evaluated formerly.

Material and methods

Collecting and extracting DPP sample

Fresh DPP powder was prepared from a local herbal market in April 2019, and its identity was confirmed by an herbal pharmacist. The sample dried and stored at laboratory temperature (21°C) for up to two weeks until extraction. The extraction process was performed based on the method described in detail in previous studies (Mohamadi yarijani et al., 2019). The yield of the extraction process was 40%.

Phytochemical analysis

After extraction, the phytochemical analysis was carried out on the crude sample of the extract according to standard protocols (Mohamadi yarijani et al., 2019; Mohan and Gupta, 2017). The compounds whose presence was examined included tannins, alkaloids, flavonoids, triterpenoids, saponins, sterols, anthraquinones, mucilage, anthocyanins and coumarins. Any change in color or formation of sediment was considered as a positive response and their rate was graded.

Studied animals and humane endpoints

In order to do the animal study section, 35 male Wistar rats (200-250g) were prepared by the Laboratory Animal Breeding Center of Kermanshah University of Medical Sciences. The animals were kept in polypropylene cages, temperature of 23±2°C, normal light/darkness cycle and a humidity of 55%, where drinking water and standard food were provided *ad libitum* at all stages of the experiment. During the experiment, the animals were monitored twice a day. Meticulous care was taken throughout the study to ensure that the studied animals suffered the least amount of pain and once abnormal symptoms (restlessness, decreased mobility and abnormal condition) occurred, deep anesthesia induced using sodium pentobarbital (Sigma-Aldrich) and were euthanized. All experimental procedures were in conformity with the European Economic Community Guidelines for the care and use of laboratory animals (EEC Directive of 1986; 86/609/EEC). Also, the study

protocols were approved by local ethics committee (Approval number: IR.KUMS.REC.1398.961).

Experiment protocol

Animals were randomly allocated into 5 groups, each group includes 7 rats. The first group, the control one, in which no intervention was performed. In the second group (sham), the extract solvent (2% of Tween 80 solution) was injected intraperitoneally (IP) during all 9 days of study, and from 3rd day, normal saline was given. The third group (Gentamicin or GM), received the extract solvent from the beginning until the end of the period, but from the third to the ninth day, GM was injected (100mg/kg, IP). The fourth group (GM + DPP200) injected daily with the hydroalcoholic extract of DPP for nine days (200mg/kg, IP) and from the third to the ninth day, GM (100mg/kg, IP) was injected. In the fifth group (GM + DPP400), the protocol was quite similar to the fourth one, except of DPP extract that was injected at 400mg/kg, IP. On the tenth day of the experiment and after induction of deep anesthesia (sodium pentobarbital, 55mg/kg), abdominal cavity was *opened* in the *midline*. Blood samples were collected from the abdominal aorta and after centrifugation, the plasma was isolated and stored in a -20°C freezer to measure creatinine and urea concentrations. Oxidative stress was assessed in right kidney tissue after immediate freezing in liquid nitrogen. In addition, inflammatory factors and tissue damages were measure in the next kidney (Mohamadi yarijani et al., 2019). Finally, rats were euthanized using an overdose of sodium pentobarbital.

Evaluation of renal functional parameters and oxidative stress

Renal functional parameters (creatinine and urea) were estimated in the plasma using an Auto-analyzer. To evaluate oxidative stress, the catalase (CAT) and superoxide dismutase (SOD) enzymes activities, the amount of membrane phospholipids peroxidation (by measuring the level of malondialdehyde: MDA) as well as ferric reducing antioxidant power (FRAP) were estimated through colorimetric assay. CAT and SOD activities and MDA level were measured using a kit (Kiazist, Iran) based on the manufacturer's protocol. FRAP measurements were performed by the Benzie method, as described previously (Najafi et al., 2017; Changizi-Ashtiyani et al., 2016).

Measurement of inflammation

Inflammation was assessed by investigating the rate of leukocytes infiltration into the interstitium along with the expression levels of NF-κB, TNF-α and intercellular adhesion molecule 1 (ICAM-1) in renal cortical tissue by the quantitative real-time PCR (qRT-PCR), as previously explained (Mohammadi et al., 2019).

The sequence of primers used is as follows: NF-κB: (F) 5′-GATCATCAACATGAGAAACGATCTGT-3′, (R) 5′- TAGCGGTCCAGAAGACTCAG-3′; TNF-α: (F) 5′-AAATGGGCTCCCTCTATCAGTTC-3′, (R) 5′- TGCTTGGTGGTTTGCTACGAC -3′; ICAM-1: (F) 5′- GGGATGGTGAAGTCTGTCAA-3′, (R) 5′- GGCGGTAATAGGTGTAAATGG-3′; B-actin: (F) 5′-TGCTATGTTGCCCTAGACTTC-3′, (R) 5′- GTTGGCATAGAGGTCTTTACGG-3′.

Measurement of tissue damages

Renal histopathologic damages were assessed through studying hematoxylin-eosin stained 5µm thick tissue sections. For this purpose, the renal histopathologic damages were studied in 10 fields of the light microscope and classified as previously explained (Najafi et al., 2017; Changizi-Ashtiyani et al., 2016).

Statistical analysis

For the statistical analysis we used SPSS-23 software and data were expressed as mean±SEM. After checking of the normal distributions of the data by Shapiro–Wilk test, one-way ANOVA followed by Duncan's post hoc test was used in normally distributed data. Otherwise, Kruskal-Wallis and Mann-Whitney tests were used. $P<0.05$ was considered as statistically significant.

Results

Qualitative phytochemical analysis of DPP extract

The constituents of the extract mostly were flavonoids and triterpenoids (++++). Then the highest amount was related to sterols (+++) and alkaloids (++). The reactions indicate the presence of coumarins as (+), but the presence of tannins were found to be suspicious. Besides, our study showed that the studied DPP extract lacked anthocyanins, anthraquinones, mucilage and saponins.

Effects on renal function parameters

Figure 1 shows that the increase in plasma creatinine and urea concentrations following gentamicin admin-

TABLE 1: Scores of histopathologic damages induced by gentamicin and the effect of DPP extract on them.

Histopathologic damages	Experimental groups				
	Sham	Control	GM	GM+DPP200	GM+DPP400
Bowman's space enlargement	0.2	0	5	2.1	2.9
Necrosis of tubular cells	0	0	2.1	0.7	1.8
Congestion of vessels	0	0	3.6	0.8	1.3
Intra-tubular proteinaceous casts	0.2	0	3.1	1.1	1.2
Leukocyte infiltration	0.5	0	4.5	0.9	1.5
Total histopathologic score	**0.9**	**0**	**18.3** ***	**5.6** * †††	**8.7** ** ††

Histopathologic scores in rats without any intervention (control), received normal saline (sham), gentamicin (GM), or gentamicin plus date palm pollen extract at 200 or 400mg/kg (GM+DPP200 and GM+DPP400). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to sham group. $^{††}P<0.01$ and $^{†††}P<0.001$ compared to gentamicin group.

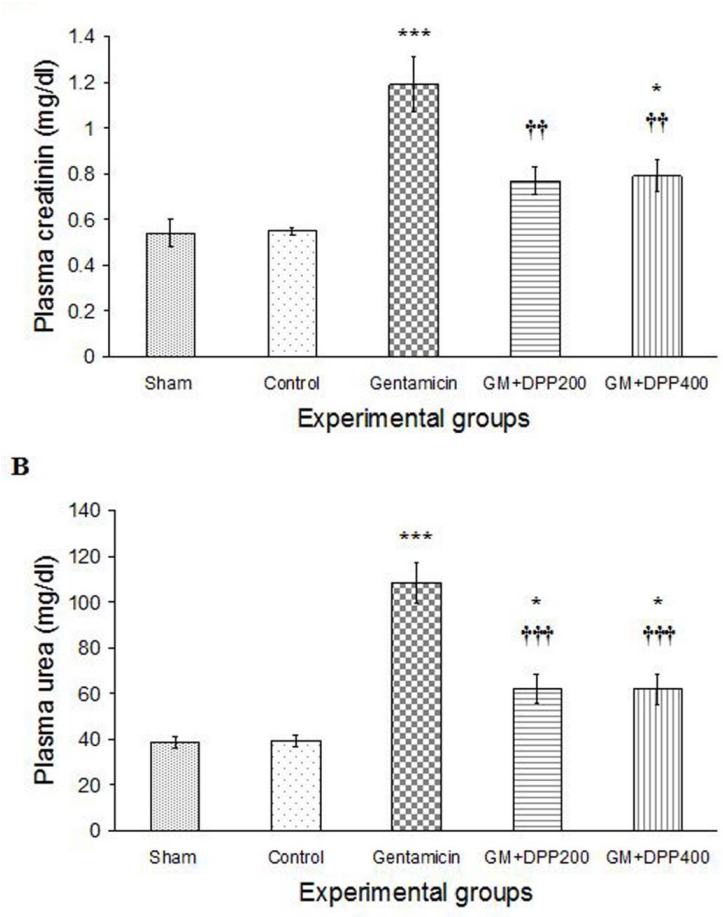

FIGURE 1. The concentrations of plasma creatinine (A) and urea (B) in rats without any intervention (Control), received normal saline (sham), gentamicin and gentamicin plus date palm pollen extract at 200 or 400mg/kg (GM+DPP200 and GM+DPP400). Data are presented as mean±SEM (n=7). $^{*}P<0.05$ and $^{***}P<0.001$ compared to sham group. $^{††}P<0.01$ and $^{†††}P<0.001$ compared to gentamicin group.

istration was significant compared to the sham group ($P<0.001$). However, DPP extract significantly reduced plasma creatinine values following its increase by gentamicin (Figure 1A). Also, co-administration of DPP extract and gentamicin significantly reduced the plasma urea values in both DPP studied doses as compared to

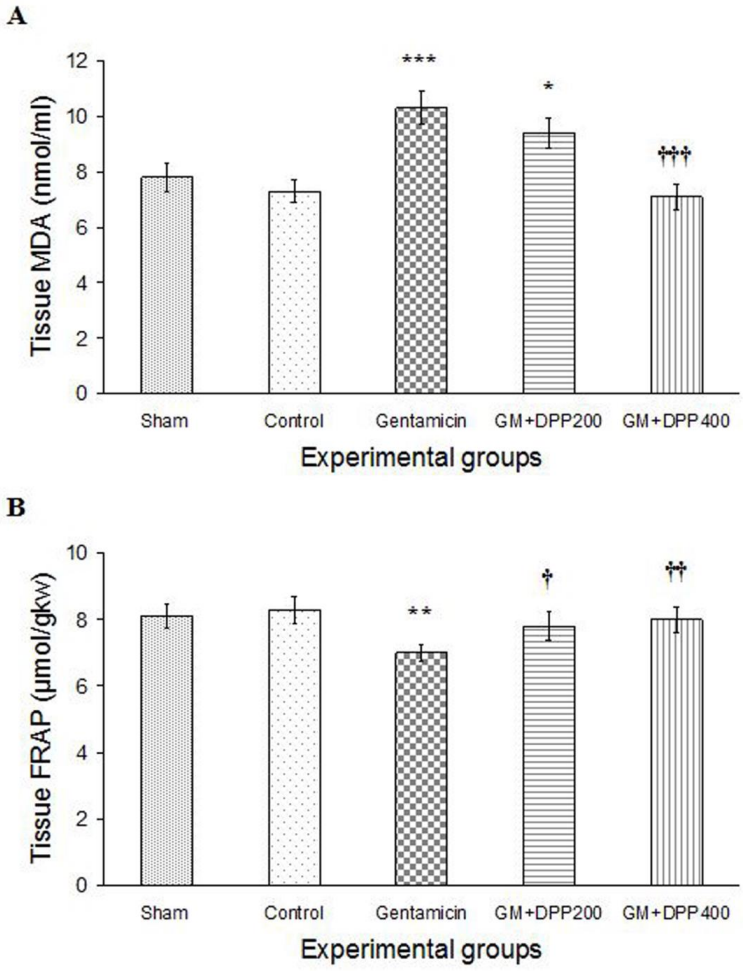

FIGURE 2. A: Renal malondialdehyde (MDA) and B: Ferric reducing antioxidant power (FRAP) in experimental groups. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to sham group. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ and $^{\dagger\dagger\dagger}P<0.001$ compared to gentamicin group.

the group that received gentamicin alone ($P<0.001$). But their amounts were still higher than urea concentration in the sham group (Figure 1B).

Effects on renal oxidative stress

As shown in Figure 2, gentamicin increased lipid peroxidation and decreased total antioxidant capacity of kidney tissue, which has shown itself as an increase in MDA ($P<0.001$) and a decrease in FRAP ($P<0.01$) levels compared to their values in the sham group. DPP extract pretreatment significantly reduced MDA in the GM + DPP400 group ($P<0.001$, Figure 2A). Also, the DPP extract increased renal tissue FRAP levels after its reduction in the GM group (Figure 2B).

Moreover, GM significantly reduced the catalase and superoxide dismutase activity in the kidney tissue ($P<0.001$), which was improved partially by pretreatment with the DPP extract, but was still less than their values in the sham group (Figure 3). It is noteworthy that the efficiency of 200mg/kg dose was higher on both parameters.

Effects on renal inflammation

For inflammation study, the gene expression level of pro-inflammatory factors NF-κB and TNF-α alongside adhesive factor ICAM-1 was measured in the renal cortex tissue. The obtained results are shown in Figures 4 and 5. Gentamicin increased the expression of the TNF-α gene by about 4 times ($P<0.001$), the expression of the ICAM-1 gene by about 3.5 times ($P<0.01$) and the expression of the NF-κB gene by about 2.8 times ($P<0.01$), in compare to the sham group. As it can be seen, DPP extract pretreatment was able to partially but significant modify the expression levels of all three genes, although they are still significantly different from their values in the sham group. Moreover, the level of

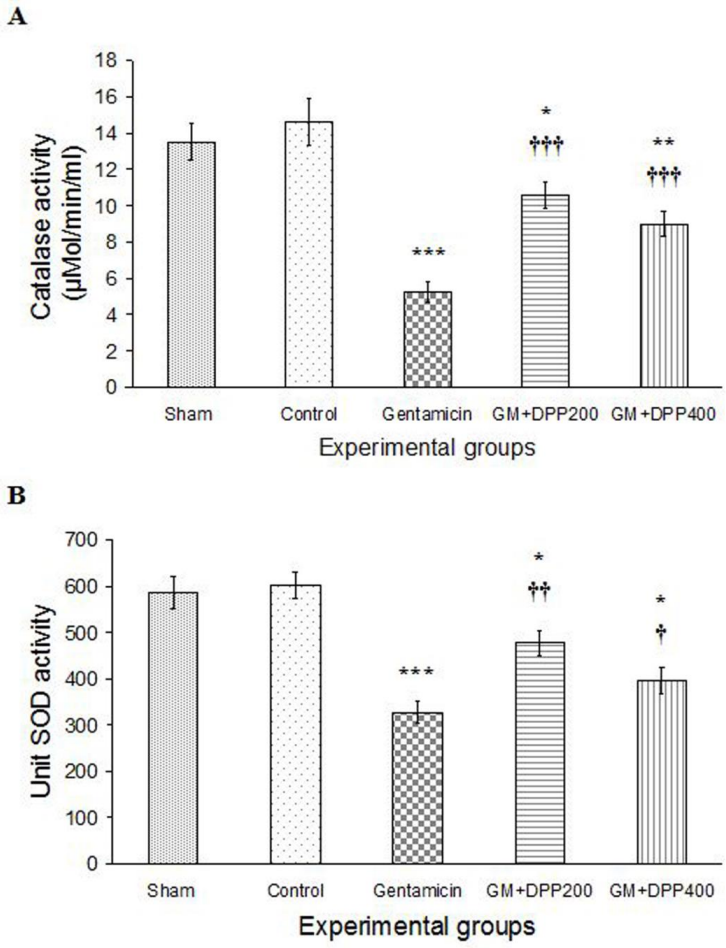

FIGURE 3. A: Renal tissue catalase and B: superoxide dismutase (SOD) activities in experimental groups. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to sham group. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ and $^{\dagger\dagger\dagger}P<0.001$ compared to gentamicin group.

the leukocytes infiltration to interstitium increased after GM administration, which was reduced by the DPP extract treatment (Figure 6 and Table 1).

Effects on renal histopathological damages

Renal histopathological damages are shown in Figure 7 and the degrees of these damages are summarized in Table 1. Gentamicin increased the size of the Bowman's space by degree 5, tubular cell necrosis by degree 2.1, vascular congestion by degree 3.6, intratubular proteinaceous cast formation by degree 3.1 and infiltration of leukocytes by degree 4.5. Therefore, the gentamicin group had a higher total histopathologic score than the sham one, which is equal to 18.3 ($P<0.001$). The DPP extract with both studied doses improved all of these damages partially such that the total histopathological score was 5.6 and 8.7 in the groups GM + DPP200 and GM + DPP400 respectively, both of the values were significantly less than that of the GM group.

Discussion

About 20% of cases of nephrotoxicity are developed by drugs, a score which increases in older people up to about 66% (Kohli et al., 2000; Naughton, 2008) and GM is one of the most important drugs that induce nephrotoxicity. One way to reduce these side effects is to use common natural and herbal supplements. Therefore, present study investigated the protective activity of DPP hydroalcoholic extract on GM-induced nephrotoxicity. Our results indicated that the DPP extract can reduce oxidative stress, inflammation, functional disturbances as well as tissue damages caused by GM, the data hitherto unreported.

In our study, GM impaired renal function, which was associated with increased creatinine and urea concentrations of plasma. In the proximal tubule, gentamicin enters the lysosomes of the epithelial cells and following their destruction, leads to the release of hydrolase enzymes and causes cellular necrosis. This, in turn,

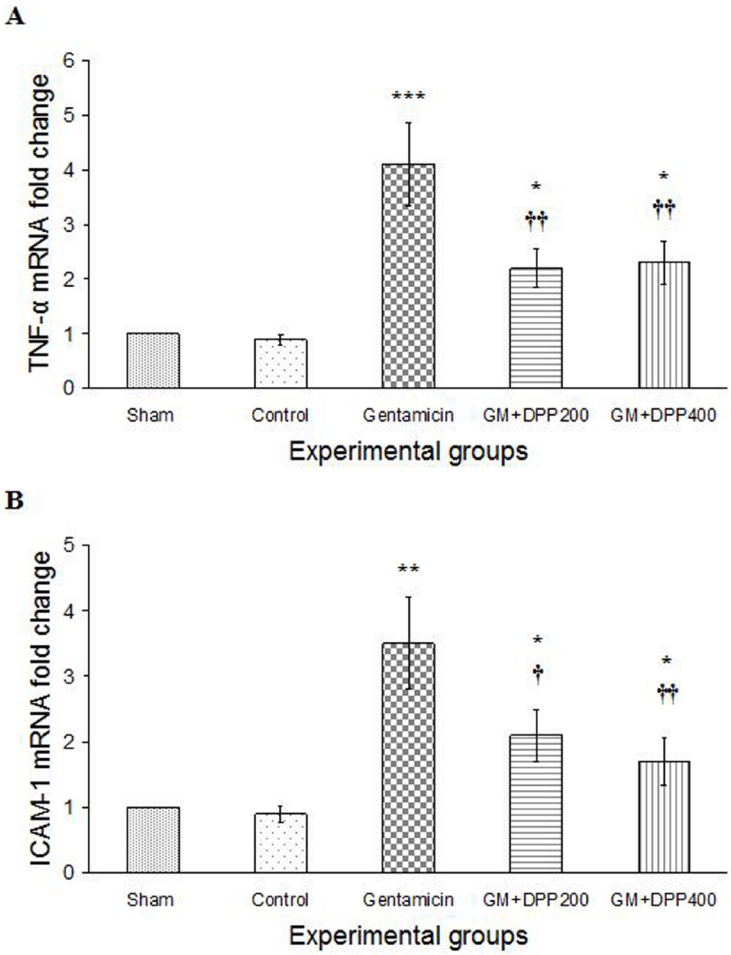

FIGURE 4. Fold change expression for A: TNF-α and B: ICAM-1 mRNA in the renal cortex tissue of experimental groups. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to sham group. $^{\dagger}P<0.05$ and $^{\dagger\dagger}P<0.01$ compared to gentamicin group.

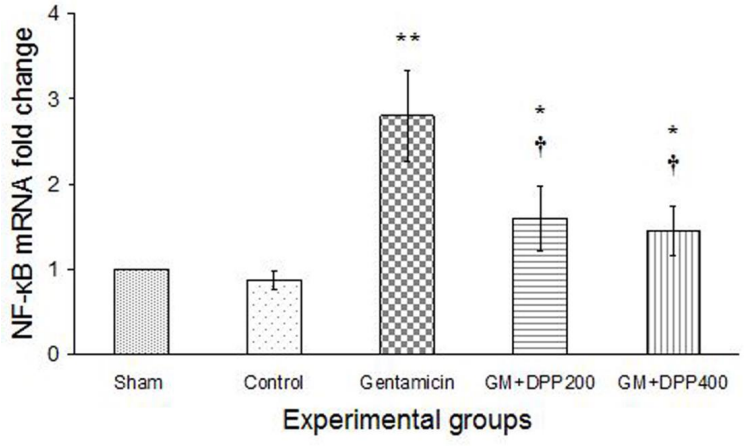

FIGURE 5. Fold change expression for NF-κB mRNA in the renal cortex tissue of experimental groups. $^{*}P<0.05$ and $^{**}P<0.01$ compared to sham group. $^{\dagger}P<0.05$ compared to gentamicin group.

leads to the proximal tubule obstruction (Hanslik et al., 1994). The obstruction of the proximal tubule reduces the amount of glomerular filtration rate (GFR) by increasing the hydrostatic pressure of the Bowman's capsule. Moreover, GM releases vasoconstrictor hormones by disrupting the cellular membrane structure (Tavafi, 2012; Valipour et al., 2016), along with the platelet aggregation factor as well as lesions resulting from cell destruction, can cause nephron obstruction and thereby reduce the ultrafiltration coefficient (Moreira Galdino

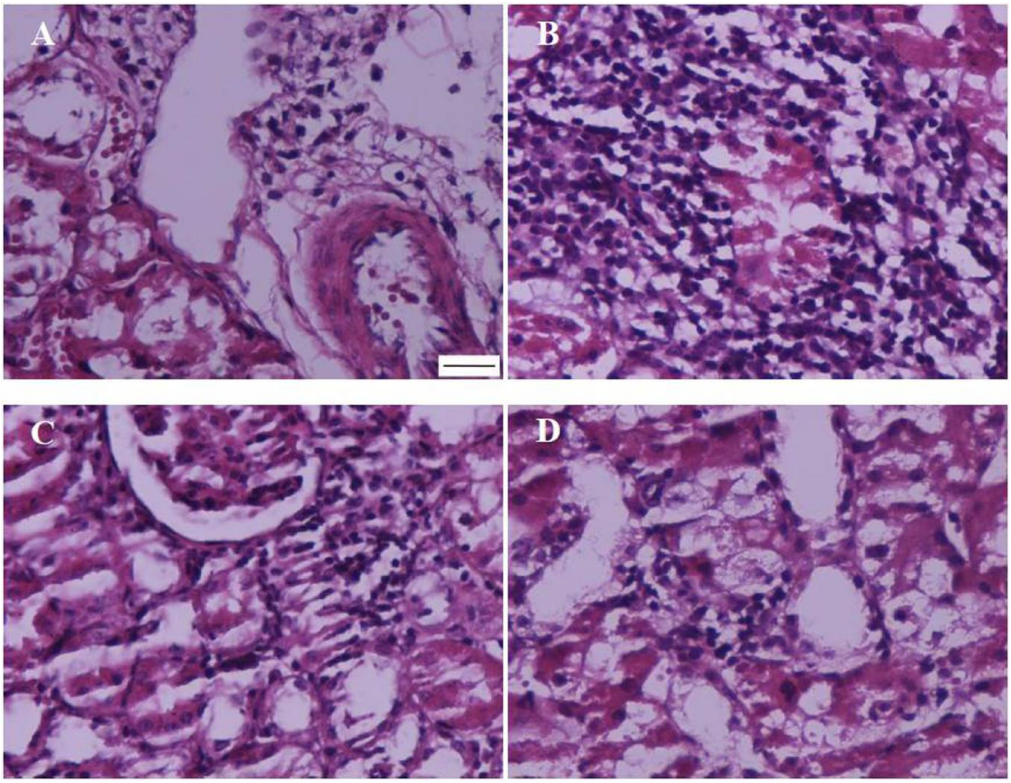

FIGURE 6. Leukocyte infiltration to renal interstitium of rats received normal saline (A), gentamicin (B) and gentamicin plus date palm pollen extract at 200 (C) or 400mg/kg (D). Haematoxylin-Eosin, magnification 400x, scale bar: 200μm.

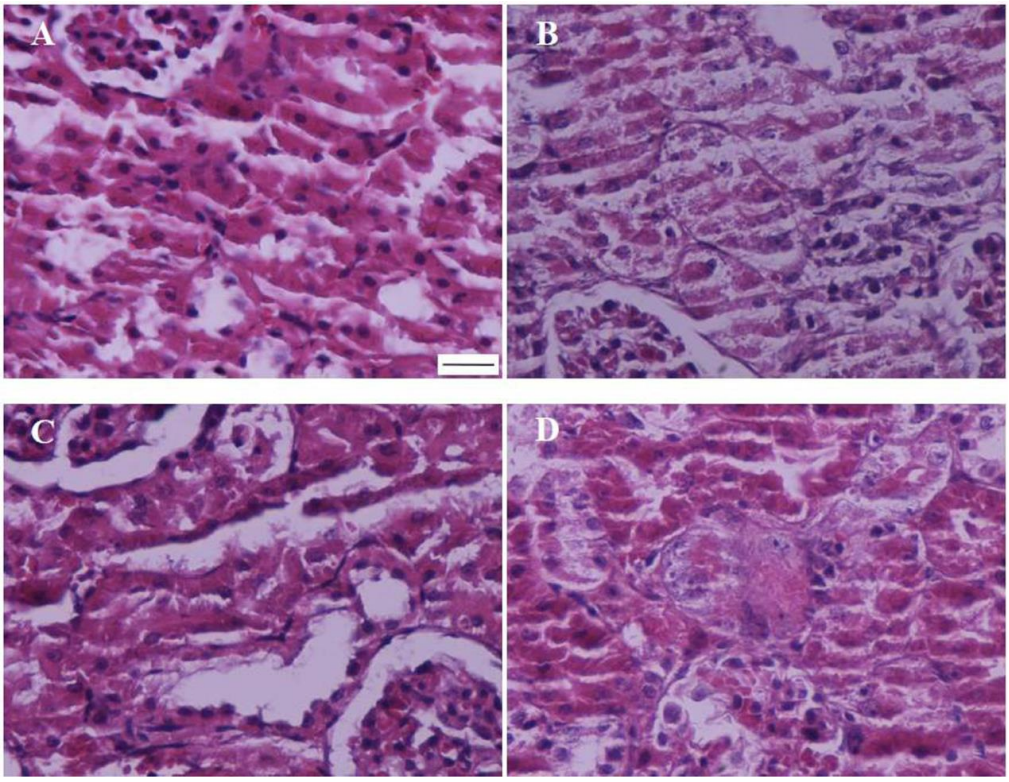

FIGURE 7. Renal tissue damages in rats received normal saline (A), gentamicin (B) and gentamicin plus date palm pollen extract at 200 (C) or 400mg/kg (D). Haematoxylin-Eosin, magnification 400x, scale bar: 200μm.

et al., 2017). In addition, through glomerular hypertrophy and changes in the glomerular basement membrane (Stojiljkovic et al., 2008), and contractions of mesangial cells as well as a reduction in the size and number of endothelial cell pores, GM reduce the ultrafiltration coefficient and thereby GFR (Randjelovic et al., 2017). Gentamicin has also been shown to contract the mesangial cells and stimulate the production of vasoconstrictors by activating the renin-angiotensin system. Angiotensin II (AT2) also stimulates the production of ROSs, so AT2 receptor blockers as well as angiotensin-converting enzyme (ACE) inhibitors protect the kidney against gentamicin (Mahi-Birjand et al., 2020; Kobori et al., 2013). Meanwhile, structural damage to proximal tubular epithelial cells reduces reabsorption and thus increases the delivery of water and electrolytes to the macula densa, which in turn reduces GFR by activating tubuloglomerular feedback (Blantz et al., 2007). Therefore, GM reduces GFR in various ways and thereby increases the concentration of nitrogenous wastes in the blood.

In present study the DPP extract administration prevented the increase in plasma creatinine and urea concentrations by GM. Many researchers have provided evidence that DPP extract has anti-inflammatory and antioxidant activities (Abbas and Abdel-Monem; 2011; El-Neweshy et al., 2012; Elberry et al., 2011), which may protect cells and thus prevent the release of vasoconstrictor hormones in these ways. Also, Daoud et al. (2017) showed that the DPP extract had ACE inhibitory properties in the heart. Therefore; it is likely that the DPP extract increases glomerular filtration and thus decreases the plasma creatinine and urea concentrations by inhibiting ACE in the kidney and also reducing cell damages, a hypothesis that needs further investigation.

The results showed that GM induces oxidative stress in the kidney tissue, which is in line with the results of the others (Moreira Galdino et al., 2017; Ansari et al., 2016). It has been shown that GM is released into the cytoplasm by lysosomes destruction and after damaging other organs including mitochondria, inhibits electron transport and impairs ATP production. These changes stimulate the production of ROS including hydrogen peroxide, superoxide anion and hydroxyl radical (Mahmoud, 2017). These oxidants also damage cellular proteins and nucleic acids and lead to the membrane lipids peroxidation and their subsequent instability, which manifests as increased MDA levels (Katary and Salahuddin, 2017; Martinez-Salgado et al., 2004). In the present study, the MDA levels were increased by GM as well. Gentamicin also increases iNOS expression, the resulting NO combines with ROSs and leads to nitrosative stress (Mahmoud et al., 2014), and the inhibition of iNOS reduces renal impairment induced by gentamicin (Famurewa et al., 2019).

Cells have an antioxidant defense mechanism including catalase, superoxide dismutase and glutathione peroxidase, which scavenge free radicals. GM reduces these antioxidant reserves and the rate of damage is proportional to the degree of depletion (Manikandan et al., 2011). The results showed that the activity of catalase and superoxide dismutase in the GM group was decreased. Therefore, GM induces oxidative and nitrosative stress and reduces the antioxidant reserves of the cells.

The results of our investigation indicated that DPP extract reduced MDA and increased catalase, superoxide dismutase and FRAP levels, indicating its proper antioxidant effects. These findings are in line with the other studies showing that the DPP extracts have antioxidant properties and scavenging of free radical activity. It also reduces iNOS activity and NO level as well (El-Kashlan et al., 2015; Abbas and Abdel-Monem, 2011; El-Neweshy et al., 2012). Therefore, the DPP extract may be exert its antioxidant properties through ROS scavenging activity and interfere with the production of free radicals. In all of these studies, the antioxidant properties of the DPP extracts have been attributed to flavonoids content as well as the vitamins it contains. Previous studies have shown that vitamin C or E, or a combination of both protects the kidneys against GM (Mahi-Birjand et al., 2020). Indeed in the phytochemical analysis, the extract studied in the current research contained a high level of flavonoids.

In this study, GM increased the gene expression of NF-κB and TNF-α, adhesion molecule ICAM-1 and the leukocytes infiltration, indicating the induction of inflammation. One way for GM to induce inflammation is to activate NF-κB. GM increases NF-κB and causes the NF-κB translocation to the nucleus by reducing the level of NF-κB inhibitory protein. NF-κB then stimulates the proinflammatory cytokines production, including TNF-α (Katary and Salahuddin, 2017; Ansari et al., 2016). TNF-α also stimulates vasoconstriction, reduces blood flow, infiltrates leukocytes, produces ICAM-1 and

thereby impairs renal function (Donnahoo et al., 1999). It has been shown that NF-κB inhibition protects the kidneys against gentamicin (Famurewa et al., 2019; Abdelrahman and Abdelmageed, 2020). Therefore, TNF-α both directly and by stimulating ICAM-1 production causes the leukocytes infiltration into the interstitium.

Another way through which toxins, ischemia or trauma can cause inflammation is damage to kidney cells. Necrotic cells release damage-associated molecular patterns into the extracellular space, which activate pattern recognition receptors. These receptors are found mainly on dendritic cells and macrophages and also slightly on non-immune cells. Activation of these receptors also releases pro-inflammatory mediators (Kurts et al., 2013).

In the current study, we found that the DPP extract reduced mRNA gene expression of pro-inflammatory and adhesive factors, and leukocytes infiltration after GM administration, which is consistent with the result of previous studies (Elberry et al., 2011; Jaikumkao et al., 2016). It has been shown that DPP have anti-inflammatory and leukocyte-reducing effects (Elberry et al., 2011), and also attenuate the inflammatory response in animals (Kimura et al., 1986). In their study, Elberry et al. (2011) showed that DPP extract reduced the expression level of IL-6, IL-8 and TNF-α in atypical prostate hypertrophy. Therefore, DPP extract because of its anti-inflammatory properties, prevents the activation of inflammatory pathways in the kidney, which in the present study is confirmed by the reduction of pro-inflammatory and adhesive factors expression, as well as reduced leukocytes infiltration. Besides, inhibition of inflammation by DPP extract can also prevent vasoconstriction, which in turn reduces the concentration of nitrogenous wastes.

Our histological studies have shown that the GM receiving group had multiple histopathological damages, including enlargement of Bowman's space, necrosis of epithelial cells, blood vessels congestion and formation of intra-tubular proteinaceous casts. In one study, Sassen et al. (2006) showed that GM disrupted cellular homeostasis, caused inflammation and thus produced cellular necrosis by inhibiting several membrane transport proteins, including the sodium-potassium pumps. As mentioned earlier, GM causes damage to cells and necrosis by destroying lysosomes and producing ROSs. The use of the DPP extract reduced these damages, indicating that DPP extract can protect renal tissue against GM-induced histopathological damages. The DPP extract may reduce GM-induced damages in the kidney tissue through its anti-congestive (Elberry et al., 2011; Osborn et al., 1981), antioxidant and anti-inflammatory effects, which requires further research.

Conclusion

In summary, this study revealed that GM impairs renal function and induces oxidative stress, inflammation and tissue damage. The administration of DPP hydroalcoholic extract protects the kidneys against the adverse effects of GM and improves its functional and histopathological damages as well. The mechanism of its protective effect appears to be decreasing NF-κB expression and subsequently TNF-α and ICAM-1, reduced leukocyte infiltration and oxidative stress, and an increase in the cellular antioxidant reserves.

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Conflict of interest

The authors declare that they have no conflicting interests.

References

Abbas FA, Abdel-Monem A. Estradiol, esteriol, estrone and novel flavonoids from date palm pollen. Aust J Basic Appl Sci 2011; 5: 606-614.

Abdelrahman RS, Abdelmageed ME. Renoprotective effect of celecoxib against gentamicin-induced nephrotoxicity through suppressing NFκB and caspase-3 signaling pathways in rats. Chem Biol Interact 2020; 315: 108863. https://doi.org/10.1016/j.cbi.2019.108863

Ansari MA, Raish M, Ahmad A, Ahmad SF, Mudassar S, Mohsin K, et al. Sinapic acid mitigates gentamicin-induced nephrotoxicity and associated oxidative/nitrosative stress, apoptosis, and inflammation in rats. Life Sci 2016; 165:1-8. https://doi.org/10.1016/j.lfs.2016.09.014

Ashtiyani SC, Seddig A, Najafi H, Hossaini N, Avan A, Akbary A, et al. Pimpinella anisum L. ethanolic extract ameliorates the gentamicin-induced nephrotoxicity in rats. Nephrology 2017; 22: 133-38. https://doi.org/10.1111/nep.12953

Balakumar P, Rohilla A, Thangathirupathi A. Gentamicin-induced nephrotoxicity: do we have a promising therapeutic approach to blunt it?. Pharmacol Res 2010; 62: 179-86. https://doi.org/10.1016/j.phrs.2010.04.004

Basuny AM, Arafat SM, Soliman HM. Chemical analysis of olive and palm pollen: antioxidant and antimicrobial activation properties. Herald J Agric Food Sci Res 2013; 2: 91-7.

Baylis C, Rennke HR, Brenner BM. Mechanisms of the defect in glomerular ultrafiltration associated with gentamicin administration. Kidney Int 1977; 12: 344-53. https://doi.org/10.1038/ki.1977.121

Blantz RC, Deng A, Miracle CM, Thomson SC. Regulation of kidney function and metabolism: a question of supply and demand. Trans Am Clin Climatol Assoc 2007; 118: 23-43.

Changizi-Ashtiyani S, Alizadeh M, Najafi H, Babaei S, Khazaei M, Jafari M, et al. Physalis alkekengi and Alhagi maurorum ameliorate the side effect of cisplatin-induced nephrotoxicity. Cancer Gene Ther 2016; 23: 235-40. https://doi.org/10.1038/cgt.2016.24

Com E, Boitier E, Marchandeau JP, Brandenburg A, Schroeder S, Hoffmann D, et al. Integrated transcriptomic and proteomic evaluation of gentamicin nephrotoxicity in rats. Toxicol Appl Pharmacol 2012; 258: 124-33. https://doi.org/10.1016/j.taap.2011.10.015

Daoud A, Ben mefteh F, Mnafgui K, Turki M, Jmal S, Ben amar R, et al. Cardioprotective effect of ethanolic extract of date palm pollen against isoproterenol induced myocardial infarction in rats through the inhibition of the angiotensin-converting enzyme. Exp Toxicol Pathol 2017; 69: 656-65. https://doi.org/10.1016/j.etp.2017.06.004

de-Barros-e-Silva ML, Varanda WA, Lachat JJ, Alves-da-Silva CG, Coimbra TM. Glomerular permeability to macromolecules in gentamicin-treated rats. Braz J Med Biol Res 1992; 25: 409-17.

Donnahoo KK, Shames BD, Harken AH, Meldrum DR. Review article: the role of tumor necrosis factor in renal ischemia-reperfusion injury. J Urol 1999; 162: 196-203. https://doi.org/10.1097/00005392-199907000-00068

Elberry A, Mufti S, Al-Maghrabi J, Abdel-Sattar E, Ashour O, Ghareib S, et al. Anti-inflammatory and antiproliferative activities of date palm pollen (Phoenix dactylifera) on experimentally-induced atypical prostatic hyperplasia in rats. J Inflam 2011; 8: 40-53. https://doi.org/10.1186/1476-9255-8-40

El-Kashlan AM, Nooh MM, Hassan WA, Rizk SM. Therapeutic potential of date palm pollen for testicular dysfunction induced by thyroid disorders in male rats. PLoS ONE 2015; 10: e0139493. https://doi.org/10.1371/journal.pone.0139493

El-Neweshy MS, El-Maddawy ZK, El-Sayed YS. Therapeutic effects of date palm (Phoenix dactylifera L.) pollen extract on cadmium-induced testicular toxicity. Andrologia 2012; 45: 369-78. https://doi.org/10.1111/and.12025

Eraslan G, Kanbur M, Silici S, Liman B, Altinordulu S, Karabacak M. Evaluation of protective effect of bee pollen against propoxur toxicity in rat. Ecotoxicol Environ Saf 2008; 72: 931-37. https://doi.org/10.1016/j.ecoenv.2008.06.008

Famurewa AC, Maduagwuna EK, Folawiyo AM, Besong EE, Eteudo AN, Famurewa OA, et al. Antioxidant, anti-inflammatory, and antiapoptotic effects of virgin coconut oil against antibiotic drug gentamicin-induced nephrotoxicity via the suppression of oxidative stress and modulation of iNOS/NF-κB/caspase-3 signaling pathway in wistar rats. J Food Biochem 2019; 44: e13100. https://doi.org/10.1111/jfbc.13100

Hanslik T, Blanchet F, Nochy D, Pirotzky E, Guilmard C, Seta N, et al. Effect of the platelet activating factor antagonist BN52021 in rabbits: role in gentamicin nephrotoxicity. Toxicol Appl Pharmacol 1994; 128: 111-15. https://doi.org/10.1006/taap.1994.1187

Hassan HMM. Chemical composition and nutritional value of palm pollen grains. Global J Biotech Biochem 2011; 6: 1-7.

Jaikumkao K, Pongchaidecha A, Thongnak L-o, Wanchai K, Arjinajarn P, Chatsudthipong V, et al. Amelioration of renal inflammation, endoplasmic reticulum stress and apoptosis underlies the protective effect of low dosage of atorvastatin in gentamicin-induced nephrotoxicity. PLoS ONE 2016; 11: e0164528. https://doi.org/10.1371/journal.pone.0164528

Katary M, Salahuddin A. Ameliorative effect of gossypin against gentamicin-induced nephrotoxicity in rats. Life Sci 2017; 176: 75-81. https://doi.org/10.1016/j.lfs.2017.03.009

Khan SA, Priyamvada S, Farooq N, Khan S, Khan MW, Yusufi AN. Protective effect of green tea extract on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Pharmacol Res 2009; 59: 254-62. https://doi.org/10.1016/j.phrs.2008.12.009

Kimura M, Kimura I, Nakase K. Micturition activity of pollen extract: contractile effects on bladder and inhibitory effects on urethral smooth muscle of mouse and pig. Planta Med 1986;

2: 148-51. https://doi.org/10.1055/s-2007-969102

Kobori H, Mori H, Masaki T, Nishiyama A. Angiotensin II blockade and renal protection. Curr Pharm Des 2013; 19: 3033-3042. https://doi.org/10.2174/1381612811319170009

Kohli HS, Bhaskaran MC, Muthukumar T, Thennarasu K, Sud K, Jha V, et al. Treatment-related acute renal failure in the elderly: a hospital-based prospective study. Nephrol Dial Transplant 2000; 15: 212-17. https://doi.org/10.1093/ndt/15.2.212

Kurts C, Panzer U, Anders HJ, Rees AJ. The immune system and kidney disease: basic concepts and clinical implications. Nat Rev Immunol 2013; 13: 738-53. https://doi.org/10.1038/nri3523

Mahi-Birjand M, Yaghoubi S, Abdollahpour-Alitappeh M, Keshtkaran Z, Bagheri N, Pirouzi A, et al. Protective effects of pharmacological agents against aminoglycoside-induced nephrotoxicity: a systematic review. Expert Opin Drug Saf 2020; 19: 167-86. https://doi.org/10.1080/14740338.2020.1712357

Mahmoud AM, Ahmed OM, Galaly SR. Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. EXCLI J 2014; 13: 98-110.

Mahmoud YI. Kiwi fruit (Actinidia deliciosa) ameliorates gentamicin-induced nephrotoxicity in albino mice via the activation of Nrf2 and the inhibition of NF-κB (Kiwi & gentamicin-induced nephrotoxicity). Biomed Pharmacother 2017; 94: 206-18. https://doi.org/10.1016/j.biopha.2017.07.079

Manikandan R, Beulaja M, Thiagarajan R, Priyadarsini A, Saravanan R, Arumugam M. Ameliorative effects of curcumin against renal injuries mediated by inducible nitric oxide synthase and nuclear factor kappa B during gentamicin-induced toxicity in Wistar rats. Eu J Pharmacol 2011; 670: 578-85. https://doi.org/10.1016/j.ejphar.2011.08.037

Martinez-Salgado C, Eleno N, Morales AI, Perez-Barriocanal F, Arevalo M, Lopez-Novoa JM. Gentamicin treatement induces simultaneous mesengial prolifration and apoptosis in rat. Kidney Int 2004; 65: 2161-71. https://doi.org/10.1111/j.1523-1755.2004.00642.x

Metwaly M, Dkhil M, Al-Quraishy S. Anti-coccidial and anti-apoptotic activities of palm pollen grains on Eimeria papillata-induced infection in mice. Biologia (Bratisl) 2014; 69: 254-9. https://doi.org/10.2478/s11756-013-0297-9

Miller CJ, Dunn EV, Hashim IB. The glycaemic index of dates and date/yoghurt mixed meals. Are dates 'the candy that grows on trees'?. Eur J Clin Nutr 2003; 57: 427-30. https://doi.org/10.1038/sj.ejcn.1601565

Mohammadi M, Najafi H, Mohamadi Yarijani Z, Vaezi G, Hojati V. Protective effect of piperine in ischemia-reperfusion induced acute kidney injury through inhibition of inflammation and oxidative stress. J Trad Complement Med 2019; 10: 570-6. https://doi.org/10.1016/j.jtcme.2019.07.002

Mohamadi yarijani Z, Najafi H, Shackebaei D, Madani SH, Modarresi M, Jassemi SV. Amelioration of renal and hepatic function, oxidative stress, inflammation and histopathologic damages by malva sylvestris extract in gentamicin induced renal toxicity. Biomed Pharmacother 2019; 112: 108635. https://doi.org/10.1016/j.biopha.2019.108635

Mohamadi Yarijani Z, Najafi H, Madani SH. Protective effect of crocin on gentamicin-induced nephrotoxicity in rats. Iran J Basic Med Sci 2016; 19: 337-43.

Mohan S, Gupta D. Phytochemical analysis and differential in vitro cytotoxicity assessment of root extracts of Inula racemosa. Biomed Pharmacother 2017; 89: 781-95. https://doi.org/10.1016/j.biopha.2017.02.053

Mokhtar B, Samar Y. Comparative study of the nutritional value of four types of Egyptian palm pollens. J Pharm Nutr Sci 2012; 2: 50-6.

Moreira MA, Nascimento MA, Bozzo TA, Cintra A, da Silva SM, Dalboni MA, et al. Ascorbic acid reduces gentamicin-induced nephrotoxicity in rats through the control of reactive oxygen species. Clin Nutr 2014; 33: 296-301. https://doi.org/10.1016/j.clnu.2013.05.005

Moreira Galdino P, Nunes Alexandre L, Fernanda Pacheco L, de Souza Lino Junior R, de Paula JR, Rodrigues Pedrino G, et al. Nephroprotective effect of rudgea viburnoides (Cham.) benth leaves on gentamicin-induced nephrotoxicity in rats. J Ethnopharmacol 2017; 201:100-7. https://doi.org/10.1016/j.jep.2017.02.035

Najafi H, Mohamadi Yarijani Z, Changizi-Ashtiyani S, Mansouri K, Modarresi M, Madani SH, et al. Protective effect of Malva sylvestris L. extract in ischemia-reperfusion induced acute kidney and remote liver injury. PloS ONE 2017; 12: e0188270. https://doi.org/10.1371/journal.pone.0188270

Naughton CA. Drug-induced nephrotoxicity. Am Fam Physician 2008; 78: 743-50.

Osborn DE, George NJR, Rao PN. Prostatodynia-physiological characteristics and rational management with muscle relaxants. Br J Urol 1981; 53: 621-3. https://doi.org/10.1111/j.1464-410X.1981.tb03275.x

Randjelovic P, Veljkovic S, Stojiljkovic N, Sokolovic D, Ilic I. Gentamicin nephrotoxicity in animals: current knowledge and future perspectives. EXCLI J 2017; 16: 388-99.

Sahu BD, Kuncha M, Sindhura GJ, Sistla R. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. Phytomedicine 2013; 20: 453-60. https://doi.org/10.1016/j.phymed.2012.12.001

Sassen MC, Kim SW, Kwon TH, Knepper MA, Miller RT, Frokiaer J, et al. Dysregulation of renal sodium transporters in gentamicin-treated rats. Kidney Int 2006; 70: 1026-37. https://doi.org/10.1038/sj.ki.5001654

Stojiljkovic N, Mihailovic D, Veljkovic S, Stoiljkovic M, Jovanovic I. Glomerular basement membrane alterations induced by gentamicin administration in rats. Exp Toxicol Pathol 2008; 60: 69-75. https://doi.org/10.1016/j.etp.2008.02.007

Tahvilzadeh M, Hajimahmoodi M, Rahimi R. The role of date palm (phoenix dactylifera L) pollen in fertility: A comprehensive review of current evidence. J Evid Based Complementary Altern Med 2016; 21: 320-4. https://doi.org/10.1177/2156587215609851

Tavafi M. Inhibition of gentamicin-induced renal tubular cell necrosis. J Nephropathol 2012; 1: 83-6. https://doi.org/10.5812/nephropathol.7512

Uzbekova D, Makarova V, Khvoynitskaya L, Slepnev A. Evaluation of bee collected pollen influence on lipid peroxidation, antioxidant system and liver function in old animals. Hepatology 2003; 38, 203-8. https://doi.org/10.1016/S0168-8278(03)80078-8

Valipour P, Heidarian E, Khoshdel A, Gholami-Arjenaki M. Protective effects of hydroalcoholic extract of ferulago angulata against gentamicin-induced nephrotoxicity in rats. Iran J Kidney Dis 2016; 10: 189-96.