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## FULL PAPER

# A novel approach to contrast-induced nephrotoxicity: the melatonergic agent agomelatine

<sup>1</sup>ADEM KARAMAN, MD, <sup>2</sup>BUSRA DIYARBAKIR, MD, <sup>3</sup>IRMAK DURUR-SUBASI, MD, <sup>2</sup>DUYGU KOSE, MD, <sup>2</sup>ASLI ÖZBEK-BILGIN, MD, <sup>4</sup>ATILLA TOPCU, MD, <sup>5</sup>CEMAL GUNDOGDU, MD, Prof, <sup>6</sup>AFAK DURUR-KARAKAYA, MD, <sup>7</sup>ZAFER BAYRAKTUTAN and <sup>1</sup>FATIH ALPER, MD, PhD

<sup>1</sup>Department of Radiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

<sup>2</sup>Department of Pharmacology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

<sup>3</sup>Department of Radiology, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey

<sup>4</sup>Department of Pharmacology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey

<sup>5</sup>Department of Pathology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

<sup>6</sup>Department of Radiology, Istanbul Medipol University, Istanbul, Turkey

<sup>7</sup>Department of Biochemistry, Erzurum Regional Training and Research Hospital, Erzurum, Turkey

Address correspondence to: Associate Professor Irmak Durur-Subasi

E-mail: [irmakdurur@yahoo.com](mailto:irmakdurur@yahoo.com)

**Objective:** To study the potential nephroprotective role of agomelatine in rat renal tissue in cases of contrast-induced nephrotoxicity (CIN). The drug's action on the antioxidant system and proinflammatory cytokines, superoxide dismutase (SOD) activity, levels of glutathione (GSH) and malondialdehyde (MDA) and the gene expression of interleukin-6 (IL-6), tumour necrosis factor (TNF)- $\alpha$  and nuclear factor kappa B (NF- $\kappa$ B) was measured. Tubular necrosis and hyaline and haemorrhagic casts were also histopathologically evaluated.

**Methods:** The institutional ethics and local animal care committees approved the study. Eight groups of six rats were put on the following drug regimens: Group 1: healthy controls, Group 2: GLY (glycerol), Group 3: CM (contrast media—iohexol 10 ml kg<sup>-1</sup>), Group 4: GLY+CM, Group 5: CM+AGO20 (agomelatine 20 mg kg<sup>-1</sup>), Group 6: GLY+CM+AGO20, Group 7: CM+AGO40 (agomelatine 40 mg kg<sup>-1</sup>) and Group 8: GLY+CM+AGO40. The groups were evaluated by one-way analysis of variance and Duncan's multiple comparison test.

**Results:** Agomelatine administration significantly improved the serum levels of blood urea nitrogen (BUN) and creatinine, SOD activity, GSH and MDA. The use of agomelatine had

substantial downregulatory consequences on TNF- $\alpha$ , NF- $\kappa$ B and IL-6 messenger RNA levels. Mild-to-severe hyaline and haemorrhagic casts and tubular necrosis were observed in all groups, except in the healthy group. The histopathological scores were better in the agomelatine treatment groups.

**Conclusion:** Agomelatine has nephroprotective effects against CIN in rats. This effect can be attributed to its properties of reducing oxidative stress and inhibiting the secretion of proinflammatory cytokines (NF- $\kappa$ B, TNF- $\alpha$  and IL-6).

**Advances in knowledge:** CIN is one of the most important adverse effects of radiological procedures. Renal failure, diabetes, malignancy, old age and non-steroidal anti-inflammatory drug use pose the risk of CIN in patients. Several clinical studies have investigated ways to avoid CIN. Theophylline/aminophylline, statins, ascorbic acid and iloprost have been suggested for this purpose. Agomelatine is one of the melatonin ligands and is used for affective disorders and has antioxidant features. In this study, we hypothesized that agomelatine could have nephroprotective, antioxidant and anti-inflammatory effects against CIN in rats.

## INTRODUCTION

Contrast-induced nephrotoxicity (CIN) is the worsening of renal function (an increase in levels of creatinine by more than one-fourth or 44  $\mu$ mol l<sup>-1</sup>) that occurs within 72 h after the administration of a contrast material in the absence of any other aetiology.<sup>1</sup> Although its incidence has been reported to be low,<sup>2</sup> it is one of the most important adverse effects of radiological procedures<sup>3</sup> and is related to an increase in morbidity and mortality.<sup>4</sup> This iatrogenic

complication following the administration of contrast materials is a common cause of hospital-acquired renal insufficiency and accounts for about 11% of cases,<sup>5</sup> occurring during diagnostic and interventional procedures.<sup>6</sup> Renal failure, diabetes, malignancy, old age and non-steroidal anti-inflammatory drug use have all been found to be associated with a higher risk of CIN.<sup>2</sup> CIN occurs more commonly in patients with underlying non-symptomatic kidney pathologies. It has become the third leading cause of hospital-acquired

acute renal failure with the increasing use of contrast media (CM) for diagnostic and interventional procedures. In this regard, it is important to thoroughly investigate this issue in animal models.

Pre-treatment with glycerol can be used to induce pre-existing renal damage in rats.<sup>7</sup> By administering glycerol, we induced CIN in the rats to mimic any underlying pathology. Iohexol is a low-osmolar non-ionic radiocontrast agent that has improved safety and tolerability compared with classic radiocontrast agents. For these reasons, iohexol became the most common radiocontrast agent used in clinical and experimental “contrast-induced nephropathy” research.<sup>8,9</sup> We therefore chose to use this contrast agent in our experimental model.

Several clinical studies have investigated ways to avoid CIN. Drugs such as theophylline/aminophylline, statins, ascorbic acid and iloprost have been suggested to be helpful in reducing the occurrence of this condition; nevertheless, a detailed evaluation of their impact is needed.<sup>10–13</sup>

Agomelatine is a melatonergic receptor agonist and a selective serotonin 5HT<sub>2C</sub> receptor antagonist that has shown antidepressant efficacy. Melatonin receptor type (MT)1 and MT2 receptors are predominating in the human foetal kidney cortex area and have been disclosed as renoprotective agents, as they are generally attributed to have the debated antioxidant capability.<sup>14,15</sup> Melatonin has several applications related to the renal system and its production is impaired in chronic renal failure and it cannot protect the kidneys from inflammation which can cause renal damage.<sup>16,17</sup> In this context, to our knowledge, there has not been a study to date on the nephroprotective role of agomelatine for CIN aggravated by glycerol in rats.

In this study, we aimed to investigate the possible nephroprotective role of agomelatine. To appreciate the relationship between the nephroprotective action of agomelatine, the antioxidant system and proinflammatory cytokines, we measured the superoxide dismutase (SOD) activity, levels of glutathione (GSH) and malondialdehyde (MDA) and the gene expression of interleukin-6 (IL-6), tumour necrosis factor (TNF)- $\alpha$  and nuclear factor kappa B (NF- $\kappa$ B) in the renal tissue of rats with CIN.

## METHODS AND MATERIALS

The study protocol was approved by the institutional ethics and local animal care committees. Animal experimentations and trials were implemented in a manner that was consistent with the national guiding principles.

### Animals

In total, 48 female albino Wistar rats (250–300 g) were used in the study. They were obtained from our university experimental animal laboratory. The rats were stored in typical plastic cages on sawdust bedding in a well-ventilated room at 22°C under precise light conditions (14/10 h light/dark cycle). Standard rat chow and tap water were provided *ad libitum*.

### Chemicals

Clinical substances were obtained from Sigma Chemical Company (Germany), agomelatine (Valdoxan® 25 mg, 28 tablets) was

obtained from Les Laboratories Servier (Istanbul, Turkey), iohexol (Omnipaque™ 300 mg/ml–1, 100 ml) from Opakim Medical Products (Istanbul, Turkey) and 100% glycerol from Bikar (Istanbul, Turkey).

### Experimental design

Eight sets of six rats in separate cages were administered the following drugs:

- (1) Group 1: healthy control group
- (2) Group 2 (GLY): glycerol only
- (3) Group 3 (CM): contrast media (iohexol 10 ml kg<sup>-1</sup>)
- (4) Group 4 (GLY+CM): glycerol + contrast media (iohexol 10 ml kg<sup>-1</sup>)
- (5) Group 5 (CM+AGO20): contrast media (iohexol 10 ml kg<sup>-1</sup>) + agomelatine 20 mg kg<sup>-1</sup> (po)
- (6) Group 6 (GLY+CM+AGO20): glycerol + contrast media (iohexol 10 ml kg<sup>-1</sup>) + agomelatine 20 mg kg<sup>-1</sup> (po)
- (7) Group 7 (CM+AGO40): contrast media (iohexol 10 ml kg<sup>-1</sup>) + agomelatine 40 mg kg<sup>-1</sup> (po)
- (8) Group 8 (GLY+CM+AGO40): glycerol + contrast media (iohexol 10 ml kg<sup>-1</sup>) + agomelatine 40 mg kg<sup>-1</sup> (po).

### The contrast media-induced nephrotoxicity model

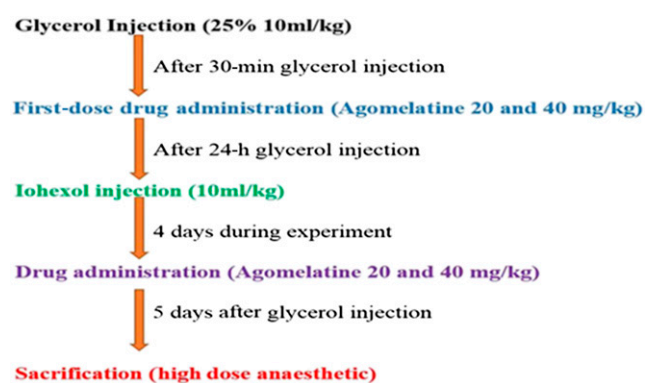
Kidney injury was induced in Groups 2, 4, 6 and 8 by intramuscular administration of 25% glycerol as a single dose of 10 ml kg<sup>-1</sup> body weight after 24-h water deprivation. The dose was divided among the legs. After glycerol injection, drinking water was resumed *ad libitum*.

24 h after glycerol injection, nephrotoxicity was induced with intravenous administration of iohexol (contrast media, CM) as a single dose of 10 ml kg<sup>-1</sup> body weight over a period of 2 min to the animals in Groups 3–8 *via* the tail vein.<sup>8</sup>

30 min after glycerol injection, agomelatine (dissolved in isotonic sodium chloride solution) was administered orally to the animals in Groups 5–8. Agomelatine administration was repeated on the second, third and fourth days (Figure 1).

Following the drug administration period, the study was finished on the fifth day after glycerol injection by an overdose of a general anaesthetic (thiopental sodium, 50 mg kg<sup>-1</sup>). Under anaesthetic conditions, blood samples were obtained by

Figure 1. Summary of experimental procedures.



intracardiac method. Kidney tissues were dissected out immediately. Half of one kidney was kept at  $-80^{\circ}\text{C}$  for biochemical evaluation; the rest was kept in an RNA stabilization reagent for molecular analysis, and the other kidney was fixed in formalin for pathological examination. Serum was instantly separated by centrifugation at 4000 rpm for 10 min at  $4^{\circ}\text{C}$  and stored at  $-80^{\circ}\text{C}$  until it was analysed.

#### Determination of serum BUN and creatinine concentrations

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).

#### Biochemical examination of kidneys

After surgery, the tissues were stored at  $-80^{\circ}\text{C}$ . All samples were first perfused with PBS/heparin and then pulverized in liquid nitrogen using a TissueLyser II (Qiagen, Ankara, Turkey) grinding jar set. Nearly 100 mg of the pulverized tissue was homogenized in 1 ml of PBS homogenate buffer in an Eppendorf tube® using the TissueLyser II. Samples were then centrifuged. SOD,<sup>18</sup> GSH<sup>19</sup> and MDA<sup>20</sup> levels from the supernatant of each sample and standard were measured at room temperature in duplicate *via* modified methods with an enzyme-linked immunosorbent assay reader. The average absorbance of each sample and standard was calculated. 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. The SOD, GSH and MDA levels in the tissues were expressed as  $\text{U mg}^{-1}$  protein,  $\text{nmol mg}^{-1}$  protein and  $\text{nmol mg}^{-1}$  protein, respectively. Obtained data were presented as mean  $\pm$  standard deviation from 1 mg of protein.

#### Molecular investigations of kidney tissues

##### Total RNA extraction and cDNA synthesis

Tissues (20 mg) were briefly stabilized in an RNA stabilization reagent (RNAlater®; Qiagen, Hilden, Germany) and then disrupted using the TissueLyser II ( $2 \times 2$  min for kidney tissue;  $2 \times 5$  min, Qiagen). Total RNA was purified in Qiacube using the RNeasy Mini Kit (Qiagen) according to manufacturer instructions. The RNA samples were reverse transcribed into complementary DNA (cDNA) using a high-capacity cDNA reverse transcription kit (Applied Biosystem®; Hernakim, Istanbul, Turkey). From  $10 \mu\text{l}$ , total RNA was treated with  $2 \mu\text{l}$   $10\times$  RT buffer,  $0.8 \mu\text{l}$   $25\times$  dNTPs mix,  $2 \mu\text{l}$   $10\times$  RT Random Primers,  $1 \mu\text{l}$  MultiScribe® reverse transcriptase and  $4.2 \mu\text{l}$  DEPC-H<sub>2</sub>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 Biosystem). The cDNA concentration and quality were assessed and quantified using the Epoch™ Spectrophotometer System and Take3 plate (Bio Tek®; Pera Medikal, Istanbul, Turkey).

##### Relative quantification of gene expression

Relative TNF- $\alpha$ , IL-6 and NF- $\kappa$ B expression analyses were performed with StepOne Plus™ Real Time PCR System

technology (Applied Biosystems) using synthesized cDNA from rat kidney RNAs. A quantitative polymerase chain reaction (qPCR) was run using a TaqMan® probe mix based on the TaqMan probe-based technology (Applied Biosystems). A real-time PCR was performed using primers generated for rat TNF- $\alpha$  (Rn00562055\_m1), rat IL-6 (Rn01410330\_m1), rat NF- $\kappa$ B (Rn01399583\_m1) and rat  $\beta$ -actin (Rn00667869\_m1). The results are expressed as relative-fold, compared with control groups. Expression data for  $\beta$ -actin in each tissue were used as endogenous controls. For each tissue, triplicate determinations were performed 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 polymerase chain reaction (PCR) Master mix (Qiagen) in each  $20\text{-}\mu\text{l}$  reaction. The plates were heated for 2 min at  $50^{\circ}\text{C}$  and for 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 applied. All data are expressed as fold change in expression, compared with the expression in other animal groups, using the  $2^{-\Delta\Delta\text{Ct}}$  method.<sup>21</sup>

#### Histological procedure

Kidneys from the rats in all groups were obtained, sectioned frontally and fixed in 10% neutral formalin for 48–72 h. Tissues were then processed routinely and embedded in paraffin wax, from which 4–5- $\mu\text{m}$ -thick serial sections were cut. All tissue sections were stained with haematoxylin and eosin for histopathology and periodic acid–Schiff (PAS) stain for basement membrane assessment and examined under a light microscope (BX51, Olympus, Japan). For histopathological assessment, tubular necrosis, hyaline casts and haemorrhagic casts were evaluated and scored independently by a pathologist blinded to the experiment. A minimum of five fields for each kidney slide at  $100\times$  magnification was evaluated and assigned scores for severity of changes as follows: Grade 0: – (negative), Grade 1: +1 (mild), Grade 2: +2 (moderate), Grade 3: +3 (severe) and Grade 4: +4 (most severe). The other sections were stored for assessing the immunohistochemistry.

Figure 2. The serum blood urea nitrogen (BUN) levels ( $\text{mg dl}^{-1}$ ) 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. AGO20 (agomelatine  $20 \text{ mg kg}^{-1}$ ); AGO40 (agomelatine  $40 \text{ mg kg}^{-1}$ ); CM, contrast media; GLY (glycerol).

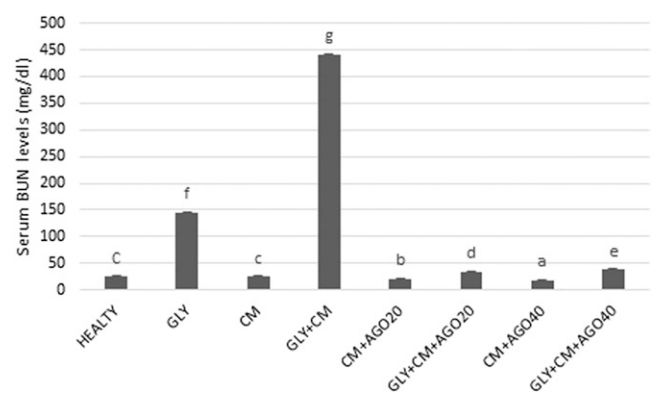
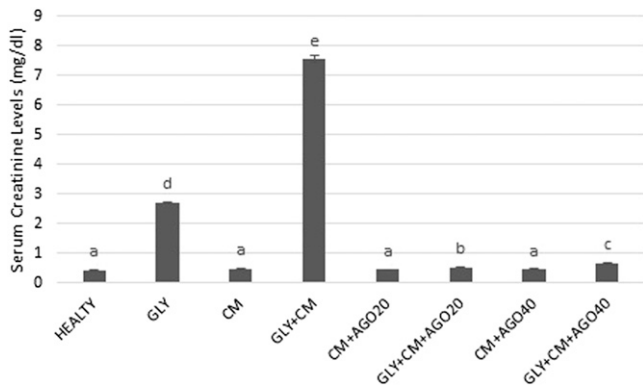


Figure 3. The serum creatinine levels of all the experimental groups. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).



### Statistical analysis

For statistical analysis, we used SPSS® v. 20.0 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). Results are presented as means ± standard deviation. Comparisons between groups were performed using one-way analysis of variance and Duncan's multiple comparison test. Significance was accepted at  $p < 0.05$ .

## RESULTS

### Serum concentrations of BUN and creatinine

It was observed that BUN and creatinine serum levels significantly increased in the rats with GLY+CM-induced nephrotoxicity compared with the other groups ( $p < 0.05$ ). Agomelatine administration in groups of 5–8 significantly decreased the activity of the serum enzymes compared with the GLY+CM group

Figure 4. The tissue superoxide dismutase (SOD) activities 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. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).

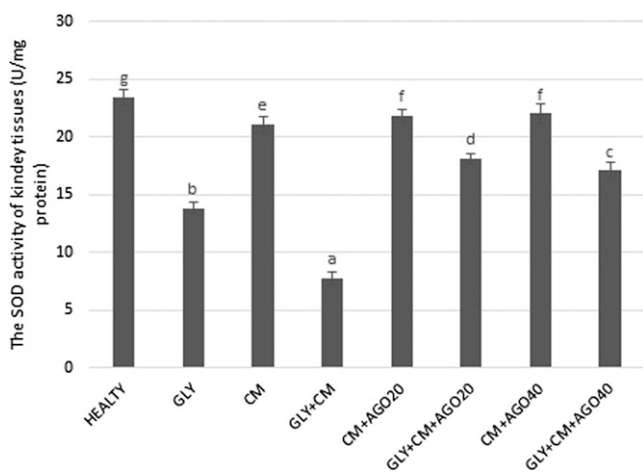
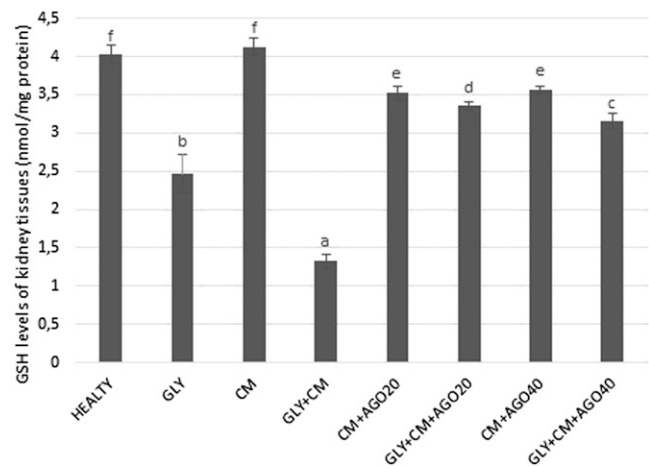


Figure 5. The tissue glutathione (GSH) levels of all the experimental groups. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).



( $p < 0.05$ ). The changes in the serum BUN and creatinine levels are shown in Figures 2 and 3, respectively.

### Biochemical results for kidney tissue

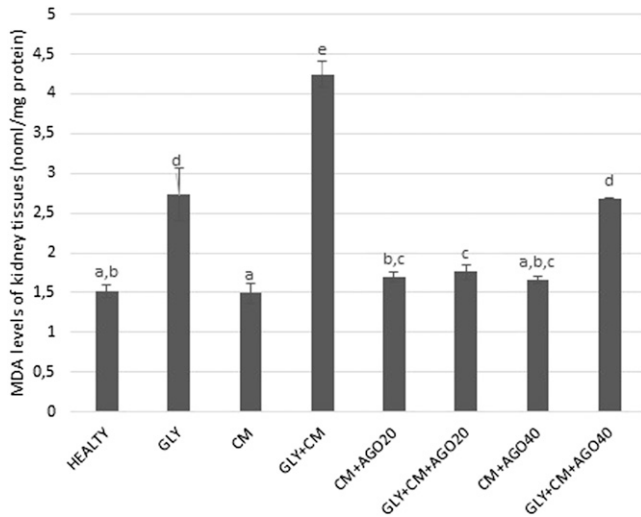
In the present study, SOD activity and levels of GSH and lipid peroxidation (MDA) were evaluated in all rat kidneys. SOD activity was significantly decreased in the GLY+CM group compared with that in the healthy group ( $p < 0.05$ ). In addition, there were significant differences between the GLY and GLY+CM groups ( $p < 0.05$ ). In the treatment groups, SOD activity was significantly improved compared with the GLY+CM group, as shown in Figure 4 ( $p < 0.05$ ). GSH levels significantly decreased in the GLY+CM group compared with that in the healthy group ( $p < 0.05$ ). There were also significant differences between the GLY and GLY+CM groups ( $p < 0.05$ ). As seen in Figure 5, GSH levels were significantly ameliorated in the drug treatment groups when compared with that in the GLY+CM group ( $p < 0.05$ ).

As shown in Figure 6, the MDA levels were significantly increased in the GLY+CM group compared with the healthy group ( $p < 0.05$ ). In addition, there were significant differences between the GLY and GLY+CM groups ( $p < 0.05$ ). The MDA levels in groups of 5–8 were significantly improved with the administration of agomelatine compared to the GLY+CM group ( $p < 0.05$ ).

### Molecular results

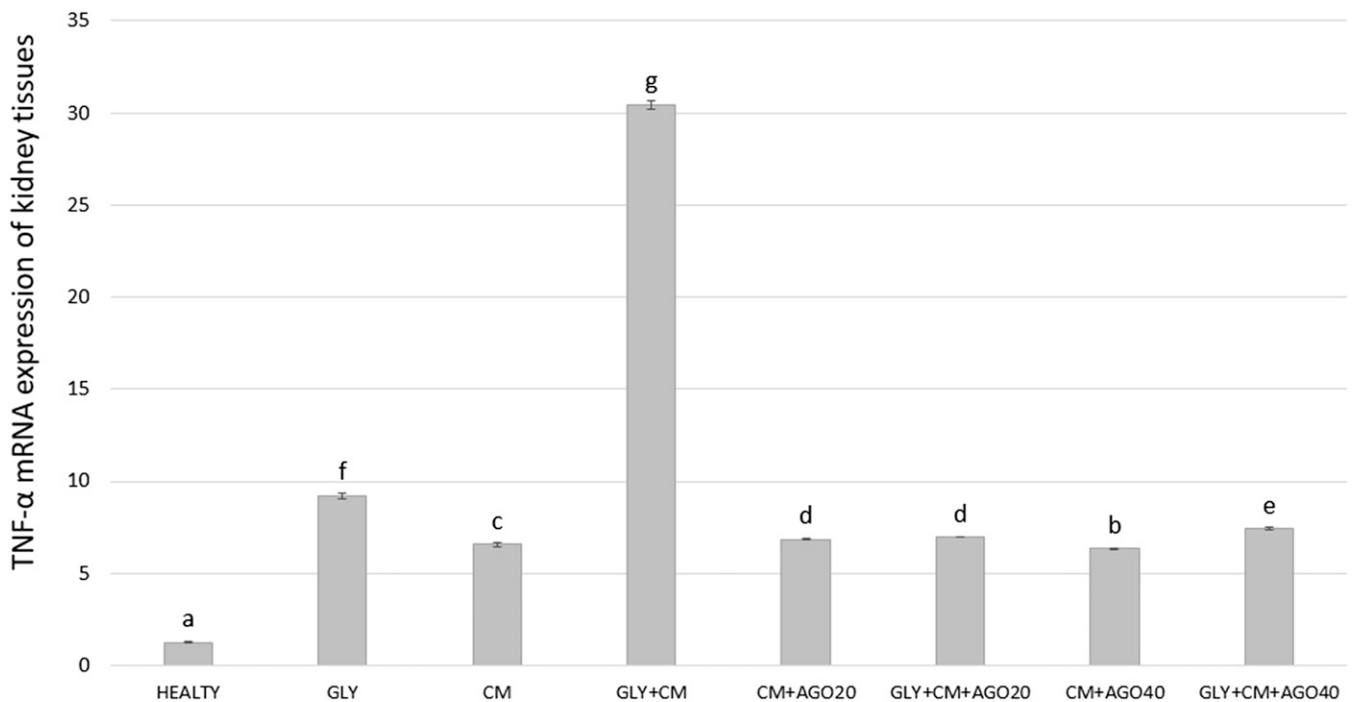
We investigated TNF- $\alpha$ , IL-6 and NF- $\kappa$ B messenger RNA (mRNA) expressions in the kidney tissue of rats using real-time PCR. As shown in Figure 7, TNF- $\alpha$  gene expression increased in the GLY+CM groups (30.46-fold) compared with that in the healthy group ( $p < 0.05$ ). Agomelatine had a significant down-regulatory effect on TNF- $\alpha$  mRNA expression, by 6.95-fold after 20 mg kg<sup>-1</sup> doses and 7.40-fold for 40 mg kg<sup>-1</sup> doses ( $p < 0.05$ ).

Figure 6. The tissue malondialdehyde (MDA) 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. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).



As shown in Figure 8, compared with the control group, the NF- $\kappa$ B mRNA level was significantly higher in the GLY+CM group, by 22.37-fold ( $p < 0.05$ ). NF- $\kappa$ B expression in the rat kidney tissues decreased by 5.82-fold and 7.45-fold in the treatment groups compared with that in the control group to nearly healthy levels ( $p < 0.05$ ).

Figure 7. The tissue TNF- $\alpha$  messenger RNA (mRNA) expression 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. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).



Compared with the control group, the IL-6 mRNA level was significantly higher (22.55-fold;  $p < 0.05$ ) in the GLY+CM groups (Figure 9). Agomelatine had a significant downregulatory effect on IL-6 mRNA expression in the treatment groups when compared with the GLY+CM group (5.36- and 7.21-fold, respectively;  $p < 0.05$ ).

#### Histopathological results

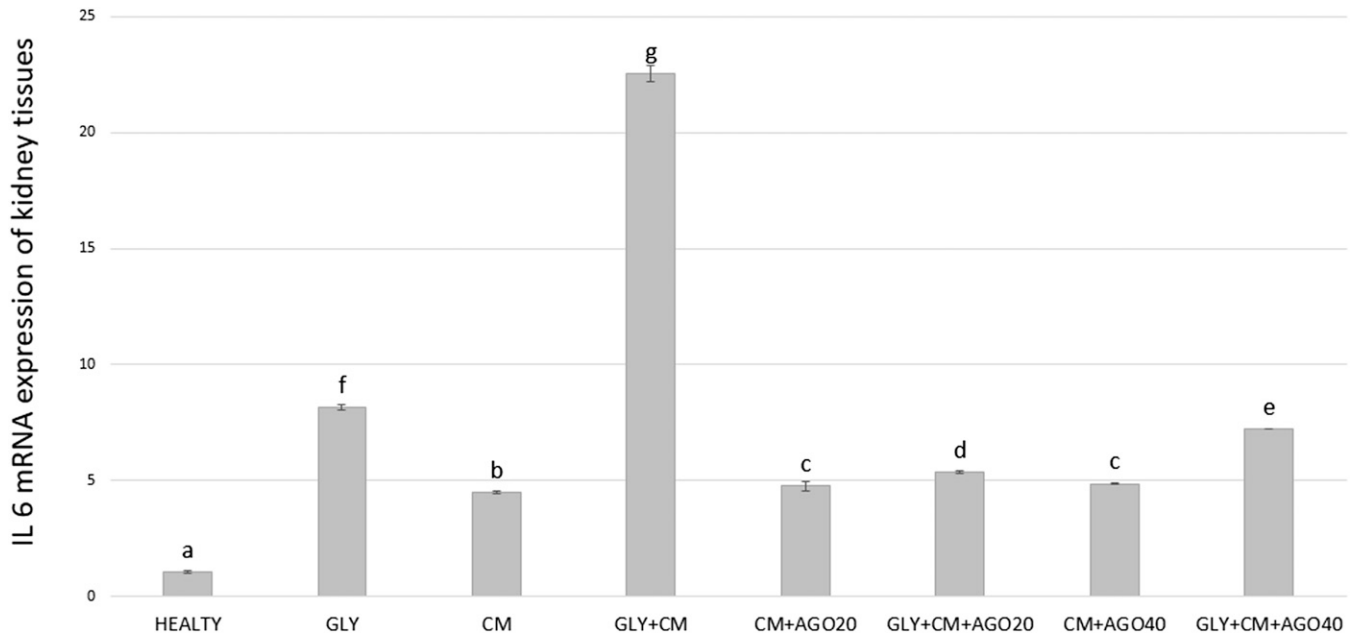
The histopathology of the kidneys of the animals in all groups was examined and scored; the results are given in Figure 10. Rats in the healthy group did not have any histopathological lesions (Figure 11), but severe lesions were seen in both the cortex and medulla of the glycerol-treated rats. Mild-to-severe hyaline and haemorrhagic casts and tubular necrosis were observed in all groups, except in the healthy group (Figure 11). The histopathological scores were better in the agomelatine treatment groups when compared with that in the GLY+CM groups.

#### DISCUSSION

In our study, we focused on the restorative effects of agomelatine for rats with contrast-induced nephropathy; we found that agomelatine administration significantly decreased the elevated serum concentrations of both BUN and creatinine to nearly healthy levels. Biochemical results for SOD activity, GSH and MDA levels in the kidney tissue, as well molecular results for TNF- $\alpha$ , NF- $\kappa$ B and IL-6, showed the ameliorative effects of agomelatine on kidney function. Histological scores in terms of hyaline and haemorrhagic casts and tubular necrosis were also improved by the administration of agomelatine.



Figure 8. The tissue NF $\kappa$ - $\beta$  messenger RNA (mRNA) expression 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. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).



Three different pathophysiological mechanisms lead to CIN: the first being haemodynamic effects (renal medullary hypoxia); the second, the direct effects of the contrast medium molecule causing tubular cell toxicity; and the third being endogenous biochemical changes such as induction in oxygen-free

radicals and/or a reduction in antioxidant enzyme activity. In addition, it is thought that activation of cytokine-induced inflammatory mediators by reactive free radicals may be a possible mechanism,<sup>22-27</sup> and apoptosis may also play a part in the development of CIN.<sup>22,28</sup> However, the main mechanism by which

Figure 9. The tissue IL-6 messenger RNA (mRNA) expression 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. AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol).

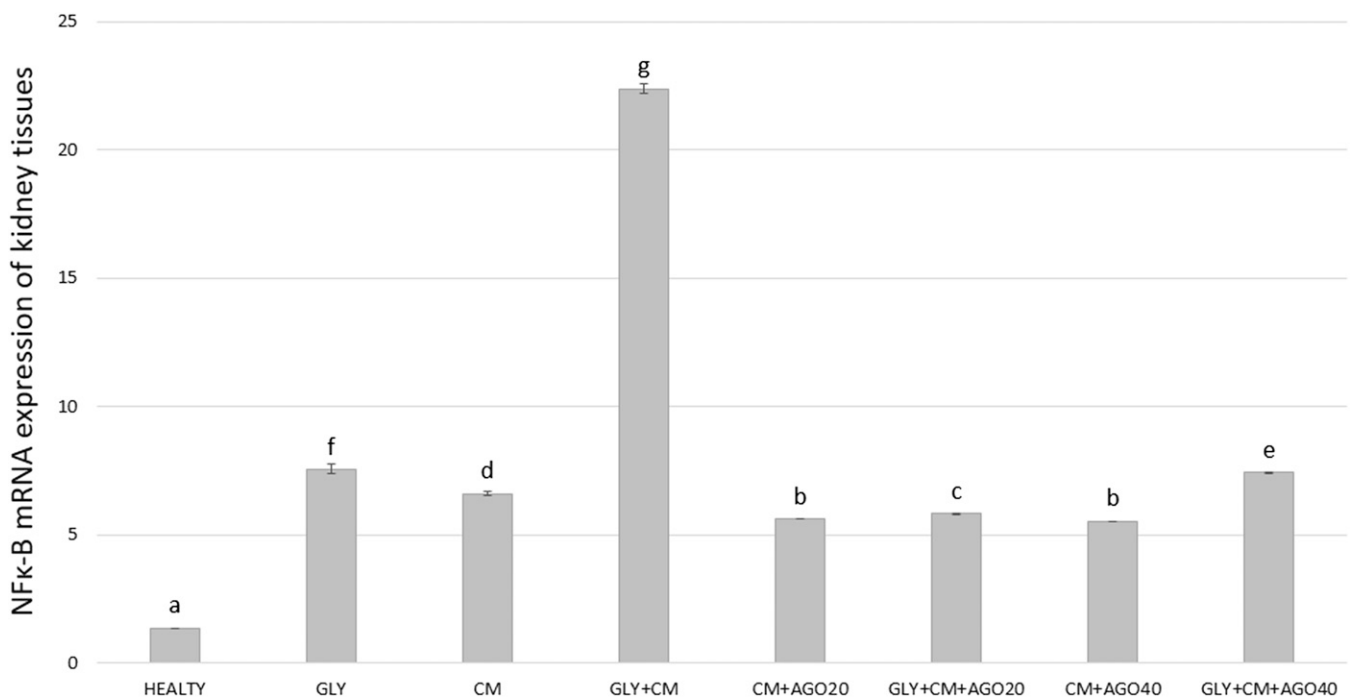
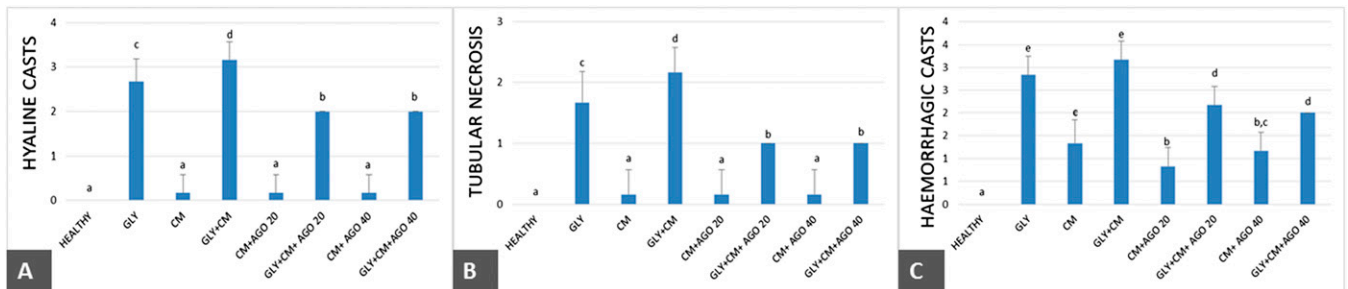


Figure 10. (a-c) Effects of agomelatine on histopathological hyaline cast (a) tubular necrosis (b) and haemorrhagic cast (c) scores in rats' kidneys. Means in the same column by the same letter are not significantly different from Duncan's multiple comparison test ( $p = 0.05$ ). Results are expressed as means  $\pm$  SD ( $n = 6$ ). AGO20 (agomelatine 20 mg kg<sup>-1</sup>); AGO40 (agomelatine 40 mg kg<sup>-1</sup>); CM, contrast media; GLY (glycerol); SD, standard deviation.



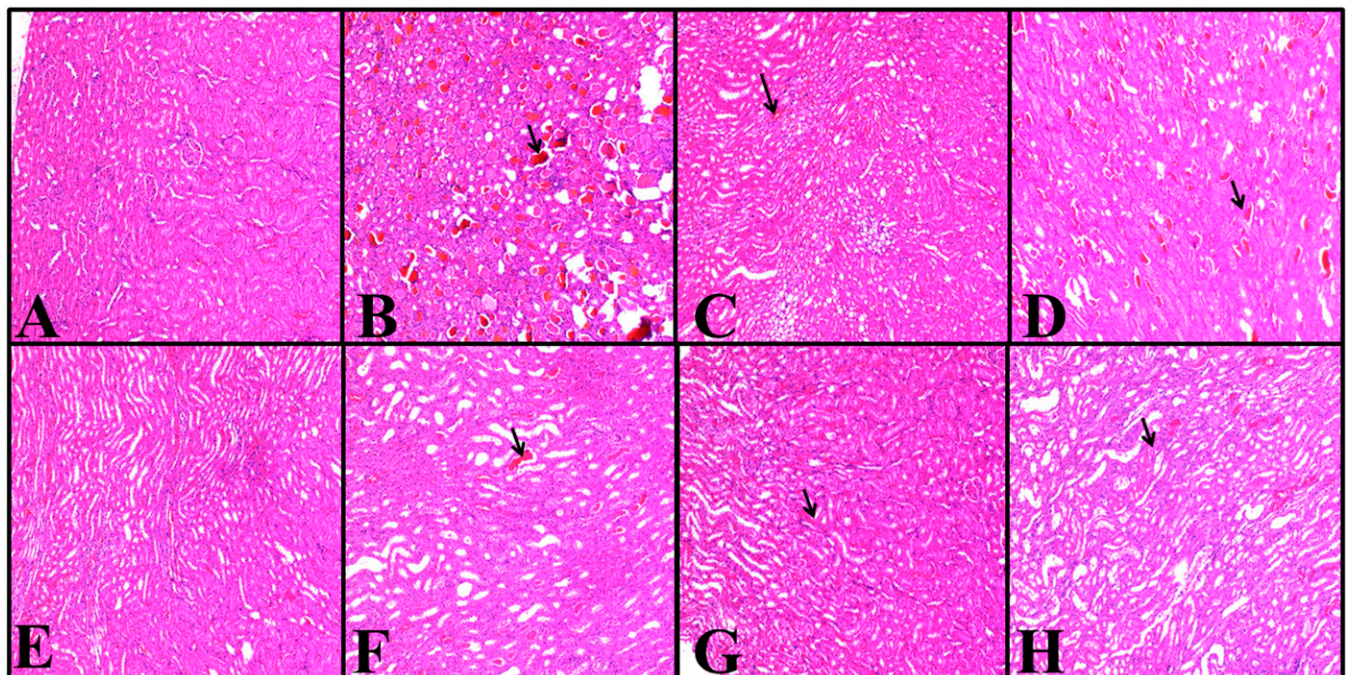
CIN develops is still unclear; so, in clinical examinations, a variety of ways of avoiding CIN has been investigated.

Agomelatine is a selective melatonergic (MT1/MT2) agonist and a 5-HT2C antagonist.<sup>29</sup> It has been reported to have antioxidant,<sup>30</sup> anti-inflammatory<sup>31</sup> and memory-facilitating effects.<sup>32</sup> Agonistic regulation of melatonin receptors has been said to improve locomotor activity and protect the cholinergic system,<sup>32</sup> as well as attenuate mitochondrial oxidative damage.<sup>32</sup> Melatonin has both lipophilic and hydrophilic properties. It can diffuse without difficulty into subcellular partitions and shield against free radical-

mediated damage. The latest literature states that melatonin averts nephrotoxicity triggered by several drugs.<sup>33-38</sup> In particular, Gazi et al<sup>39</sup> demonstrated the defensive effect of melatonin administration in rat radiocontrast nephrotoxicity by reducing BUN and creatinine levels.<sup>40</sup> However, these studies have not measured oxidative and molecular parameters to indicate whether regeneration of the kidneys occurred after injury. In the present literature, there is no study about CIN related to agomelatine.

Serum creatinine is the most frequently used parameter for evaluating kidney function, along with BUN.<sup>41</sup> In previous

Figure 11. (a-h) Haematoxylin and eosin results in rats' kidney tissues; magnification 100X. Healthy group (a), GLY (glycerol) group (b), CM (contrast media) group (c), GLY+CM group (d), CM+AGO20 (agomelatine 20 mg kg<sup>-1</sup>) group (e), GLY+CM+AGO20 group (f), CM+AGO40 (agomelatine 40 mg kg<sup>-1</sup>) group (g) and GLY+CM+AGO 40 group (h). (a) Normal structure of the kidney in the control group. (b) Hyaline and haemorrhagic casts are seen severely and moderately; tubular necrosis is also present. (c) Only haemorrhagic casts are seen moderately. Hyaline and tubular necrosis are observed mildly. (d) Haemorrhagic and hyaline casts are seen severely. Moderate tubular necrosis is present. (e) Only haemorrhagic casts are seen mildly. (f) Both hyaline and haemorrhagic casts are seen moderately. Tubular necrosis is also present mildly. (g) Only haemorrhagic casts are seen mildly. (h) Hyaline and haemorrhagic casts are seen moderately; tubular necrosis is observed mildly.



studies, in which nephrotoxicity was created by glycerine and CM, increased levels of serum BUN and creatinine were shown in parallel with elevated oxidative stress and inflammation.<sup>7</sup> In our study, we detected large increases in serum BUN and creatinine levels in the GLY and GLY+CM groups compared with the healthy group. We also showed that agomelatine, in both groups, ameliorated the serum BUN and creatinine levels of nephrotoxic rats. These decreases in the levels of serum BUN and creatinine, which are important clinical findings of CIN, suggested that agomelatine may have prevented CM-induced nephrotoxicity.

Another possible marker of CIN is oxidative stress. Changes in SOD activity, GSH and MDA levels develop owing to generation of free radicals.<sup>42</sup> Glycerol, when injected intramuscularly, causes renal GSH depletion.<sup>43</sup> Baykara et al<sup>42</sup> in their study on propolis, a potential nephroprotective agent against a specific contrast agent (diatrizoate), claimed that propolis protects the renal tissue against diatrizoate toxicity, free radicals and other adverse effects by restoring MDA, GSH and SOD levels. In another CIN study, decreases in SOD levels in nephrotoxicity groups increased with ebselen treatment.<sup>44</sup> In cisplatin-induced nephrotoxicity, significantly increased MDA levels were observed in groups administered cisplatin.<sup>45</sup> In this study, MDA levels dramatically increased in the GLY and GLY+CM groups compared with that in the healthy group. In parallel with this, SOD activity and GSH levels for the groups of 5–8 in the GLY and GLY+CM groups dramatically decreased. Agomelatine administration improved SOD activity and GSH levels compared with the GLY and GLY+CM groups. Similar antioxidant effects of agomelatine have been demonstrated in previous studies.<sup>46</sup>

The inflammatory process is a factor acting in the pathogenesis of nephrotoxicity. In this process, macrophages released due to inflammation increase the production of proinflammatory cytokines in addition to oxidant release.<sup>47–49</sup> Previous studies demonstrated that contrast media administration led to increased levels of proinflammatory cytokines in the kidneys.<sup>43</sup> IL-6 and TNF- $\alpha$  are associated with CIN severity<sup>35,39</sup> and it has been shown that radiocontrast media administration increases NF- $\kappa$ B.<sup>50</sup> TNF- $\alpha$  is a proinflammatory cytokine that further

recruits numerous mediators associated with tissue damage. In the animal model of nephrotoxicity, TNF- $\alpha$  is of great importance in the activation of inflammatory response.<sup>35</sup> Another important inflammatory cytokine, IL-6, exhibits many physiological effects.<sup>36</sup> Contrast media administration was shown to lead to an increase in the levels of IL-6 in the kidney,<sup>38</sup> while TNF- $\alpha$  led to an increase in NF- $\kappa$ B.<sup>51,52</sup>

In our study, NF- $\kappa$ B upregulation, in parallel with contrast media and glycerol administration, correlated with the increasing levels of TNF- $\alpha$  and IL-6. However, doses of agomelatine caused significant downregulation of NF- $\kappa$ B, TNF- $\alpha$  and IL-6 expression, in comparison with GLY and GLY+CM groups. In a previous study, we demonstrated that agomelatine has an anti-inflammatory effect in cases of paracetamol hepatotoxicity; agomelatine administration also significantly improved TNF- $\alpha$  and IL-6 levels.<sup>46</sup> The results of the present study indicate that agomelatine has a potential nephroprotective effect against CIN.

In various studies, tubular necrosis and epithelial injury have been shown to be significant indicators in nephrotoxicity injury;<sup>53</sup> tubular epithelial damage, tubular necrosis, hyaline and haemorrhagic casts have been found in these investigations.<sup>54</sup> In the development of nephropathy, significant damage to haemorrhagic renal tubules with hyaline occurs and is related to tubular injury. We determined positive effects of agomelatine administration on tubular structure.

In conclusion, our study showed that agomelatine has nephroprotective, antioxidant and anti-inflammatory effects against CIN in rats. This effect is brought about by a reduction in oxidative stress and inhibition of the secretion of proinflammatory cytokines (NF- $\kappa$ B, TNF- $\alpha$  and IL-6) that trigger the initiation of inflammation in the kidney. This animal model can be a reflection of CM-induced nephropathy in patients with underlying renal insufficiency. We can suggest that agomelatine can be a drug of choice for both radiologists and nephrologists before CM administration in patients with renal insufficiency. However, more detailed future clinical studies are required to better clarify the protective effects of agomelatine in CIN.

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