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Protective effect of vitamin D and melatonin on adriamycin-induced nephrotoxicity in a rat model: A renal scintigraphic and biochemical study

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Abstract

The aim of the present study was to evaluate the renal protective effect of melatonin and vitamin D treatments in adriamycin-induced nephrotoxic rats using both ^{99m}Tc-DMSA renal scintigraphic imaging and biochemical methods. Forty-nine male rats were randomly separated into seven groups: control(CON), adriamycin-induced nephrotoxicity (ADR, 18 mg/kg), substance control of melatonin (40 mg/kg) and vitamin D (60.000 IU/kg), ADR+MEL, ADR+Vit D, ADR+MEL+Vit D. Nephrotoxicity was induced by intraperitoneal administration of adriamycin (ADR) at a cumulative dose of 18 mg/kg, administered over three consecutive days (days 15–17). Vitamin D pretreatments were applied as single dose injections of 60.000 IU/kg/i.p, on the first day of experiment, melatonin (40 mg/kg/i.p/day) were administered for 17 days. The renal functions were examined on 18th days of experiment. Adriamycin significantly reduced ^{99m}Tc-DMSA uptake levels in the kidney area (43%, $p<0.001$) but increased blood urea nitrogen (951%, $p<0.001$) and creatinine levels (806%, $p<0.001$) compared to control group. Pretreatment with melatonin, vitamin D, and their combination significantly improved ^{99m}Tc-DMSA uptake and reduced BUN and creatinine levels compared with the ADR group ($p<0.001$). The results demonstrated a strong correlation between scintigraphic findings and biochemical parameters. In addition, it was concluded that 99mTc DMSA could be used as a non-invasive technique to determine kidney damage induced by adriamycin.

Keywords: Adriamycin; melatonin; nephrotoxicity; ^{99m}Tc DMSA; vitamin D

Introduction

Adriamycin (ADR), also called doxorubicin, is a widely used first-line anthracycline antibiotic as an anticancer agent. It is used in patients suffering from solid tumors, breast cancer, and hematological malignancies [1,2]. In spite of its high antitumor activity, clinical use of adriamycin is limited because of its toxic effect on several organs and tissues, including the kidney, liver, heart, and testicles [3]. Adriamycin may cause kidney complications, and in cumulative doses, it might lead to nephrotoxicity [4]. In rodent tests, it was shown that a single dose of adriamycin-induced proteinuria causes to tubulointerstitial injury [5].

The mechanisms underlying the adriamycin-induced organ damage have been examined extensively. Adriamycin-induced organ toxicity is multifactorial and has not been fully characterized yet. However, the most plausible mechanism for the nephrotoxic effects of adriamycin is increased membrane lipid peroxidation and free-radical formation, which ultimately increase oxidative stress [6]. In kidney toxicity, oxidative stress in cells is induced by the reactive oxygen species (ROS) [6]. ROS are among the important causes of renal failure [7]. Previous studies indicated that oxygen-free radicals lead to nephrotoxicity in animals. A number of studies showed that renal toxicity induced by oxidative stress could be prevented by antioxidants [8,9].

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Melatonin (MEL) is an important biological antioxidant and bio-regulatory molecule produced by the pineal gland. It regulates numerous physiological functions involving the reduction of oxidative stress and free radicals [10]. Melatonin limits lipid peroxidation and reduces oxidative damage in cells [11]. In previous studies, melatonin has been shown to limit adriamycin-induced renal injury in the rat kidney and a rat model of cardiorenal syndrome [12]. Montilla et al. [12] revealed the renal protective effect of melatonin in hyperlipidemic nephropathy and oxidative stress induced by adriamycin in rats.

Vitamin D (Vit D) is a significant nutrient due to its character maintaining the homeostasis of phosphorus and calcium levels in the body. It also has diverse systemic impacts on metabolism.

Studies have demonstrated that vitamin D may affect the immune, muscular, endocrine, central nervous, cardiovascular, and excretory systems [13]. Malluche et al. [14] reported that vitamin D is beneficial in patients with chronic renal failure. Many studies have shown that vitamin D treatment inhibits extracellular matrix accumulation, reduces albuminuria, and reduces the release of inflammatory mediators [15,16]. Recent reports have indicated that vitamin D can inhibit podocyte hypertrophy and alleviate podocyte loss in the subtotally nephrectomized rats [17].

In another study, we demonstrated that pharmacological concentrations of melatonin and vitamin D decrease adriamycin-induced cardiac damage in rats. This present study was designed to explore the renal protective effects of melatonin and vitamin D on adriamycin-induced nephrotoxicity in kidneys using technetium-99m labeled dimercaptosuccinic acid (^{99m}Tc DMSA) renal scintigraphically and biochemical methods.

Material and Methods

Animal Tests and Experimental Design

Forty-nine adult male Wistar albino rats (body weight 250 ± 10 g and approximately two to three months old) were used. Rats were obtained from Tokat Gaziosmanpasa University Center for Experimental Animals. Rats were kept under standard room temperature (20 ± 3 ºC), a 12-hour dark/light cycle, and free access to standard laboratory rodent food and tap water. The experimental procedure was approved by the Ethical Committee of Tokat Gaziosmanpasa University (HADYEK-2017/22).

The groups were established with seven rats per group. The experimental groups were as follows:

1. Control group (CON): Sterile physiological saline (4 mL/kg, i.p.) was administered.
2. Adriamycin group (ADR): a cumulative dose of 18 mg/kg i.p. was administered for three consecutive days (days 15–17).
3. Melatonin group (MEL): 40 mg/kg i.p. melatonin.
4. Vitamin D group (Vit D): 60.000 IU/kg i.p. vitamin D administration.
5. Adriamycin and melatonin group (ADR+MEL): 18 mg/kg i.p. adriamycin and 40 mg/kg i.p. melatonin.
6. Adriamycin and vitamin D group (ADR+Vit D): 18 mg/kg i.p. adriamycin and 60.000 IU/kg i.p. vitamin D administration.
7. Adriamycin, melatonin and vitamin D group (ADR+MEL+Vit D): 18 mg/kg i.p. adriamycin, 40 mg/kg i.p. melatonin and 60.000 IU/kg i.p. vitamin D administration.

Drug Administration and Induction of Nephrotoxicity

Adriamycin and vitamin D were purchased from a pharmacy in Tokat, Türkiye. Melatonin was purchased from Sigma Chemical Co. Adriamycin was used at the dose of 18 mg/kg, while melatonin dose was 40 mg/kg and vitamin D dose was 60.000 IU/kg. All drug doses were determined based on previous studies [18,19]. Adriamycin was dissolved in sterile physiologic saline. Melatonin was dissolved in 5% ethanol and 95% sterile physiological saline. Adequate doses of all drugs were injected (i.p.) as 1 mL solution.

In the adriamycin group, nephrotoxicity was induced by intraperitoneal administration of adriamycin in divided doses over three consecutive days (days 15–17), resulting in a total cumulative dose of 18 mg/kg body weight. As a control for melatonin treatment, rats were administered only melatonin at a dose of 40 mg/kg/day, i.p., for 17 days. The vitamin D substance control was a single dose of vitamin D (60.000 IU/kg, i. p.) injected on the first day of the experiment. In the melatonin treatment group, rats were treated with melatonin (40 mg/kg/day, i.p.) for 17 days, followed by adriamycin injection (18 mg/kg, i.p.) on the 15th, 16th, and 17th days. Rats in the vitamin D group received a single dose of vitamin D (60.000 IU/kg, i. p.) on the first day of the experiment and were injected with adriamycin (cumulative dose of 18 mg/kg, i.p.) on the 15th, 16th, and 17th days. Rats in the MEL+Vit D group received melatonin (40 mg/kg/day, i.p.) for 17 days and a single dose of vitamin D (60.000 IU/kg, i.p.) on the first day of the study, followed by adriamycin injection (cumulative dose of 18 mg/kg, i. p.) on the 15th, 16th, and 17th days. Biochemical parameters and ^{99m}Tc DMSA renal scintigraphy were performed on the 18th day of the study.

Renal Scintigraphic Imaging

Renal scintigraphy was performed 1 hour after the injection of 1 mCi (37 MBq) of ^{99m}Tc DMSA through the tail vein using a dual-head gamma camera detector with a collimator to acquire layered static images over a 10-min period with a matrix of 64×64 pixels and a 140 keV±20% energy window (Symbia, Siemens Medical Solutions, USA). The 1-hour imaging timepoint was selected because it allows sufficient cortical binding of ^{99m}Tc-DMSA and adequate background clearance, thereby providing a reliable semiquantitative assessment of renal cortical uptake, as reported in previous experimental studies [20]. Regions of interest (ROIs) were determined around the rat kidneys and background areas for both anterior and posterior images [20]. For the semiquantitative

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analyses, ROIs were drawn around each kidney. DMSA uptake was expressed by subtracting the background level. Thereafter, after background correction, the geometric mean of the posterior and anterior images was used to calculate the left and right renal ^{99m}Tc DMSA uptake [20]. Semiquantitative ^{99m}Tc DMSA uptake was determined for each kidney by calculating the radioactivity count per minute (cpm). The sum of the ^{99m}Tc DMSA uptake values of the right and left kidneys was calculated for each rat (Figure 1).

Biochemical Assays

After scintigraphic imaging, all animals were anesthetized under mild anesthesia. Blood was collected from the inferior vena cava, and the rats were sacrificed by cervical dislocation. The blood samples were immediately centrifuged at 3500 rpm for 10 min. Biochemical analyses of serum blood urea nitrogen and creatinine levels were performed using an automatic biochemical analyzer.

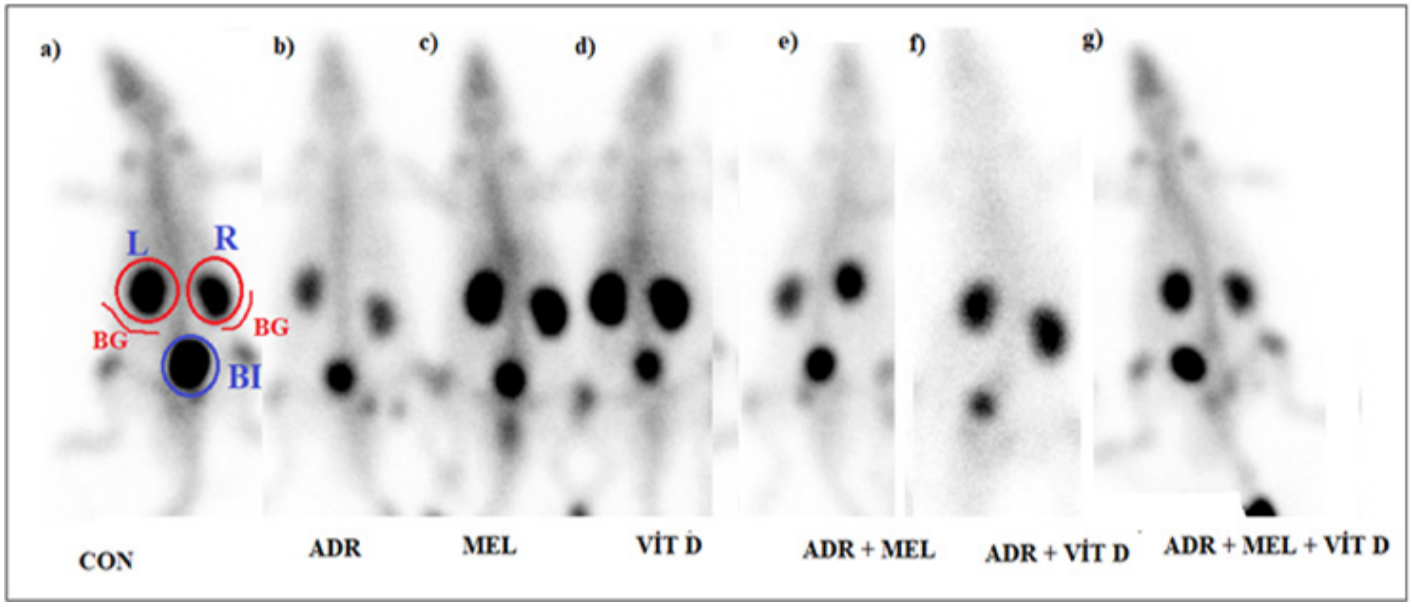

Figure 1. Static images in posterior position of all groups. L: Left Kidney, R: Right Kidney, BG: Background and Bl: Bladder. CON: control group; ADR: adriamycin substance control group; MEL: melatonin substance control group; Vit D: vitamin D substance control group; ADR+MEL: adriamycin and melatonin group; ADR+Vit D: adriamycin and vitamin D group; and ADR+MEL+Vit D: adriamycin, melatonin, and vitamin D group. The ^{99m}Tc DMSA uptake in the renal proximal tubules was higher in the control group. In contrast, in the ADR group, ^{99m}Tc DMSA uptake was low because of the development of damage. ^{99m}Tc DMSA uptake increased in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups

Statistical Analyses

SPSS software (version 18.0) and GraphPad Prism software (version 7.0) were used for statistical analyses. Statistical differences between groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was set at $p<0.05$. Values are expressed as mean±standard error (SEM). Graphics were prepared using GraphPad Prism.

Result

Biochemical Assays

As shown in Table 1, plasma blood urea nitrogen (BUN) and creatinine levels were 18.01±2.28 and 0.40±0.04 mg/dL in the control group and 171.3±11.08 and 3.22±0.15 mg/dL in the ADR group, respectively. Accordingly, BUN and creatinine levels in the ADR group corresponded to 951% and 806% of the control values, respectively ($p<0.001$).

Melatonin and vitamin D alone did not significantly alter BUN (83% and 94% of the control, respectively) or creatinine levels (95% and 105% of the control, respectively) compared with the control group (Figure 2).

Plasma blood urea nitrogen and creatine levels were 18.01±2.28 and 0.40±0.04 mg/dL in the control group, 171.3±11.08 and 3.22±0.15 mg/dL in the ADR group, 73.25±6.68 and 1.14±0.07 mg/dL in the ADR+MEL group, 87.61±8.24 and 1.35±0.15 mg/dL in the ADR+Vit D group, and 75.91±7.15 and 1.22±0.06 mg/dL in the ADR+MEL+Vit D group, respectively. In the pretreatment groups, BUN levels corresponded to 406%, 486%, and 421% of the control values in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups, respectively ($p<0.001$). Similarly, creatinine levels corresponded to 286%, 337%, and 305% of the control values in these groups.

However, compared with the ADR group, pretreatment with melatonin, vitamin D, or their combination significantly reduced BUN levels by 42%, 51%, and 44%, respectively ($p<0.001$), and creatinine levels by 35%, 41%, and 37%, respectively ($p<0.001$) (Table 1 and Figure 2).

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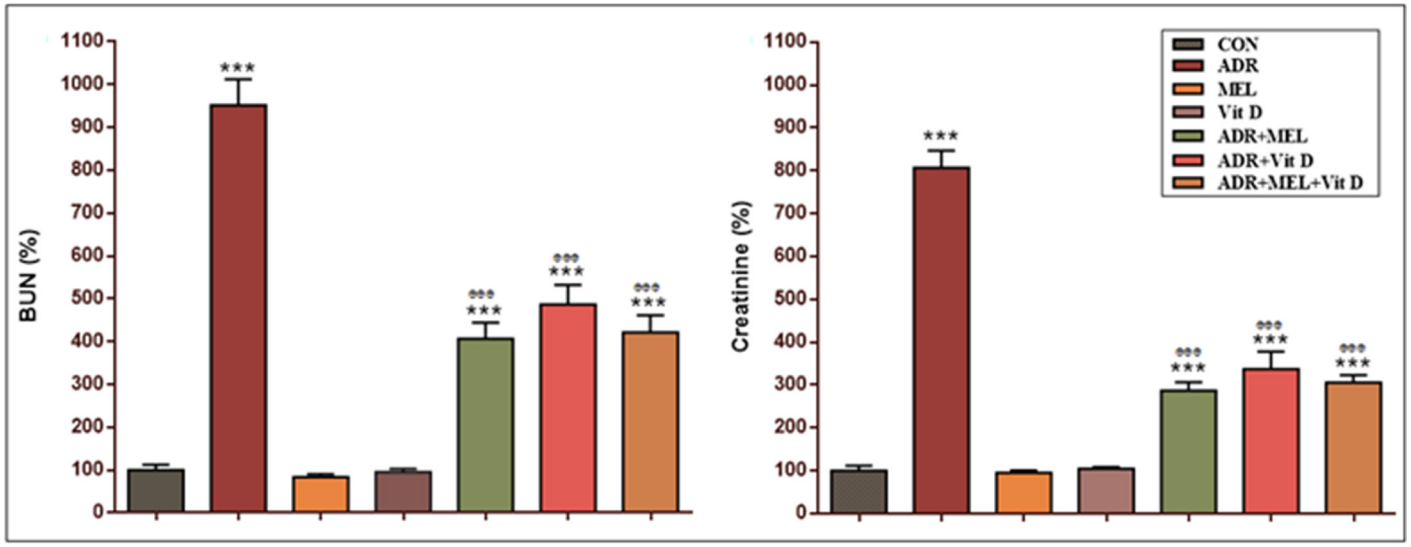

Figure 2. CON: control group; ADR: adriamycin group; MEL: melatonin group; Vit D: vitamin D group; ADR+MEL: adriamycin and melatonin group; ADR+Vit D: adriamycin and vitamin D group; and ADR+MEL+Vit D: adriamycin, melatonin, and vitamin D group. One-way ANOVA was performed, followed by Tukey's post-hoc test. Data are presented as mean±SEM. Blood urea nitrogen and creatinine levels were significantly higher in the ADR, ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups than in the control group (***: p<0.001). Pre-treatment groups of ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D significantly decreased the blood urea nitrogen and creatinine levels compared to the ADR group (⊕⊕⊕ : p<0.001). The blood urea nitrogen level (%) was calculated as follows: blood urea nitrogen = [(blood urea nitrogen value of the group after the application of the substance/average blood urea nitrogen value of the control group)×100]. Creatinine level (%) was calculated as follows: creatinine level = [(creatinine value of the group after the application of the substance/average creatinine value of the control group)×100].

Table 1. Summary of the biochemical parameters of all study groups

Groups	Blood urea nitrogen (mg/dL)	Creatinine (mg/dL)
CON	18.01±2.28	0.40±0.04
ADR	171.3±11.08[a]	3.22±0.15[a]
MEL	15.05±1.34	0.38±0.02
Vit D	17.10±1.50	0.42±0.01
ADR+MEL	73.25±6.68[a,b]	1.14±0.07[a,b]
ADR+Vit D	87.61±8.24[a,b]	1.35±0.15[a,b]
ADR+MEL+Vit D	75.91±7.15[a,b]	1.22±0.06[a,b]

CON: control group; ADR: adriamycin substance control group; MEL: melatonin substance control group; Vit D: vitamin D substance control group; ADR+MEL: adriamycin and melatonin group; ADR+Vit D: adriamycin and vitamin D group; and ADR+MEL+Vit D: adriamycin, melatonin, and vitamin D group. One-way ANOVA was performed, followed by Tukey's post-hoc test. Data are presented as mean±SEM. Blood urea nitrogen and creatinine levels were significantly higher in the ADR, ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups than in the control group ([a]: p<0.001)

Renal Scintigraphy Imaging

As demonstrated in Table 2 and Figure 3, ^{99m}Tc DMSA uptake in the kidney was 352,264±125 cpm in the control group and 151,790±109 cpm in the ADR group. Accordingly, ^{99m}Tc DMSA uptake in the ADR group corresponded to 43% of the control value (p<0.001).

As substance controls, melatonin and vitamin D alone did not significantly alter renal ^{99m}Tc DMSA uptake, corresponding to 97% and 96% of the control values, respectively (Figure 3).

The ^{99m}Tc DMSA uptake levels were 352,264±125, 151,790±109, 238,754±109, 230,500±713, and 254,932±107 cpm in the CON, ADR, ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups, respectively. In the pretreatment groups, ^{99m}Tc DMSA uptake corresponded to 67%, 65%, and 72% of the control values in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups, respectively (p<0.001).

However, compared to the ADR group, ^{99m}Tc DMSA uptake was significantly higher in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups, corresponding to 157%, 151%, and 167% of the ADR values, respectively (p<0.001) (Table 2 and Figure 3).

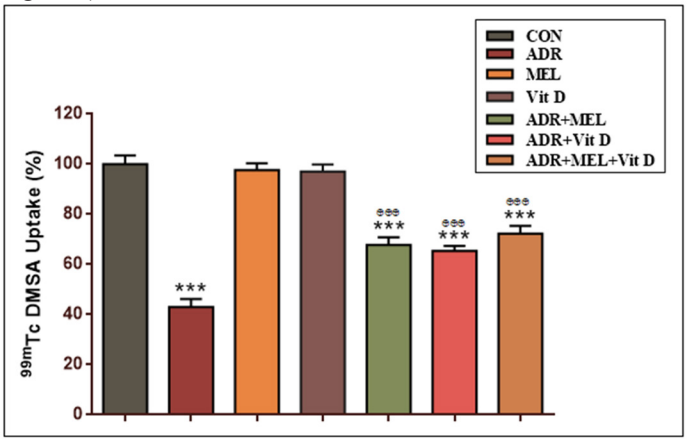

Figure 3. CON: control group; ADR: adriamycin group; MEL: melatonin group; Vit D: vitamin D group; ADR+MEL: adriamycin and melatonin group; ADR+Vit D: adriamycin and vitamin D group; ADR+MEL+Vit D: adriamycin, melatonin, and vitamin D group. One-way ANOVA was performed, followed by Tukey's post-hoc test. Data are presented as mean±SEM. ^{99m}Tc DMSA uptake was significantly lower in the ADR group than in the control group (***:p<0.001). Although uptake values in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups remained lower than control, they were significantly higher than those of the ADR group (p<0.001). (⊕⊕⊕ : p<0.001).

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Table 2. Statistical analysis of scintigraphic data for the study groups

Groups	^{99m}Tc DMSA Uptake (cpm)
CON	352264±125
ADR	151790±109[a]
MEL	344108±967
Vit D	342112±983
ADR+MEL	238754±109[a,b]
ADR+Vit D	230500±713[a,b]
ADR+MEL+Vit D	254932±107[a,b]

CON: control group; ADR: adriamycin substance control group; MEL: melatonin substance control group; Vit D: vitamin D substance control group; ADR+MEL: adriamycin and melatonin group; ADR+Vit D: adriamycin and vitamin D group; and ADR+MEL+Vit D: adriamycin, melatonin, and vitamin D group. One-way ANOVA was performed, followed by Tukey's post-hoc test. Data are presented as mean±SEM. The ADR, ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups were compared to the control group ([a]:p<0.001). The ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups were compared to the ADR group ([b]:p<0.001).

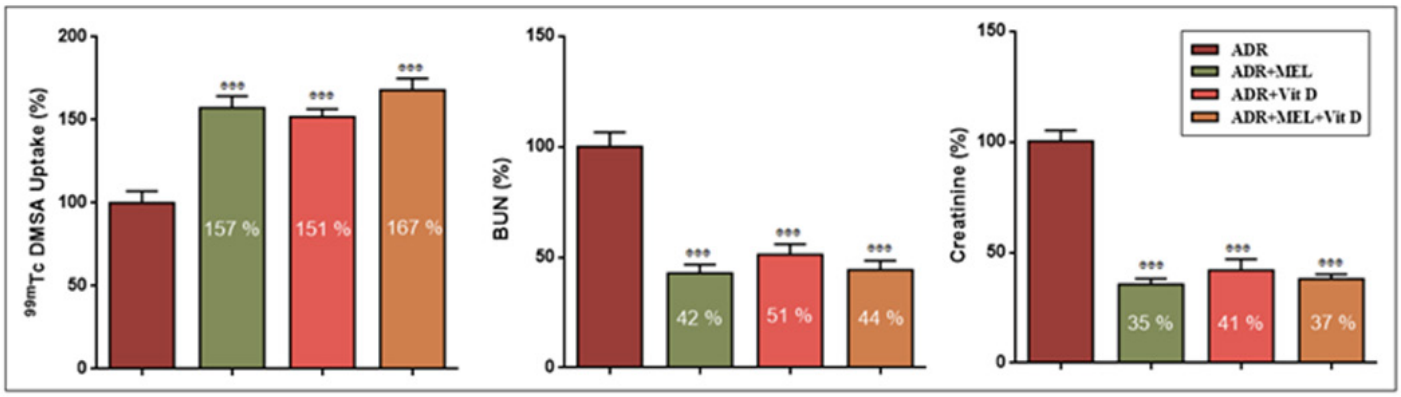

Figure 4. ADR: adriamycin group; ADR + MEL: adriamycin and melatonin group; ADR+Vit D: adriamycin and vitamin D group; ADR+MEL+Vit D: adriamycin, melatonin, and vitamin D group. The 99mTc DMSA radiopharmaceutical uptake was significantly higher in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups than in the ADR group (p<0.001). In contrast, blood urea nitrogen and creatinine levels were significantly lower in the ADR+MEL, ADR+Vit D, and ADR+MEL+Vit D groups than in the ADR group (p<0.001)

Discussion

In this study, we aimed to determine the renoprotective effects of melatonin and vitamin D treatments in Adriamycin-induced nephrotoxic rats using both ^{99m}Tc-DMSA renal scintigraphy and biochemical parameters, such as blood urea nitrogen and creatinine levels, to evaluate renal function. We demonstrated that Adriamycin administration led to renal damage. ^{99m}Tc-DMSA uptake was strongly correlated with biochemical results, indicating that Adriamycin administration reduced ^{99m}Tc-DMSA uptake and increased plasma blood urea nitrogen and creatinine levels.

Adriamycin is widely used to treat various cancer types [1,2]. However, clinical application of adriamycin is limited owing to its toxicity on several organs including the kidney [3]. Oxidative stress is among the most significant factors responsible for the development of adriamycin-induced nephrotoxicity [8]. It degrades the glomerular cell membrane, leading to alterations in cell membrane permeability and leakage of cellular enzymes into the plasma [21]. These enzymes (BUN and creatinine) can be measured in the serum. Many studies have suggested that blood urea nitrogen and creatinine increases are important indicators of the nephrotoxicity and they indicate a decrease in the glomerular filtration [22-23]. The biochemical findings of the present study clearly showed that creatinine and blood urea nitrogen levels were significantly higher in the adriamycin group than in the control group, indicating adriamycin-induced nephrotoxicity. These findings are consistent with previous studies reporting that, in adriamycin-induced nephrotoxicity, elevated plasma urea nitrogen and creatinine levels [8]. It is important to note that elevated levels of these biomarkers were markedly reversed after the administration of melatonin or vitamin D, and the combination of melatonin and vitamin D treatments further increased this reversal. It has been demonstrated that adriamycin-induced oxidative stress in cells results from the generation of ROS and a decrease in renal endogenous antioxidants [24]. In literature, melatonin was shown to be a potent antioxidant, and its use in adriamycin-induced nephrotoxic animals increased

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the antioxidant capacity of kidneys, reduced lipid peroxidation, blood urea nitrogen, and creatinine levels, and restored renal functions [3,25].

The present study demonstrated that, similar to melatonin, vitamin D has a nephroprotective effect on the kidney through reducing blood urea nitrogen and creatinine levels. Similar to our findings, vitamin D has been shown to decrease blood urea nitrogen and creatinine levels in various experimental models of the nephropathy [26]. 1,25-dihydroxycholecalciferol is an active metabolite of vitamin D. It was shown to remedy podocytopenia in adriamycin-induced nephropathic rats and protect podocytes from adriamycin-induced apoptosis. This protective mechanism may alleviate oxidative stress, since vitamin D has been shown to inhibit the synthesis of free radicals and reactive oxygen species in other experimental nephropathy models [27]. Vitamin D also increases gamma glutamyl transpeptidase and glutathione, thereby triggering innate antioxidant pathways [28]. In a recent study, Aljohri et al. [29] reported that vitamin D is a natural antioxidant that can reduce the levels of various biomarkers implicated in oxidative stress. The mechanism for this protective effect of melatonin and vitamin D in adriamycin-induced nephrotoxicity might be due to antioxidant properties and stabilization of the membrane action.

Histological techniques and biochemical markers, such as creatinine and blood urea nitrogen, are currently used to evaluate renal function [30]. However, histological techniques are invasive, take long time and are inadequate for diagnosis. Biochemical parameters of plasma blood urea nitrogen and creatinine levels are among the standard methods used to assess renal function. However, it has been demonstrated that any changes in blood urea nitrogen and creatinine levels in plasma occur only after at least 75% of functional nephrons are lost [23,23]. Thus, the development of non-invasive methods is necessary for early diagnosis of renal dysfunction. Renal scintigraphic imaging with ^{99m}Tc DMSA is preferred for functional imaging because the ^{99m}Tc DMSA gives high-quality images [31]. ^{99m}Tc DMSA scintigraphy has high sensitivity and negative predictive value for detecting cortical defects [32]. DMSA scintigraphy is a standard imaging modality for pediatric renal scars [33]. Therefore, we used ^{99m}Tc-DMSA renal scintigraphy, a noninvasive technique, to assess renal dysfunction in Adriamycin-induced nephrotoxicity in rats.

In a previous study, Fatemikia et al. [20] demonstrated that ^{99m}Tc DMSA uptake level decreased in the gentamicin-induced renal dysfunction nephrotoxicity model. In addition, Yamada (34] evaluated cisplatin-induced nephrotoxicity using ^{99m}Tc DMSA renal scintigraphic imaging. Also, Yamada [34] demonstrated that ^{99m}Tc DMSA uptake level significantly decreased compared to control group. Similar to the previous studies, we observed a decrease in ^{99m}Tc DMSA uptake level in adriamycin-induced nephrotoxic group compared to control group [35]. However, melatonin and vitamin D treatments significantly increased ^{99m}Tc DMSA uptake levels of kidney in adriamycin-induced nephrotoxic rat model.

Doxorubicin-induced nephrotoxicity, although less frequently encountered than cardiotoxicity in routine clinical oncology practice, represents one of the well-characterized experimental models of oxidative stress–mediated renal injury [8]. This model reliably reproduces the glomerular and tubular damage mechanisms observed in drug-induced nephropathies [5]. In the present study, an acute dosing protocol was employed to induce controlled renal injury and to evaluate the protective potential of melatonin and vitamin D under pronounced oxidative stress conditions. Therefore, this model provides a meaningful translational framework for investigating renoprotective strategies against chemotherapy-related renal injury.

Although the administered doses of vitamin D and melatonin may appear high compared to routine human supplementation, both were selected based on established pharmacological and translational evidence. Single high-dose vitamin D regimens (≥200,000 IU) have generally been well tolerated in adults, with 300,000 IU producing mean 25(OH)D levels around 60 ng/mL without hypercalcemia [36], and systematic reviews reporting no serious adverse effects below 600,000 IU [37,38[. Similarly, melatonin at 40 mg/kg falls within the widely used experimental range (10–50 mg/kg) shown to exert antioxidant and cytoprotective effects, including in doxorubicin-induced injury via NRF2/p53–SIRT1 pathways [6,39]. Collectively, these data support the pharmacological feasibility and mechanistic relevance of our dosing strategy.

Limitations

A limitation of the present study is the absence of histopathological evaluation. Although biochemical parameters and ^{99m}Tc-DMSA scintigraphic findings consistently indicated significant renal injury, direct structural confirmation of glomerular and tubular damage was not performed. Future studies incorporating histological assessment would allow a more comprehensive evaluation of renal injury severity and further strengthen the translational relevance of the findings.

Conclusion

In this study, we demonstrated the renoprotective effects of melatonin and vitamin D against adriamycin-induced nephrotoxicity in rats using ^{99m}Tc DMSA scintigraphic imaging and biochemical analysis. Melatonin and vitamin D treatments were found to remedy renal dysfunction due to adriamycin-induced nephrotoxicity in rats by increasing ^{99m}Tc DMSA uptake levels and reducing creatinine and blood urea nitrogen levels. Furthermore, we observed a significant positive correlation between ^{99m}Tc DMSA uptake and biochemical results. These results suggest that melatonin and vitamin D may be clinically useful agents for preventing adriamycin-induced nephrotoxicity. Moreover, the present study showed that ^{99m}Tc DMSA renal scintigraphy may be useful for diagnosing nephrotoxicity.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
The experimental procedure was approved by the Ethical Committee of Tokat Gaziosmanpasa University (HADYEK-2017/22).

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