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Effects of the *Portulaca oleracea* Extract on Gentamicin-Induced Nephrotoxicity in Male Rats

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Abstract

Background: Some data suggest the protective effect of *Portulaca oleracea* against renal failure and its association with the preservation of the renal antioxidant status and the regulation of apoptosis.
Objectives: The current study aimed to examine the renoprotective effects of treatment with *Portulaca oleracea* (PO), a prominent hydrogen sulfide donor, in a 5/6 nephrectomy animal model.
Methods: In this experimental study, 32 adult male Wistar rats, initially weighting 200 - 250 g, were housed under standard conditions since the beginning of April 2017 until the end of January 2018. The effect of PO extract on renal dysfunction induced by gentamicin was studied. The male rats were treated with two selected doses of PO (i.e., 200 and 600 mg/kg p.o.) and normal saline (5 mL/kg p.o.) for 28 consecutive days. Gentamicin (80 mg/kg/day intraperitoneally [i.p.]) was administered to two groups for seven days, and they were considered as the PO200 and PO600 gentamicin groups, respectively, while the group administered normal saline (5 mL/kg p.o.) for 35 consecutive days was considered as the control group. The rats were anesthetized on day 36. Then, they were sacrificed under deep anesthesia, and plasma and tissue samples were obtained.
Results: Treatment with PO decreased the renal histological damages and apoptosis induced by gentamicin and enhanced renal function parameters compared to the gentamicin group.
Conclusions: The present findings provide strong evidence to support the traditional medicinal use of this herb by the tribal people in the treatment of renal impairment. Finally, these results support the therapeutic effect of PO in preventing the development of renal dysfunction.

Keywords: Acute Kidney Injury, Apoptosis, Gentamicin, Nephrotoxicity, *Portulaca oleracea*, Renal Insufficiency, Traditional Medicine

1. Background

Acute kidney injury (AKI) is a major clinical complication resulting in mortality and morbidity (1). One of the main causes of AKI is toxin-induced nephrotoxicity, which occurs in 10% - 20% of therapeutic regimens. Gentamicin (GM), as one of the pivotal aminoglycoside antibiotics, is widely used in clinics for its potent antibiotic activity against several Gram-negative microorganisms. The cochlea, kidneys, and the vestibular apparatus are the crucial parts of the body affected by the use of this drug. In addition, its application is limited due to the potential nephrotoxic and ototoxic properties (2). Furthermore, its relationship with mitochondrial impairment in proximal tubules has been well studied (3).

The release of mitochondrial cytochrome c, the intrinsic or mitochondrial pathway, and mitochondrial antioxidant status imbalance have been reported in tubular cells and in rat kidney injuries induced by GM (3). GM can directly trigger inflammation and apoptosis pathways as indicated by the increase in expressions of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), TNF-α receptor type1 (TNF-αR1), COX-2 inhibitor, inducible nitric oxide synthase (iNOS), and caspase-3 as well as the decrease in the expression of B-cell lymphoma-extra-large (Bcl-XL) (4). On the other hand, some data suggest the protective effect of *Portulaca oleracea* (PO) against renal failure and its association with the preservation of the renal antioxidant status and the regulation of apoptosis (5-7).

PO is a wild plant which has been applied as a tradi-

tional medicine in various countries. It has been shown to play major pharmacological roles, such as antibacterial, analgesic, anti-aging, anti-inflammatory, antioxidant, wound-healing, and neuroprotective activities (8-10). Some studies have indicated that PO can also be used to treat diabetic nephropathy by the prevention of renal fibrosis and inflammation (9, 11). It also has the ability to defend ischemia/reperfusion by the inhibition of inflammation (12). However, its mechanism of action has not been clarified.

2. Objectives

The present study was designed to investigate whether the PO can prevent AKI through the restoration of renal function and apoptosis in gentamycin-induced nephrotoxicity in male rats.

3. Methods

3.1. Experimental Animals

This experimental study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran) and was in accordance with the international guidelines for the care and use of laboratory animals (Code of Ethics Committee: 66001656). In this study, 32 adult male Wistar rats (provided from Pasteur Institute of Iran), initially weighting 200 - 250 g, were housed under standard conditions (12 h light/dark cycle; 20 - 22°C) with food and water *ad libitum* since the beginning of April 2017 until the end of January 2018.

3.2. Drugs

Gentamicin was obtained as a gift sample from a pharmaceutical company based in Tehran, Iran.

3.3. Experimental Design

The rats were randomly divided into four groups of eight animals using simple randomization. The following treatments were administered: Group 1: normal saline (5 mL/kg p.o.) for 35 consecutive days, and it was considered as the control group. Group 2: normal saline (5 mL/kg p.o.) for 28 consecutive days. After gentamicin (80 mg/kg/day intraperitoneally [i.p.]) was administered for seven days, and it was considered as the gentamicin group. Group 3: PO extract (200 mg/kg p.o.) for 28 consecutive days. After gentamicin (80 mg/kg/day intraperitoneally [i.p.]) was administered for seven days, and it was considered as the PO 200 group. Group 4: the PO extract (600 mg/kg p.o.) for 28 consecutive days. After gentamicin (80 mg/kg/day intraperitoneally [i.p.]) was administered for seven days, and it was considered as the PO 600 group.

3.4. Preparation of the Plant's Extract

The fresh plant was collected in June 2016 from Varamin city, Tehran Province, Iran. For the extract preparation, 400 g of shadow-dried PO leaves powder was macerated at a ratio of 1:10 (W/V) in distilled water at laboratory temperature for 72 hours. The mixture was filtered and concentrated in an oven at 55°C until dryness of the extract was achieved, and then it was kept at 4°C. The extract concentration was adjusted to 20 mg/mL by adding distilled water to the dried extract.

3.5. Assessment of Nephroprotective Effects

3.5.1. Urine Parameters

The animals were separately kept in metabolic cages. Urine samples were collected 24 hours after the last oral administration on day 28 for the determination of urine volume and fractional excretion of sodium (FENa) based on previous studies (13, 14). In all the groups, the volume of water intake was also measured daily.

3.5.2. Measurement of Blood Creatinine and Urea Concentration

In all the groups, the animals were anesthetized with the intraperitoneal (i.p.) injections of ketamine (75 mg/kg) and xylazine (10 mg/kg) on day 36. Then, the rats were sacrificed under deep anesthesia. Blood samples were collected and serum was collected after 15 minutes of centrifugation at 12000 rpm. Blood creatinine level and blood urea nitrogen (BUN) concentrations were measured by colorimetric methods as described previously (15).

3.5.3. Histopathological Examinations

The right kidney of each animal was dissected and checked for gross morphological abnormalities. Then, the standard procedure for the histopathological assay was performed. Kidney tissues were fixed in a buffered formalin solution and embedded in paraffin, and 3-μm sections were cut with a microtome and stained with Hematoxylin and Eosin (H & E) stain according to standard procedures and the result was examined under a light microscope (16). For each sample, at least eight random non-overlapping areas were observed. The assay was carried out at 100$\times$ magnification for the presence of capillary congestion, leukocyte infiltration, tubular degeneration, and the formation of casts and luminal debris. According to previous studies, sections were scored as follows:

0: minimal or no lesions;

1: $<$ 25% of tubules were involved;

2: 25% - 50% of tubules were involved;

3: $>$ 50% of tubules were involved (17).

3.5.4. TUNEL Staining

Cellular apoptosis was analyzed by performing a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay using an in situ cell death detection kit (TUNEL; In Situ Cell Death Detection Kit, 11684817910 Roche) following the kit protocol. The numbers of TUNEL-positive and total cells were counted under a fluorescence microscope.

3.5.5. Statistical Analysis

Departure from normality assumption was assessed by the Kolmogorov-Smirnov test. Mean ± standard error of mean (MEN) or median (inter-quintile range) was used for presenting the data with normal or non-normal distribution, respectively. Statistical differences between the groups were compared by one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. P value of less than 0.05 was considered statistically significant.

4. Results

4.1. Experimental Animals

Renal function parameters were enhanced due to the treatment with the PO extract in gentamicin-induced kidney injury.

The biochemical assay results showed that the PO extract caused a significant decrease in urine creatinine level compared to the gentamicin group ($P < 0.01$; Figure 1A). Also, there was no significant difference between the two extract-treated groups and between these two groups and the control group. As shown in Figure 1C, an increase in FENa could be detected in the gentamicin group; thus, there was a significant reduction in FENa level due to the extract treatment in both 200 and 600 PO groups. In line with this finding, a significant decrease in BUN level in the extract-treated groups compared to the gentamicin group was observed ($P < 0.01$; Figure 1A). Moreover, the findings showed that in the PO200 and PO600 groups, there were more renal function improvements compared to the gentamicin group as the water intake volume decreased in the extract-treated groups (Figure 1D). Long-term treatment with PO extract reduced histopathological damages in gentamicin-induced kidney injury.

As exhibited in Figure 2, the histological results determined that gentamicin treatment for seven days in adult rats led to the widening of the Bowman's space in the glomeruli, capillary congestion, leukocyte infiltration, and tubular degeneration in the kidneys. In addition, tubular debris was present and leukocytes infiltrations were also frequently detected in these tissues. The results indicated that PO treatment for four weeks in adult rats attenuated the effects of gentamicin on renal histological scoring, which is showed in Figure 2. PO extract treatment at both 200 and 600 mg/kg doses led to lower degrees of Bowman's space widening, and less tubular casts were present in this group as compared to the gentamicin-induced kidney injury group.

The PO extract treatment prevented renal tubular cell apoptosis induced by gentamicin in rats.

The results of the TUNEL assay further confirmed that gentamicin induced apoptosis in renal tubular cells in adult rats. As shown in Figure 3, the percentage of TUNEL-positive cells dramatically increased in the gentamicin group compared to the control group. However, the results confirmed the protective effect of the PO extract on gentamicin-induced apoptosis. As presented in Figure 3, it was determined that the number of apoptotic cells was significantly lower in the PO200 and PO600 groups compared to the gentamicin group.

5. Discussion

In the present study, it was aimed to evaluate the effectiveness of the PO extract as a potential candidate for preventing gentamicin-induced nephrotoxicity. The key findings of the current study were: 1) the PO extract reduced BUN, Cr, and water intake in nephrotoxicity with gentamicin-treated Wistar rats, which was accompanied by the reduction of FENa level, 2) in the rats' model of gentamicin-induced nephrotoxicity, PO extract treatment reduced apoptosis, and 3) these results were supported by the histological improvements and reduced kidney injury in gentamicin-induced rats because the rats were treated after four weeks of *Portulaca oleracea* extract treatment.

In this study, it was demonstrated that the aqueous extract of PO prevented the development of gentamicin nephrotoxicity symptoms including renal impairment through the improvement of renal function and apoptosis in the gentamicin-induced nephrotoxicity rats. Nephrotoxicity was considered as one of the most common complications of the use of aminoglycosides characterized by increased apoptosis and loss of renal functions (9, 18). Rodent models of gentamicin-induced renal injury are of prime importance in understanding the mechanisms of nephrotoxicity and may assist with providing an effective therapy for the management of renal failure in clinical settings.

The loss of renal function in gentamicin-induced rats implied the development of renal failure, which may increase morbidity and mortality in these subjects (19). Hence, a significant increase in apoptosis may stimulate

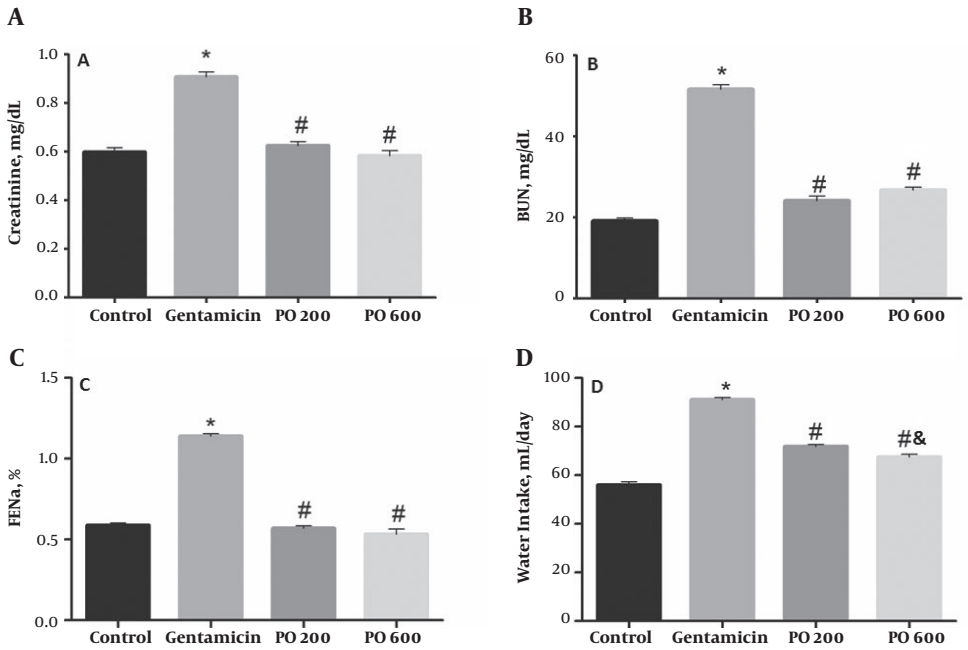

Figure 1. Effects of the *Portulaca oleracea* extract [(PO), 200 and 600 mg/kg p.o.] on renal function markers including: Blood Creatinine (A), Blood Urea Nitrogen (BUN; B), Fractional excretion of sodium (FENa; C), and daily water intake volume (D). Data represent mean ± SEM (n = 6); * P < 0.05 vs. Control group, # P <0.05 vs. Gentamicin group and & P < 0.05 vs. PO200 group. PO group rats were treated with the PO extract before gentamicin injections.

inflammatory-cell infiltration and, when severe, can lead to necrosis (20). Such inflammatory processes, especially in the presence of ROS, may induce cytotoxic effects and cause apoptotic cell death. Renal cell necrosis and apoptosis are the crucial pathological changes that take place following renal damages. Likewise, the decrement of cell necrosis and apoptosis are considered to be beneficial (21).

In the TUNEL assay, when DNA strands are disported by nucleases, 30-hydroxyl terminals are exposed. Hydroxyl groups may then be present as a starting point for terminal deoxynucleotidyl transferase (TdT) enhancing deoxyribonucleotides in a template-independent manner (22). Apoptosis induced by aminoglycoside nephrotoxicity has become an important topic.

Apoptosis has recently been considered as a pivotal mechanism of cell death interposing chemical and hypoxic damages to epithelial cells. Apoptosis has been proved to take place in the kidney ensuing ischemic injury (23, 24). The actions leading to apoptosis are so complicated that they may initiate the apoptosis process and are suspected to be present in the deprivation of growth factors, mitochondrial damage, ROS, F-actin cytoskeletal injury, and the disturbance of intracellular Ca^{2+} homeostasis (25, 26).

The responsible mechanism(s) for apoptosis in GM-induced injury is not well known. The competency of GM to change mitochondrial respiration has been well reported in evidence from both in vitro and in vivo studies (27). Gentamicin leads the cell to apoptosis by the p53 pathway. One possible mechanism clarifying the enhancement of p53 levels induced by gentamicin is reactive oxygen species production (28). Gentamicin-induced nephrotoxicity is defined functionally by enhanced serum creatinine and blood urea nitrogen.

The PO extract significantly reduced BUN and creatinine concentrations in gentamicin-induced nephrotoxic animals. This observation was in line with the findings of Tavafi et al. (29) who stated that the enhancement of serum creatinine and urea were reversed significantly in animals treated with olive leaf extract in comparison with gentamicin-treated animals. In addition, the present study's findings on PO confirm the protective effect of grape seed extract on gentamicin-induced AKI suggested by Ezejiofor et al. (30) and Safa et al. (31).

In recent years, it has been reported that anti-apoptosis agents protect the kidney against nephrotoxicity by reducing renal injury through the inhibition of apoptosis factors like caspase 3 (32, 33). Herein, the results showed that plasma concentrations of BUN, Cr, and FENa were significantly higher in the rat model of gentamicin-induced

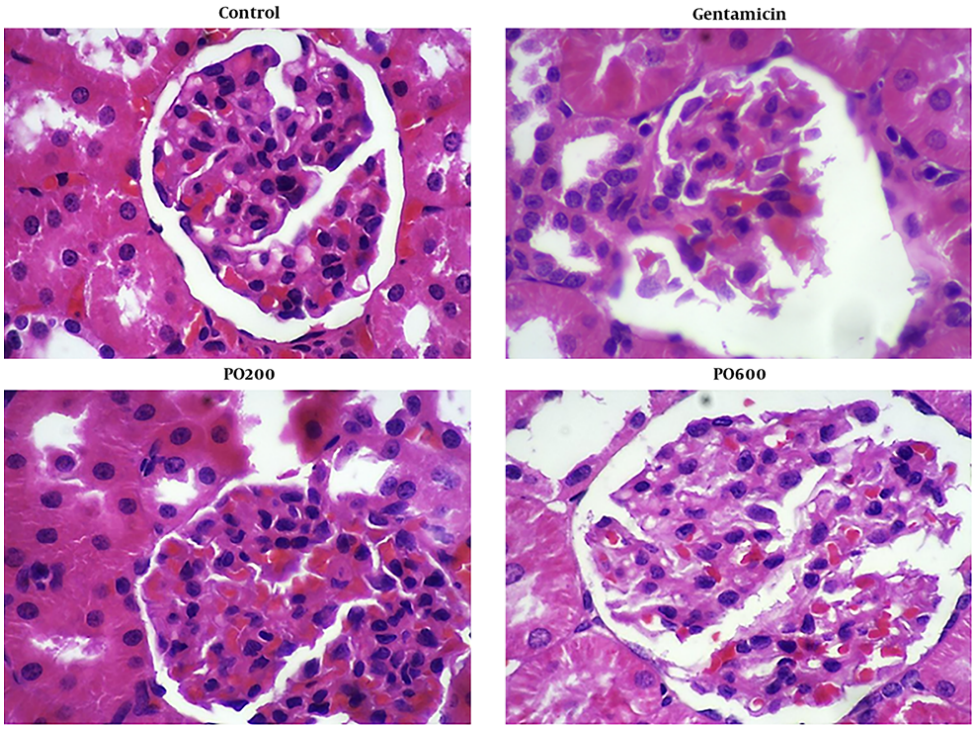

Figure 2. Effects of the *Portulaca oleracea* (PO) extract on renal histological changes induced by gentamicin in the control group, Gentamicin group, PO200 group, and PO600 group. * Kidney sections were stained with hematoxylin and eosin (H & E) and examined by a light microscope (400 × magnification).

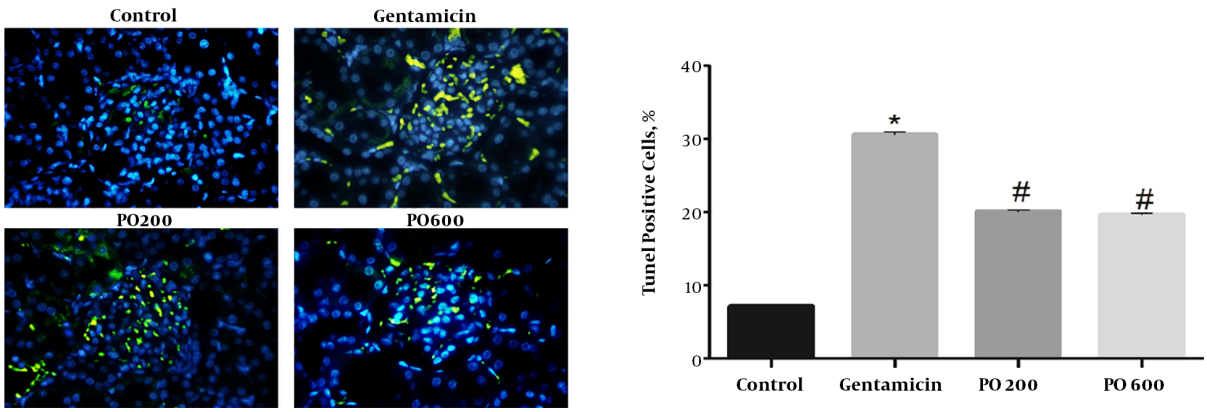

Figure 3. Effects of the *Portulaca oleracea* extract (PO) on gentamicin-induced apoptosis in the control group, gentamicin group, PO200 group and PO600 group. Cell apoptosis was determined by the TUNEL assay. Apoptotic cell nuclei were stained by the TUNEL assay with green, and all nuclei were stained by DAPI with blue (original magnification, ×100). TUNEL-positive cells percentage was calculated. Data are shown as mean ± SEM (n = 6); * P < 0.05 vs. Control group and # P < 0.05 vs. Gentamicin group.

nephrotoxicity as compared to sham subjects. Furthermore, water intake considerably increased in these subjects. It seems that the overexpression of apoptotic cells is in accordance with our observations about the histology findings regarding gentamicin-induced nephrotoxicity in rats, which could contribute to the deterioration of renal function.

In the present study, PO treatment ameliorated renal function markers such as plasma creatinine, BUN, FENa, and water intake. This suggests a correlation between these markers and feeling thirsty in the present model. Furthermore, PO extract improved renal apoptosis, sug-

gesting the possible beneficial role of PO against renal nephrotoxicity in the gentamicin-induced rats. PO is used as a vegetable and administered pharmaceutically for different conditions. In the present study, PO had an anti-apoptotic effect and a preventive effect against the pathological mechanism of gentamicin-induced nephrotoxicity through the inhibition of renal apoptosis and leukocyte infiltration in the gentamicin-induced rats.

The present findings provide intense evidence supporting the traditional medicinal use of this herb by the tribal people in the treatment of renal impairment. These results also substantiate the therapeutic effect of PO in preventing the development of renal dysfunction.

Footnotes

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Approval: This experimental study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran) and was in accordance with the international guidelines for the care and use of laboratory animals (Code of Ethics Committee: 66001656).

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References

1. Chen Q, Cui Y, Ding G, Jia Z, Zhang Y, Zhang A, et al. PEA3 protects against gentamicin nephrotoxicity: Role of mitochondrial dysfunction. *Am J Transl Res*. 2017;**9**(5):2153–62. [PubMed: 28559968]. [PubMed Central: PMC5446500].
2. Kaplan HM, Singirik E, Erdogan KE, Doran F. Protective effect of alpha-linolenic acid on gentamicin-induced ototoxicity in mice. *Somatosens Mot Res*. 2017;**34**(3):145–50. doi: 10.1080/08990220.2017.1356283. [PubMed: 28760048].
3. Negrette-Guzman M, Garcia-Nino WR, Tapia E, Zazueta C, Huerta-Yepez S, Leon-Contreras JC, et al. Curcumin attenuates gentamicin-induced kidney mitochondrial alterations: Possible role of a mitochondrial biogenesis mechanism. *Evid Based Complement Alternat Med*. 2015;**2015**:917435. doi: 10.1155/2015/917435. [PubMed: 26345660]. [PubMed Central: PMC4541007].
4. Arjinajarn P, Chueakula N, Pongchaidecha A, Jaikumkao K, Chatsudthipong V, Mahatheeranont S, et al. Anthocyanin-rich Riceberry bran extract attenuates gentamicin-induced hepatotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. *Biomed Pharmacother*. 2017;**92**:412–20. doi: 10.1016/j.biopha.2017.05.100. [PubMed: 28558354].
5. Abd El-Azime AS, Hussein EM, Ashry OM. Synergestic effect of aqueous purslane (Portulaca oleracea L.) extract and fish oil on radiation-induced damage in rats. *Int J Radiat Biol*. 2014;**90**(12):1184–90. doi: 10.3109/09553002.2014.926040. [PubMed: 24882390].
6. Jaradat NA, Zaid AN, Al-Ramahi R, Alqub MA, Hussein F, Hamdan Z, et al. Ethnopharmacological survey of medicinal plants practiced by traditional healers and herbalists for treatment of some urological diseases in the West Bank/Palestine. *BMC Complement Altern Med*. 2017;**17**(1):255. doi: 10.1186/s12906-017-1758-4. [PubMed: 28482898]. [PubMed Central: PMC5422913].
7. Abdel Moneim AE. The neuroprotective effects of purslane (Portulaca oleracea) on rotenone-induced biochemical changes and apoptosis in brain of rat. *CNS Neurol Disord Drug Targets*. 2013;**12**(6):830–41. [PubMed: 23844694].
8. Kaveh M, Eidi A, Nemati A, Boskabady MH. Modulation of lung inflammation and immune markers in asthmatic rats treated by Portulaca oleracea. *Avicenna J Phytomed*. 2017;**7**(5):409–16. [PubMed: 29062802]. [PubMed Central: PMC5641415].
9. Lee AS, Lee YJ, Lee SM, Yoon JJ, Kim JS, Kang DG, et al. An aqueous extract of Portulaca oleracea ameliorates diabetic nephropathy through suppression of renal fibrosis and inflammation in diabetic db/db mice. *Am J Chin Med*. 2012;**40**(3):495–510. doi: 10.1142/S0192415X12500383. [PubMed: 22745066].
10. Wang PP, Sun HX, Liu CJ, Hu MH, He XQ, Yue S, et al. Racemic oleracein E increases the survival rate and attenuates memory impairment in D-galactose/NaNO(2)-induced senescent mice. *Phytomedicine*. 2016;**23**(5):460–7. doi: 10.1016/j.phymed.2016.02.014. [PubMed: 27064004].
11. Dehghan F, Soori R, Gholami K, Abolmaesoomi M, Yusof A, Muniandy S, et al. Purslane (Portulaca oleracea) seed consumption and aerobic training improves biomarkers associated with atherosclerosis in women with type 2 diabetes (T2D). *Sci Rep*. 2016;**6**:37819. doi: 10.1038/srep37819. [PubMed: 27917862]. [PubMed Central: PMC5137030].
12. Zheng C, Liu C, Wang W, Tang G, Dong L, Zhou J, et al. Ethanol extracts from Portulaca oleracea L. attenuated ischemia/reperfusion induced rat neural injury through inhibition of HMGB1 induced inflammation. *Am J Transl Res*. 2016;**8**(11):5016–24. [PubMed: 27904702]. [PubMed Central: PMC5126344].
13. Hammad FT, Lubbad L. The effect of thymoquinone on the renal functions following ischemia-reperfusion injury in the rat. *Int J Physiol Pathophysiol Pharmacol*. 2016;**8**(4):152–9. [PubMed: 28078054]. [PubMed Central: PMC5209444].
14. Elshiekh M, Kadkhodaee M, Seifi B, Ranjbaran M, Askari H. Up-regulation of nitric oxide synthases by erythropoietin alone or in conjunction with ischemic preconditioning in ischemia reperfusion injury of rat kidneys. *Gen Physiol Biophys*. 2017;**36**(3):281–8. doi: 10.4149/gpb_2016058. [PubMed: 28471344].
15. Askari H, Seifi B, Kadkhodaee M. Evaluation of renal-hepatic functional indices and blood pressure based on the progress of time in a rat model of chronic kidney disease. *Nephrourol Mon*. 2016;**8**(3). e37840. doi: 10.5812/numonthly.37840. [PubMed: 27570756]. [PubMed Central: PMC4983449].
16. Ramasamy A, Jothivel N, Das S, Swapna A, Albert AP, Barnwal P, et al. Evaluation of the protective role of glycine max seed extract (soybean oil) in drug-induced nephrotoxicity in experimental rats. *J Diet Suppl*. 2018;**15**(5):583–95. doi: 10.1080/19390211.2017.1358792. [PubMed: 28956655].
17. Jablonski P, Howden BO, Rae DA, Birrell CS, Marshall VC, Tange J. An experimental model for assessment of renal recovery from warm ischemia. *Transplantation*. 1983;**35**(3):198–204. [PubMed: 6340272].
18. 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. doi: 10.1016/j.lfs.2016.09.014. [PubMed: 27664833].
19. Ross AD, Boscainos PJ, Malhas A, Wigderowitz C. Peri-operative renal morbidity secondary to gentamicin and flucloxacillin chemoprophylaxis for hip and knee arthroplasty. *Scott Med J*. 2013;**58**(4):209–12. doi: 10.1177/0036933013507850. [PubMed: 24215038].
20. Ueda N, Shah SV. Tubular cell damage in acute renal failure-apoptosis, necrosis, or both. *Nephrol Dial Transplant*. 2000;**15**(3):318–23. [PubMed: 10692516].
21. Prozialeck WC, Edwards JR, Lamar PC, Liu J, Vaidya VS, Bonventre JV. Expression of kidney injury molecule-1 (Kim-1) in relation to necrosis and apoptosis during the early stages of Cd-induced

proximal tubule injury. *Toxicol Appl Pharmacol*. 2009;**238**(3):306–14. doi: 10.1016/j.taap.2009.01.016. [PubMed: 19371613]. [PubMed Central: PMC2709705].
22. Lee S, Kim KH, Park C, Lee JS, Kim YH. Portulaca oleracea extracts protect human keratinocytes and fibroblasts from UV-induced apoptosis. *Exp Dermatol*. 2014;**23 Suppl 1**:13–7. doi: 10.1111/exd.12396. [PubMed: 25234830].
23. Savill J. Apoptosis and the kidney. *J Am Soc Nephrol*. 1994;**5**(1):12–21. [PubMed: 7948779].
24. Haghi-Aminjan H, Asghari MH, Goharbari MH, Abdollahi M. A systematic review on potential mechanisms of minocycline in kidney diseases. *Pharmacol Rep*. 2017;**69**(4):602–9. doi: 10.1016/j.pharep.2017.02.001. [PubMed: 28500967].
25. Hagar H, Ueda N, Shah SV. Role of reactive oxygen metabolites in DNA damage and cell death in chemical hypoxic injury to LLC-PK1 cells. *Am J Physiol*. 1996;**271**(1 Pt 2):F209–15. doi: 10.1152/ajprenal.1996.271.1.F209. [PubMed: 8760263].
26. Ueda N, Shah SV. Apoptosis. *J Lab Clin Med*. 1994;**124**(2):169–77. [PubMed: 8051480].
27. Choi KH, Kim TI, Chong DL, Lee HY, Han DS. Gentamicin induced apoptosis of renal tubular epithelial (LLC-PK1) cells. *Korean J Intern Med*. 2000;**15**(3):218–23. [PubMed: 11242810]. [PubMed Central: PMC4531773].
28. Denamur S, Boland L, Beyaert M, Verstraeten SL, Fillet M, Tulkens PM, et al. Subcellular mechanisms involved in apoptosis induced by aminoglycoside antibiotics: Insights on p53, proteasome and endoplasmic reticulum. *Toxicol Appl Pharmacol*. 2016;**309**:24–36. doi: 10.1016/j.taap.2016.08.020. [PubMed: 27568863].
29. Tavafi M, Ahmadvand H, Toolabi P. Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. *Iran J Kidney Dis*. 2012;**6**(1):25–32. [PubMed: 22218116].
30. Ezejiofor AN, Orish CN, Orisakwe OE. Costus afer ker gawl leaves against gentamicin-induced nephrotoxicity in rats. *Iran J Kidney Dis*. 2014;**8**(4):310–3. [PubMed: 25001137].
31. Safa J, Argani H, Bastani B, Nezami N, Rahimi Ardebili B, Ghorbanihaghjo A, et al. Protective effect of grape seed extract on gentamicin-induced acute kidney injury. *Iran J Kidney Dis*. 2010;**4**(4):285–91. [PubMed: 20852368].
32. Ozkok A, Edelstein CL. Pathophysiology of cisplatin-induced acute kidney injury. *Biomed Res Int*. 2014;**2014**:967826. doi: 10.1155/2014/967826. [PubMed: 25165721]. [PubMed Central: PMC4140112].
33. Askari H, Raeis Abdullahi E, Poortahmasebi V, Foad Abazari M, Asghari MH, Sadeghi S, et al. Molecular mechanisms underlying the nephrotoxicity of cisplatin, lead acetate and cyclosporine: Key roles of myc and smad4. *Biol Syst Open Access*. 2017;**6**(1). doi: 10.4172/2329-6577.1000176.