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Protective Effects of Pomegranate Juice on Nephrotoxicity Induced by Captopril and Gentamicin in Rats

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Introduction. Nephrotoxicity is an important side effects of captopril and gentamicin. This study investigated the prophylactic and protective effects of pomegranate juice (PJ) on the kidney exposed to nephrotoxicity induced by these medications.
Materials and Methods. Wistar male rats received drinking water (groups 1 to 3) or PJ at doses of 4 mL/kg (group 4), 10 mL/kg (groups 5 and 7), and 15 mL/kg (group 6) for 14 days. Captopril and gentamicin were administrated on days 10 and 14 to groups 1 and 2, respectively, while groups 3 to 6 received both. Group 7 did not receive anything. The serum, urine, and renal tissue parameters were measured after the experiment.
Results. Group 1 (captopril) had a higher malondialdehyde level than groups 4, 5, 6, and 7 with PJ (P <0.05), and group 3 (captopril and gentamicin) showed the most significant malondialdehyde level compared to other groups (P < .001). Group 5 (captopril, gentamicin, and PJ, 10 mL/kg) had the most significant sodium excretion compared to other groups (P < .001), and group 2 (gentamicin) showed the highest potassium absolute excretion (P < .001). The instability of the renal index was observed during the experiment for the groups receiving drinking water, while no significant changes were observed in the groups receiving PJ.
Conclusions. The prophylactic consumption of PJ for 14 days could show nephroprotective effects by reducing oxidative stress and potassium depletion. It could also lead to the stabilization of kidney function during this period despite using captopril and gentamicin.

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INTRODUCTION

Nephrotoxicity is an adverse effect of some chemicals and medications. It can be more profound in patients already suffering from kidney impairment. Nephrotoxicity causes approximately 20% of community- and hospital-acquired episodes of acute kidney failure. Analgesics, antidepressants, mood stabilizers, aminoglycosides, amphotericin B, and angiotensin-converting enzyme (ACE) inhibitors are the drugs that can induce nephrotoxicity.[1]

Angiotensin-converting enzyme inhibitors like captopril cause kidney impairment through altering intraglomerular hemodynamics. Thus, they eliminate the angiotensin II-induced vasoconstriction of efferent arteriole of the kidney.[2] Underlying renal insufficiency, bilateral and unilateral artery stenosis, intravascular volume depletion, an age greater than 60 years, and concomitant use of other potential nephrotoxic agents are known as risk factors.[1,2] There are several reports regarding ACE inhibitors-induced acute kidney failure and nephrotoxicity,[3-8] which result in the adjustment

of ACE inhibitors dosage during kidney failure.[2]

In contrast, some studies suggest a beneficial protective role of captopril in the nephrotoxicity induced by cisplatin,[9] adriamycin,[10] doxorubicin,[11] and gentamicin[12] in rats. Captopril may exert its beneficial effects through cutting the impact of the renin-angiotensin system on the efferent arteriole resistance and contracting mesangial cells. By another mechanism, captopril may weaken the vasodilatory quinine and increase vasodilatory prostaglandins.[12]

On the other hand, ACE inhibitors and aminoglycosides have a high potential of nephrotoxicity. Approximately, 8% to 26% of the patients receiving aminoglycosides for several days develop mild kidney impairment. Toxicity correlates with the total amount of the drug administered and is more likely to be encountered with longer courses of exposure. Cationic aminoglycosides interact and accumulate with anionic phospholipids of the proximal tubular cells. It leads to cell membrane perturbation; synthesis of membrane-derived autacoids and intracellular second messengers such as prostaglandins, inositol phosphates, and diacylglycerol; inhibition of phospholipases, sphingomyelinases, and ATPases; and alteration of the function of mitochondria and ribosomes. The excretion of tubular brush border enzymes, hyaline, and granular casts, mild proteinuria, reduction of glomerular filtration rate (GFR), and a mild rise in plasma creatinine are among the manifestations of these damages. A reduced sensitivity of the collecting duct epithelium to antidiuretic hormone and a nonoliguric phase result in the effects of aminoglycosides on the distal portion. Other drugs, such as amphotericin B, vancomycin, ACE inhibitors, cisplatin, and cyclosporine, may potentiate aminoglycoside-induced nephrotoxicity.[1,3,13,14]

Punica granatum (pomegranate) is one of the oldest edible fruits and belongs to the family of *Punicacea*. Pomegranate is extensively cultivated in the Mediterranean and the Near and Far East countries. This kind of plant has a unique biochemistry. Eighty percent of pomegranate seed oil is consisted of 18-carbon fatty acids (punicic acid). Polyphenols, flavonoids, and anthocyanins are the compounds in pomegranate regarded as strong antioxidants.[15] Pomegranate has had so many applications in traditional medicine as well. Its juice contains several substances with antioxidant and renal protective effects.[16-23] Beside its matrix metalloproteinases inhibitory effects (MMPs),[18,20,24,25] it has anti-inflammatory, antimutationary, antiviral, and skin bleaching effects. Different parts of pomegranate, that is, its leaf, stem, root, fruit, and seed, as well as its juice, contain active ingredients with antimicrobial and anti-oxidant effects. Its fruit and juice not only have antifungal and anti-inflammatory properties, but also are active in reducing blood pressure. The main phenolic compound of the extract of pomegranate flower is gallic acid, which has protective effects against kidney disorders.[15]

In accordance with the potential synergism of nephrotoxicity induced by ACE inhibitors such as captopril and aminoglycosides like gentamicin, it seems that the prophylactic administration of some substances used in traditional medicine would be helpful. This study aimed at investigating the potential protective effects of pomegranate juice (PJ) against synergistic nephrotoxicity induced by captopril and gentamicin.

MATERIALS AND METHODS

Animals and Ethics Considerations

Fifty-six locally bred male Wistar rats (250g to 300g) were included in the study. Animal studies were conducted according to the guidelines for the care and handling of animals prepared by the Iranian Ministry of Health (ethics board approval number: 90-116-1, Arak University of Medical Sciences) and the internationally accepted principles for laboratory animal use and care as found in the European Community Guidelines (EEC Directive of 1986; 86/609/EEC).

Pomegranate Juice

Pomegranates were collected from the orchards of Saveh (Markazi province, Iran), in autumn, and were dried and washed. The Physicochemical properties of pomegranate with this origin were studied previously.[20,23,26] The juice of the ruby-colored seeds together with all the white pulps was taken by using a juice maker (Pars Khazar, JBG-610P, Iran). The juice was made on a daily basis and was refrigerated until use.[27]

Experimental Design

The rats were divided into 7 groups (8 rats

in each group) as follows: group 1, drinking water and captopril; group 2, drinking water and gentamicin; group 3, drinking water and captopril and gentamicin; group 4, PJ (4 mL/kg body weight) and captopril and gentamicin; group 5, PJ (10 mL/kg body weight) and captopril and gentamicin; group 6, PJ (15 mL/kg body weight) and captopril and gentamicin; and group 7, PJ (10 mL/kg body weight). The drinking water and PJ were administrated for 14 days. Captopril (Exir Pharmaceutical, Iran), 25 mg/kg, and gentamicin (Alborzdarou, Iran), 100 mg/kg, were administrated intraperitoneally and as gavage, respectively, on the 10th and 14th days.

Urine samples were taken in a metabolic cage after the animals were kept for 24 hours and the blood samples were taken from the tails and aorta on days 4, 11, and 15. Serum levels of creatinine, glucose, urea nitrogen, ferric-reducing ability of plasma (FRAP), lactate dehydrogenase (Auto-analyzer, Selectra, Netherlands) of aorta and tail as well as sodium, potassium (Flame Photometer, Seak, Italy), and urine volume were measured. The left kidney was weighed and malondialdehyde and FRAP tests were performed on renal tissue on day 15.

Measurement of Malondialdehyde and Ferric-Reducing Ability

Malondialdehyde. To do the test, these solutions were prepared: the phosphate buffer (NaCl, 8 g; KCl, 0.2 g; Na_2HPO_4, 1.044 g; and KH_2PO_4, 0.24 g), sodium dodecyl sulfate (8.1%), tetraethoxypropane (5 mM, 10 mM, 20 mM, and 40 mM), thiobarbituric acid (0.8% solved in Na_2SO_4, 2 M, at 60°C), and 20% acetic acid (pH lowered to 3.5 by NaOH; Merck, Germany). Thiobarbituric acid produces a pink complex with malondialdehyde as a final marker of oxidative stress at high temperature and acidic pH. The measurement of this reaction and its light absorbance is regarded as lipid peroxidation.

The rats were anesthetized by ketamine (Rotexmedica, Germany) and xylazine (Alfasan, Netherlands) on day 15. After perfusion, the kidneys were weighed, kept at -20°C, and then were homogenized (Heidolph, Germany). The tubes were centrifuged (Zentrifugen, Tuttlingen, Germany) and the malondialdehyde test was done in accordance to previous studies.[14,15,28] The light absorbance of upper layer was read at 532 nm by spectrophotometer (Biowave II, Biochrom, Cambridge, UK).

Ferric-reducing ability. During FRAP measurement, the concentration of Fe^{2+}-TPTZ (2, 4, 6-Tri (2-pyridyl)-1, 3, 5-triazine; Merck, Germany) was considered as a function of antioxidant ability.[14,23,29]

Statistical Analysis

The analysis of data was done using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA). All values of urine and plasma renal parameters were expressed as mean ± standard error of mean. Data were analyzed by the 1-way analysis of variance followed by the LSD test and repeated measures as well. The significance level was set at a *P* value less than .05.

RESULTS

Figure 1 shows the mean renal tissue malondialdehyde. The results showed that group 1 (captopril) had more malondialdehyde value than groups 4, 5, 6, and 7 with PJ (P <0.05). Also, group 3 (captopril and gentamicin) showed the most significant malondialdehyde value compared to other groups (P < .001). Moreover, groups 2 (gentamicin) and 7 (PJ) had significant differences (P < .05). As it is shown in Figures 2 and 3, the renal FRAP and the mean GFR on day 15 did not reveal any significance differences among the groups.

Figures 4 and 5 display the mean for urinary sodium and potassium absolute excretion values

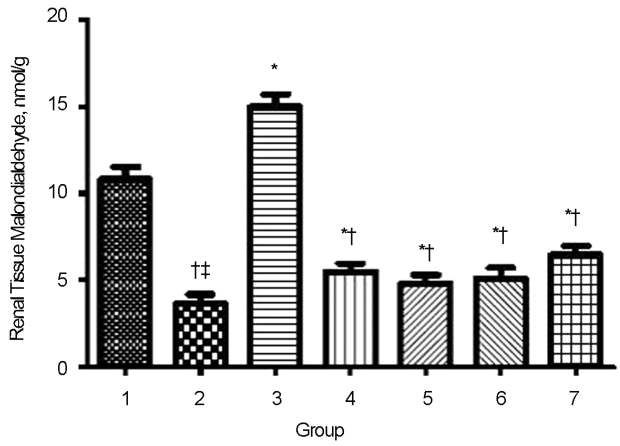

Figure 1. Mean renal malondialdehyde in the studied groups.
*P < .05 compared to group 1
†P < .001 compared to group 3
‡P < .05 compared to group 7

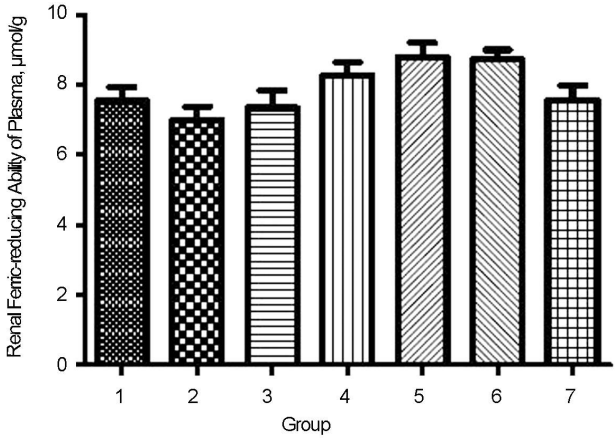

Figure 2. Mean renal ferric-reducing ability of plasma in the studied groups.

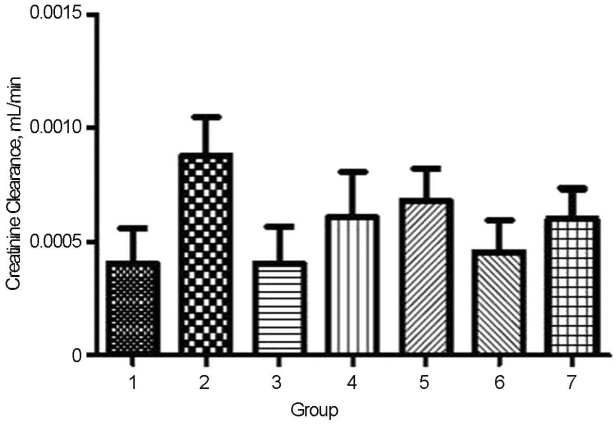

Figure 3. Mean creatinine clearance on the 15th day in the studied groups.

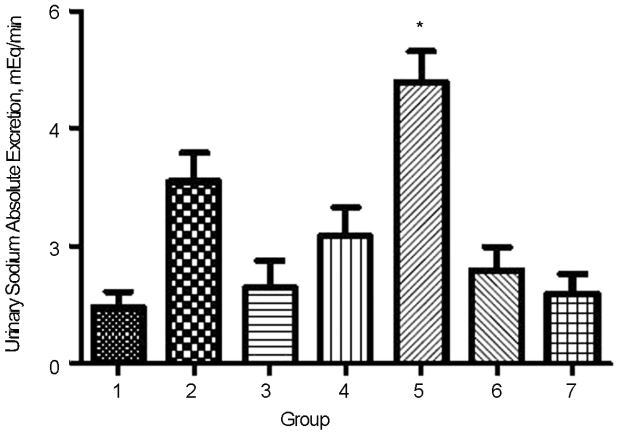

Figure 4. Mean of urinary sodium absolute excretion on the 15th day in the studied groups.
*P < .001 compared to all other groups, except group 2

on the 15th day of the experiment. It represented that group 5 (captopril, gentamicin, and PJ, 10 mL/kg) had the most significant sodium excretion

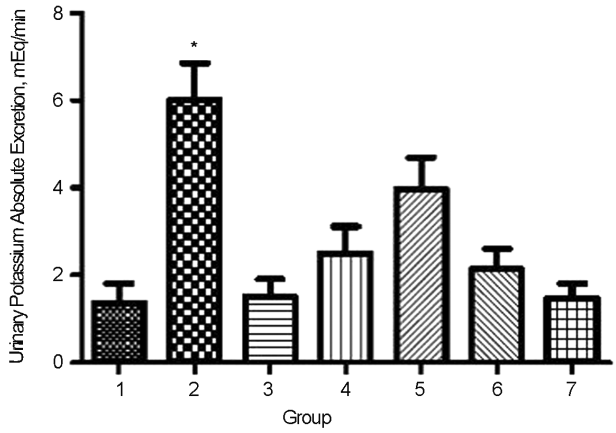

Figure 5. Mean of urinary pottasium absolute excretion on the 15th day in the studied groups.
*P < .001 compared to all other groups, except group 5

compared to other groups (P < .001). Accordingly, group 2 (gentamicin) showed the highest potassium absolute excretion (P < .001).

DISCUSSION

Using plants with phenols and flavonoids is of great interest due to their high capacity for scavenging free radicals and their antioxidant properties to cure various diseases. They showed the benefits on different aspects of acute renal damages like renal reperfusion injury.[30,31] Pomegranate is an important source of tannins, punicaline, anthocyanins, punicalagin, and gallic acids and ellagic acid as well as vitamin C. The antioxidant properties of pomegranate in scavenging free radicals and its vitamin C and pomegranate phenolic compounds have also been reported in other studies. On the other hand, the results of experimental and clinical studies have shown that oxidative stress may be involved in a variety of chronic kidney damages, and kidney is considered as an organ susceptible to oxidative stress. In this regard, reactive oxygen species play an important role in a variety of kidney diseases, such as proteinuria, glomerulonephritis, and tubulointerstitial nephritis.[15]

The present study showed that the prophylactic administration of PJ (10 and 15 ml/Kg/day) for 14 days could prevent the nephrotoxicity induced by captopril and gentamicin. This prescription could also prevent captopril and gentamicin-induced changes of renal functions during this period. Captopril, as an ACE inhibitor, causes acute kidney failure in patients with severe bilateral renal artery

stenosis. The vasoconstrictive effect of angiotensin II on the efferent compared to afferent arteriole of the kidney leads to reducing GFR and acute kidney failure.[2-8] However, there are some contradictory reports regarding the protective effects of captopril in some models and specific conditions of renal toxicity such as the nephrotoxicity induced by doxorubicin.[11,32] It seems that in these cases, captopril by reacting with free radicals increases the enzymatic function of superoxide dismutase and selenium-dependent glutathione peroxidase.[11]

Gentamicin, like other aminoglycosides, causes nephrotoxicity by inhibiting protein synthesis in renal cells. It accumulates in the proximal tubule cells resulting in acute tubular necrosis. Gentamicin can excrete apical membrane enzymes, protein, and hyaline, and reduces GFR after a few days. The nonoliguric phase of kidney failure is related to gentamicin effects on the distal tube. This family reacts with membrane phospholipid (negative charge), which leads to the disruption of the production of membrane-derivated autacoids and second messengers such as prostaglandins, phosphatidylinositol trisphosphate, and diacylglycerol.[3,33] Gentamicin can lead to increased serum concentrations of urea and creatinine. There are reports of decreased kidney damage caused by gentamicin with the help of ingredients like garlic, vitamin E, selenium, and melatonin.[33]

A synergism for nephrotoxicity was observed when captopril and gentamicin were taken together.[2,3,5,34,35] It may be due to the incitation of kinin and thromboxane B2. Captopril increases thromboxane production as vasoconstrictor via the inhibition of kinase II-converting enzyme. The enhancement of oxidative-nephrotoxicity of aminoglycoside is more obvious in potassium depleted cases.[35] It is seen that the use of gentamicin and captopril could lead to increased malondialdehyde and decreased FRAP and increased plasma urea and creatinine and decreased GFR. It seems that reactive oxygen species cause mesangial cell contraction as well as filtration level changes and filtration coefficient adjustment.[7]

The synergism of nephrotoxicity of captopril and gentamicin was studied in our study, too. Accordingly, the enhancement of serum urea and creatinine levels were more in rats which had taken gentamicin and captopril together in comparison to the groups which had taken only one of these drugs or none of them (data not shown). Furthermore, we observed more potassium wasting while the most increase of malondialdehyde for the groups receiving gentamicin (Figures 5 and 1, respectively). Our results showed that the administration of PJ could exert the prophylactic effects on this kind of nephrotoxicity. Thus, the groups receiving PJ revealed significant malondialdehyde decrease compared to the groups taking only water, especially on days 4 and 11. However, when oxidative stress reaches to a certain amount, antioxidant defense mechanism becomes inefficient, leading to a reduction in the concentration of intracellular glutathione and antioxidant enzymes. In this study, we observed that the administration of gentamicin and captopril caused a significant increase in the production of malondialdehyde as an indicator of lipid peroxidation in the kidney, which could be amended by PJ.

Pomegranate juice is rich in compounds with potential antioxidant and anti-inflammatory effects, especially if it is prepared freshly.[16,36] Anthocyanins, β-carotene, and phenolic compounds such as punicalagin, gallagic acid, ellagic acid, and tannin compounds like protocatechuic acid, punicalagin, o- and p-coumaric acids, ferulic acid, caffeic acid, chlorogenic acid, phloridzin, and quercetin provide the potent antioxidant properties of PJ. The antioxidant agents in PJ can stop chain reaction of free radical production and delay plasma lipid peroxidation via combination with cupper.[18-22] Also, flavonoids, vitamin E, gallic acid, catechin, epicatechin, iron,[18,23] and ascorbic acid present in PJ contribute to antioxidant activity[19,20] and scavenging properties of free radicals.[37] They also decrease the systemic oxidative stress index such as superoxide dismutase and catalase activity, and protect against protein and DNA oxidation as well.[17] The hydroxyl groups as the main phenolic hydrogen donors would explain the scavenging property of free radicals such as 1,1-diphenyl-2-picrylhydrazyl[19] and the inhibition of macrophages ability to low-density lipoprotein and lipid oxidation. They scavenge oxygen and nitrogen free radicals, as well.[24,38] The increases of glutathione transferase, reductase, and peroxidase activity as well glutathione level have important roles in the high antioxidant potency of PJ.[15 28] According to the potential antioxidant properties of pomegranate, it has been successfully tested

to reduce the nephrotoxicity induced by some agents such as: carbon tetrachloride,[15] gentamicin,[37] hexachlorobutadiene,[16] ethylene glycol,[39] oxalate crystals,[40] adenine,[41] and chemotherapeutic agents such as cisplatin,[28] in such a way that the renal histopathological indexes, such as the levels of urea and creatinine, showed improvement.[15,33,37] There are also reports on the positive effects of PJ on improving renal histopathological oxidative damage caused by other nephrotoxic models like ischemic reperfusion.[33] It is noteworthy that there is evidence of increased levels of malondialdehyde and even exacerbation of gentamicin-induced tissue damage resulted from the administration of PJ. However, these effects have also been observed at high doses of some antioxidants, including PJ at doses of 50 mg/kg and more.[15,33]

According to our study, due to the protective effects of the active antioxidant ingredients of PJ on kidney disease, it might also affect malondialdehyde as the indicator of the lipid peroxidation induced by gentamicin and captopril. It seemed that PJ could stabilize and improve the kidney function during this period, despite the continual prescription of gentamicin or captopril. Pomegranate juice had also positive effects on the changes of plasma glucose and urine volume induced by these two drugs.

Pomegranate juice can exert anti-inflammation effects, as well. The ellagitannins compounds present in PJ such as punicalin and punicalagin exert their anti-inflammatory cell signaling effects through a decline in the activation of the nuclear factor-kB and mitogen-activated protein kinases. Procanthocyanidins and anthocyanidins can inhibit cyclooxygenase and inducible nitric oxide synthase.[18,39,40,42] As a result, these ingredients interfere with the inflammation signaling pathway such as PI3K/AKT, mTOR, PI3K, Bcl-X, Bax, Bad, ERK1/2, P38, JNK, and caspase which lead to the inhibition of ornithinedecarboxylase, carbonic anhydrase, 17 beta-hydroxysteroid dehydrogenase, and serine protease.[19,24] Flavonols such as kaempferol present in PJ decrease the expression of tumor necrosis factor-α and interleukin-1-β. Pomegranate juice inhibits phospholipase A2, arachidonic acid production, and fibroblast, as well. It also acts through the inhibition of matrix metalloproteinase, which has an important role in destroying structural proteins. This will reinforce interstitial spaces to help maintain the normal cellular architecture.[18,42] The alterations in the structure and composition of the extracellular matrix, inhibition of endothelial dysfunction, matrix remodeling, and gelatinases activity are the suggested mechanisms for these functions of PJ.[25]

The quality of pomegranate compounds accounts for its antioxidant and anti-inflammatory superiority compared to other fruits.[36] These potential abilities can be involved in the improvement of gentamicin- and captopril-induced renal toxicity and the neutralization of free radicals as seen in this study. It is obvious that a detailed analysis of the pomegranate or its juice ingredients can help introduce its natural agents to attenuate adverse effects of the used drugs at clinic. Since the priority of present work was only the evaluation of pomegranate juice effects on a part of nephrotoxicity-induced captopril and gentamicin features, some indexes like stress-oxidative parameters, blood urea, urinary sodium and potassium, and serum creatinine were considered. There must be more accurate markers like neutrophil gelatinase-associated lipocalin or interleukins, which were not the aim of present work. Also to best conclude, the renal pathological assessment is necessary. This matter was not designed for present report as well. The measurement of other more sensitive markers and the pathophysiology evaluations are the limitations of present work. They will be considered at the subsequent project during the next step of our study.

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CONFLICT OF INTEREST

None declared.

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