Protective effect of hydroalcoholic extract of *Pistacia vera* against gentamicin-induced nephrotoxicity in rats

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ABSTRACT

**Purpose:** *Pistacia vera* is a plant of the family Anacardiaceae found in Central and West Asia. *P. vera* nut (Pistachio) possess multiple pharmacological effects such as antimicrobial, anti-hyperlipidemia, antioxidant and anti-inflammatory. This study is designed to evaluate the protective effect of the hydroalcoholic extract of pistachio on gentamicin-induced nephrotoxicity in rats.

**Methods:** Nephrotoxicity was induced in rats by intraperitoneal injection of gentamicin (100 mg/kg/day for 7 days). Hydroalcoholic extract of pistachio (10, 50 and 100 mg/kg/p.o) was administered for 7 days. The nephroprotective activity was evaluated by determining creatinine clearance, serum creatinine, urine volume, urine glucose and blood urea nitrogen (BUN) levels. The kidneys were processed for histopathological examinations and all specimens were examined for morphologic parameters involving tubular degeneration, tubular necrosis and tubule interstitial nephritis.

**Results:** Results showed a significant increase in the levels of serum creatinine, urine volume, urine glucose and BUN and decrease of creatinine clearance by gentamicin (GA) administration. Co-administration with pistachio extract showed reduction in the levels of serum creatinine, urine volume, urine glucose and BUN and increase of creatinine clearance in all doses but the most significant alteration was observed in doses of 100 mg/kg. Also, the nephroprotective effect of the GA was confirmed by the histological examination of the kidneys.

**Conclusion:** The study revealed the nephroprotective effect of the hydroalcoholic extract of pistachio. These findings suggest that pistachio treatment may attenuate renal dysfunction and structural damage through the reduction of oxidative stress and inflammation in the kidney.

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Introduction

Acute renal failure (ARF) refers to a sudden and usually reversible decrease in kidney function [1]. The pathogenesis of ARF is complex, however ischemia or toxins are known as the major underlying factors [2]. Nephrotoxic drugs such as cisplatin and aminoglycoside antibiotics are the main causes for nearly 20% of all ARF cases in intensive care units [3]. Aminoglycoside antibiotics – such as gentamicin (GM) – are used as effective agents against gram-negative bacteria infections [4]. About 30% of the patients, undergone GM treatment for more than seven days, display signs of nephrotoxicity [5]. The cellular mechanism/s of GM-induced nephrotoxicity is still poorly understood. Reactive oxygen species (ROS) have important role in pathological mechanisms of GM-induced ARF. Production and accumulation of ROS results in induction of apoptosis, tubular necrosis and increased infiltration of leukocyte [6]. This GM-induced ARF is clinically characterized by an increase in serum creatinine levels and urea nitrogen, a reduction in the glomerular filtration rate (GFR) and urine osmolality [7].

Several lines of evidence support the use of plant extracts for the prevention and attenuation of ARF [8]. *Pistacia vera (P. vera)* (family: Anacardiaceae) is native of arid zones of Central and West Asia and has commonly been used in traditional herbal medicine [9]. Pistachio (*P. vera* nut) have a valuable nutrient profile. It is a unique source of unsaturated fatty acids and numerous...
antioxidants, including α-tocopherol, β-carotene, lutein, selenium, flavonoids and phytoestrogens [10]. Previous studies have provided evidence suggesting various pharmacological properties for *P. vera* including antioxidant [11], anti-microbial [12], anti-nociceptive, anti-inflammatory [13] and hepatoprotective effect [14]. It has been shown that pistachio consumption has positive effects on serum lipid profile and CVD risk factors in hypercholesterolemic humans [15]. In a recent study in humans, it was observed that pistachio diet significantly improved oxidative status and decreased circulating inflammatory biomarkers [16].

Inflammation and ROS play significant roles in pathophysiology of ARF [17]; therefore, administration of compounds with antioxidant and anti-inflammatory properties induces ameliorative effects. The present study was designed to investigate the effect of hydroalcoholic extract of *P. vera* in a rat model of GM-induced ARF.

**Materials and methods**

**Plant material and extraction method**

Dried Pistachio from *Akbari* species with genetic code of M30 were purchased from an herbal pharmacy in Rafsanjan, Iran. In order to prepare the required extract, dried and finely powdered fruits (100 g) were macerated in 1 L of methanol (80%) for 72 h to obtain the whole extract using the percolation method. Extract vehicle was evaporated in a rotary under low pressure. The extract was then frozen and stored at −20°C. For administration, the frozen pistachio extract (PE) was freshly dissolved in dimethyl sulfoxide 10% (DMSO, Sigma-Aldrich, Germany).

**Animals**

Forty-nine male Wistar rats (250–300 g) were obtained from the animal house of School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Animals were housed in polycarbonate cages under 24 ± 2°C room temperature with a 12-h light/dark cycle and *ad libitum* access to food and water. All experiments were performed in accordance with the guidelines set by the ethical committee of Rafsanjan University of Medical Sciences and the European Communities Council Directive 24 November 1986 (86/609/EEC).

**Experimental design**

Animals were divided into seven experimental groups as follows: group 1 (Control group) did not receive any solvent or drug during experiments and received a usual diet; group 2 (GM group) received 100 mg/kg of GA (Alborz Co, Tehran, Iran) intraperitoneally (i.p.) for 7 days; group 3 (DMSO group) received i.p. injections of 100 mg/kg of GA and DMSO 10% orally for 7 days; group 4 (D10 group) received i.p. injections of 100 mg/kg of GA and PE orally at the dose of 10 mg/kg for 7 days; group 5 (D50 group) received i.p. injections of 100 mg/kg of GA and PE orally at the dose of 50 mg/kg for 7 days; group 6 (D100 group) received i.p. injections of 100 mg/kg of GA and PE orally at the dose of 100 mg/kg for 7 days and group 7 (Extract 100 group) received PE orally at the dose of 100 mg/kg for 7 days to assess the possible toxic effects of PE.

**Sample collection and biochemical assays**

On day 7 of experiment, 24-h urine samples were collected for measurement of urine volume and glucose concentration. Animals were sacrificed on day 8 of experiment, using ether anesthesia. Blood samples were taken by cardiac puncture and kept for 1 h at 4°C. These were then centrifuged at 3000 rpm for 15 min to separate serum. The serum samples were stored for measurement of the blood urea nitrogen (BUN) and serum creatinine. The GFR (mL/24 h) was estimated by creatinine clearance. The serum and urine creatinine concentrations were determined by Jaffe’s method. BUN was measured colorimetrically using Autoanalyzer (Technicon RA-1000, London, England) and urea kit (Man Lab Company, Tehran, Iran). Urinary glucose concentration was measured by the enzymatic assay (glucose oxidase) and protein concentration was assessed via turbidimetric method.

**Histopathological examination**

Both kidneys were immediately removed and fixed in 10% neutral buffered formalin for histopathological examinations. The kidney tissues were dissected out, washed by normal saline solution (0.9%) and then fixed in 10% formalin solution for 48 h. The kidneys were processed for dehydration using absolute ethanol, cleaned in xylene, embedded in paraffin and sectioned for histopathological evaluations. The prepared sections were stained with haematoxylin and eosin and were then visually observed under light microscope. All specimens were examined for three morphologic parameters, including tubular degeneration (TD), tubular necrosis (TN) and tubule interstitial nephritis (TIN) on a semiquantitative score from 0 to 4 [1]. The score of zero was assigned to the normal tissue with no damage.
Statistical analyses

Statistical analyses were performed by Excel 2007 (Microsoft Corporation, Seattle, WA) and SPSS 18 software (SPSS Inc, Chicago, IL). Results are presented as mean ± standard error of the mean (SEM). Differences between groups were determined using ANOVA followed by the Tukey post hoc test. Values of $p < .05$ were considered significant.

Results

Biochemical assays

PE induced a significant nephroprotective effect and most GM-induced renal alterations were not observed following co-administration of PE + GM (Figure 1). In animals treated with 100 mg/kg PE, the concentrations of BUN ($p < .05$), serum creatinine ($p < .05$), urine volume ($p < .05$) and urine glucose ($p < .001$) were significantly decreased compared to the GM group, however creatinine clearance ($p < .05$) showed a significant increase compared to the GM group. In rats treated with 50 mg/kg PE, the concentrations of urine glucose ($p < .001$) were significantly decreased compared to the GM group, but creatinine clearance ($p < .01$) showed a significant increase compared to the GM group. In groups treated with 10 mg/kg PE, the concentrations of serum creatinine ($p < .01$) and urine glucose ($p < .001$) were significantly decreased compared to the GM group. In addition, administration of 100 mg/kg PE did not elicit any clinical sign of toxicity, renal dysfunction and mortality for a period of 7 days.

Histopathology

In order to evaluate the effect of PE on the histological changes in the kidney, H&E staining was performed (Figure 2). Histopathological scores of TD, TN and TIN in all experimental groups were graded (Figure 3). Sections from kidney tissues of GM treated rats showed massive TD, TN and TIN (Figure 3), while co-administration of PE + GM reduced these parameters in renal tissues compared to the GM group in a dose–response manner. Inanition, administration of 100 mg/kg PE did not cause any detectable alteration in the renal structure of the normal rat.

Figure 1. Effect of PE (10, 50 and 100 mg/kg) on concentrations of BUN (A), serum creatinine (B), creatinine clearance (C), urine volume (D) and urine glucose (E) in rats with GM-induced ARF. Data are expressed as mean ± SEM and analyzed by one-way ANOVA followed by post hoc Tukey tests. *$p < .05$, **$p < .01$, ***$p < .001$ as compared with the GM group and #$p < .05$, ##$p < .01$, ###$p < .001$ as compared with the control group.
Figure 2. Effect of PE on the morphology of the rat kidneys with GM-induced ARF. Control group: healthy kidney structure was seen. The glomerulus (arrow) and tubules (bent arrow) are normal (A: ×20 and B: ×40). GM group: kidney is severely damaged. Acute tubular necrosis (filled arrow) and extensive tubular degeneration (thick arrow) were seen. Sever leukocyte infiltrations in intertubular area were also found (star) (C: ×20 and D: ×40). D50: minimal tubular necrosis and tubular degeneration (thick arrow) were observed. Slight leukocyte infiltrations in intertubular area are still seen (E: ×20 and F: ×40). D100: showed dramatic improvement in the morphologic appearance. Tubular degeneration (thick arrow) and leukocyte infiltrations have been recovered (G: ×20 and H: ×40).
Discussion

In the present study, the effect of PE on GM-induced ARF was investigated in rats. Results indicated that intraperitoneal administration of GM (100 mg/kg) results in significant nephrotoxicity as evidenced by increase in serum creatinine, urine volume, urine glucose and BUN levels as well as sever TD, TN and TIN which was consistent with previous reports [5,18,19]. Treatment with PE increased the GM-induced attenuation of creatinine clearance and decreased the GM-induced enhancement of serum creatinine, urine volume, urine glucose and BUN levels. Moreover, we found that administration of PE for 7 days significantly decreased the TD, TN and TIN scores. We also demonstrated that administration of PE (100 mg/kg) in normal rats for 7 days did not alter the kidney morphologically and functionally. The results of the present study, for the first time, indicated that oral administration of PE had a significant and to some extent dose-dependent protective effect on GA-induced nephrotoxicity in rats.

Aminoglycosides are commonly used against gram-negative pathogens [20]. In recent years, the consumption of these drugs has been reduced due to the induction of nephrotoxicity and ototoxicity. Among the aminoglycoside antibiotics GA has been used worldwide due to its availability, effectiveness and cost especially in developing countries [21].

Currently, it is well established that the most important mechanism of GM-induced nephrotoxicity is overproduction of ROS like hydroxide and hydrogen peroxide causing renal cell damage [22]. This overproduction of ROS is associated with depletion of renal antioxidant enzymes [23]. ROS damage the protein molecules and alter the cellular membrane integrity via lipid peroxidation processes which in turn results in morphological and functional changes [24]. The nephroprotective effects of antioxidant compounds have been reported. Sahu et al. have shown that naringin attenuates renal dysfunction and GM-induced structural damage via reducing the oxidative stress. They suggested that antioxidative effect of naringin reduces the inflammation and apoptosis in the kidney. In another study, Jafarey et al. suggested that the antioxidative effect of calcium dobesilate mitigates the nephrotoxicity caused by GA [25]. In addition, pistachio has been ranked among the 50 antioxidant-rich foods [26]. Pistachio have some component with high antioxidant activity such as polyphenols, tocopherols, lutein, phytosterols, vitamin B6, gallic acid and carotenoids [27]. Kocyigit et al. have shown that the consumption of pistachio significantly decreases oxidative stress and improves plasma lipid profile in healthy volunteers [28]. Also, Shahrai et al. have reported the hepatoprotective effect of PE against ROS formation and lipid peroxidation. They have demonstrated that methanolic extract of pistachio has ROS and carbonyl scavenging activity and inhibits lipid peroxidation process [9]. Moreover, in a recent study in humans Gentile et al. showed that pistachio significantly improves the oxidative status and reduces the circulating inflammatory biomarkers in inflammatory bowel diseases [16]. These observations support the hypothesis that the nephroprotective effect of PE might be attributed to direct attenuation of ROS (antioxidant activity) and reinforcement of the antioxidant system.

Our results showed that the concurrent administration of PE significantly decreases the histopathological scores compared to the GM-treated group. Accumulation of GM in the renal tubules is another mechanism underlying GM-induced nephrotoxicity [6]. This accumulation could result in tubular degeneration and necrosis as well as stimulating inflammatory events and promoting the migration of monocytes and macrophages at the site of renal injury [29]. Attenuating the inflammatory processes and leukocytes recruitment have been shown to improve the GFR and renal functional parameters [22]. It has been reported that treatment with GA increases NF-κB activation, cyclooxygenase-2 expression [2] and levels of pro-inflammatory cytokines such as TNF-α and IL-6 [22]. On the other hand, the anti-inflammatory effects of P. vera have
been previously demonstrated. Gentile et al. have shown that pistachio decreases cyclooxygenase-2 expression, IL-6 and IL-8 release and NF-kB activation [16]. Accordingly, *P. vera* may possibly improve histopathological scores and decrease leukocytes infiltration through the suppression of inflammatory process.

Treatment with several herbal extracts has been extensively studied and shown to be useful for either the prevention or amelioration of drug-induced nephrotoxicity [30–33]. Boroushaki and Sadeghnia demonstrated the protective effect of Safranal (the main constituents of saffron extract) against GM-induced nephrotoxicity in rat [34]. In another study, Kang et al. suggested that *Houttuynia cordata* induces renoprotection by reduction of oxidative stress in GM-induced ARF [35]. Moreover, Nasri et al. reported the protective effect of Garlic against GM-induced nephrotoxicity [36]. The renoprotective activities of these plants have been attributed to the antioxidant properties of the plants. Other antioxidants have also revealed renoprotection against nephrototoxic agents [30,37–39]. Hence, the renoprotective property of *P. vera*, at least in part, might be related to its antioxidant activity. There are a lot of other plants with antioxidant activity [40–42], and, if we accept this conclusion, they should also have renoprotective activity, which worth examining.

**Conclusion**

The data gathered in the present study suggest that the methanolic extract of *P. vera* possesses potential protective activity against GM-induced ARF. We also found that treatment with PE increases creatinine clearance and attenuates the serum creatinine, urine volume, urine glucose, BUN levels and histopathological scores. This may stand to reason that PE has antioxidant and anti-inflammatory effects. However, further investigations are required to unveil the precise underlying cellular mechanisms.

**Disclosure statement**

The authors declare no conflict of interest relevant to this article.

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**References**

[1] Hur E, Garip A, Camyar A, et al. The effects of vitamin d on gentamicin-induced acute kidney injury in experimental rat model. Int J Endocrinol. 2013;2013:313528.
[2] Jain A, Nahata A, Singhai AK. Effect of *Tephrosia purpurea* (L.) pers. Leaves on gentamicin-induced nephrotoxicity in rats. Sci Pharm. 2013;81:1071–1087.
[3] Uchino S, Kellum JA, Bellomo R, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005;294:813–818.
[4] Nagai J, Takano M. Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokinet. 2004;19:159–170.
[5] Abdel-Rahem IT, Abdel-Ghany AA, Mohamed GA. Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats. Biol Pharm Bull. 2009;32:61–67.
[6] Martinez-Salgado C, Eleno N, Tavares P, et al. Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. Kidney Int. 2002;62:1682–1692.
[7] Kidwell DT, McKeown JW, Grider JS, et al. Acute effects of gentamicin on thick ascending limb function in the rat. Eur J Pharmacol Environ Toxicol Pharmacol. 1994;270:97–103.
[8] Nasri H, Ardalan M-R, Rafieian-Kopaei M. Mechanistic impacts of medicinal plants in diabetic kidney disease. Iran J Public Health. 2014;43:1311.
[9] Shahraki J, Zareh M, Kamalinejad M, et al. Cytoprotective effects of hydrophilic and lipophilic extracts of *Pistacia vera* against oxidative versus carbonyl stress in rat hepatocytes. Iran J Pharm Res. 2014;13:1263.
[10] Tokuşoğlu Ö, Unal MK, Yemis F. Determination of the phytoalexin resveratrol (3,5,4’-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatographic diode array (HPLC-DAD) and gas chromatography – mass spectrometry (GC–MS). J Agric Food Chem. 2005;53:5003–5009.
[11] Tomaino A, Martorana M, Arcoraci T, et al. Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L. variety Bronte) seeds and skins. Biochimie. 2010;92:1115–1122.
[12] Benhammou N, Bekkara FA, Panovska TK. Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts. Afr J Pharm Pharmacol. 2008;2:022–028.
[13] Ahmad NS, Waheed A, Farman M, et al. Analgesic and anti-inflammatory effects of *Pistacia integerrima* extracts in mice. J Ethnopharmacol. 2010;129:250–253.
[14] Parvardeh S, Niapoor M, Hosseinzadeh H. Hepatoprotective activity of *Pistacia vera* L. gum extract in rats. J Med Plants. 2002;4:27–34.
[15] Gebauer SK, West SG, Kay CD, et al. Effects of pistachios on cardiovascular disease risk factors and potential mechanisms of action: a dose–response study. Am J Clin Nutr. 2008;88:651–659.
[16] Gentile C, Perrone A, Attanzio A, et al. Sicilian pistachio (*Pistacia vera* L.) nut inhibits expression and release of inflammatory mediators and reverses the increase of paracellular permeability in IL-1β-exposed human intestinal epithelial cells. Eur J Nutr. 2015;54:811–821.
[17] Rafieian-Kopaei M, Nasri H. Re: Erythropoietin ameliorates oxidative stress and tissue injury following renal ischemia/reperfusion in rat kidney and lung. Med Princ Pract. 2013;23:95–95.

[18] Al-Majed AA, Mostafa AM, Al-Rikabi AC, et al. Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. Pharmaco Res. 2002;46:445–451.

[19] Tavafi M, Ahmadvand H, Toolabi P. Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. Iran J Kidney Dis. 2012;6:25.

[20] Xie J, Talaska AE, Schacht J. New developments in aminoglycoside therapy and ototoxicity. Hear Res. 2011;281:28–37.

[21] Riff LJ, Jackson GG. Pharmacology of gentamicin in man. J Infect Diseases. 1971;124(Supplement 1):S98–S105.

[22] Sahu BD, Tatireddy S, Koneru M, et al. Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis and inflammation in rats: possible mechanism of nephroprotection. Toxicol Appl Pharmacol. 2014;277:8–20.

[23] Alarifi S, Al-Doaiss A, Alkahtani S, et al. Blood chemical changes and renal histological alterations induced by gentamicin in rats. Saudi J Biol Sci. 2012;19:103–110.

[24] Sahu BD, Kuncha M, Putcha UK, et al. Effect of metformin against cisplatin induced acute renal injury in rats: a biochemical and histoarchitectural evaluation. Exp Toxicol Pathol. 2013;65:933–940.

[25] Jafarey M, Changizi Ashtiyani S, Najafi H. Calcium dobesilate for prevention of gentamicin-induced nephrotoxicity in rats. Iran J Kidney Dis. 2014;8:46–52.

[26] Halvorsen BL, Carlsen MH, Phillips KM, et al. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am J Clin Nutr. 2006;84:95–135.

[27] Phillips KM, Ruggio DM, Ashraf-Khorassani M. Phytosterol composition of nuts and seeds commonly consumed in the United States. J Agric Food Chem. 2005;53:9436–9445.

[28] Kocyigit A, Koylu AA, Keles H. Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers. Nutr Metab Cardiovas Dis. 2006;16:202–209.

[29] Balakumar P, Rohilla A, Thangathirupathi A. Gentamicin-induced nephrotoxicity: do we have a promising therapeutic approach to blunt it? Pharmaco Res. 2010;62:179–186.

[30] Baradaran A, Nasri H, Nematbakhsh M, et al. Antioxidant activity and preventive effect of aqueous leaf extract of Aloe Vera on gentamicin-induced nephrotoxicity in male Wistar rats. Clin Ter. 2013;165:7–11.

[31] Amini FG, Rafieian-Kopaei M, Nematbakhsh M, et al. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci. 2012;17:621–625.

[32] Rafieian-Kopaei M, Baradaran A, Merrikhi A, et al. Efficacy of co-administration of garlic extract and metformin for prevention of gentamycine-renal toxicity in Wistar rats: a biochemical study. Int J Prev Med. 2013;4:258–264.

[33] Boroushaki MT, Asadpour E, Sadeghnia HR, et al. Effect of pomegranate seed oil against gentamicin-induced nephrotoxicity in rat. J Food Sci Technol. 2014;51:3510–3514.

[34] Boroushaki MT, Sadeghnia HR. Protective effect of safranal against gentamicin-induced nephrotoxicity in rat. Iran J Med Sci. 2015;34:285–288.

[35] Kang C, Lee H, Hah DY, et al. Protective effects of Houttuynia cordata Thunb. on gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicol Res. 2013;29:61–67.

[36] Nasri H, Nematbakhsh M, Rafieian-Kopaei M. Ethanolic extract of garlic for attenuation of gentamicin-induced nephrotoxicity in Wistar rats. Iran J Kidney Dis. 2013;7:376–382.

[37] Nasri H, Baradaran A, Ardalan MR, et al. Bright renoprotective properties of metformin: beyond blood glucose regulatory effects. Iran J Kidney Dis. 2013;7:423.

[38] Nasri H, Rafieian-Kopaei M. Protective effects of herbal antioxidants on diabetic kidney disease. J Res Med Sci. 2014;19:82–83.

[39] Rafieian-Kopaei M, Nasri H. Comment on: is the renoprotective effect of erythropoietin in chronic kidney disease a myth? J Formos Med Assoc. 2014;113:62.

[40] Gupta A, Chaphalkar SR. Anti-inflammatory and antimicrobial activities of aqueous leaves extract of Butea frondosa. J HerbMed Pharmacol. 2016;5:85–88.

[41] Pourianezhad F, Tahmasebi S, Abdusi V, et al. Review on feverfew, a valuable medicinal plant. J HerbMed Pharmacol. 2016;5:45–49.

[42] Chinedu E, Arome D, Jacob D. Preliminary assessment of the antiproliferative potential of Ananas comosus (pineapple) fruit juice. J Herbmed Pharmacol. 2016;5:50–53.