

# Ameliorative Effect of Green Tea Against Contrast-induced Renal Tubular Cell Injury

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**Keywords.** contrast media, green tea, renal injury, nephrotoxicity

**Introduction.** Reactive oxygen species are a mediator of kidney damage by contrast media, and green tea is a potent-free radical scavenger. This study was designed to examine whether green tea could protect against the nephrotoxicity induced by contrast media.

**Materials and Methods.** Forty rats were randomly divided into 4 groups. Group 1 was control; group 2 received contrast medium (intravenous iodixanol, 10 mL/kg, as a single dose); group 3 received contrast medium and then green tea extract for 3 days (10 mg/kg/d, intraperitoneal); and group 4 first received green tea and then contrast medium. Histological changes (degeneration, vacuolization of tubular renal cells, dilatation of tubular lumen, and presence of debris in the lumens) were assessed and recorded as scores from zero to 4. The sum of scores were used as the overall renal injury level.

**Results.** Groups 3 and 4 with green tea treatment had significantly higher overall scores than the control group, but significantly lower scores than group 2 with contrast medium only. A similar trend was seen for dilatation and degeneration levels. Vacuolization level was not significantly lower in the green tea groups as compared to the contrast medium group. Debris level was not significantly lower in group 3 than group 2. The differences were not significant between groups 3 and 4.

**Conclusions.** We observed beneficial effect of green tea against nephrotoxicity of contrast media. Green tea extract may offer an inexpensive and nontoxic intervention strategy in patients with a risk for nephrotoxicity with contrast media.

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## INTRODUCTION

Contrast media are nephrotoxic iodinated materials and injury is associated with an increase in serum creatinine level.<sup>1,2</sup> Contrast media are the 3rd most common cause of kidney dysfunction.<sup>3</sup> They increase adenosine and free radicals, causing an increase in vasoconstriction while reducing nitric oxide and prostaglandin that lead to decreased vasodilation in the renal medulla. In addition, they can lead to acute tubular necrosis and hypoxia.<sup>2-5</sup>

Contrast agents have effects on the inhibition of mitochondrial enzyme activity and increase adenosine by adenosine triphosphate hydrolysis. Catabolism of adenosine releases reactive oxygen species and reduces nitric oxide which have direct toxic effects on renal tubular cells.<sup>6-11</sup> The effects of contrast media on renal tubules include DNA fragmentation, vacuolization of proximal tubular epithelial cells, and necrotic cells in the medullary thick ascending loop of Henle.

Green tea is one of the plants which prevent or alleviate many human diseases and has a wide range of pharmacological effects. It is a source of flavonoids and polyphenols. Antioxidant activity of green tea has an important role in the treatment and prevention of various complications.<sup>12-15</sup> Green tea polyphenols act as metal chelators and scavengers of free radicals. The antioxidant activities are protective effects against renal damage.<sup>16</sup> Moreover, green tea polyphenols decrease ischemia and chemical toxicity in different tissues of the body.<sup>17</sup> Green tea is an inexpensive nontoxic agent which protects the nephrotoxicity induced by gentamicin.<sup>18</sup> Considering the safety profile of green tea and its ability to reduce renal injury due to other nephrotoxic drugs, this investigation was designed to test whether green tea would protect against the nephrotoxicity induced by contrast media.

## MATERIALS AND METHODS

### Green Tea Extract Preparation and Chemicals

Iodixanol (Healthcare, Ireland) was purchased and green tea (Golestan, Iran) was purchased from a local supermarket in Shahrekord, Iran. A herbarium specimen of green tea was deposited in the Herbarium Unit of Shahrekord University of Medical Sciences (code 349).

The maceration method was used to prepare green tea extract.<sup>19</sup> One liter of ethanol (70%) was added to 100 g of green tea and was left in laboratory temperature for 2 days. The solution was then filtered using a filter paper and the pulp was squeezed to discharge. The filtered extract was concentrated by a rotary evaporator.<sup>20</sup> Then, it was frozen and stored at -20°C until use, which was reconstructed with distilled water when needed.<sup>21</sup>

### Experiments

To identify the protective effect of green tea against the nephrotoxicity of contrast media, 40 adult male Wistar rats (6 weeks old) with a body weight of 200 g to 250g were used. The estimated required sample size was 9 in each group for 1-sample comparison of mean values to the hypothesized value with an alpha of 0.05 (2-sided), a power of 0.95, and a mean  $\pm$  standard deviation of degeneration of  $25 \pm 4$ . To elevate the precision, we selected 10 rats in each group. The rats were designated randomly into 4 experimental groups as follow: group 1, the control group (sham

group) that did not receive any drugs; group 2, the contrast medium group that received intravenous iodixanol, 10 mL/kg, as a single dose<sup>22</sup>; group 3, green tea group that first received the same dose of iodixanol and then 10 mg/kg/d of green tea extract,<sup>23</sup> for 3 days; and group 4, green tea pretreatment group that first received green tea, 10 mg/kg/d, for 3 days and then 10 mL/kg of iodixanol on the 3rd day.

### Experimental Study Protocol

All rats were given unlimited access to standard rat chow and water. After 20 minutes, rats of group 4 were given 10 mg/kg of green tea by daily intraperitoneal injection for 3 days. On the 3rd day, 10 mL/kg of contrast medium was injected to animals in groups 2 and 4 via the lateral tail vein. After 20 minutes, rats of group 3 were given 10 mg/kg of green tea by intraperitoneal injection daily, for 3 days. On the 5th day, all of the rats were killed using ketamine. The kidneys were dissected out immediately after sacrificing and fixed with 10% formalin for histological examinations. The kidney paraffin sections (2- to 3- $\mu$ m thick) were prepared by a microtome and stained with hematoxylin-eosin for histological examinations.<sup>22</sup>

The hematoxylin-eosin-stained sections were evaluated by light microscope for severity of renal injury (degeneration, vacuolization of tubular renal cells, dilatation of tubular lumen, and presence of debris in the lumens), using conventional protocols.<sup>21,22</sup> The slides were coded and evaluated by a nephropathologist who was blinded to the animal groups. Each morphologic lesion was scored from 1 to 4, while the score zero was assigned to the normal tissue without any pathological damage. Therefore, score zero was considered as normal; score 1, up to 19% involvement; score 2, 20% to 49% involvement; score 3, 50% to 69% involvement; and score 4, 70% to 100% involvement.<sup>24,25</sup>

### Ethics

The experiment was approved by the Ethical Committee of Shahrekord University of Medical Sciences. We followed the ethics principles of working on animals to impose the lowest possible stress on animals.

### Data Analysis

All continuous variables expressed as

mean  $\pm$  standard deviation, and categorical variables frequency and percentage. Given the normality of data distributions, the 1-way analysis of variance and the post hoc Scheffe test were used for the comparison of mean values between the four groups. *P* values less than .01 were considered significant. Data analysis was done using Stata software (Stata Statistical Software, version 12, Stata Corp LP, College Station, TX, USA).

## RESULTS

The mean levels of dilatation, degeneration, vacuolization, and debris of each group are shown in Table 1. The mean of score value was significantly lower in the control group when compared to the other groups ( $F = 81.62, P < .001$ ), while it was highest in group 2 (contrast medium), and groups 3 and 4 with green tea treatment had significantly lower scores than that. A similar trend was seen for dilatation and degeneration levels (Table 2). However, vacuolization level was not significantly lower in the green tea groups as compared to the contrast medium group, and debris level was not significantly lower than that in group 2 when green tea was administered after contrast medium (group 3). Although renal injury and overall scores were higher in group 3 than the scores of group 4, the differences were not significant (Table 2).

## DISCUSSION

In this study, the renal histological changes due to contrast medium were documented in the rats that received iodixanol only (group 2), which was significantly greater compared to the controls and the rats that also received green tea. Renal injury scores in the groups 3 and 4 (green tea and contrast medium) were higher than in the control group, they were lower as compared to group 2. Comparing the two intervention strategies with green tea, although renal injury scores were higher in the rats that received green tea after the contrast medium (group 3) than the scores in the rats that received green tea first (group 4), the difference was not significant.

Ameliorative effect of green tea extract, as an antioxidant, had been tested against various nephrotoxic agents. Ryu and colleagues showed the protective effects of green tea extract on cyclosporine -induced structural and functional alternations of the kidney.<sup>17</sup> They concluded that this ameliorative efficiency might be the blockage of renin-angiotensin-aldosterone system by green tea.<sup>17</sup> Likewise, in an investigation on rats, Rehman and coworkers observed green tea polyphenols markedly abolished cyclosporine-induced kidney injury and improved kidney function. They concluded that green tea polyphenols attenuated cyclosporine-induced renal damage, at least in part, through

**Table 1.** Levels of Dilatation, Degeneration, Vacuolization, and Debris of Renal Tubular Cells

Injury Level	Group*				All
	1	2	3	4	
Dilatation	0	26.8 $\pm$ 6.5	13.8 $\pm$ 7.0	13.8 $\pm$ 4.7	13.6 $\pm$ 10.9
Degeneration	1.9 $\pm$ 1.5	22.6 $\pm$ 5.0	12.6 $\pm$ 7.3	12.0 $\pm$ 2.3	12.2 $\pm$ 8.6
Vacuolization	1.2 $\pm$ 1.0	12.3 $\pm$ 2.8	12.7 $\pm$ 6.0	11.1 $\pm$ 1.4	9.3 $\pm$ 5.9
Debris	0	21.6 $\pm$ 4.7	15.3 $\pm$ 8.2	11.1 $\pm$ 1.4	12.0 $\pm$ 9.2
Overall score	3.1 $\pm$ 1.4	83.5 $\pm$ 8.5	54.6 $\pm$ 22.5	48.1 $\pm$ 4.7	47.3 $\pm$ 31.0

\*Group 1 is the control group; group 2, the contrast medium; group 3, contrast medium and green tea; and group 4, contrast medium and green tea pretreatment.

**Table 2.** Differences in Scores for Dilatation, Degeneration, Vacuolization, and Debris of Renal Tubular Cells Between Rat Groups

Injury Level	Differences Between Groups*					
	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4
Dilatation	26.8†	13.8†	13.8†	13.0†	13.0†	0
Degeneration	20.7†	10.7†	10.2†	10.0†	10.6†	0.6
Vacuolization	11.1†	11.5†	9.9†	0.4	1.3	1.6
Debris	21.6†	15.3†	11.1†	6.3	10.5†	4.2
Overall score	80.3†	51.5†	45.0†	28.9†	35.3†	0.8

\*Group 1 is the control group; group 2, the contrast medium; group 3, contrast medium and green tea; and group 4, contrast medium and green tea pretreatment.

†*P* < .01

the stimulation of mitochondrial biogenesis.<sup>18</sup> Accordingly, the antiproteinuric effect of green tea extract on cyclosporine-induced acute kidney injury was tested in rats by Shin and colleagues.<sup>24</sup> They found that green tea extract showed significant antiproteinuric effects through antioxidative activity in kidneys from cyclosporine-induced acute kidney injury.<sup>26</sup> Salem and colleagues investigated whether green tea extract could attenuate the nephrotoxic effects of gentamicin in uninephrectomized rats.<sup>27</sup> They studied 40 uninephrectomized rats and found that green tea ameliorated gentamicin-induced nephrotoxicity and oxidative damage through improving antioxidant defense and tissue integrity.<sup>27</sup> Also in the study conducted by Inoue and colleagues, the protective effect of low and medium doses of green tea polyphenols was shown on dextran sodium sulfate-induced nephrotoxicity.<sup>28</sup> Other studies also revealed the beneficial properties of green tea extract on nephrotoxicity and diabetic kidney disease.<sup>29-36</sup>

Studies concerning the effect of green tea extract on contrast nephropathy are quite scarce, and to the best of our knowledge, this is the first preclinical study which aimed to investigate the green tea effect on contrast-induced nephrotoxicity.<sup>37-42</sup> It is well known that green tea polyphenols have a beneficial effect on pathological states related to oxidative stress of the kidney.<sup>1,12,13</sup> Also, green tea polyphenols have been shown to act as metal chelators, preventing the metal-catalyzed formation of radical species, antioxidant enzyme modulators, and scavengers of free radicals, including the hydroxyl radical.<sup>13,42-45</sup>

## CONCLUSIONS

In our study, beneficial effect of green tea was documented against contrast-induced nephrotoxicity. Moreover, we observed that damage score was less when green tea was administered before exposure to the contrast medium as compared to its administration afterwards, while their difference was not significant. Preclinical studies are necessary to demonstrate whether pretreatment of green tea against contrast-induced nephrotoxicity is more effective than concurrent treatment with this herb and administration of contrast media. Indeed, green tea extract may offer an inexpensive, nontoxic, and effective intervention strategy in patients with a high risk for contrast-

induced nephrotoxicity,<sup>45-55</sup> and further preclinical and human studies on the efficacy of this substance against various injurious insults on renal tubular cells are necessary.

## CONFLICT OF INTEREST

None declared.

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