Research Article

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Pomegranate attenuates kidney injury in cyclosporine-induced nephrotoxicity in rats by suppressing oxidative stress

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Abstract: To investigate the effect of pomegranate juice (PJ) on the cyclosporine (CsA)-induced nephrotoxicity in rats, 80 rats were divided into four groups. The first group was regarded a negative control group, and the others were as follows: group 2 (CsA group) received CsA in a dose of 25 mg/kg/day orally, group 3 (treated group) received CsA in a dose of 25 mg/kg/day plus 2.5 mL/day of PJ, and group 4 (PJ group) received 2.5 mL of PJ daily. By the end of the 21st day, plasma creatinine, blood urea nitrogen (BUN), creatinine clearance, urinary KIM-1, and NGAL were determined. Histopathological investigation and the determination of malondialdehyde and antioxidant enzymes were analyzed in kidney tissues. The results show that plasma creatinine, BUN, creatinine clearance, kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin were significantly altered in the CsA group. The supplement of PJ attenuated the alteration in these parameters. The treatment with PJ also prohibits the CsA-induced alteration in the histopathology, lipid peroxidation, and antioxidant enzymes. We can conclude that PJ protects against CsA-induced nephrotoxicity due to its antioxidant effects.

Keywords: pomegranate, cyclosporine, nephrotoxicity, kidney injury, oxidative stress

1 Introduction

Acute kidney injury (AKI) is a diverse disease characterized by a substantial decrease in glomerular filtration rate (GFR), resulting in the retention of metabolic waste products such as urea and creatinine, as well as dysregulation of fluid, electrolyte, and acid–base balance. The causes of AKI can be categorized into three classes: pre-renal, intrinsic, and post-renal. Examples of pre-renal causes are hypovolemia, impaired cardiac function, systemic vasodilatation, and drugs that cause renal vasoconstriction such as cyclosporine (CsA). Intrinsic causes include renal ischemia, nephrotoxic agents, and glomerulonephritis. Prostate hypertrophy, improperly placed catheter, some types of cancers, and retroperitoneal fibrosis are examples of post-renal causes [1]. Coronavirus disease 2019, a recent pandemic infectious disease, can cause AKI either directly through angiotensin-converting enzyme 2 and dipeptidyl peptidase 4 receptors or indirectly through the development of cytokine storm [2].

CsA is an immunosuppressive medication that has improved the quality of life and survival rate of transplant recipients, as well as being used to treat autoimmune disorders [3]. The use of CsA after kidney transplantation is thought to be linked to the development of chronic allograft nephropathy, which results in a progressive and irreversible loss of graft function and is a leading cause of
redialysis [4]. Although the mechanism of CsA-induced nephrotoxicity is unknown, the findings suggest that CsA reduces GFR due to vasoconstriction in the afferent preglomerular arteriole [5]. Progressive glomerulosclerosis, tubulointerstitial fibrosis associated with mononuclear cell infiltration, and tubular atrophy are the general histological manifestations of CsA-induced nephrotoxicity [6]. The devastating effects of reactive oxygen species (ROS) and lipid peroxidation are also announced in the CsA nephrotoxicity [7,8]. Many experimental studies demonstrated that CsA increases the production of ROS and lipid peroxidation products in kidney tissues [9]. Other studies found that CsA induces tubular cell apoptosis by inhibiting their DNA synthesis [10]. A recent article suggested that CsA induces the synthesis of an inflammation promotor, namely, osteopontin, which induces renal tubular cell damage [11].

Medicinal plants with antioxidant activities have been used for the treatment and prevention of several human disorders [12]. These natural products have little or no side effects compared to allopathic drugs and therefore are safe for long-term use. The bioactive constituents in natural products can attain the same potency as synthetic drugs, although they are taken in larger quantities [13,14]. Nowadays, successful attempts have been performed to explore the effects of several natural products for the prevention of CsA nephrotoxicity. The experiments included curcumin [8], Nigella sativa oil [15], amino acids [16,17], fatty acids [18,19], and vitamin C [20].

Pomegranate (*Punica graminatum*) is an ancient fruit that is grown in many locations in the world. It is a rich source of bioactive ingredients, especially polyphenols and flavonoids that have antioxidant properties [21]. Pomegranate juice (PJ) was found to have higher antioxidant capacity compared to green tea, vitamin E, β-carotene, and ascorbic acid [22]. Literature survey displayed that pomegranate has potent health effects. It can prevent and assist treatment of many disorders including hyperglycemia [23], hypertension [24], hyperlipidemia [25], inflammatory diseases [26], arthritis [27], Alzheimer’s disease [28], and malignancies [29–31]. Pomegranate extract also has antimicrobial [32], antimalarial [33], and dental antiplaque effects [34] and improves wound healing [35]. Recent studies suggested that pomegranate is effective against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infections [36,37].

As a result, the current study was intended to investigate the nephroprotective effect of PJ against CsA-induced nephrotoxicity in rats by measuring biomarkers of kidney injury and oxidative stress. According to our knowledge, this is the first study that investigates the effect of PJ on CsA nephrotoxicity by measuring sensitive markers of kidney injury (urinary excretion of kidney injury molecule-1 [KIM-1] and neutrophil gelatinase-associated lipocalin [NGAL]) in addition to the classical markers, that is, serum creatinine, blood urea nitrogen (BUN), and creatinine clearance. Moreover, the oxidative stress and lipid peroxidation markers were estimated to predict the possible mechanism of nephroprotective effect of PJ.

## 2 Experimental

### 2.1 Chemicals

Double distilled water and analytical grade solvents (Sigma-Aldrich, MO, USA) were used throughout the work. CsA was purchased from Novartis (Novartis Pharmaceuticals, Australia) as oral solution (100 mg mL\(^{-1}\)) commercially known as Neoral\textsuperscript{®}. Freshly prepared solution of CsA (20 mg mL\(^{-1}\)) was prepared daily by dilution with olive oil.

### 2.2 PJ preparation

Fresh pomegranate fruits were purchased from a local market at Mansoura City, Egypt. Pomegranates were washed and manually peeled. The seeds were separated and ground using an electrical blender. The mixture was filtered, and the obtained PJ was stored in a dark container at \(-20^\circ C\) for a period not exceeding 3 days. The juice was characterized by the determination of total phenolic compounds (mg gallic acid equivalent [GAE] per liter) by a modified Folin–Ciocalteu [38] and total flavonoids (mg rutin equivalent [RE] per liter) by aluminum chloride method [39]. The total antioxidant activity was determined by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay [40] and by ferric reducing ability of plasma (FRAP in mol Trolox equivalents [TE] per L) method [41]. The concentrations of gallic acid, caffeic acid, ellagic acid, and catechin were estimated by a Waters 2690 Alliance HPLC system (Milford, MA, USA) equipped with C18 thermo column (4.6 mm × 250 mm, 5 μm) and a Waters 996 photodiode array detector. The results of five independent measurements are summarized in Table 1.

### 2.3 Animals

The study was conducted on 80 Sprague Dawley male rats (9–10 weeks old, initially weighing about 200–220 g). The animals were housed in individual metabolic cages at a temperature of 25 ± 1°C, a relative humidity of about
60–70%, with a 12-h light–dark cycle, and fed ad libitum standard pellet chow diet (23% protein, 5.75% fat, 3.41% fiber, 3,150 kcal kg−1). Experiments were performed in accordance with the Committee for the Urology and Nephrology Center of Experimental Animals, Mansoura University, following the guide for care and use of laboratory animals [42].

2.4 Experimental design

The rats were randomly distributed into four groups (n = 20 in each group) and treated for 21 consecutive days as follows:

- **Group 1 “control group”:** rats were treated by gavage with olive oil as vehicle for CsA (0.25 mL/day).

- **Group 2 “CsA group”:** rats received CsA at a dose of 25 mg/kg/day of body weight in 0.25 mL of olive oil.

- **Group 3:** rats received CsA (25 mg/kg/day) in addition to 2.5 mL of PJ, equivalent to 20 gallic acid/kg body weight/day, respectively. PJ was administered immediately after CsA intake.

- **Group 4 “PJ group”:** rats received oral 2.5 mL of PJ.

2.5 Sampling

On the 22nd day, 24 h urine samples were collected by a metabolic cage. The samples were collected into plastic tubes and centrifuged at 1,500 rpm for 10 min at 4°C. The rats were anesthetized with ketamine–xylazine mixture [43], and about 3.0 mL of blood was withdrawn into plain red Vacutainer tubes (Becton Dickinson Vacutainer Systems, NJ, USA) by retro-orbital puncture. The serum samples were obtained by centrifugation at 3,000 rpm for 10 min. After that, the rats were sacrificed by cervical decapitation. The right kidneys for all animals were separated, washed with phosphate-buffered saline (pH 7.4), weighed, and homogenized with ice-cold phosphate buffer.

The protein content of the homogenate was determined, and the samples were centrifuged (1,500 rpm, 15 min, 4°C). Finally, the supernatant was collected and stored at −80°C in an Eppendorf tube until the analysis of oxidative stress parameters. The left kidney was fixed in a 10% solution of buffered formalin (pH 7.4). The tissue was embedded in paraffin, and sections of 5 μm were taken using MAC microtome (Macro Scientific Works, Delhi) and stained with hematoxylin–eosin (H&E). The slides were examined for the histological variations using light microscope by a pathologist in blinded manner.

### Table 1: Levels of some bioactive components of PJ

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic compounds</td>
<td>1872.2 ± 70.7 mg GAE L−1</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>182.7 ± 6.0 mg RE L−1</td>
</tr>
<tr>
<td>Total antioxidant activity – DPPH (IC50)</td>
<td>25.3 ± 0.2 g L−1</td>
</tr>
<tr>
<td>Total antioxidant activity – FRAP</td>
<td>6.6 ± 0.1 mol TE L−1</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>11.7 ± 1.1 mg L−1</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>6.4 ± 0.2 mg L−1</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>71.7 ± 3.3 mg L−1</td>
</tr>
<tr>
<td>Catechin</td>
<td>103.6 ± 4.6 mg L−1</td>
</tr>
</tbody>
</table>

2.6 Biochemical parameters

Serum creatinine (Scr), urine creatinine (Ucr), and BUN levels were measured by commercial spectrophotometric kits (Biodiagnostic Co., Giza, Egypt). Creatinine clearance (Ccr) was estimated using the following equation:

\[
C_{cr} (\text{mL min}^{-1}) = \frac{U_{cr} (\text{mg dL}^{-1}) \times \text{urine volume (mL)}}{S_{cr} (\text{mg dL}^{-1}) \times 1,440}
\]

The urinary activities of KIM-1 and NGAL were assayed using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions (SunLong Biotech, Hangzhou, Zhejiang, China). The levels of urinary KIM-1 and NGAL were normalized to urinary creatinine to compensate for the effect of flow rate.

Markers of lipid peroxidation, that is, malondialdehyde (MDA), and antioxidant enzymes, catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), were measured in kidney tissues according to the protocol of the supplier (Bio-Diagnostic, Giza, Egypt). The results were expressed per milligram tissue protein.

2.7 Statistical analysis

All statistical calculations were performed and analyzed using SPSS statistical package, version 25 software (MAS Medical & Scientific Eq. Co, IL, USA) by the one-way analysis of variance. The results were expressed as mean ± SD, and values were considered statistically significant at P < 0.05.

3 Results

3.1 Body and kidney weight changes

The oral administration of CsA (25 mg/kg/day) caused a decrease in body weight (Figure 1) and kidney weight
(Figure 2) compared to the controls ($P < 0.05$). However, the oral co-administration of PJ with CsA significantly reduced these effects by maintaining the body and kidney weight to the same levels ($P > 0.05$) of the control group. During the experiment, the increase in animal body weight was lower in rats treated with

**Table 2: Results of the histopathological study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>CsA group</th>
<th>CsA + PJ group</th>
<th>PJ group</th>
<th>$P1$</th>
<th>$P2$</th>
<th>$P3$</th>
<th>$P4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilated irregular tubules</td>
<td>0 (20)</td>
<td>0 (0)</td>
<td>0 (10)</td>
<td>0 (20)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>+ (0)</td>
<td>+ (1)</td>
<td>+ (10)</td>
<td>+ (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++ (0)</td>
<td>++ (8)</td>
<td>++ (0)</td>
<td>++ (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++ (0)</td>
<td>+++ (11)</td>
<td>+++ (0)</td>
<td>+++ (0)</td>
<td></td>
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<tr>
<td>Attenuated and shedding</td>
<td>0 (20)</td>
<td>0 (0)</td>
<td>0 (10)</td>
<td>0 (20)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>+ (0)</td>
<td>+ (3)</td>
<td>+ (10)</td>
<td>+ (0)</td>
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<td></td>
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<tr>
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<td>++ (10)</td>
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<td>+++ (0)</td>
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</tr>
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<td>Increased nuclear-cytoplasmic ratio</td>
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<td>0 (0)</td>
<td>0 (9)</td>
<td>0 (20)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>and prominent nucleoli</td>
<td>+ (0)</td>
<td>+ (5)</td>
<td>+ (9)</td>
<td>+ (0)</td>
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<tr>
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<td>+++ (5)</td>
<td>+++ (0)</td>
<td>+++ (0)</td>
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<td></td>
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<tr>
<td>Interstitial edema</td>
<td>0 (20)</td>
<td>0 (0)</td>
<td>0 (12)</td>
<td>0 (20)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
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<tr>
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<tr>
<td></td>
<td>+++ (0)</td>
<td>+++ (5)</td>
<td>+++ (0)</td>
<td>+++ (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>0 (20)</td>
<td>0 (1)</td>
<td>0 (11)</td>
<td>0 (20)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>+ (0)</td>
<td>+ (8)</td>
<td>+ (9)</td>
<td>+ (0)</td>
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</tr>
<tr>
<td></td>
<td>+++ (0)</td>
<td>+++ (3)</td>
<td>+++ (0)</td>
<td>+++ (0)</td>
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</tr>
</tbody>
</table>

0 (absent), + (mild), ++ (moderate), +++ (marked). $P1$: between the four groups. $P2$: Controls vs CsA groups. $P3$: Controls vs CsA + PJ groups. $P4$: CsA vs CsA + PJ groups.
CsA (6.3%) compared to that in the control group (12.3%), and the difference reached the significant level ($P < 0.05$). The percent increase in the body weight in the other groups is statistically comparable to the control group ($P > 0.05$).

### 3.2 Histopathological findings

The histopathological investigation displayed significant variations between the studied groups (Table 2). These alterations were principally observed in inner renal cortex and medulla of the CsA-treated groups (Figure 3). All kidneys from the control and PJ groups had normal architecture without any signs of glomerular or tubular lesions. In contrast, all rats of the CsA-treated group showed dilated irregular tubules with markedly thinned lining epithelium and intraluminal casted degenerated cells, tubular necrosis, interstitial edema, and inflammation. The nonsheaded remaining tubular epithelial cells show increased nuclear-cytoplasmic ratio and prominent nucleoli. In the rats that were treated with both CsA and PJ, mild tubular injury in focal areas was found with dilated irregular tubules, attenuated lining, and focal shedding into lumen in few tubules.

### 3.3 Changes in serum and urinary biochemistry

Plasma creatinine elevated in the CsA-treated group, and the increase is statistically significant compared to the controls ($P < 0.05$). Levels of creatinine clearance were significantly decreased in the group that treated with CsA when compared to the controls ($P < 0.01$). A statistically significant increment ($P < 0.01$) in the level of BUN was observed in animals treated with CsA alone, when compared to the controls. The treatment with PJ significantly ameliorated the alterations in plasma creatinine, creatinine clearance, and BUN as presented in Table 3. The results also indicated that the urinary excretions of KIM-1 and NGAL were significantly higher than those among the controls ($P < 0.01$), and the supplement of PJ corrected the excretion of these urinary markers toward normal in the group of animals that treated with both CsA and PJ.

### 3.4 Lipid peroxidation and antioxidant enzymes

There was a significant elevation in the concentration of MDA in kidney tissues in the rats that treated with CsA compared with the controls ($P < 0.01$). The supplement of PJ together with CsA attenuated the CsA-induced lipid peroxidation in kidney tissues (Figure 4). In contrast, the activities of CAT, GPx, and SOD in kidney tissues were significantly decreased among the CsA group compared with the controls. Treatment with PJ also significantly prohibits the CsA-induced reduction in the antioxidant enzymes.

### 4 Discussion

The present study demonstrated that pomegranate exhibits antioxidant and nephroprotective properties, which
in turn ameliorates CsA-induced nephrotoxicity in experimental rats and preserves the renal function. Table 4 briefly summarizes a comparison of the main findings in our study with previously published results of similar materials.

CsA has been used in the treatment of various diseases, but unfortunately it leads to nephrotoxicity. The mechanism of the nephrotoxicity is not clearly known, but this could be induced by generating ROS as suggested in some studies [50]. According to our literature review, this is the broadest study reporting that PJ attenuates the detrimental effects of CsA in kidney tissues as shown by histologic and biochemical data. Moreover, our study is the first that demonstrates the antioxidant effect of PJ on the CsA nephrotoxicity and uses AKI novel markers to confirm its protective effect. Pomegranate has shown its antioxidant effect in ameliorating the toxicities of other drugs like cisplatin and gentamycin [51,52].

We noticed that the weight of the rats in the CsA group was significantly reduced compared to the control and the PJ + CsA groups at the end of the study. This is similar to what Tutanc et al. found in their study, which evaluated the effects of erdosteine on CsA-induced nephrotoxicity [53]. This may be due to the decreased water, food consumption, and decreased mobility associated with the deteriorating renal function due to the CSA nephrotoxicity in this group.

This study demonstrated that PJ has advantageous histological and biochemical effects on the CsA nephrotoxicity. Ali et al. reported a potential nephroprotective effect of PJ against CsA-induced nephrotoxicity. Histological assessments and biochemical changes confirmed the nephrotoxic

**Table 3: Effect of CsA and pomegranate treatment on parameters of kidney function**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>CsA group</th>
<th>CsA + PJ group</th>
<th>PJ group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg dL⁻¹)</td>
<td>0.41 ± 0.03</td>
<td>0.59 ± 0.04*</td>
<td>0.45 ± 0.04†</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>Creatinine clearance (mL min⁻¹)</td>
<td>1.32 ± 0.23</td>
<td>0.38 ± 0.11**</td>
<td>0.88 ± 0.19††</td>
<td>1.34 ± 0.18</td>
</tr>
<tr>
<td>BUN (mg dL⁻¹)</td>
<td>15.6 ± 1.6</td>
<td>35.4 ± 9.4**</td>
<td>22.7 ± 2.0††</td>
<td>16.3 ± 1.8</td>
</tr>
<tr>
<td>Urinary KIM-1 (ng mgCr⁻¹)</td>
<td>0.44 ± 0.10</td>
<td>33.5 ± 5.9**</td>
<td>12.4 ± 4.0**. ††</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>Urinary NGAL</td>
<td>32.7 ± 6.3</td>
<td>268.4 ± 31.3**</td>
<td>141.0 ± 19.6**. ††</td>
<td>29.4 ± 5.5</td>
</tr>
</tbody>
</table>

*Significantly different from the control group at P < 0.05. **Significantly different from the control group at P < 0.01. †Significantly different from the CsA-treated group at P < 0.05. ††Significantly different from the CsA-treated group at P < 0.01. CsA, cyclosporine A; PJ, pomegranate juice; BUN, blood urea nitrogen; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin.

**Figure 4: Effect of CsA and pomegranate treatment on markers of oxidative stress.**

**Significantly different from the control group at P < 0.01. ††Significantly different from the CsA-treated group at P < 0.01.**
Table 4: Comparison of the effect of PJ on CsA-induced nephrotoxicity with other similar materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Animal model</th>
<th>Main conclusion</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigella sativa oil</td>
<td>Wistar albino rats</td>
<td>Nigella sativa oil protects the kidney, potentially against oxygen-free radicals, reducing plasma urea and morphological changes caused by prolonged CsA treatment</td>
<td>[15]</td>
</tr>
<tr>
<td>Hydroxytyrosol</td>
<td>Sprague Dawley rats</td>
<td>Administration of hydroxytyrosol prevented the increase in superoxide level in renal artery and a mild effect on histological damages and does not impact the change in GFR. Hydroxytyrosol entirely prevents the oxidative stress caused by CsA in the arteries and kidneys, and it has only a moderate effect on the accompanying tubular damage and interstitial fibrosis without changing the arteriolopathy. It is unable to alleviate the blood pressure elevation and GFR decrease associated with CsA.</td>
<td>[44]</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>Wistar albino rats</td>
<td>The increase in serum creatinine and urea due to the administration of CsA was prevented by co-administration of N-acetylcysteine with CsA. Milder histopathological changes in the kidney were observed in the treated group compared to the CsA group.</td>
<td>[45]</td>
</tr>
<tr>
<td>Caffeic acid phenethyl ester</td>
<td>Wistar albino rats</td>
<td>Caffeic acid phenethyl ester inhibits CAT and lipid peroxidation-mediated nephrotoxicity by inhibiting the oxidative process.</td>
<td>[46]</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>Wistar albino rats</td>
<td>Epicatechin reduced the toxicity of CsA by scavenging free radicals and increasing antioxidant activity. It prevented the increase in hydroperoxides and thiobarbituric acid reactive substances, while it enhanced the activities of antioxidant enzymes (SOD, CAT, and GPx). No markers of kidney damage were measured in this study.</td>
<td>[47]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Wistar albino rats</td>
<td>Curcumin markedly modified the alteration in serum creatinine, BUN, creatinine, and urea clearance that were induced due to CsA administration. It also decreased high levels of thiobarbituric acid reactive substances, improved antioxidant enzyme levels (GHX, SOD, and CAT) in CsA-treated rats, and corrected the abnormal kidney morphology.</td>
<td>[48]</td>
</tr>
<tr>
<td>Aqueous leaf extract of Costus afer</td>
<td>Wistar albino rats</td>
<td>Treatment with the extract at various dosages attenuated CsA-induced nephrotoxicity and oxidative kidney impairments, as demonstrated by considerably lower plasma creatinine, BUN, K+, and renal MDA levels. Furthermore, all dosages significantly increased renal GSH levels as well as plasma SOD activity, CAT, and glutathione-S-transferase.</td>
<td>[49]</td>
</tr>
<tr>
<td>PJ</td>
<td>Sprague Dawley rats</td>
<td>Treatment with PJ prevents CsA-induced changes in markers of kidney damage (plasma creatinine, creatinine clearance, BUN, KIM-1, and NGAL), lipid peroxidation (MDA), antioxidant enzymes (CAT, GPx, and SOD), and histopathology.</td>
<td>Our study</td>
</tr>
</tbody>
</table>

Effect of CsA in rats following repeated daily exposure to CsA for 28 days [54]. Similar to our study, Ali et al. showed the structural damage of the renal tubules and the rise of BUN have been improved significantly in the CsA + PJ group. The same histological benefits of pomegranate on other drug toxicities like cisplatin and gentamycin have been reported by Karwasa et al. and Cekmen et al., respectively [51,52]. Plasma creatinine was significantly higher in the CsA group, and the creatinine clearance was significantly lower in the CsA group. Moreover, the urinary KIM and NGAL were significantly lower in the CsA + PJ group compared with the CsA group in our study. The administration of PJ corrects these changes toward the levels of normal controls. It has been documented in the literature that pomegranate significantly reduced inflammatory and renal tubular injury biomarkers (KIM-1 and NGAL) due to its nephroprotective effect and attenuation of the toxicities of other drugs. For example, Alkuraishy et al. have demonstrated that pomegranate has a protective effect against the gentamycin nephrotoxicity. They found a significant reduction of the KIM-1 and NGAL levels in the sera of rats in the group of pomegranate [55]. KIM-1 and NGAL are more sensitive markers of nephrotoxicity compared to plasma creatinine and BUN [56].

Lipid peroxidation is initiated as a result of ROS-induced abstraction of hydrogen from polyunsaturated fatty acids of cellular membrane, which results in the formation of relatively stable compounds such as MDA. Increased levels of lipid peroxides and conjugated dienes...
were shown previously in the renal tissue of CsA-treated rats [53,57]. Similarly, in our study the MDA levels of the CsA-administered group were significantly higher than those of the controls. This showed that CsA caused lipid peroxidation in renal tissue, thus leading to oxidative damage, which is significantly ameliorated in the CsA + PJ group. The beneficial effect of pomegranate on reducing the renal MDA level has been reported in other drug toxicities such as cisplatin-induced AKI, and this is augmenting the role of pomegranate in the inhibition of oxidative stress [51]. In our study, we found that CsA significantly reduces the antioxidant enzymes in the renal tissue. This was not significant in the study by Tutanc et al. [53]. This may be explained by the short period of the study and the lower dose of CsA. Tutanc et al. used 20 mg/kg/day and was given for only 7 days, which may not be enough to reflect the detrimental effect of CsA on the inhibition of the antioxidant enzymes. Moreover, the number of rats in each group was only seven. We have reported that pomegranate supplementation suppresses the renal MDA level and increases antioxidant enzyme activities (CAT, SOD, and GPx) in renal tissue. These findings support the antioxidant activity of pomegranate, which is established in other nephrotoxic models such as cisplatin and gentamicin-induced nephrotoxicity [51,52,58].

5 Conclusion

Pomegranate has a protective role against CsA-induced nephrotoxicity in rats as shown by our biochemical and histological findings. Oral supplement of PJ significantly improved renal dysfunction and protected the kidneys from free radical-mediated injury caused by CsA by protecting the marker enzymes, as well as further strengthening the cell’s antioxidant status. The findings indicate that PJ is beneficial in preventing functional impairment in a rat model of CsA-induced nephrotoxicity. Future studies are suggested to determine the benefit of co-administration of PJ with CsA in kidney transplant patients.

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Ethical approval: The Institutional Review Board of Urology and Nephrology Center (Mansoura University) approved the protocol of the study.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References


