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RESEARCH ARTICLE

Protective Effect of Melatonin and Mycophenolate Mofetil Against Nephrotoxicity Induced by Tacrolimus in Wistar Rats

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Abstract

Although Tacrolimus (TAC) is a potent and well-tolerated drug, it has some side effects. Melatonin and mycophenolate mofetil (MMF) have some protective properties against drug-induced damage. We aimed to evaluate TAC-induced nephrotoxicity and the protective effect of melatonin and MMF against this injury in rats. The animals were divided into five equal groups (n=6): Control group (untreated), group II TAC, group III as the TAC + melatonin, group IV as the TAC + MMF, and group V as the TAC + melatonin + MMF. TAC was applied orally, 2 mg/kg once daily. Melatonin and MMF were applied orally 10 mg/kg once and 40 mg/kg once daily, respectively. In the TAC group, kidney tissue malondialdehyde (MDA), total oxidative status (TOS), interleukin-1, and tumor necrosis factor-alpha levels were higher, and catalase and total antioxidant status (TAS) levels were lower. Severe histopathologic changes such as glomerular congestion, intertubular hemorrhage, hyaline formation, degenerative-necrotic tubules epithelium, and mononuclear cell infiltration were seen in the TAC group. There was a clear improvement in the groups in which melatonin and MMF were used together with TAC. It was shown that TAC causes nephrotoxicity through oxidative stress. Melatonin and MMF together or separately protect the kidney against oxidative stress damage caused by TAC.

Keywords: *Tacrolimus, Melatonin, Mycophenolate mofetil, Nephrotoxicity, Oxidative stress*

Melatonin ve Mikofenolat Mofetilin Wistar Sıçanlarında Takrolimus Tarafından İndüklenen Nefrotoksisiteye Karşı Koruyucu Etkisi

Öz

Takrolimus güçlü ve iyi tolere edilen bir ilaç olmasına rağmen; bazı yan etkileri vardır. Melatonin ve mikofenolat mofetil (MMF), ilaca bağlı hasara karşı bazı koruyucu özelliklere sahiptir. Biz bu çalışmada sıçanlarda Takrolimus kaynaklı nefrotoksisiteye karşı melatonin ve MMF'nin koruyucu etkisini değerlendirmeyi amaçladık. Hayvanlar beş eşit gruba ayrıldı (n=6): Kontrol grubu (tedavi edilmemiş), grup II Takrolimus, grup III Takrolimus +melatonin, grup IV Takrolimus + MMF ve grup V Takrolimus + melatonin + MMF. Takrolimus, günde bir kez 2 mg/kg oral olarak uygulandı. Melatonin ve MMF, sırasıyla günde bir kez 10 mg/kg ve 40 mg/kg oral olarak uygulandı. Takrolimus grubunda böbrek dokusu malondialdehit (MDA), toplam oksidatif stress (TOS), interlökin-1 ve tümör nekroz faktör-alfa düzeyleri daha yüksek, katalaz ve toplam antioksidatif stress (TAS) düzeyleri daha düşüktü. Takrolimus grubunda glomerüler konjesyon, intertübüler kanama, hiyalin oluşumu, dejeneratif-nekrotik tübül epiteli ve mononükleer hücre infiltrasyonu gibi ciddi histopatolojik değişiklikler görüldü. Takrolimus ile birlikte melatonin ve MMF kullanılan gruplarda belirgin bir iyileşme oldu. Takrolimusun oksidatif stres yoluyla nefrotoksisiteye neden olduğu gösterilmiştir. Melatonin ve MMF birlikte veya ayrı ayrı böbreği Takrolimusun neden olduğu oksidatif stres hasarına karşı korumaktadır.

Anahtar sözcükler: *Tacrolimus, Melatonin, Mycophenolate mofetil, Nefrotoksisite, Oksidatif stres*

INTRODUCTION

The prevalence of chronic renal failure increases daily, leading to a cause of health problems worldwide. Kidney transplantation (KT) is the most effective and advanced treatment option for these patients [1]. However, graft rejection is still a major problem and sometimes can result in graft loss. During the rejection, the recipient

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immune system cells attack and destroy the graft [2]. Immunosuppressive agents such as tacrolimus (TAC), cyclosporine, mycophenolate mofetil (MMF) are used alone or combined to prevent rejection after transplantation [3]. Thanks to these immunosuppressive agents, success rates in the field of organ transplantation have increased significantly. TAC is an important immunosuppressive agent and greatly improves grafts and patients' survival rates after solid-organ transplantation [4]. Although TAC has strong potency and is well tolerated, serious side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, glucose intolerance, gastrointestinal toxicity, posttransplant lymphoproliferative disease have been reported in previous studies [5]. Among them, nephrotoxicity and hepatotoxicity continue to be serious problems [6,7]. A previous study found that TAC caused glomerular damage by disrupting the balance in the oxidant-antioxidant defense system [8,9].

Melatonin, a protein hormone, plays a role in many hormone-related reactions endocrine functions such as sleep, sexual development and reproduction, mood, immune functions, and aging [10,11]. Although melatonin has been found in different parts of the body, such as the gastrointestinal tract, retina, bile, skin, bone marrow, and some leucocytes, it is mainly concentrated in several brain areas [12]. Melatonin not only acts as an antioxidant by stimulating antioxidant enzymes but also has free radical scavenging properties. It also has antiapoptotic and anti-inflammatory effects [13].

Mycophenolate mofetil (MMF) inhibits inosine monophosphate dehydrogenase and, consequently, blocks T and B's proliferation and clonal expansion [14]. MMF is one of the most common immunosuppressive agents used in combination therapy, mostly with TAC [15]. During the last years, it has been proven that MMF has a cytoprotective effect by reducing the production of free radicals [16-18].

This study's main objective is to investigate the role of TAC in kidney injury and the protective effect of melatonin and MMF against this damage. Specifically, the TAC-induced nephrotoxicity rat model was constructed, and each kidney sample was divided, and histopathological changes were evaluated by using light microscopy. Furthermore, the biochemical analyses were performed for measuring superoxide dismutase (SOD), catalase (CAT), malonyl dialdehyde (MDA) total antioxidant status (TAS), total oxidative status (TOS), interleukin-1 (IL-1), and tumor necrosis factor (TNF) alpha, contents in kidney tissues and blood urea nitrogen (BUN), creatinine, albumin, total protein, and uric acid levels in serum.

Material and Methods

Animals

For this experimental animal study, approval was provided from Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (decision no.65202830-050.04.04-310). All the experimental procedures were performed following the institution's ethical standards at which the studies were conducted. We used 30, 220-300 g, 10-12-week-old male Wistar rats purchased from the Sivas Cumhuriyet University Experimental Animal Production Application and Research Center (Sivas, Türkiye). Before starting experimental work, all animals were preserved for 14 days under the same laboratory conditions of a 12 h day -12 h night period, temperature (22±3°C), relative humidity (55±5%) and fed a standard diet (DSA Poultry, Kırıkkale, Türkiye).

Experimental Design

The subjects were classified into five groups, 6 in each group, for 14-day regimens of control or experimental groups. Control group: animals were untreated. The treatments in the experimental groups were as follows: TAC group: 2 mg/kg once daily TAC (Prograf®; Astellas Phama Inc. Tokyo, Japan) was given by gavage for 14 days starting on day 1; TAC + melatonin group: TAC as above plus 10 mg/kg/day melatonin (Bio Basic, Canada) was given by gavage for 14 days; TAC + MMF group: TAC as above plus 40 mg/kg once daily MMF (Cellcept® 250 mg tablets; Roche) were administered by gavage for 14 days starting on day 1; and the TAC + melatonin + MMF group were given TAC as above plus melatonin and MMF as described previously. The choice of medication, dosage, and administration was done under the guidance of previous studies [18-20]. At the end of the study, prior to euthanasia, blood samples were obtained by cardiac puncture, then all the animals were sacrificed using anesthesia overdose ketamine HCl (Ketalar®; Eczacibasi Warner-Lambert, Levent, Istanbul, Türkiye), and tissue samples were obtained. Blood was centrifuged at 2.058xg at 4°C for 15 min to obtain serum, which then was stored at -80°C. Each kidney tissue was prepared for histopathologic evaluation by light microscopy, and biochemical analyses were performed for measuring SOD, CAT, MDA, TAS, TOS, IL-1, and TNF alpha contents.

A priori power analysis was conducted using G-Power Version 3.1.9.7 to test the difference between five independent groups using a two-tailed test, a medium-large effect size (f=0.70), and an alpha of 0.05. Results showed that a total sample of 30 participants with five equal-sized groups of 6 rats was required to achieve a power of 0.80.

Histopathologic Evaluations

Samples were taken from the kidneys of the animals in all groups and fixed in 10% formalin. The specimens were processed in an auto-technician device, later embedded in paraffin blocks. The blocks were cut at 5 µm thickness, deparaffinized, rehydrated using standard techniques, and sections were stained with Haemotoxylen-Eosin (H&E) stain using standard protocols for analysis by light microscopy (Eclipse E 600; Nikon, Tokyo, Japan). The histopathological

scoring method was modified and used in histopathological examinations, and changes in kidney tissues were graded [21,22]. The kidney's main histopathological lesions that were considered include glomerular congestion, intertubular hemorrhage, hyaline formation, degenerative-necrotic tubules epithelium, mononuclear cell infiltration. The histopathological harm's degree was evaluated for each kidney group *(Table 1, Table 2)* is classified according to the severity.

Biochemical Analyses

- Preparation of Kidney Tissue Homogenates

The tissue samples were mixed with a cold phosphate-buffered saline solution (PBS, pH: 7.4) and homogenized using a mechanical homogenizer (Analytic Jena speed mill plus, Jena, Germany). The homogenates were centrifuged at 2.957xg for 10 min at a temperature of 4°C. Then, the supernatants were received and preserved in a glacial environment until evaluation. Bradford protein assay kit (Cat no:39222.03, Serva, Heidelberg, Germany) was used to determine the amount of protein in samples [23].

- Measurement of SOD, CAT, MDA, IL-1, and TNF alpha

SOD, CAT, MDA, IL-1, and TNF-alpha levels from kidney supernatants were measured using rat ELISA commercial kits (Sunred Biological Technology, Shanghai, China). Standard and tissue samples were added to the plate and incubated at 37°C for 60 min. Plates with standard and tissue samples added were incubated at 37°C for 60 min. Following the washing process, the staining solution was added and incubated in the same conditions (37°C for 60 min). Finally, a stop solution was added and read at 450 nm. The coefficients of variation between and within plates were found below 10%. During these measurements, the manufacturer's guidelines were followed.

Table 1. *Histopathological scoring for renal cortex damage among groups*

Pathological State	Score	Definition
Glomerular congestion	0	No
	1	1 of 10 glomeruli
	2	1 to 3 out of 10 glomeruli
	3	4 or more of 10 glomeruli
Intertubular hemorrhage	0	No
	1	2 or less of 10 tubules
	2	3 to 5 out of 10 tubules
	3	5 or more of 10 tubules
Hyaline formations	0	No
	1	2 or less of 10 tubules
	2	3 to 5 out of 10 tubules
	3	5 or more of 10 tubules
Degenerative-necrotic tubules epithelium	0	No
	1	10 to 20% of the tubules
	2	20 to 50% of 10 tubules
	3	50% or more of 10 tubules

The degree of kidney damage was rated according to a grading system described as follows; 0: None, 1: mild, 2: moderate, 3: severe

Table 2. *Histopathological scoring for renal medulla damage among groups*

Pathological State	Score	Definition
Intertubuler hemorrhage	0	No
	1	2 or less of 10 tubules
	2	3 to 5 out of 10 tubules
	3	5 or more of 10 tubules
Mononuclear cell infiltration	0	No
	1	2 or less of 10 tubules in the intertubular area
	2	Between 3 and 5 of 10 tubules in the intertubular area
	3	5 or more of 10 tubules in the intertubular area

The degree of kidney damage was rated according to a grading system described as follows; 0: None, 1: mild, 2: moderate, 3: severe

- Measurement of TAS and TOS

The rate of TAS in the kidney supernatants was determined according to the novel assay method. This method is based on finding the reaction rate during the free radical reaction by measuring the absorption of colored dianisidyl radicals [24]. The results were expressed in micromolar Trolox equivalents per milligram tissue protein (µmol Trolox Eq/mg protein).

The amount of TOS in the kidney tissue was measured using the automated assay method of Erel [25]. This method is based on quantifying TOS levels by measuring tissue ferric ion concentration using xylenol orange. The ferrous ion is oxidized to ferric ion when enough oxidants are in the medium. Calibration of the assay results was performed using hydrogen peroxide [25]. The assay data were expressed with micromolar hydrogen peroxide equivalents per milligram tissue protein (µmol H_2O_2 Eq/mg protein).

- Measurement of Serum Biochemical Parameters

Serum BUN, creatinine, albumin, total protein, and Uric acid levels were measured with the spectrophotometric method (Roche Cobas 8000, Germany, Mannheim).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 20.0 was used for the analysis of the data obtained. One-way analysis of variance (ANOVA) was applied to compare the groups' laboratory parameters ($P<0.001$). The Kruskal-Wallis test was applied for histopathologic statistical evaluation to determine all groups' effects on each experimental parameter. $P<0.05$ was considered to indicate a statistically significant difference among groups.

Results

Effects of TAC on Oxidative Stress

We measured biochemical parameters such as SOD, CAT, MDA, TAS, and TOS in kidney tissues to evaluate oxidative stress-mediated nephrotoxicity. A comparison of laboratory parameters among groups is shown in *Table 3*. No statistically significant differences were found when comparing group I (control) to group II (TAC) in SOD levels. Increased SOD levels were determined in group V compared to group I and group II. The difference between group III and group IV was not statistically significant in terms of SOD. It was observed that CAT, which is an endogenous antioxidant, significantly decreased in the TAC group. Higher CAT activity was found in group III (TAC + melatonin), group IV (TAC + MMF), and group V (TAC + melatonin + MMF) compared to group II.

The MDA level was measured to evaluate the lipid peroxidation status. In the TAC group, kidney tissue MDA level was higher than group II, III, IV, and V ($P<0.05$). MDA level was significantly decreased in rats treated with combined drugs, especially in the group where TAC, melatonin, and MMF are applied together. Decreased TAS and increased TOS levels were found in group II concerning group I. Increased TAS and decreased TOS levels were detected in groups III, IV, and V compared to group II.

We examined IL-1 and TNF-alpha to determine whether melatonin and MMF could reduce inflammation caused by TAC. As shown in the results, the expressions of these different cytokines were elevated in the TAC-treated groups. Increased IL-1 and TNF-alpha levels were determined in group II compared to group I ($P<0.001$). Compared to group II, decreased IL-1 and TNF-alpha levels were determined in group IV and group V ($P<0.001$) *(Table 3)*. There was no significant difference between the groups in terms of the serum BUN, creatinine, albumin, total protein, and uric acid results *(Table 4)*.

Histopathologic Findings

Light microscope examination of group I showed normal renal cortex architecture. Group II (TAC) revealed significant renal cortex changes with severe glomerular congestion, intertubular hemorrhage, hyaline formation, degenerative-necrotic tubules epithelium, and mononuclear cell infiltration. These changes were found to be moderate in TAC + Melatonin and TAC + MMF groups. On the other hand, histopathological examination of group V (TAC + melatonin + MMF) had a near-normal appearance. Only milddegenerative-necrotic tubule epithelium was seen in group V. Histopathological examination findings of the renal medulla were similar to those of the renal cortex mentioned above. While histopathological findings of both renal cortex and renal medulla were severe in the TAC group, these findings

Table 3. *Comparison of kidney homogenates oxidative-antioxidative enzyme activity between groups*

Parameters	Control (n=6)	TAC (n=6)	TAC+Melatonin (n=6)	TAC+MMF (n=6)	TAC+Melatonin+MMF (n=6)	P
SOD/TP (U/mg protein)	11.56±4.86^a	9.65±1.04^a	8.22±1.56^b	8.28±1.66^b	15.08±1.38^c	P<0.001
CAT/TP (U/mg protein)	20.81±2.44^a	8.82±1.44^b	14.98±1.17^c	20.57±2.43^a	28.48±1.68^d	P<0.001
MDA (nmol/mg protein)	8.56±0.54^a	12.44±0.55^b	9.98±1.29^c	7.92±0.81^a	8.68±0.71^a	P<0.001
TAS (µmol Trolox Eq/mg protein)	0.68±0.03^a	0.48±0.03^b	0.48±0.05^b	0.55±0.03^c	0.55±0.02^c	P<0.001
TOS (µmol H_2O_2 Eq/mg protein)	2.93±0.59^a	7.92±1.76^b	3.10±0.44^a	3.93±0.51^a	3.00±0.23^a	P<0.001
IL-1/TP (pg/mg protein)	1113.47±91.12^a	1537.74±228.73^b	1261.66±78.22^a	1222.51±122.06^a	1154.61±104.51^a	P<0.001
TNF-alpha/TP (pg/mg rotein)	385.23±22.17^a	474.65±25.06^b	380.33±60.55^a	381.55±23.19^a	382.70±44.71^a	P<0.001

SOD: *Superoxide dismutase,* ***CAT:*** *Catalase,* ***MDA:*** *Malonyl dialdehyde,* ***TAS:*** *Total antioxidative status,* ***TOS:*** *Total oxidative status,* ***IL-1:*** *Interleukin-1,* ***TNF:*** *Tumor necrosis factor-alpha,* ***TAC:*** *Tacrolimus,* ***MMF:*** *Mycophenolate mofetil. Results were given as mean±standard deviation. Different upper superscripts indicate statistical differences among groups*

Table 4. *Comparison of laboratory parameters among groups*

Parameters	Control (n=6)	TAC (n=6)	TAC+Melatonin (n=6)	TAC+MMF (n=6)	TAC+Melatonin+MMF (n=6)	P
BUN	18.41±2.25^a	25.81±4.74^a	17.65±2.43^a	16.38±3.56^a	22.85±10.72^a	NS
Kreatinin	0.39±0.05^a	0.41±0.03^a	0.38±0.06^a	0.33±0.02^a	0.32±0.07^a	NS
Albumin	39.71±1.91^a	40.81±2.48^a	40.16±0.99^a	39.18±2.26^a	37.75±4.33^a	NS
T.Protein	68.23±2.06^a	68.46±3.57^a	66.61±1.53^a	59.15±6.36^b	58.50±5.56^b	P<0.001
Uric acid	1.06±0.37^a	1.40±0.35^a	1.13±0.36^a	0.88±0.24^a	0.96±0.19^a	NS

BUN: *Blood urea nitrogen,* ***ALT:*** *Alanine aminotransferase,* ***ALP:*** *Alkaline phosphatase,* ***TAC:*** *Tacrolimus,* ***MMF:*** *Mycophenolate mofetil,* ***NS:*** *Not significance. Results were given as mean±standard deviation. Different upper superscripts indicate statistical differences among groups*

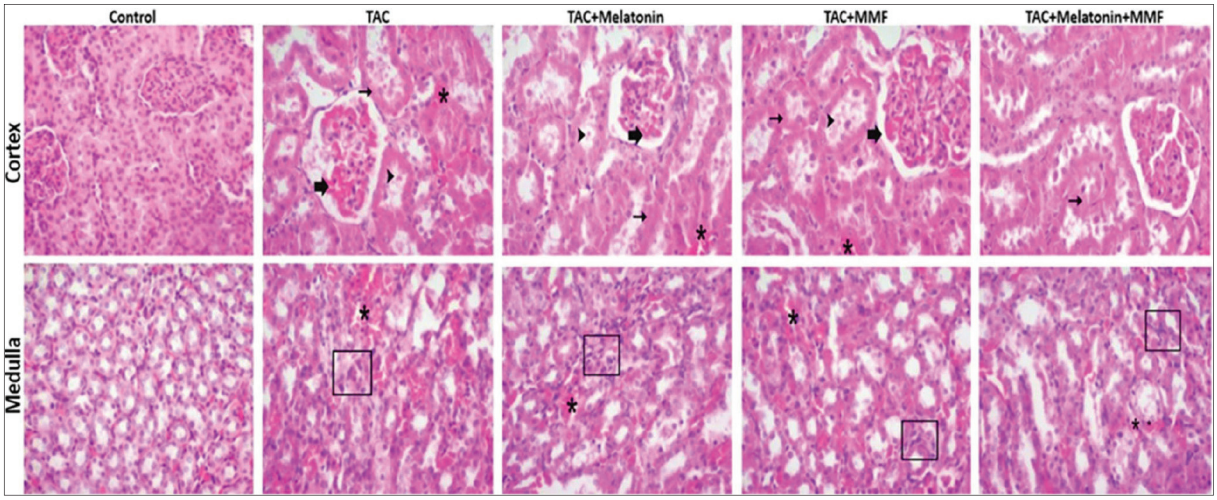

Fig 1. Renal cortex. A photomicrograph of sections in the renal cortex of different studied groups. In the control group, normal renal cortex appearance. In group II (TAC group), severe glomerular congestion (thick arrow), intertubular hemorrhage (*), hyaline formation (arrowhead), and degenerative-necrotic tubules epithelium *(arrow)* were observed. In group III (TAC + melatonin) and group IV (TAC+ MMF), moderate glomerular congestion, intertubular hemorrhage, hyaline formation, and degenerative-necrotic tubules epithelium were observed. Only mild degenerative-necrotic tubules epithelium was observed in group V (TAC + melatonin + MMF) (H&Ex40); **Renal medulla.** A photomicrograph of sections in the renal medulla of different studied groups. In the control group, normal renal medulla appearance. In group II (TAC group), severe intertubular hemorrhage (*) and mononuclear cell infiltration (□) were observed. In group III (TAC + melatonin) and group IV (TAC+ MMF), moderate intertubular hemorrhage and mononuclear cell infiltration were observed. In group V (TAC + melatonin + MMF), only mild intertubular hemorrhage was observed (H&Ex40). TAC: Tacrolimus, MMF: Mycophenolate mofetil

Table 5. *Comparison of histopathological changes in renal cortex among groups*

Parameters	Control (n=6)	TAC (n=6)	TAC+Melatonin (n=6)	TAC+MMF (n=6)	TAC+Melatonin+MMF (n=6)
Glomerular congestion	0.16±0.40^{a}	2.83±0.40^{b}	1.83±0.40^{c}	2.16±0.40^{c}	0.33±0.51^{a}
Intertubuler hemorrhage	0.16±0.40^{a}	2.83±0.40^{b}	2.00±0.63^{c}	2.33±0.51^{c}	0.33±0.81^{a}
Hyaline formation	0.33±0.51^{a}	3.00±0.00^{b}	2.00±0.63^{c}	2.00±0.63^{c}	0.33±0.81^{a}
Degenerative-necrotic tubulus epithelium	0.33±0.51^{a}	3.00±0.00^{b}	2.16±0.40^{c}	2.16±0.40^{c}	1.16±0.40^{d}

TAC: *Tacrolimus,* ***MMF:*** *Mycophenolate mofetil. Results were given as mean±standard deviation. Different upper superscripts indicate statistical differences among groups (P<0.05)*

Table 6. *Comparison of histopathological changes in renal medulla among groups*

Parameters	Control (n=6)	TAC (n=6)	TAC+Melatonin (n=6)	TAC+MMF (n=6)	TAC+Melatonin+MMF (n=6)
Intertubuler hemorrhage	0.33±0.51^{a}	3.00±0.00^{b}	1.83±0.40^{c}	1.66±0.51^{c}	0.83±0.40^{d}
Mononuclear cell infiltration	0.33±0.51^{a}	2.83±0.40^{b}	2.83±0.40^{b}	1.66±0.51^{c}	1.66±0.51^{c}

TAC: *Tacrolimus,* ***MMF:*** *Mycophenolate mofetil. Results were given as mean±standard deviation. Different upper superscripts indicate statistical differences among groups (P<0.05)*

were significantly regressed in group V *(Fig. 1)*. Comparison of histopathological parameters among groups was demonstrated in *Table 5* and *Table 6*. The histopathological changes such as glomerular congestion, intertubular hemorrhage, hyaline formation, degenerative-necrotic tubule epithelium, mononuclear cell infiltration were more pronounced in group II than group III, IV and V (P<0.05 in all cases). These changes were significantly improved in group V.

Discussion

Living or cadaveric kidney transplantation is the gold treatment for chronic kidney failure due to various reasons [1]. Thanks to immunosuppressive agents such as TAC, which are used to prevent rejection, good progress has been made in organ transplantation in the last few decades. Unfortunately, the use of TAC has side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, glucose intolerance, and gastrointestinal toxicity [26].

The mechanism of TAC-induced nephrotoxicity remains unclear. Therefore, we aimed to biochemically and histopathologically investigate the toxic effects of TAC on the kidney and examine the cytoprotective effect of melatonin and MMF against TAC-induced kidney injury. Our study demonstrated that melatonin and MMF effectively reduced

TAC-induced histopathological changes such as glomerular congestion, intertubular hemorrhage, hyaline formation, degenerative-necrotic tubule epithelium, mononuclear cell infiltration in the kidney tissue. Moreover, activation and up-regulation of pro-inflammatory cytokines such as IL-1 and TNF-alpha and the generation of oxidative stress products were reduced. Our study revealed that melatonin and MMF could protect against oxidative stress-mediated acute kidney injury in a rat model induced by TAC.

Our findings in this study revealed that TAC alone caused a significant increase in MDA, TOS, and pro-inflamatuar cytokines such as IL-1 and TNF alpha in rats' kidneys. We thought that increased MDA production due to TAC nephrotoxicity in kidney tissues of rats might be accompanied by induction of lipid peroxidation. The obtained results are parallel to the results of lipid peroxidation caused by TAC reported in previous studies [27-30]. Besides, CAT activity was significantly reduced after treatment with TAC alone. Hence, decreased CAT activities in renal tissues may be due to ROS/lipid peroxidation's excess production. Disruption in the oxidative system has been reported as one of the main causes of TAC-induced renal dysfunction [9,27,31,32].

Melatonin, thanks to its antioxidant and cell-protective properties, prevents the formation of free radicals and neutrophil accumulation during oxidative damage induced by various events, such as drug-induced injury, ischemia-reperfusion, and prevents the destruction of kidney tissue [33-35]. Our study results revealed that melatonin has antioxidant potential to prevent oxidative stress and lipid peroxidation, which likely contributed to its protection against TAC-induced kidney injury in rats. This protective effect of melatonin may be due to suppressing lipid peroxidation and activation of SOD and CAT. These findings suggest that MMF could effectively treat the tissue damage induced by TAC.

Combination therapy is an ideal treatment option for providing adequate immunosuppression after organ transplantation and minimizing graft rejection. MMF, a novel immunosuppressive drug, is often used in combination therapy with TAC [15]. The previous study showed that MMF has a protective effect against drug-induced renal injury [18]. In this study, we investigated the protective effect of MMF against TAC-induced oxidative damage by the measure of MDA level, biomarkers of the prooxidant system, and CAT activities, an indicator of the antioxidant system in the kidney of rats. Oxidative stress and lipid peroxidation mediated by oxygen free radicals are important causes of damage to the cell and mitochondrial membranes [29,36,37]. The histological examination of the kidney sections confirmed the results mentioned above; MMF co-administration with TAC can restore the kidney's nearly normal cellular architecture and reverse TAC-induced histopathological effects *(Fig. 1)*. These results demonstrated the potential beneficial effects of MMF to counteract the oxidative stress induced by TAC administration.

We also used histological scoring methods to evaluate the histopathological changes, which are oxidative stress injury features increased due to TAC-induced kidney damage. These two features were decreased after both melatonin and MMF treatment in the TAC-treated groups. Moreover, histopathological examination of the kidney of group V (TAC+melatonin+MMF) was close to normal appearance, with only mild degenerative-necrotic tubules epithelium in the cortex and mild intertubular hemorrhage in the renal medulla. These findings suggest that melatonin and MMF together can protect effectively against oxidative stress -mediated kidney injury in a rat model induced by TAC.

Although TAC, which is used for immunosuppressive purposes after organ transplantation, prevents rejection, it also causes nephrotoxicity through oxidative stress. This kidney injury can be assessed by demonstrating an increase in ROS and lipid peroxidation marker MDA levels and decreased TAS. We have seen that melatonin and MMF, especially together or separately, protect the kidney against oxidative stress damage caused by TAC not only biochemically but also histopathologically by reducing glomerular congestion, intertubular hemorrhage hyaline formation, degenerative-necrotic tubules epithelium, mononuclear cell infiltration in the kidney tissue.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author (S. Koc) on reasonable request.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

SK, AA, BS and MO conceived and supervised the study. SK, AA and MO collected and analyzed data. AA and BS made laboratory measurements. MO applied the histopathological examination of the study. The first draft of the manuscript was written by SK, and all authors contributed to the critical revision of the manuscript and have read and approved the final version.

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