EXPERIMENTAL STUDY

Protective effect of melatonin and agomelatine on adriamycin-induced nephrotoxicity in rat model: a renal scintigraphy and biochemical study

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ABSTRACT

OBJECTIVE: We aimed to determine the possible protective effects of melatonin and agomelatine on an animal model of adriamycin nephrotoxicity by ^{99m}Tc DMSA renal scintigraphy and biochemical methods.
METHODS: Ten weeks old 49 male Wistar rats were randomly separated into seven groups; namely control (CON), adriamycin (ADR), melatonin (MEL), agomelatine (AGO), melatonin + adriamycin (MEL+ADR), agomelatine + adriamycin (AGO+ADR) and melatonin + agomelatine + adriamycin (MEL+AGO+ADR) groups. Nephrotoxicity was induced by a three-dose of 18 mg/kg adriamycin, i.p. at a 24 h interval on the 5th, 6th and 7th days. A dose of melatonin and agomelatine (40 mg/kg/i.p, the same doses) were injected for 7 days before and after the injected of ADR (18 mg/kg, i.p.), respectively. On the 8th day of the experiment, all animals were evaluated and scintigraphic and biochemical parameters were assessed, respectively.
RESULTS: ADR significantly increased blood urea nitrogen (1040 %) and plasma creatinine (1020 %), and decreased ^{99m}Tc DMSA uptake levels (59 %) compared to the control (p < 0.001). Pretreatment with MEL, AGO, MEL+AGO mitigated these abnormalities produced by ADR in the kidney (p < 0.001).
CONCLUSION: ^{99m}Tc DMSA for the early determination of ADR-induced nephrotoxicity had an important role. Also, a significant correlation was found between biochemical and scintigraphy parameters. Adriamycin caused significant damages to kidneys that were reduced with MEL and AGO *(Tab. 2, Fig. 3, Ref. 39)*. Text in PDF *www.elis.sk*.
KEY WORDS: adriamycin, melatonin, agomelatine, rat, ^{99m}Tc DMSA, nephrotoxicity.

Introduction

Adriamycin (ADR), is commonly used as an antitumoral anthracycline antibiotic in humans (1). Disappointingly, the usage of ADR was limited due to its toxic effects in several organs including its effects on kidneys, liver, and heart (2, 3, 4). In rats, a single ADR dose, causes tubulointerstitial injury characterized by severe glomerulosclerosis (5).

The mechanisms of ADR-induced organ damage were studied extensively. In the most plausible mechanism of ADR-induced renal toxicity, there is increasing clinical and experimental data, suggesting a prominent role at an increase of free oxygen radicals, which induced membrane lipid peroxidation and oxidative stress in cells (6, 7, 8). This idea suggests that the use of synthetic or natural antioxidants may have potential protective effects on ADR-induced renal toxicity by reducing oxidative stress.

Melatonin is a powerful biological antioxidant and the main product of the pineal gland. Many experimental studies demonstrated that melatonin reduced oxidative stress by neutralizing ROS production in cell (9, 10). The protective effect of melatonin on the ADR-induced nephropathy in rats was previously reported (11, 12, 13). Agomelatine is a selective melatonin M1/M2 receptor agonist, and selective serotonin 5HT-2C receptor antagonist as well and has a higher affinity for M1/M2 receptors (14). Many studies demonstrated that AGO acts as a potent antioxidant and a free radical scavenger, which is this case prevents cellular damage in rats.

In this study, we investigated the possible protective effect MEL and AGO on ADR-induced nephrotoxicity by biochemical and technetium 99m labeled dimercaptosuccinic acid (^{99m}TcDMSA) renal scintigraphically. In many studies, histologic and biochemical methods have been used to determine ADR-induced nephrotoxicity. For the first time in the present study, ADR-induced nephrotoxicity was determined by scintigraphic nuclear imaging.

Materials and methods

Animal groups

Ten weeks old 49 male Wistar Albino rats weighing 200–240 g were used. All experimental procedures of this study were approved by the local animal ethics commission of University of Gaziosmanpasa Experimental Research Centre, Turkey, Protocol No. 2017/21. The rats were randomly separated into seven groups

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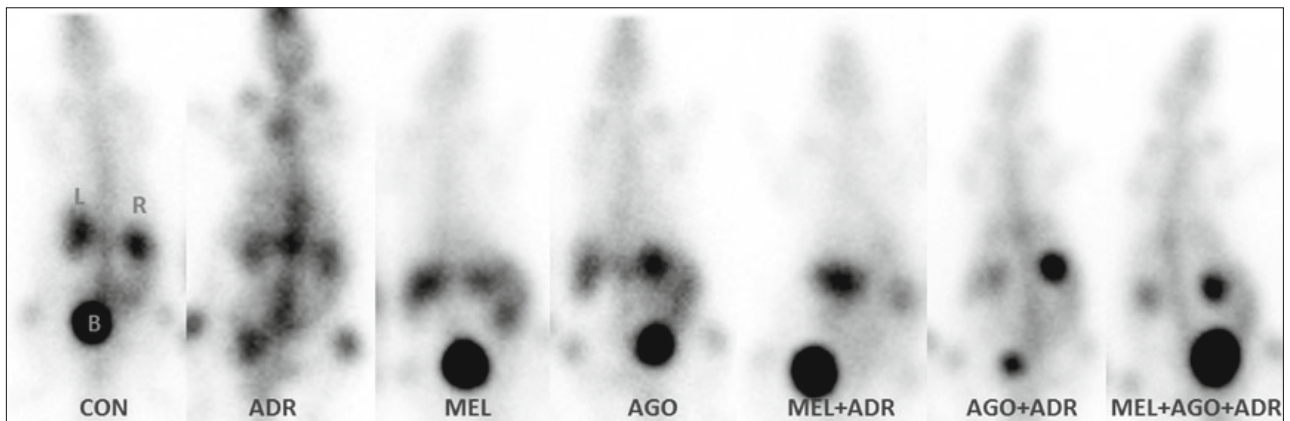
Fig. 1. Static images in posterior position of all the groups. L: Left Kidney R: Right Kidney B: Bladder. CON = control, ADR = adriamycin, MEL = melatonin, AGO = agomelatine, and MEL+ADR = melatonin + adriamycin, AGO+ADR = agomelatine + adriamycin, and MEL +AGO +ADR = melatonin + agomelatine + adriamycin. It was observed that ^{99m}Tc DMSA uptake in renal proximal tubules was higher in the CON group. On the other hand, in DOX group, ^{99m}Tc DMSA uptake appeared to be lower because of the developing damage.

of seven rats each. Animals were housed with regular 12:12-h light–dark cycle (light on at 18:00 a.m., light off at 18: p.m.), under controlled humidity (60 ± 12 %), and temperature (23 ± 4 °C). All animals were given standard chow and water.

Experimental design

The rats were randomly separated into seven groups of seven rats each (n = 7x7) and the experimental groups were arranged as follows: 1 – control (CON), 2 – adriamycin (ADR), 3 – melatonin (MEL), 4 – agomelatine (AGO), 5 – melatonin + adriamycin (MEL+ADR), 6 – agomelatine + adriamycin (AGO+ADR), 7 – melatonin + agomelatine + adriamycin (MEL + AGO + ADR).

Group I (Control) served as the control group and animals received only normal saline for 7 days.

Group II (ADR) served as ADR group (nephrotoxic group) and rats were administered three doses of 18 mg/kg adriamycin, i.p. at a 24 h interval on the 5th, 6th and 7th days.

Group III (MEL), rats received only melatonin for (40 mg/kg/i.p.) for 7 days.

Group IV (AGO), rats received only agomelatine for (40 mg/kg/i.p.) for 7 days.

Group V (MEL + ADR), rats pretreatment with melatonin (40 mg/kg/i.p.) for 7 days and were administrated ADR (18 mg/kg, i.p.) at a 24 h interval on the 5th, 6th and 7th days.

Group VI (AGO + ADR), rats pretreatment with agomelatine (40 mg/kg/i.p.) for 7 days and were administrated ADR (18 mg/kg, i.p.) at a 24 h interval on the 5th, 6th and 7th days.

Group VII (MEL + AGO + ADR), rats pretreatment with melatonin and agomelatine (40 mg/kg/i.p., the same doses) for 7 days and then followed by three doses of 18 mg/kg ADR, i.p. at a 24 h interval on the 5th, 6th and 7th days.

Drugs and drug administration

Normal saline, ethanol, ketamine, hydrochloride (HCl) and xylazine hydrochloride, Melatonin (Sigma Chemical Co., St. Louis, MO, USA) and agomelatine (Tablet form) of agomelatine was

used. Valdoxan tablets containing 25 mg agomelatine were taken from the local pharmaceutical company. Melatonin and agomelatine were dissolved in 1 % ethyl alcohol. The required doses were administered i.p. in a volume of 1 mL for 7 days. The doses of the agomelatine and melatonin were determined in accordance with the previous studies (15, 16, 17).

Scintigraphic imaging

Renal scintigraphy was accomplished 7 days after drug induction of nephrotoxicity under a general anesthesia using Symbia (Siemens Medical Solutions, USA), a double-headed gamma camera scans. The imaging system was equipped with two gamma camera detector heads fitted with parallel-hole collimators and CsI (Na) crystal with 64x64 matrix. Energy window was centered at 140 keV and discriminator window was 20 % of the total width. According to the results of the previous studies (18), the recommended image acquisition time for renal scintigraphy with ^{99m}Tc DMSA in the rat is 1 h post-injection. Therefore, 1 h after injection of 1 mCi (37 MBq) of ^{99m}Tc DMSA through the tail vein, two opposed planar images (anterior and posterior) were collected in a 64x64 matrix. Regions of interest (ROIs) were selected around the kidneys and background areas for both anterior and posterior images. For semiquantitative analysis, ROIs were drawn around each kidney. DMSA uptake was expressed after a background subtraction. After background correction, the geometric mean of the anterior and posterior images was used for the calculation of left and right renal ^{99m}Tc DMSA uptake. Semiquantitative ^{99m}Tc DMSA uptake was determined for each kidney by the calculation of the photon counts per pixel. The sum of the ^{99m}Tc DMSA uptake values of the right and left kidneys was obtained for each rat (Fig. 1).

Blood sampling and biochemical parameters in the serum

After scintigraphic imaging, in the deeply anesthetized rats, needles were inserted into the heart ventricular chamber. Blood samples collected from the heart were put on non-heparin containing tubes. These tubes were centrifuged at 3000 rpm for 10 min;

serum samples were separated and transferred to Eppendorf for biochemical analysis of blood urea nitrogen (BUN) and creatinine levels. At the end of the experimental protocol, rats were killed by cervical dislocation under the anesthesia.

Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM) and were tested using one-way analysis of variance (ANOVA) followed by Tukey post hoc test (HSD) test. A probability of p-value less than .05 (p < 0.05) was considered statistically significant.

Results

Scintigraphic imaging assays

As shown in Figure 2 and Table 1, ^{99m}Tc DMSA uptake levels in kidney were 362264 ± 111 in CON rats and 150290 ± 105 in ADR, reduction by 59 %, (p < 0.001) when compared to the CON groups. MEL and AGO alone did not change ^{99m}Tc DMSA uptake levels as compared to the CON groups (Fig. 2, Tab. 2). The ^{99m}Tc DMSA uptake levels in kidney were 362264 ± 111; 150290 ± 105; 227088 ± 790; 230500 ± 713; 299932 ± 220 and CON, ADR injected, MEL pretreatment, AGO pretreatment, MEL+AGO
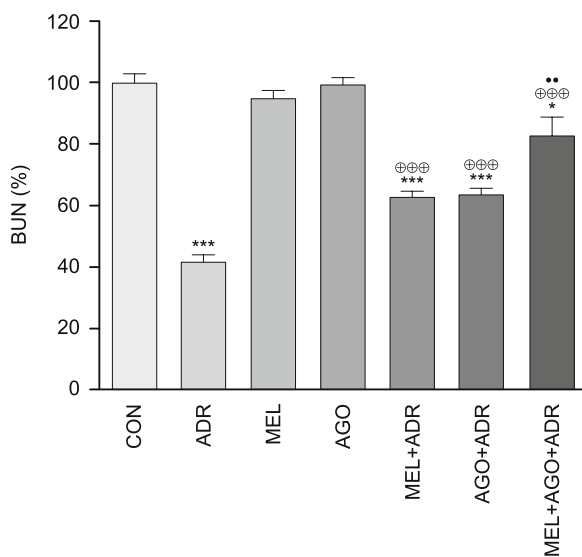
Fig. 2. CON = control, ADR = adriamycin, MEL = melatonin, AGO = agomelatine, and MEL+ADR = melatonin + adriamycin, AGO+ADR = agomelatine + adriamycin, and MEL+AGO+ADR = melatonin + agomelatine + adriamycin. One-way ANOVA and followed by Tukey post hoc test. Data are as the mean ± SEM. The ADR, MEL+ADR, AGO+ADR, MEL+AGO+ADR groups significantly decreased the ^{99m}Tc DMSA radiopharmaceutical uptake compared with the CON group (* p<0.05, ** p<0.01, *** p<0.001). Pre-treatment groups of MEL+ADR, AGO+ADR, MEL+AGO+ADR significantly decreased the ^{99m}Tc DMSA radiopharmaceutical uptake compared to the ADR group (⊕ = p< 0.05; ⊕⊕ = p<0.01; ⊕⊕⊕ = p < 0.001), Pre-treatment groups of MEL+ADR, AGO+ADR significantly increased the ^{99m}Tc DMSA radiopharmaceutical uptake compared to combination pretreatment groups of MEL+AGO+ADR (• = p<0.05, •• = p<0.01, ••• = p<0.001).

Tab. 1. The statistical analysis of the scintigraphic data for the all groups.

Groups	^{99m}Tc DMSA Uptake (cpm)
CON	362264±111
ADR	150290±105 c
MEL	344108±894
AGO	360445±807
MEL+ADR	227088±790 c,f
AGO+ADR	230500±713 c,f
MEL+AGO+ADR	299932±220 c,f,*

CON = control, ADR = adriamycin, MEL = melatonin, AGO = agomelatine, and MEL+ADR = melatonin+ adriamycin, AGO+ADR = agomelatine+adriamycin, and MEL+AGO+ADR = melatonin + agomelatine + adriamycin. One-way ANOVA and followed by Tukey post hoc test. Data are as mean± SEM. A = p<0.05, b = p<0.01, c = p<0.001 all groups as compared to the CON groups, d = p<0.05, e = p<0.01, f = p<0.001 MEL+ADR, AGO+ADR, MEL+AGO+ADR groups as compared to the ADR groups (* p<0.05, ** p<0.01, *** p<0.001). MEL+ADR, AGO+ADR groups as compared to MEL+AGO+ADR groups.

Tab. 2. The statistical analysis of the biochemical data for all groups.

Groups	BUN (mg/dL)	Creatinine (mg/dL)
CON	14.78±0.61	0.35±0.01
ADR	153.8±4.28 c	3.64±0.19 c
MEL	14.48±0.55	0.37± 0.01
AGO	15.67±0,83	0.40±0.01
MEL+ADR	62.25±5.15 c,f	1.24±0.14 c,f
AGO+ADR	64.18±4.91 c,f	1.40± 0.15 c,f
MEL+AGO+ADR	57.48±4.97 c,f	1.35±0.10 c,f

CON = control, ADR = adriamycin, MEL = melatonin, AGO = agomelatine, and MEL+ADR = melatonin + adriamycin, AGO+ADR = agomelatine+adriamycin, and MEL+AGO+ADR = melatonin + agomelatine + adriamycin. One-way ANOVA and followed by Tukey post hoc test. Data are as the mean ± SEM. a = p<0.05, b = p<0.01, c = p<0.001 The ADR, MEL+ADR, AGO+ADR, MEL+AGO+ADR as compared to the CON groups, d = p<0.05, e = p<0.01, f = p<0.001 MEL+ADR, AGO+ADR, MEL+AGO+ADR groups as compared to the ADR groups.

pretreatment groups, respectively (Tab. 1, Fig. 2). Pretreatment of ADR-induced nephrotoxicity with MEL, AGO, MEL combination with AGO decreased the ^{99m}Tc DMSA uptake levels (Fig. 2,Tab. 2) (62, 69 and 82 %; p < 0.001, p < 0.001, p < 0.05 respectively) when compared to the CON group, but all pretreatment groups significantly increased the levels of ^{99m}Tc DMSA uptake when compared to the ADR diseased group (Fig. 2 and Tab. 1) (p < 0.001; p < 0.001, p < 0.001 respectively).

Pretreatment groups of MEL+ ADR, AGO+ADR significantly decreased the ^{99m}Tc DMSA radiopharmaceutical uptake compared to combination pretreatment groups of MEL+AGO+ADR (uptake (Fig. 2 and Tab. 1) (p < 0.01; p < 0.01, respectively)

Biochemical assays

As shown in Table 2 and Figure 3. The BUN and creatinine in plasma were 14.78 ± 0.61; 0.35 ± 0.01 and 153.8 ± 4.28; 3.64 ± 0.19 in CON and ADR groups, respectively. ADR injection significantly increased BUN level by 1040 % and creatinine level by 1020 % in plasma when compared to the CON group (Fig. 2). MEL and AGO alone did not change BUN (97 and 106 %, respectively) and creatinine (105 and 113 %, respectively) in plasma level when compared to the CON groups.

The BUN and creatinine in plasma levels 14.78 ± 0.61, 0.35 ± 0.01; 153.8 ± 4.28, 3.64 ± 0.19; 62.25 ± 5.15, 1.24 ± 0.14; 64.18
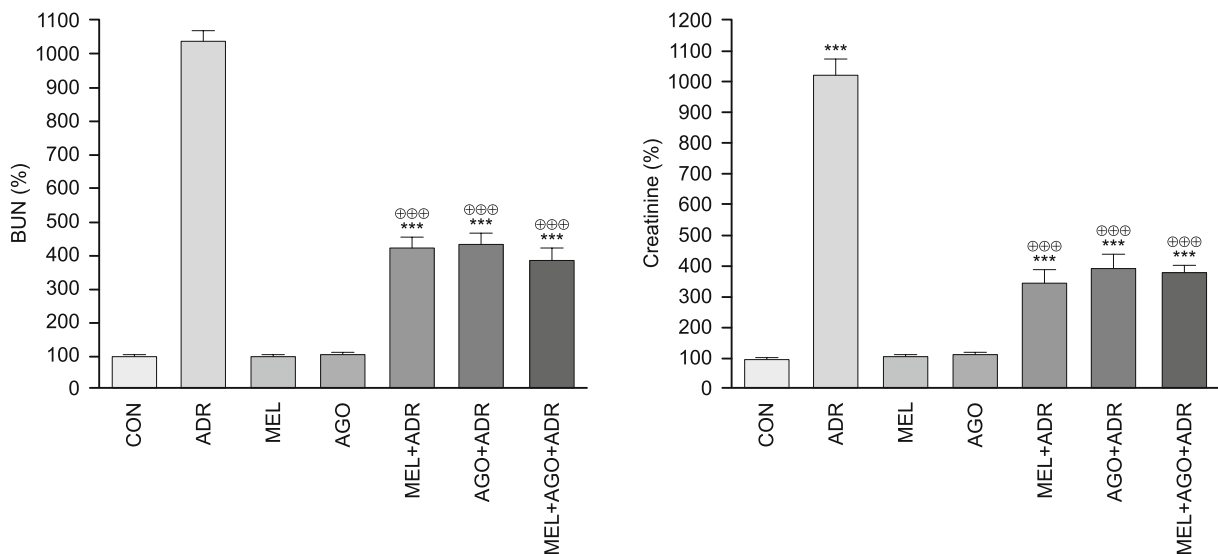
Fig. 3. CON = control, ADR = adriamycin, MEL = melatonin, AGO = agomelatine, and MEL+ADR = melatonin + adriamycin, AGO+ADR = agomelatine + adriamycin, and MEL+AGO+ADR = melatonin + agomelatine + adriamycin. One-way ANOVA and followed by Tukey post hoc test. Data are as the mean± SEM. The ADR, MEL+ADR, AGO+ADR, MEL+AGO+ADR groups significantly increased the BUN, creatinine in comparison to the CON group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Pre-treatment groups of MEL+ADR, AGO+ADR, MEL+AGO+ADR significantly decreased the BUN, creatinine compared to the ADR group (⊕= $p<0.05$; ⊕⊕= $p<0.01$; ⊕⊕⊕= $p<0.001$).

± 4.91, 1.40± 0.15; 57.48 ± 4.97, 1.53 ± 0.10 and CON, ADR injected, MEL- pretreatment, AGO pretreatment, MEL+AGO pretreatment groups, respectively (Tab. 1; Fig. 2). Pretreatment of ADR-induced toxic rat with MEL, AGO, MEL combination with AGO increased the levels of both of BUN (421.432 and 388 %, respectively) and creatinine (349.392, 431 %, respectively) when compared to the CON group levels, but this pretreatment groups significantly decreased the levels of both of BUN (Figure 2 and Table 1, $p<0.001$; $p<0.001$, $p<0.001$ respectively), and creatinine (Fig. 2 and Tab. 1) ($p < 0.001$; $p < 0.001$, $p < 0.001$ respectively), when compared to the ADR toxic group.

Discussion

In the present study, we investigated the renal protective effect of MEL and AGO treatment in an ADR induced nephrotoxicity model in rat. Renal scintigraphic imaging and biochemical parameters such as: BUN and creatinine were used for the assessment of renal function. A strong correlation was found between [99mTc] DMSA uptake and biochemical finding, which in the present study indicate that ADR increased BUN, creatinine and decreased [99mTc] DMSA uptake levels. Furthermore, a MEL and AGO treatment attenuated the renal dysfunction in ADR-induced nephrotoxicity in rats by reducing BUN, creatinine and elevated [99mTc] DMSA uptake levels.

ADR-induced nephrotoxicity is well-known accompanied by renal dysfunction (19, 20). Biochemical markers such as: serum creatinine and blood urea nitrogen (BUN) along with histological examinations are used for the comment of renal function (21, 22, 23). However, biochemical assays lack sensitivity to relay acute

renal injury. This is proven by the fact that any changes in the levels of creatinine and BUN require the loss of at least 75 % of functional nephrons. Histopathological methods are invasive, inadequate to be fully diagnosed and taking too much time. Thus, the development of non-invasive methods with a high accuracy and sensitivity is of interest for the early diagnosis of renal dysfunction (23, 24, 25). DMSA renal scan may be a decent tool for an early non-invasive diagnosis of acute tubulointerstitial nephritis in children and particularly useful in those patients, who are not candidates for a kidney biopsy. Furthermore, DMSA scan allows monitoring of the evolution of acute renal parenchymal inflammation with the potential risk of renal scar formation and gives an accurate follow-up evaluation (26). Therefore, we evaluated the [99mTc] DMSA renal scintigraphy, a non-invasive method, to compare renal dysfunction in ADR-induced nephrotoxic rats by biochemical markers. In our study, we showed that [99mTc] DMSA uptake level was decreased in nephrotoxic groups as compared to the CON group. Treatment with MEL and AGO led to an important increase in the levels of [99mTc] DMSA uptake on kidney area in the ADR-induced rat. In this respect, our results are an inconsistency with Fatemikia et al (18), who investigated the renal damage in two nephrotoxicity animal models, which is gentamicin-induced and unilateral ureteral ligation using [99mTc] DMSA scintigraphy and biochemical parameters. They showed a significant decrease in [99mTc] DMSA uptake and increased BUN and creatinine in two nephrotoxicity group (18). Also, our results were similar to Yamada, who assessed the [99mTc] DMSA renal uptake in cisplatin-induced nephrotoxicity. The author found that [99mTc] DMSA uptake level reduced in cisplatin-induced nephrotoxicity, when compared the control group (27). This finding suggests that the nephrotoxicity caused by ADR can be determined

scintigraphically, and this toxicity in the kidney can be reduced by the treatment of MEL and AGO melatonin.

Renal cortical scintigraphy with ^{99m}Tc DMSA is used for functional imaging of the proximal renal tubular mass, which depends on the renal blood flow and proximal tubular cell membrane transport function. Provoost and friends' experimental findings apparently demonstrated that a generalized proximal tubular dysfunction considerably affected the renal handling of ^{99m}Tc DMSA (28). ^{99m}Tc DMSA provides high-quality renal images due to preferential cortical accumulation and allows quantification of separate kidney functions (29). Several studies showed that determination of injected dose per gram of ^{99m}Tc DMSA in tissue could be a good technique for assessment of nephrotoxicity and/or nephroprotective effect in the animal model (18, 30). In addition, ^{99m}Tc DMSA uptake is found to correlate with renal blood flow, glomerular filtration rate, and creatinine clearance (18). In our study, we displayed the kidney damage with ^{99m}Tc DMSA renal scintigraphy similar to literature. In our study, we found that ^{99m}Tc DMSA uptake was significantly lower in the ADR group compared to the control group due to proximal tubule damage. In the treatment groups, ^{99m}Tc DMSA uptake was close to the control group.

In other words, in order to visualize the kidneys to study renal morphology, and to assess individual kidney function, ^{99m}Tc DMSA was used. ^{99m}Tc DMSA is mostly used to determine differential renal function. Both clinically and experimentally, good interrelationships were found between the differential renal ^{99m}Tc DMSA uptake and indices of the renal function (28). A study conducted by Vidal et al. proposed that in the diagnostic process of acute tubulointerstitial nephritis in children, DMSA scan might be a useful device. Furthermore, comparing the DMSA scan images in the acute phase with those gathered from a follow-up scintigraphy allows controlling of the evolution of renal parenchymal inflammation with possible risk of the chronic damage because of the renal scarring (26). In this study, we were able to identify a peculiar DMSA renal scan pattern in the acute phase of ATIN and we also demonstrated the usefulness of DMSA scintigraphy in the follow-up evaluation.

Toxic effect of ADR is related to the production of ROS in the kidney, which results in oxidative stress (5). The oxidative stress destructs glomerular cells membrane, causing rupture of the membrane, alteration in cell membrane permeability and leakage of cellular enzymes into plasma (31). These enzymes can be estimated in serum. Blood urea nitrogen (BUN) and serum creatinine are known to be the most important biomarkers showing renal function (24, 32). In the current study, the result demonstrated that ADR increased the levels of BUN and creatinine significantly, which is an indicator of nephrotoxicity and indicating a decrease in glomerular filtration (19). Our results are very similar to that of the reported previously in literature. A lot of studies demonstrated that ADR caused a rise in levels of biomarker enzymes (5, 33). Any change in BUN and creatinine levels has been proven by the fact that the functional nephron needs to be lost by at least 75 % (24, 32). Interestingly, the level of BUN and creatinine decreased with MEL, AGO and MEL combination with AGO treatment in the ADR-induced nephrotoxic rat. Melatonin is a free radical scaven-

ger and effective antioxidant (34). ADR-induced renal injury improvement MEL by reduced lipid peroxidation, ROS production and increased the antioxidant renal capacity and improved renal function (6, 12, 35). Agomelatine is a melatonin receptor agonist (M1/M2) and serotonin receptor antagonist (5 HT-2C), known to be more potent than melatonin receptor (36). These findings are in accordance with several other types of research, which reported that AGO decreased in BUN and creatinine in sepsis-related acute kidney injury by reducing oxidative stress (37). The nephrotoxicity of ADR, also including sepsis-related acute kidney injury, is related to the oxidative stress in kidney (6, 35, 37). A relationship between oxidative stress and nephrotoxicity has been confirmed in many experimental models (8, 38, 39). The mechanism of this protective effect of MEL and AGO might be due to antioxidant properties.

Conclusion

The results of the present study indicate that ADR-induced nephrotoxicity is prevented by melatonin and agomelatine, which could enhance renal function. Likewise, our findings demonstrated that there was a good correlation between the results of scintigraphy biochemical results, which means ^{99m}Tc DMSA renal scintigraphy scan might be used for diagnosis of renal nephrotoxicity.

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