

LABORATORY STUDY

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## A new update for radiocontrast-induced nephropathy aggravated with glycerol in rats: the protective potential of epigallocatechin-3-gallate

Saziye Sezin Palabiyik<sup>a</sup>, Busra Dincer<sup>b,c</sup>, Elif Cadirci<sup>b</sup>, Irfan Cinar<sup>b</sup>, Cemal Gundogdu<sup>d</sup>, Beyzagul Polat<sup>e</sup>, Muhammed Yayla<sup>f</sup> and Zekai Halici<sup>b</sup>

<sup>a</sup>Faculty of Pharmacy, Pharmaceutical Toxicology Department, Ataturk University, Erzurum, Turkey; <sup>b</sup>Faculty of Medicine, Pharmacology Department, Ataturk University, Erzurum, Turkey; <sup>c</sup>Faculty of Pharmacy, Pharmacology Department, Erzincan University, Erzincan, Turkey; <sup>d</sup>Faculty of Medicine, Pathology Department, Ataturk University, Erzurum, Turkey; <sup>e</sup>Faculty of Pharmacy, Pharmacology Department, Ataturk University, Erzurum, Turkey; <sup>f</sup>Faculty of Medicine, Pharmacology Department, Kafkas University, Kars, Turkey

### ABSTRACT

Contrast media (CM) is known to have nephrotoxic adverse effects. Epigallocatechin-3-gallate (EGCG) is the most abundant and active catechin in green tea, and has strong antioxidant and anti-inflammatory properties. This study investigated whether EGCG can reduce contrast-induced nephrotoxicity (CIN), alone or with glycerol (GLY)-induced renal damage, and to understand its mechanisms of protection against toxicity, using models of GLY and CIN in rats. The rats were separated into eight groups ( $n = 6$  in each), as follows: Healthy, GLY, CM, GLY + CM, CM + EGCG 50 mg/kg (po), GLY + CM + EGCG 50 mg/kg (po), CM + EGCG 100 mg/kg (po), and GLY + CM + EGCG 100 mg/kg (po). Both doses of EGCG protected against CM-induced renal dysfunction, as measured by serum creatinine and blood urea nitrogen (BUN). In addition, EGCG treatment markedly improved CIN-induced oxidative stress, and resulted in a significant down-regulatory effect on tumor necrosis factor (TNF)- $\alpha$  and nuclear factor (NF)- $\kappa$ B mRNA expression. Moreover, histopathological analysis showed that EGCG also attenuated CM-induced kidney damage. Considering the potential clinical use of CM and the numerous health benefits of EGCG, this study showed the protective role of multi-dose EGCG treatment on CIN and GLY-aggravated CIN through different mechanisms.

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### KEYWORDS

Contrast media;  
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## 1. Introduction

With recent advances in medical technology, iodinated contrast media (ICM) has been increasingly used in radiographic diagnostic and interventional procedures.<sup>1,2</sup> CM may be nephrotoxic when combined with potentially nephrotoxic agents, or in patients with a comorbidity, such as diabetic nephropathy. The toxic effect of ICM results in acute kidney injury, and leads to a condition known as contrast-induced nephropathy (CIN). While the incidence of CIN is approximately 2% in patients without risk factors, it may range between 9 and >50% in people with mild-to-moderate renal impairment and diabetes mellitus.<sup>3</sup> It is now the third most common cause of hospital-acquired renal failure, and has led to increased medical care costs and increased mortality.<sup>1,2</sup> Therefore, it is important to understand the etiology of CIN, and to formulate effective prophylactic and therapeutic regimens to reduce its

incidence.<sup>3</sup> Approximately 150,000 patients annually experience CIN, and almost 1% of these individuals require dialysis and a prolonged hospital stay, with the annual cost of dialysis being estimated at ~\$32 million.<sup>1</sup>

We used a well-established acute renal failure model with glycerol (GLY), and the CM agent iohexol, which has an improved safety and tolerability profile when compared to classic radiocontrast agents. Nevertheless, a data mining analysis of the US Food and Drug Administration databases showed that iohexol has the highest association with pooled renal events among the low osmolar contrast media (OCM) agents, including iopamidol, for which the lowest association was found.<sup>4</sup> Therefore, iohexol has become the most interesting radiocontrast agent in CIN-related clinical and experimental studies.

Tea (*Camellia sinensis*, Theaceae) is second only to water in global consumption. Green tea is one of its major forms, and represents 20% of the world's tea

**CONTACT** S. Sezin Palabiyik ✉ [sezinp@gmail.com](mailto:sezinp@gmail.com) 📧 Faculty of Pharmacy, Pharmaceutical Toxicology Department, Ataturk University, TR90-25240, Erzurum, Türkiye

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consumption. It is characterized by the presence of large amounts of catechins,<sup>5</sup> with the major ones being epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate, epigallocatechin, and epicatechin.<sup>6</sup> Previous studies have suggested that green tea and green tea polyphenols, particularly EGCG, exert beneficial effects against neurodegenerative disease,<sup>7</sup> cancer,<sup>8</sup> and inflammation caused by lipid peroxidation and excessive free radical production.<sup>9</sup> Numerous mechanisms have been proposed for the biological activities in the preventive effect of EGCG, including anti-inflammatory and antioxidant mechanisms. The protective effect of EGCG has been shown with a different underlying mechanism for pathology and protection in nephrotoxicity studies.<sup>10,11</sup> However, there is gap in the literature, as EGCG has not been assessed with regard to the nephrotoxicity that results from CM. Therefore, this study is the first to examine the protective effect of EGCG against devastating/fatal CIN.

To our knowledge, in addition to evaluating its protective activity against different nephrotoxic agents, this study is the first to reveal the prominent protection that EGCG provides against devastating GLY-induced CM toxicity.

## 2. Materials and methods

### 2.1. Animals

A total of 48 male albino Wistar rats (250–300 g) obtained from Experimental Animal Laboratory of Medicinal and Experimental Application and Research Center of Atatürk University, Turkey, were used. The rats were stored on sawdust bedding in typical plastic cages in a well-ventilated room at 22 °C under precise light conditions (14/10 h light/dark cycle), which had been approved by the local animal care committee of Atatürk University (26.06.2015/122). Standard rat food and tap water were given *ad libitum*.

### 2.2. Reagents

All reagents used in our laboratory experiments were purchased from the Sigma Chemical Company (Steinheim, Germany). The EGCG was a gift from DSM Nutritional Products (Istanbul, Turkey). The iohexol (OMNIPAQUE, OPAKIM Medical Products) and the GLY (Bikar Medical Products) were purchased from Istanbul, Turkey.

### 2.3. Experimental design

Eight groups were created, with six rats in each:

- Group 1: A healthy group
- Group 2: GLY (GLY only)

Group 3 CM (iohexol 10 mL/kg)

Group 4: GLY + CM (iohexol 10 mL/kg)

Group 5: CM + EGCG 50: CM (iohexol 10 mL/kg) + EGCG (50 mg/kg, po)

Group 6: GLY + CM + EGCG 50: GLY + CM (iohexol 10 mL/kg) + EGCG (50 mg/kg, po)

Group 7: CM + EGCG 100: CM (iohexol 10 mL/kg) + EGCG (100 mg/kg, po)

Group 8: GLY + CM + EGCG 100: GLY + CM (iohexol 10 mL/kg) + EGCG (100 mg/kg, po)

The EGCG doses were selected on the basis of previously published nephrotoxicity-related studies.<sup>12,13</sup>

### 2.4. Contrast media-induced nephrotoxicity model

After 24-h water deprivation, kidney injury was induced in groups 2, 4, 6, and 8 with intramuscular administration of 25% GLY at a single dose of 10 mL/kg body weight, which was divided across all legs. Drinking water and food were then resumed *ad libitum*. In addition to the induced kidney injury, nephrotoxicity was induced 24 h after GLY injection, via intravenous administration of iohexol CM into the tail vein of the animals in groups 3–8, at a single dose of 10 mL/kg by weight over a period of 2 min.<sup>14</sup> Thirty minutes after GLY injection, the different doses of EGCG (50–100 mg/kg) was prepared in a volume of 1 mL, dissolved in 0.9% NaCl, and orally administered to the animals in groups 5–8 by gavage using an intubation needle. EGCG treatment was continued for 4 days.

Following the drug administration period, the study was completed in all groups on day 5. Blood was collected through heart puncture deep of a general anesthetic (thiopental sodium, 50 mg/kg), and serum was immediately separated by centrifugation at 4000 rpm for 10 min at 4 °C, and stored at –80 °C until being assayed. Kidney tissues were immediately dissected out. One kidney from each animal was stored at –80 °C for biochemical and molecular analyses and the remaining kidney was fixed in 10% formalin solution for pathological examination.

### 2.5. Biochemical analyses

#### 2.5.1. Serum measurements of blood urea nitrogen and creatinine concentration

Serum blood urea nitrogen (BUN) (Lot No: B0382A, S.r.i, Italy) and creatinine (Lot No: B0914A, S.r.i, Italy) levels were detected using commercially available kits. All analyses were performed in a ChemWell 2910-automated-EIA and Chemistry analyzer (Awareness Technology, Inc., Palm City, FL).

### 2.5.2. Biochemical investigation of kidney tissues

Following surgery, the tissues were stored at  $-80^{\circ}\text{C}$ . The tissue samples from each rat were first perfused with phosphate buffered saline (PBS)/heparin and then ground in liquid nitrogen using the TissueLyser II grinding jar set (Qiagen, Hilden, Germany). Approximately 100 mg of ground tissue was homogenized in 1 mL PBS homogenate buffer in an Eppendorf tube with TissueLyser II, and the samples were then centrifuged. Superoxide dismutase (SOD) activity,<sup>15</sup> glutathione (GSH) levels,<sup>16</sup> and malondialdehyde (MDA) levels<sup>17</sup> from each sample supernatant and standards were measured at room temperature in duplicate via modified methods, with an enzyme-linked immunosorbent assay (ELISA) reader. A standard curve was plotted and the equation was obtained from the absorbance of the standards. The linear SOD, GSH, and MDA concentrations were calculated according to this equation, and were expressed as U/mg protein, nmol/mg protein, and nmol/mg protein, respectively. The data obtained were presented as mean  $\pm$  standard deviation (SD) as 1 mg of protein.

## 2.6. Molecular investigations of kidney tissues

### 2.6.1. Total RNA extraction and cDNA synthesis

The tissues (20 mg) were stabilized in RNA stabilization reagent (RNAlater, Qiagen, Hilden, Germany), and then disrupted using the TissueLyser II ( $2 \times 2$  min for kidney;  $2 \times 5$  min, Qiagen). The total RNA was purified using the RNeasy Mini Kit (Qiagen) in a QIAcube (Qiagen), according to the manufacturer's instructions. The RNA samples were then reverse-transcribed into complementary DNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). From 10  $\mu\text{L}$ , total RNA was treated with 2  $\mu\text{L}$  10X RT buffer, 0.8  $\mu\text{L}$  25X dNTPs mix, 2  $\mu\text{L}$  10X RT random primers, 1  $\mu\text{L}$  multiscribe reverse transcriptase, and 4.2  $\mu\text{L}$  DEPC- $\text{H}_2\text{O}$ . Reverse transcription was carried out at  $25^{\circ}\text{C}$  for 10 min, followed by 120 min at  $37^{\circ}\text{C}$ , and finally 5 min at  $85^{\circ}\text{C}$ , using a Veriti 96-well thermal cycler (Applied Biosystems). The concentration and quality of the cDNA were assessed and quantified using the Epoch Spectrophotometer System and Take3 Plate (BioTek Inc., Winooski, VT).

### 2.6.2. Relative quantification of gene expression (real-time reverse transcriptase-polymerase chain reaction)

Relative tumor necrosis factor (TNF)- $\alpha$  and nuclear factor (NF)- $\kappa\text{B}$  expression analyses were performed with StepOne Plus Real Time PCR System technology (Applied Biosystems, Foster City, CA) using synthesized cDNA from

rat kidney RNA. A quantitative polymerase chain reaction (qPCR) was run using a TaqMan probe mix based on TaqMan probe-based technology (Applied Biosystems). A real-time PCR was performed using primers generated for rat TNF- $\alpha$  Rn00562055\_m1, rat NF- $\kappa\text{B1}$  Rn01399583\_m1, and rat  $\beta$ -actin Rn00667869\_m1. The results are expressed as relative-fold, compared with control animals. The expression data for  $\beta$ -actin in each tissue were used as the endogenous control. Each determination was performed in triplicate for each tissue in a 96-well optical plate for both targets, using 9  $\mu\text{L}$  of cDNA (100 ng), 1  $\mu\text{L}$  of Primer Perfect Probe mix, and 10  $\mu\text{L}$  of QuantiTect Probe PCR Master mix (Qiagen) in each 20- $\mu\text{L}$  reaction. The plates were heated for 2 min at  $50^{\circ}\text{C}$  and then 10 min at  $95^{\circ}\text{C}$ . Subsequently, 40 cycles of 15 s at  $94^{\circ}\text{C}$  and cycles of 60 s at  $60^{\circ}\text{C}$  were carried out. All data are expressed as the fold-change in expression compared to the expression in other animal groups, using the 2-delta-delta Ct ( $2^{-\Delta\Delta\text{Ct}}$ ) method.<sup>18</sup>

## 2.7. Histopathological analyses

Kidneys from the rats in all groups were obtained, sectioned frontally, and fixed in 10% neutral formalin for 48–72 h. The tissues were then routinely processed, and embedded in paraffin wax, and 4–5- $\mu\text{m}$  thick serial sections were cut. All tissue sections were stained with hematoxylin and eosin (H&E) for histopathologic assessment, and examined under a light microscope (Olympus BX51, Japan). Tubular necrosis, hyaline casts, and hemorrhagic casts were evaluated and scored. A minimum of five fields for each kidney slide at 100X magnification were evaluated and assigned for severity of changes, using scores on a scale of Grade 0: (negative), Grade 1: +1 (mild), Grade 2: +2 (moderate), Grade 3: +3 (severe), and Grade 4: +4 (most severe).

## 2.8. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL) software was used for the statistical analysis. The results are presented as means  $\pm$  SD. Comparisons between groups were performed using one-way analysis of variance (ANOVA) and the Duncan Multiple Comparison Test. Significance was accepted at a level of  $p < .05$ .

## 3. Results

### 3.1. Biochemical results

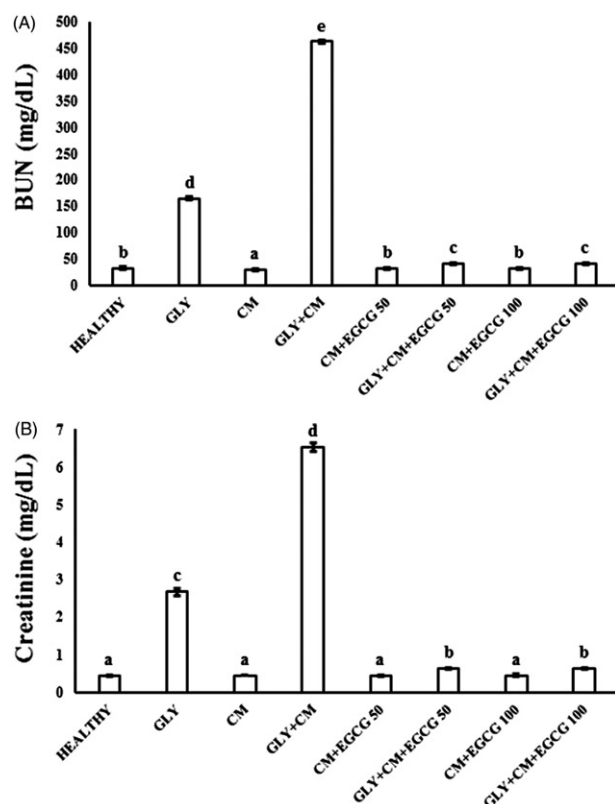
#### 3.1.1. Serum measurements of BUN and creatinine

The serum levels of BUN and creatinine in the control and experimental groups were evaluated. The effects of

nephrotoxicity on serum creatinine and BUN are shown in **Figures 1(A) and 1(B)**, respectively. BUN and creatinine serum levels significantly increased in the GLY + CM-induced nephrotoxicity compared with the other groups ( $p < .05$ ). EGCG administration significantly decreased the activity of the serum enzymes when compared with the GLY + CM group ( $p < .05$ ).

### 3.1.2. Biochemical investigation of kidney tissues

SOD activity, levels of GSH, and lipid peroxidation (MDA) were evaluated in all rat kidneys. SOD activity was significantly decreased in the GLY + CM groups compared with the healthy group ( $p < .05$ ). In addition, significant differences between the GLY and GLY + CM groups ( $p < .05$ ) were found. SOD activity was significantly improved in the treatment groups compared to the GLY + CM group ( $p < .05$ ), as shown in **Figure 2(A)**. GSH levels significantly were decreased in the CM and GLY + CM group compared to the healthy group ( $p < .05$ ), and significant differences between the GLY and GLY + CM groups ( $p < .05$ ) were observed. GSH levels were significantly ameliorated in the drug treatment groups when compared to the GLY + CM groups, as shown in **Figure 2(B)** ( $p < .05$ ). MDA levels were

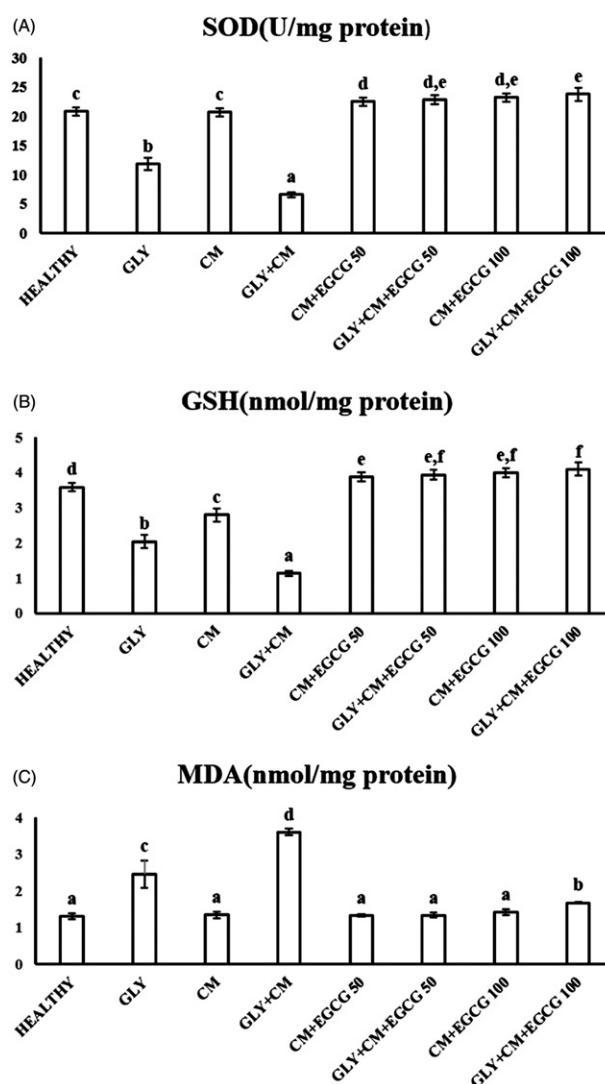


**Figure 1.** The serum BUN (A) and creatinine (B) levels of all the experimental groups. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups.

significantly increased in the GLY + CM group compared with the healthy group ( $p < .05$ ), and significant differences between the GLY and GLY + CM groups ( $p < .05$ ) were found (**Figure 2(C)**). It was observed that both doses of EGCG restored MDA levels in the healthy group ( $p < .05$ ).

### 3.2. Molecular results

We investigated TNF- $\alpha$  and NF- $\kappa$ B mRNA expression in the kidney tissue of the rats using real-time PCR. As **Figure 3(A)** shows, TNF- $\alpha$  gene expression was 10.7-fold upregulated in the GLY + CM groups compared to the healthy group ( $p < .05$ ). Both doses of EGCG provided a significant down-regulatory effect on TNF- $\alpha$  mRNA expression, by 10.9-fold for the 50 mg/kg dose and



**Figure 2.** The tissue SOD activities (A), GSH (B) and MDA (C) levels of all the experimental groups. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups.



11.6-fold for 100 mg/kg dose compared to GLY + CM group (both,  $p < .05$ ).

The NF- $\kappa$ B mRNA level was significantly higher by 8.03-fold in the GLY + CM group compared to the control group ( $p < .05$ ) (Figure 3(B)). Both doses of EGCG demonstrated a significant down-regulatory effect on NF- $\kappa$ B mRNA expression in the treatment groups compared to the GLY + CM group, by 7.2-fold for the 50 mg/kg dose and 7.5-fold for the 100 mg/kg dose compared to GLY + CM group (both,  $p < .05$ ).

### 3.3. Histopathological results

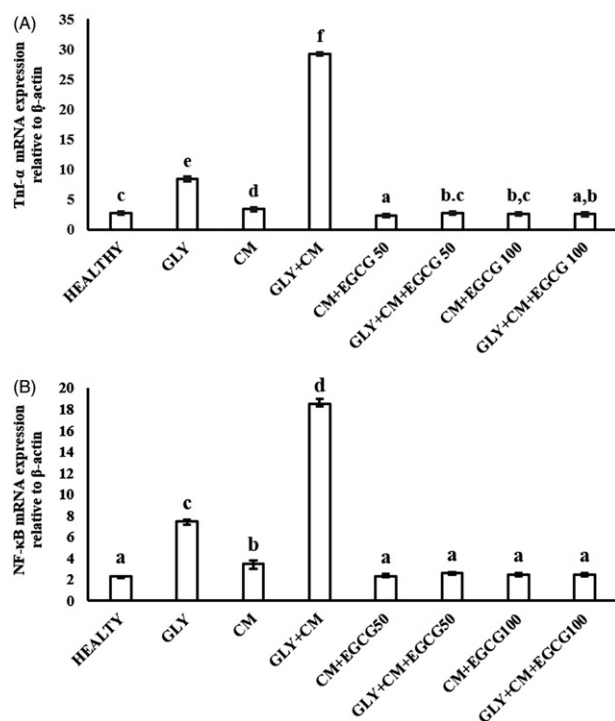
Histological evaluation of kidney sections from rats in all groups cohorts subjected to radiocontrast with aggravated GLY toxicity was examined and scored hyaline and hemorrhagic casts and tubular necrosis. The results are presented in Figures 4 and 5. The control group revealed normal morphology in 100% of tubules. In the GLY group, damaged tubules were more significant than in the healthy group ( $p < .05$ ) and tend to increase in CM group. Hyaline and hemorrhagic casts, and necrosis in the tubules were noted (Figure 4). Degenerative changes were more evident in proximal tubules. However, hyaline and hemorrhagic casts were significantly higher in CM + GLY group than healthy and CM group. The tubular necrosis was significantly

increased in group CM + GLY in comparison with CM group. The treatment with EGCG before radiocontrast application significantly improved renal morphology compared with the CM + GLY groups ( $p < .05$ ), but some tubules with edema and vacuolization remained (Figure 5). To a certain extent, acute tubular necrosis developed frequently in the CM + GLY compared with the CM + GLY + EGCG groups. However, the hyaline and hemorrhagic casts were not decreased as much as the control and CM levels.

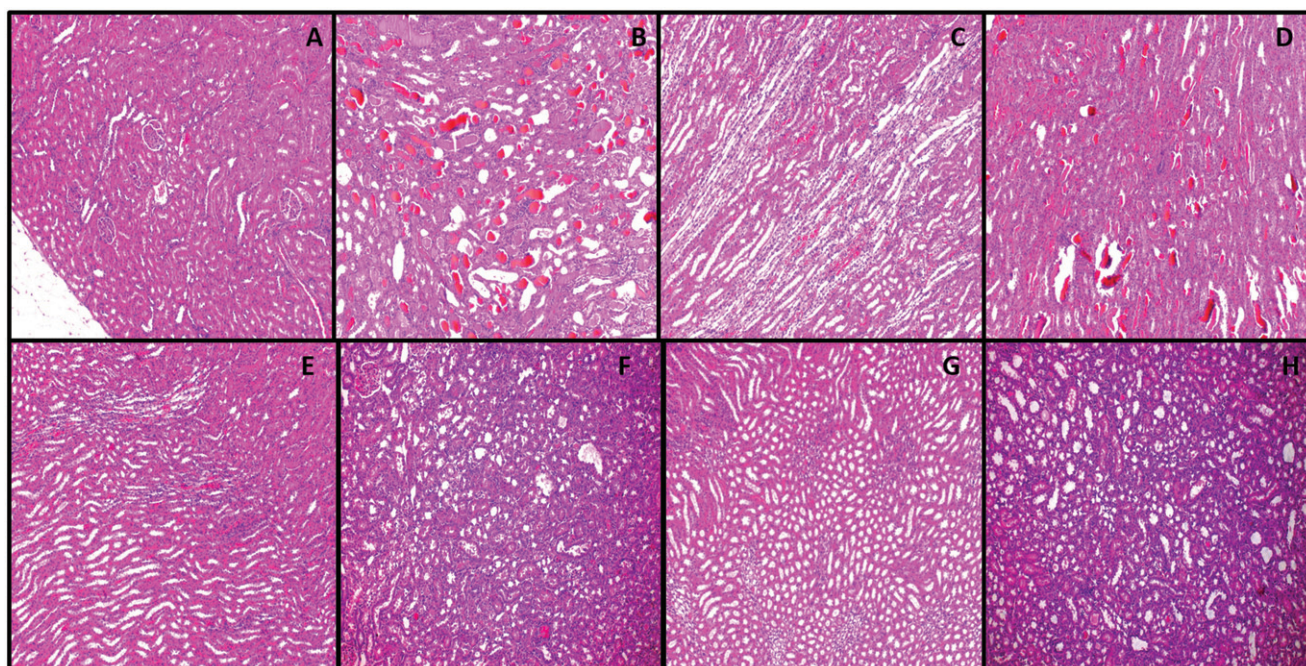
### 4. Discussion

This study investigated the protective role of the natural compound EGCG at two different doses (50 and 100 mg/kg) in CIN and CIN aggravated by GLY. We showed that EGCG reduced the increased levels of serum BUN and creatinine in rats in GLY-aggravated CIN. Furthermore, EGCG provided significant protection against increased oxidative stress and inflammation due to nephrotoxicity through NF- $\kappa$ B and TNF- $\alpha$  mRNA expression levels. In addition, histopathology showed that EGCG ameliorated the renal injury. Considering the potential clinical use of CM and the numerous health benefits of EGCG, this study was undertaken to assess the protective role of multi-dose EGCG treatment on CIN and GLY-aggravated CIN. Elevation of the serum level of creatinine after exposure to a contrast agent is most frequently used in diagnosing CIN. Filtration between the renal tubules and glomerules is represented by serum levels of creatinine.<sup>1</sup> In clinical practice, level of serum creatinine, which is a surrogate marker of glomerular filtration rate, has been used for the diagnosis of CIN.<sup>2</sup> Increased serum BUN and creatinine levels have previously been reported in several CIN studies.<sup>19</sup> The ameliorative effect of EGCG on BUN and creatinine levels has also previously been shown. It was found that BUN and creatinine decreased with EGCG treatment in the presence of various nephrotoxic drugs, such as cyclosporine and cisplatin.<sup>10-12</sup> In accordance with previous studies, we found that EGCG treatment decreased BUN and creatinine levels, which are increased in CIN and GLY-aggravated CIN, thereby indicating improved renal function. Both doses of EGCG had the same effect on BUN and creatinine levels.

The pathogenesis of CIN is not clear, but recent studies have shown that it is caused by a combination of toxic and ischemic injury to the renal tubular cells. The risk of CIN has decreased with the use of low-osmolar agents, such as iohexol.<sup>1</sup> CM reduces blood flow through the kidney areas, and increases hypoxic damage.<sup>2</sup> Direct cytotoxic effects, auto- and paracrine factors that perturb renal hemodynamics, altered



**Figure 3.** The tissue TNF- $\alpha$  (A) and NF- $\kappa$ B (B) mRNA expressions of all the experimental groups. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups.



**Figure 4.** Hematoxylin and eosin results in rats' kidney tissues; magnification 100X. A: Healthy B: GLY C: CM D: GLY + CM E: CM + EGCG 50 F: GLY + CM + EGCG 50 G: CM + EGCG 100 H: GLY + CM + EGCG 100

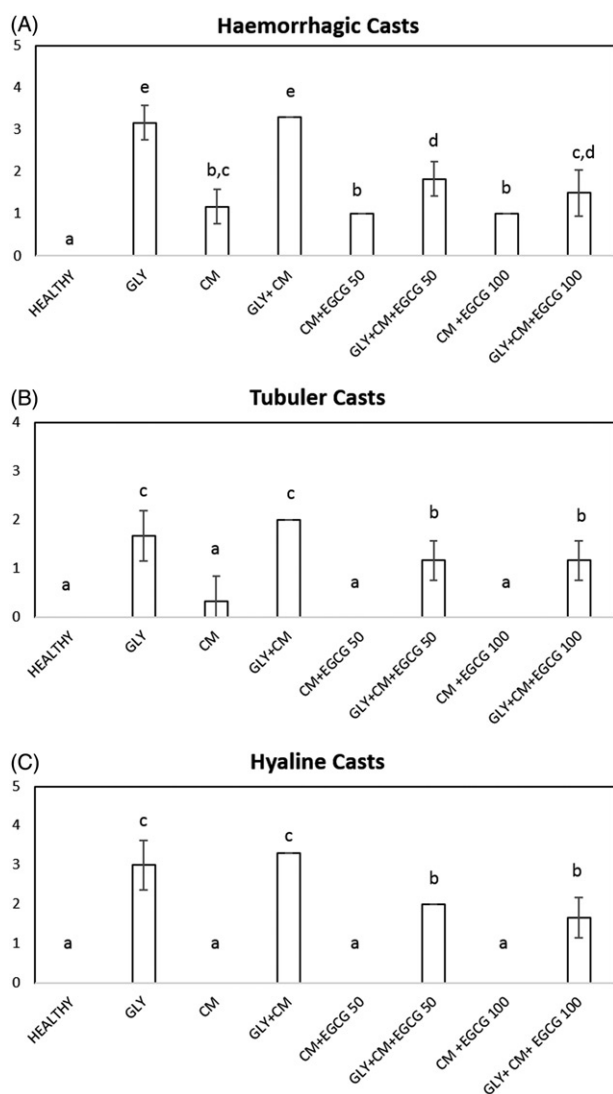
rheological properties, which affect renal hemodynamics and tubulodynamics, and regional hypoxia are the mechanisms underlying CIN and these act in concert.<sup>2</sup> However, the clinical features and the pathophysiological mechanism of CIN, alone or in the presence of other underlying renal pathologies, are significantly differentiated.<sup>20</sup> In our study, pretreatment with GLY was used to induce preexisting renal insufficiency and mimic any underlying pathology. In GLY-induced renal failure, ischemic injury or tubular nephrotoxicity occurs.<sup>21</sup>

Protection of the renal tubules from prolonged contact with CM is the goal of prevention, as permanent damage can occur at the time of contact. Sodium bicarbonate and N-acetyl cysteine (NAC) are used to prevent an acidic environment and formation of free radicals in the renal tubules. Antioxidant protection preserves at the level of the glomerulus and reduces level of systemic inflammatory factors.<sup>1</sup> Although some prevention strategies exist, including adequate hydration of the patient, discontinuation of nephrotoxic drugs before CM administration and NAC, vitamins C and E treatment,<sup>22</sup> and some compounds such as infliximab<sup>23</sup> and simvastatin<sup>24</sup> have been shown to ameliorate CIN; there is no available standard prophylactic or therapeutic agent for this condition.

EGCG is the most abundant catechin, and represents 50–80% of catechins by weight.<sup>5,6</sup> Its ability to quench free radical species, including the hydroxyl radical, superoxide anion, nitric oxide, and peroxyxynitrite, and to chelate transition metals, means that EGCG has strong

antioxidant activity.<sup>5</sup> Under optimal dose regimens, both properties of EGCG are expected to contribute to the modulation of the oxidative stress response. Although exposure to a low concentration of a pro-oxidant prior to exposure to oxidative stress induces adaptation, exposure to an antioxidant concurrently with exposure to the oxidative stress affords protection against the oxidative stress.<sup>25</sup> Tea polyphenols, particularly EGCG, have generally been regarded as antioxidants, and the emerging evidence for the protective effects of this compound is interesting and raises numerous potential questions.

Oxidative stress, the perturbation of the balance between pro-oxidants and antioxidants, may underline the pathology of CIN.<sup>23,26</sup> EGCG has antioxidant activity, which is attributed to the galloyl group at the 3 position and the o-trihydroxyl group in the B ring in its structure, and it protects cells from damage by reactive oxygen species (ROS). Thus, EGCG may have an antioxidant effect on oxidative stress-induced impaired renal function.<sup>10,13</sup> In our study, administration of GLY and GLY + CM resulted in depletion of enzymatic and non-enzymatic antioxidant defense systems, leading to a significant decrease in SOD activity and GSH levels. Although these antioxidant levels significantly changed in the GLY-aggravated CIN group, when compared to the GLY group, treatment with CM did not change SOD activity but changed GSH levels. We report that both doses of EGCG (50 and 100 mg/kg) significantly increased SOD activity and GSH levels when compared



**Figure 5.** Effects of EGCG on histopathological hemorrhagic cast (A) tubular casts (B) and hyaline cast (C) scores in rats' kidneys. Means in the same column by the same letter are not significantly different from the test of Duncan ( $p = .05$ ). Results are expressed as means  $\pm$  SD ( $n = 6$ ).

to the control group. The effect of EGCG did not change, irrespective of the dose administered, but SOD activity and GSH levels approached the GSH levels and SOD activity of the control group at both doses. Decreased SOD activity and GSH levels have previously been reported in CIN, and different treatment strategies have ameliorated oxidative stress.<sup>23,26</sup> In accordance with the results of our study, Saritemur et al. showed decreased SOD activity and GSH levels in GLY-aggravated CIN, and treatment with infliximab increased SOD activity and GSH levels.<sup>23</sup>

Oxygen free radicals lead to peroxidation of lipids and produce damage to cells and tissues, which may be another mechanism of CIN.<sup>10</sup> MDA levels increased to statistically significant levels in the GLY+CM group

compared with the control group in this study, while lipid peroxidation also increased as a result of oxidative stress in the GLY treatment group, but not in the CM treatment groups. Both doses of EGCG decreased the MDA levels, but only the 50 mg/kg dose reduced the MDA levels to control levels. These results reveal that CM increased nephrotoxicity in the presence of kidney injury via an oxidative stress mechanism, and that consumption of EGCG ameliorates this effect with its radical-scavenging activity. These data are in agreement with previous studies conducted with various antioxidant supplements. In the light of these oxidative stress parameters results, we can state that CM alone did not affect oxidative stress, but it caused nephrotoxicity via the oxidative stress mechanism in the presence of kidney pathology. In addition, treatment with EGCG can block this effect via its strong antioxidant effect.

ROS are known to enhance inflammation through the activation of NF- $\kappa$ B via phosphorylation of I- $\kappa$ B. Stimuli that activate NF- $\kappa$ B also induce oxidative stress, and some evidence has shown that NF- $\kappa$ B activation is stimulated by ROS and is inhibited by antioxidants. Antioxidants can suppress the NF- $\kappa$ B activation that is induced by ROS,<sup>27</sup> and EGCG has been shown to inhibit the constitutive activation of NF- $\kappa$ B in different cell culture studies.<sup>28</sup> Pan et al. suggested that EGCG might influence the inflammatory response in cisplatin nephrotoxicity through the NF- $\kappa$ B pathway.<sup>29</sup> In another study, Sahin et al. also showed the preventive effect of EGCG against cisplatin-induced NF- $\kappa$ B expression in rat kidney.<sup>12</sup> In accordance with these studies, NF- $\kappa$ B mRNA expression was up-regulated with GLY, CM, and GLY+CM administrations. The highest expression was found in the GLY+CM group, showing that CM is more potent in kidney injury in the presence of renal failure. Both doses of EGCG treatment diminished the increased mRNA expression of NF- $\kappa$ B in the GLY, CM, and GLY+CM groups to control group values. Therefore, EGCG is beneficial in nephrotoxicity.

The production of pro-inflammatory cytokines, such as TNF- $\alpha$ , is increased in the inflammatory process, which is another factor that plays a role in the pathogenesis of nephrotoxicity.<sup>30</sup> Increased TNF- $\alpha$  levels were observed in a study conducted on cisplatin-induced nephrotoxicity.<sup>13</sup> El-Mowafy et al. evaluated the protective effect of EGCG in cisplatin-induced kidney injury, via the role of inflammatory cytokines. Although TNF- $\alpha$  levels increased in kidney homogenates in the cisplatin treatment groups, treatment with 50 mg/kg EGCG significantly decreased these levels.<sup>13</sup> Saritemur et al. showed the mRNA expression of TNF- $\alpha$  in GLY-aggravated radiocontrast nephrotoxicity in rats, and that this was decreased by the TNF- $\alpha$  inhibitor



infliximab.<sup>23</sup> Consistent with these findings, TNF- $\alpha$  mRNA expression increased in the rats that were administered GLY, CM, or GLY + CM in this study, and these levels decreased in the rats that were administered 50 and 100 mg/kg EGCG.

Tubular necrosis, tubular epithelial damage, hyaline casts, and hemorrhagic casts have been found in various studies,<sup>31</sup> and it has been shown that tubular necrosis and epithelial injury are significant indicators in nephrotoxicity injury in these studies.<sup>32</sup> In association with the tubular injury, significant damage to hemorrhagic renal tubules occurs with hyaline in the development of nephropathy. In our histopathological studies, we determined the ameliorative effect of both EGCG doses on tubular structure, but the changes did not differ between doses.

## 5. Conclusions

In conclusion, we have demonstrated that (1) the administration of EGCG attenuates kidney injury and increases antioxidant enzymes; (2) the administration of EGCG significantly inhibits inflammation mediated by NF- $\kappa$ B and TNF- $\alpha$  in rats with GLY-aggravated kidney injury. Further clinical investigation is needed to better clarify the usefulness of EGCG in CIN in clinic.

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## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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