IJKD KIDNEY DISEASES

Ethanolic Extract of Garlic for Attenuation of Gentamicin-induced Nephrotoxicity in Wistar Rats

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Introduction. Nephrotoxicity is a serious side effect of gentamicin and is believed to be related to reactive oxygen species in the kidney. This study was aimed to find out whether garlic preparation (*Allium sativum L*) has ameliorative effects on gentamicin nephrotoxicity.
Materials and Methods. Fifty male Wistar rats were divided into 5 groups of 10 as follows: group 1, sham group (control); group 2 (positive control group), gentamicin for 10 days; group 3, garlic and gentamicin for 10 days; group 4, gentamicin for 10 days followed by garlic for 10 days; and group 5, gentamicin for 10 days followed by saline solution for 10 days. Gentamicin, 10 mg/kg, and garlic extract, 20 mg/kg, were administered intraperitoneally. Serum creatinine and concentrations were measured and the kidneys were processed for histopathological examinations. All specimens were examined for morphologic parameters involving tubular cells.
Results. Serum creatinine and BUN levels were significantly high in the gentamicin group (group 2) after the experiment. However, the levels of these parameters in group 3 (co-treatment with gentamicin and garlic) were significantly lower than those in group 2 ($P < .05$). These parameters were also lower in group 4 (consecutive treatment with gentamicin and garlic), when compared with group 5 (gentamicin and saline). The pathology damage score was high for the gentamicin group. Postadministration of garlic after gentamicin treatment (group 4) or co-administration of garlic and gentamicin (group 3) significantly attenuated the damage score.
Conclusions. Garlic has regenerative potential after tubular injury induced by gentamicin in animal models.

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INTRODUCTION

Gentamicin, an aminoglycoside antibiotic, is widely used in the treatment of infections, particularly against aerobic gram-negative bacteria.[1,2] Nephrotoxicity is a serious side effect in the use of gentamicin and is believed to be related to the generation of reactive oxygen species (ROS) in the kidney.[2,3] Reactive oxygen species induce vasoconstriction and decrease glomerular filtration rate. They also induce cellular damage and necrosis via lipid peroxidation and protein change.[4-6] While the drug is mostly excreted in the urine, a section is selectively accumulated in the renal cortex,[5-7] and leads to renal cell injury. Gentamicin also induces superoxide anion, hydrogen peroxide, and hydroxyl radical production from renal mitochondria.[7-9] In addition, hydrogen peroxide generation, lipoperoxidation and the content of nitrotyrosine, and protein carbonyl are increased and glutathione is diminished in renal cortex of gentamicin-treated rats.[8-10] Hence, the administration of compounds with antioxidant properties, ROS scavengers, or

antioxidant enzymes should ameliorate the severity of gentamicin-induced renal damage.[10-16] Moreover, the kidneys from gentamicin-treated rats are more susceptible to ROS because they are deficient in the antioxidant enzymes; manganese-superoxide dismutase,[7-13] glutathione peroxidase,[17-19] glutathione reductase, and catalase.[8,18]

Garlic (*Allium sativum L*) is an important component in the complementary and alternative medicine.[19] People traditionally believe that garlic can protect them against various diseases.[20-22] Experimental and clinical studies confirm that the ancient experience with beneficial effects of garlic holds validity even in prevention of various disorders and metabolic illnesses.[12,22] Most previous reports convincingly have pointed out that garlic reduces abnormal plasma lipids, oxidized low-density lipoproteins, abnormal platelet aggregation, and high blood pressure.[12,13,23-25] Stimulation of nitric oxide generation in endothelial cells seems to be the preventive mechanism.[24-29] Garlic also may promote an anti-inflammatory environment by cytokine modulation in human blood.[22-30] Effects of dietary garlic are mediated in large part via the generation of hydrogen sulfide. Garlic-derived organic polysulfides are converted by erythrocytes into hydrogen sulfide, which relaxes vascular smooth muscle, induces vasodilatations of blood vessels, and significantly reduces blood pressure. It is noteworthy that garlic properties are found to be due the existence of compounds such as water-soluble organosulfure compounds, S-allylcysteine, and lipid soluble compounds like diallyl sulfide.[30-32]

The first aim of the present study was to find out whether garlic preparation has ameliorative effects on gentamicin nephrotoxicity. On the other hand, most studies reported previously were designed to administer drugs before or at the same time of renal insult. Indeed most cases of acute kidney failures are not identified until the insult has already occurred. Thus, the clinical utility of any therapeutic agent for this disease would be greatly enhanced if delayed administration of the drug still proved to be renoprotective. Whether delayed treatment with garlic juice exerts similar benefits on toxic renal injury is still unknown. Thus, we secondly aimed to assess the probable posttreatment ameliorative effects of garlic predation on renal injury in rats.

MATERIALS AND METHODS

Garlic Extract Preparation

Fresh garlic was purchased at the peak of maturity from a local grocery in Hamadan, Iran. The garlic was cleaned, crushed, and macerated in 96% ethanol for 48 hours. Then, it was centrifuged at 200 g for 5 minutes. The supernatant was then filtered and rotary-evaporated at 40°C. The extract was frozen and stored at -20°C. The frozen extracts were reconstituted with normal saline to prepare final concentration when needed.[33]

Determination of Total Flavonoids

The amount of total flavonoids in the garlic extract was determined using the colorimetric method described by Bahmani and colleagues.[34] In this method, 0.5 mL of garlic extract or rutin (standard flavonoid compound) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1-M potassium acetate, and 2.8 mL of distilled water. Then, it was left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm, prepared using rutin solutions at concentrations of 25 ppm to 500 ppm in methanol. The experiment was repeated in triplicate. Total flavonoids were expressed in terms of rutin equivalents (in mg/g).

Determination of Total Phenolic Compounds

The amount of total phenolic compounds in the garlic extract was determined calorimetrically using the Folin-Ciocalteu reagent with minor modification, as described by Mirzaei and colleagues.[35] In brief, 5 mL of garlic extract or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (1:10 diluted with distilled water) and aqueous sodium carbonate (4 mL, 1 M). The mixtures were allowed to stand for 15 minutes, and the total phenols were determined by colorimetry at 765 nm. A standard curve was prepared using zero, 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, and 250 mg/L solutions of gallic acid in methanol:water (50:50 in terms of volume). Total phenol values were expressed in terms of gallic acid equivalent (in mg/g). The experiment was repeated in triplicate.

Determination of Antioxidant Activity

The ferric thiocyanate method was employed to evaluate antioxidant activity of the extract.[35] In a suitable vial, 500 µg of the extract was dissolved in

ethanol and added to a reaction mixture containing 2.88 mL of 2.5% linoleic acid and 9 mL of 40 mM phosphate buffer. The vial was incubated at 40°C for 96 hours. Every 12 hours (during incubation), 0.1 mL of the vial content was diluted with 9.7 mL of 75% ethanol, 0.1 mL of ammonium thiocyanate, and 0.1 mL of ferrous chloride. The absorbance of sample was measured at 500 nm, and the percentage inhibition (the capacity to inhibit the peroxide formation in linoleic acid) was determined using the following equation:

Percentage of inhibition = [1-(absorbance of sample/absorbance of control]) ×100

A high inhibition percentage indicates a high antioxidant activity. Ethanol within the sample and without reagents was used as the negative control.

Allicin Determination

Allicin content was measured in garlic extract using the method of Shirzad and coworkers.[36] In brief, 200 mg of extract was added to 1.0 mL (final volume) of 2-nitro-5-thiobenzoate (1.2×10^{-4}M) in 50 mM of sodium phosphate and 1 mM of ethylenediaminetetraacetic acid (pH, 7.2). The decrease in optical density at 412 nm was determined after 30-minute incubation at room temperature.[37] The concentration of allicin was calculated according to the following equation:

Allicin (mg/mL) = $\Delta A412 \times 5.72 \times 10^{-3}$

where $\Delta A412$ is the decrease in optical density compared with the initial absorption at 412 nm.

Animals

Study samples included 50 male Wistar rats with a weight range of 200 g to 250 g. The rats were purchased from Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. All animals were similarly handled in the animal house of the research center and had free access to food and water. They were housed at a controlled environment with temperature and humidity of 25 ± 3°C and 50% to 60%, respectively. In addition, they were kept with a 12-hour dark-light cycle (lights on at 7.00 AM) and allowed free access to pelleted diet and tap water. Their general health state and activity were monitored closely during the experiment. The animal experimentation was conducted in accordance with the National Institute of Health guide for the careful use of laboratory animals.[38] The project protocol was approved by the Ethical Committee of Sharekord University of Medical Sciences, Shahrekord, Iran.

Experimental Design

The animals were divided into 5 groups (10 rats in each) as follows: group 1, sham group (control); group 2 (positive control group), gentamicin for 10 days; group 3, garlic and gentamicin for 10 days; group 4, gentamicin for 10 days followed by garlic for 10 days; and group 5, gentamicin for 10 days followed by saline solution for 10 days. Gentamicin was administered intraperitoneally at a dose of 10 mg/kg. Gentamicin treatment protocol used in the present study has been reported previously.[39] Garlic extract was also administered intraperitoneally at a dose of 20 mg/kg.

All of the animals were sacrificed on the last day of their study period by intraperitoneally injecting ketamine under general anesthesia. On the first day (before experiment) and on the last day (day of sacrificing) serum samples were obtained to measure blood urea nitrogen (BUN) and serum creatinine. The kidneys were removed immediately after sacrificing for histological examinations.

Kidney Function Tests

Serum creatinine and BUN levels were measured using a colorimetric method, employing commercial kits by an auto analyzer.

Histopathological Examination

The kidneys of each animal were dissected out and then fixed in buffered formalin for 12 hours and processed for histopathological examinations. Three micrometer-thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol.[40] Histopathological studies were performed under a light microscope. Slides were coded and were examined by a histopathologist who was blinded to the treatment groups. All specimens were examined for 6 morphologic parameters, including epithelial cell vacuolization, degeneration, tubular cell flattening, hyaline cast, tubular dilatation, and debris materials in tubular lumen on a semi-quantitative score from 1 to 5. The score of zero was assigned to the normal tissue without damage.[41,42]

Statistical Analyses

Continuous data were expressed as mean ±

standard error. The paired *t* test was used to compare serum creatinine and BUN levels before and after the experiments. The 1-way analysis of variance was applied to compare the serum creatinine and BUN levels between the groups. To compare the pathology damage score between the groups, the Mann-Whitney U test and Kruskal-Wallis test were *applied*, where appropriate. Values of *P* less than .05 were considered significant.

RESULTS

Bioactive Components of Garlic Extract

The amount of flavonoids in garlic extract was 6.1 ± 0.5 mg/g (equivalent to rutin) and the amount of phenolic compounds was 12.9 ± 0.8 mg/g (equivalent to gallic acid). The amount of allicin in garlic extract was found to be 15 µg/mL and the antioxidant activity (the percentage of inhibition or the capacity to inhibit the peroxide formation in linoleic acid was 52.6%.

Kidney Function Tests

Serum creatinine and BUN levels are demonstrated in Figure 1. No significant differences were observed before the experiment between the five groups. After the experiment these parameters were significantly higher in the gentamicin group (group 2) as compared with the control group ($P < .05$). However, the levels of these parameters in group 3 (co-treatment with gentamicin and garlic) were significantly lower than those in group 2 ($P < .05$). Serum creatinine and BUN levels were also lower in group 4 (treatment with gentamicin for 10 days and garlic for the next 10 days), when compared with the ones in group 5 (gentamicin and saline; $P < .05$).

Garlic Extract and Pathology Damage Score

The pathology damage score indicated a higher score for the gentamicin group, which was significantly different from the control group ($P < .05$). However, postadministration of garlic after 10 days of gentamicin treatment (group 4) or co-administration of garlic and gentamicin (group 3) significantly attenuated the damage score ($P < .05$), when compared with groups 2 and 5, respectively.

DISCUSSION

The results of the present study indicated that co-administration or postadministration of garlic attenuated the serum creatinine and BUN levels which were increased due to gentamicin injection. Also, the pathology damage scores indicated

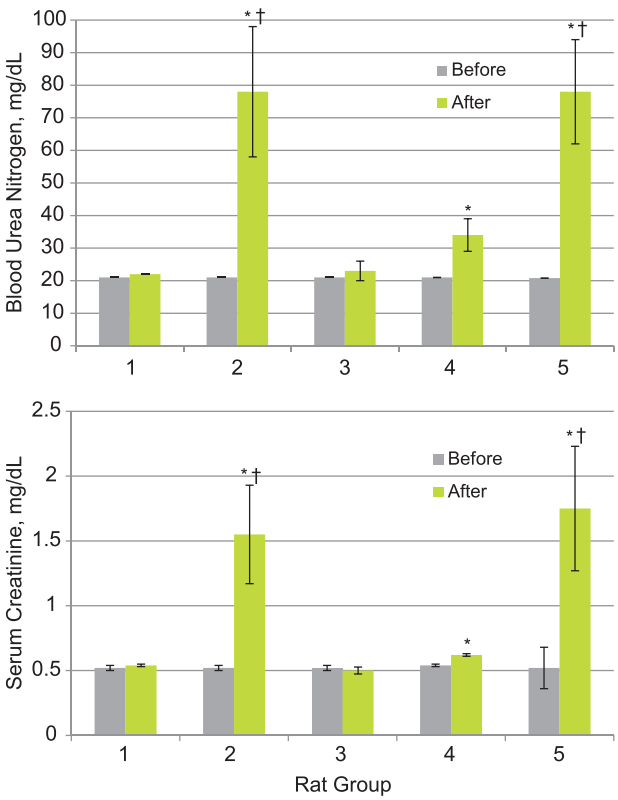

Figure 1. Serum creatinine and BUN levels before and after the experiment in 5 groups of animals. Group 1 is the control group; group 2, positive control group treated with gentamicin; group 3, rats co-treated with garlic and gentamicin for 10 days; group 4, rats treated with gentamicin for 10 days and garlic for the next 10 days; and group 5, rats treated with gentamicin for 10 days and saline for the next 10 days.
*$P < .05$, compared with before experiment
†$P < .05$, compared with groups 1, 3, and 4

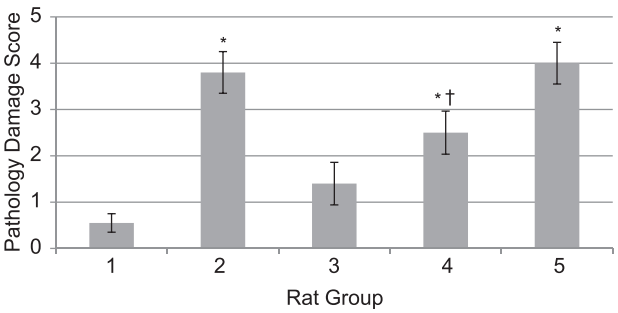

Figure 2. Pathology damage score in 5 groups of animals. Group 1 is the control group; group 2, positive control group treated with gentamicin; group 3, rats co-treated with garlic and gentamicin for 10 days; group 4, rats treated with gentamicin for 10 days and garlic for the next 10 days; and group 5, rats treated with gentamicin for 10 days and saline for the next 10 days.
*$P < .05$, compared with groups 1 and 3
†$P < .05$, compared with groups 2 and 4

that postadministration of garlic after 10 days of gentamicin administration significantly attenuated the damage score. However, the regeneration was not so obvious when garlic was administrated for 10 days, following gentamicin injection.

The aminoglycoside antibiotic gentamicin is still widely used against infections by gram-positive and gram-negative aerobic bacteria. However, its use has been limited due to renal impairment that occurs in up to 30% of treated patients.[43-49] The drug may accumulate in epithelial tubular cells causing a range of effects starting with loss of the brush border in epithelial cells and ending in overt tubular necrosis, activation of apoptosis, and massive proteolysis. Gentamicin also causes cell death by generation of free radicals, phospholipidosis, extracellular calcium-sensing receptor stimulation and energetic catastrophe, reduced renal blood flow, and inflammation.[36] Various drugs have been shown to either ameliorate or potentiate gentamicin- nephrotoxicity. Most of the nephroprotective agents have not been tested in large controlled clinical trials. Because of their relative safety and effectiveness, antioxidant agents seem to be good candidates for testing in humans.

Garlic is a commonly worldwide used food, and its medical properties have been well recognized since the ancient times. Garlic is known for its antibacterial, anticarcinogenic, hypolipidemic, hypoglycemic, antifungal, and anti-atherosclerotic properties.[50] The protective effect of the garlic-derived antioxidant S-allylcysteine on renal injury and oxidative stress induced by ischemia and reperfusion was shown by Segoviano-Murillo and coworkers.[51] The results of this study showed that garlic possess high level of antioxidant activity. The antioxidant efficacy of garlic powder was also examined in the study conducted by Pedraza-Chaverri and colleagues.[30] They found that nephrotoxicity following administration of potassium dichromate was ameliorated by 2% garlic powder diet for one month in rats. In another study, Pedraza-Chaverri and colleagues[13] showed that S-allylmercaptocysteine (one of the water soluble organo-sulfur compounds found in aged garlic extract scavenges hydroxyl radical in vitro and attenuates oxidative and nitrosative stress.[13]

Gentamicin is rapidly excreted, predominantly by glomerular filtration, and the reabsorption of a small but notable amount of drug by the proximal tubule results in accumulation within the renal cortex. This preferential binding is responsible for nephrotoxicity.[5,7,52,53] The mechanism by which gentamicin induces nephrotoxicity remains unknown; however, it has been postulated that oxidative and nitrosative stress are involved in this process.[5,54] It has been found that, hydroxide and hydrogen peroxide, are involved in renal damage induced by gentamicin.[3,42,43] Furthermore, gentamicin induces hydrogen peroxide generation by mitochondria.[16,17,54] To explore the effect of diallyl sulfide (DAS), a garlic-derived compound with antioxidant properties, on gentamicin-induced nephrotoxicity, Pedraza-Chaverri and colleagues conducted another study on 4 groups of rats including the control group, treated with olive oil as a vehicle; gentamicin group, treated subcutaneously with gentamicin (125 mg/kg/d for 4 days); DAS group, treated intragastrically with DAS (50 mg/kg/d for 4 days), and gentamicin and DAS group. Nephrotoxicity in their study was considered by the increase in creatinine and blood urea nitrogen in serum, increase in urinary excretion of N-acetyl-beta-D-glucosaminidase and total protein, and necrosis of proximal tubular cells. They found that these functional and structural alterations were prevented or ameliorated by DAS treatment. Moreover, gentamicin increased levels of renal oxidative stress markers nitrotyrosine and protein carbonyl groups which were also ameliorated by DAS in the gentamicin and DAS group. They concluded that the mechanism by which DAS has a protective effect on gentamicin-induced nephrotoxicity may be related, at least in part, to the decrease in oxidative stress in renal cortex induction of nephrotoxicity.[55]

CONCLUSIONS

The present study showed that co-administration or postadministration of garlic juice for gentamicin-induced acute kidney failure was effective. Hence, we could assume that garlic is a nephroprotective drug to ameliorate tubular damage by gentamicin or probably other nephrotoxic agents which act through the same mechanisms as this aminoglycoside does. However this study is promising, it warrants more comprehensive trials.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Nagai J, Takano M. Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokinet. 2004;19:159-70.
2. Tavafi M. Inhibition of gentamicin–induced renal tubular cell necrosis. J Nephropathol. 2012;1:83-6.
3. Rafieian-Kopaei M, Baradaran A. Teucrium polium and kidney. J Ren Inj Prev.2012;2:3-4.
4. Khajehdehi P. Turmeric: reemerging of a neglected Asian traditional remedy. J Nephropathol. 2012;1:17-22.
5. Martinez-Salgado C, Eleno N, Tavares P, et al. Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. Kidney Int. 2002;62:1682-92.
6. Rafieian-Kopaei M, Nasri H, Nematbakhsh M, Baradaran A, Gheissari A, Rouhi H, et al. Erythropoietin ameliorates genetamicin-induced renal toxicity: a biochemical and histopathological study. J Nephropathol. 2012;1:109-16.
7. Edson RS, Terrell CL. The aminoglycosides. Mayo Clin Proc. 1999;74:519-28.
8. Kadkhodaee M. Erythropoietin; bright future and new hopes for an old drug. J Nephropathol. 2012;1:81-2.
9. Assadi F. The epidemic of pediatric chronic kidney disease: the danger of skepticism. J Nephropathol 2012;1:61-4.
10. Konopska B, Warwas M. [Molecular aspects of aminoglycoside nephrotoxicity]. Postepy Hig Med Dosw (Online). 2007;61:511-8.
11. Ardalan MR, Samadifar Z, Vahedi A. Creatine monohydrate supplement induced interstitial nephritis. J Nephropathol. 2012;1:117-20.
12. Nasri H. Renoprotective effects of garlic. J Ren Inj Prev. 2012;2:27-8.
13. Pedraza-Chaverri J, Barrera D, Maldonado PD, et al. S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo. BMC Clin Pharmacol. 2004;4:5.
14. Nasri H. Preventive role of erythropoietin against aminoglycoside renal toxicity induced nephropathy; current knowledge and new concepts. J Ren Inj Prev.2012;2:29-30.
15. Tavafi M. Protection of renal tubules against gentamicin induced nephrotoxicity. J Ren Inj Prev.2012;2:5-6.
16. Guidet BR, Shah SV. In vivo generation of hydrogen peroxide by rat kidney cortex and glomeruli. Am J Physiol. 1989;256:F158-F164.
17. Gheissari A. Acute kidney injury and renal angina. J Ren Inj Prev. 2013;2:33-4.
18. Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, et al. Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidant enzymes. Free Radic Biol Med. 2000;29:602-11.
19. Nagourney RA. Garlic: medicinal food or nutritious medicine? J Med Food. 1998;1:13-28.
20. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. In vitro antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. J Med Food. 2004;7:327-33.
21. Deniz M, Sener G, Ercan F, Yegen BC. Garlic extract ameliorates renal and cardiopulmonary injury in the rats with chronic renal failure. Ren Fail. 2011;33:718-25.
22. Savas M, Yeni E, Ciftci H, et al. The antioxidant role of oral administration of garlic oil on renal ischemia-reperfusion injury. Ren Fail. 2010;32:362-7.
23. Kook S, Kim GH, Choi K. The antidiabetic effect of onion and garlic in experimental diabetic rats: meta-analysis. J Med Food. 2009;12:552-60.
24. Rafieian-Kopaei M, Nasri H. Ginger and diabetic nephropathy. J Ren Inj Prev. 2012;2:9-10.
25. Tayebi Khosroshahi H. Short history about renal transplantation program in Iran and the world: Special focus on world kidney day 2012. J Nephropathol. 2012;1:5-10.
26. Razo-Rodriguez AC, Chirino YI, Sanchez-Gonzalez DJ, Martinez-Martinez CM, Cruz C, Pedraza-Chaverri J. Garlic powder ameliorates cisplatin-induced nephrotoxicity and oxidative stress. J Med Food. 2008;11:582-6.
27. Tolou-Ghamari Z. Nephro and neurotoxicity, mechanisms of rejection: a review on tacrolimus and cyclosporin in organ transplantation. J Nephropathol. 2012;1:23-30.
28. Einollahi B. Are acquired cystic kidney disease and autosomal dominant polycystic kidney disease risk factors for renal cell carcinoma in kidney transplant patients? J Nephropathol. 2012;1:65-8.
29. Rafieian-Kopaie M. Metformin and renal injury protection. J Ren Inj Prev. 2013; 2: 91-2.
30. Pedraza-Chaverri J, Yam-Canul P, Chirino YI, et al. Protective effects of garlic powder against potassium dichromate-induced oxidative stress and nephrotoxicity. Food Chem Toxicol. 2008;46:619-27.
31. Rafieian-Kopaie M, Nasri H. Silymarin and Kidney. J Ren Inj Prev. 2012;1:3-6.
32. Chung LY. The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. J Med Food. 2006;9:205-13.
33. Rafieian-Kopaei M, Asgary S, Adelnia A, et al. The effects of Cornelian cherry on atherosclerosis and atherogenic factors in hypercholesterolemic rabbits. J Med Plants Res. 2011;5:2670-6.
34. Bahmani M, Rafieian-kopaei M, Parsaei P, Mohsenzadegan A. The anti-leech effect of Peganum harmala L. extract and some anti-parasite drugs on Limnatis nilotica. Afr J Microbiol Res. 2012;6:2586-90.
35. Mirzaei, MGh, Sewell RDE, Kheiri S, Rafieian-Kopaei M. A clinical trial of the effect of St. John's Wort on migraine headaches in patients receiving sodium valproate. J Med

Plants Res. 2012;6:1519-23.

36. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. J Med Food. 2011;14:969-74.
37. Azadmehr A, Hajiaghaee R, Afshari A, Amirghofran Z, Refieian-Kopaei M, Yousofi-Darani H and Shirzad H. Evaluation of in vivo immune response activity and in vitro anti-cancer effect by Scrophularia megalantha. J Med Plants Res. 2011;5:2365-8.
38. Gheshlaghi F. Toxic renal injury at a glance. J Ren Inj Prev. 2012;1:15-6.
39. Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci. 2012;17:621-5.
40. Nasri H, Mortazavi M, Ghorbani A, Shahbazian H, Kheiri S, Baradaran A, et al. Oxford-MEST classification in IgA nephropathy patients: a report from Iran. J Nephropathol. 2012;1:31-42.
41. Baradaran A, Mahmoud Rafieian-kopaeiM. Histopathological study of the combination of metformin and garlic juice for the attenuation of gentamicin renal toxicity in rats. J Ren Inj Prev.2012;2:15-21.
42. Nematbakhsh M, Ashrafi F, Pezeshki Z, et al. A histopathological study of nephrotoxicity, hepatoxicity or testicular toxicity: Which one is the first observation as side effect of Cisplatin induced toxicity in animal model. J Nephropathol. 2012;1:190-3.
43. Ali BH, Al ZM, Blunden G, Nemmar A. Experimental gentamicin nephrotoxicity and agents that modify it: a mini-review of recent research. Basic Clin Pharmacol Toxicol. 2011;109:225-32.
44. Gheissari A, Mehrasa P, Merrikhi A, Madihi Y. Acute kidney injury: A pediatric experience over 10 years at a tertiary care center. J Nephropathol. 2012;1:101-8.
45. Kam-Tao Li PK, Burdmann EA, Mehta RL. Acute kidney injury: global health alert. J Nephropathol. 2013;2:90-7.
46. Gheissari A, Hemmatzadeh S, Merrikhi A, Fadaei Tehrani S, Madihi Y. Chronic kidney disease in children:A report from a tertiary care center over 11 years. J Nephropathol. 2012;1:177-82.
47. Shahni N, Gupta KL. Dietary antioxidants and oxidative stress in predialysis chronic kidney patients. J Nephropathol.2012;1:134-42.
48. Kari J. Epidemiology of chronic kidney disease in children. J Nephropathol. 2012;1:162-3.
49. Solati M, Mahboobi HR. Paraoxonase enzyme activity and dyslipidemia in chronic renal failure patients. J Nephropathol. 2012;1:123-5.
50. Hassan HA, El-Agmy SM, Gaur RL, Fernando A, Raj MH, Ouhtit A. In vivo evidence of hepato- and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress. Int J Biol Sci. 2009;5:249-55.
51. Segoviano-Murillo S, Sanchez-Gonzalez DJ, Martinez-Martinez CM, Cruz C, Maldonado PD, Pedraza-Chaverri J. S-allylcysteine ameliorates ischemia and reperfusion induced renal damage. Phytother Res. 2008;22:836-40.
52. Rafieian-Kopaei M, Baradaran A, Rafieian M. Plants antioxidants: From laboratory to clinic. J Nephropathol. 2013;2:152-3.
53. Ghorbani A, Omidvar B, Parsi A. Protective effect of selenium on cisplatin induced nephrotoxicity: A double-blind controlled randomized clinical trial. J Nephropathol. 2013;2:129-34.
54. Cuzzocrea S, Mazzon E, Dugo L, et al. A role for superoxide in gentamicin-mediated nephropathy in rats. Eur J Pharmacol. 2002;450:67-76.
55. Pedraza-Chaverri J, Maldonado PD, Barrera D, Ceron A, Medina-Campos ON, Hernandez-Pando R. Protective effect of diallyl sulfide on oxidative stress and nephrotoxicity induced by gentamicin in rats. Mol Cell Biochem. 2003;254:125-30.

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