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Melatonin alleviates oxidative stress, inflammation, apoptosis, and DNA damage in acrylamide−induced nephrotoxicity in rats

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ABSTRACT

Objective: To investigate the effects of melatonin on renal inflammation, oxidative stress, apoptosis, as well as DNA and tissue damage in acrylamide-induced nephrotoxicity in rats.

Methods: Fifty male rats were randomly divided into five groups. The control group received distilled water by gastric lavage for 11 days and the acrylamide group was administered acrylamide (50 mg/kg, *i.g.*) for 11 days. The MEL10+ACR and MEL20+ACR groups received intraperitoneal melatonin 10 and 20 mg/kg, respectively, for 11 days, and acrylamide (50 mg/kg, *i.g.*) was administered 1 h after melatonin injection. The MEL20 group was injected with melatonin (20 mg/kg) for 11 days. Kidney function tests were performed and biochemical and inflammatory parameters were determined. In addition, histopathological, immunohistochemical, and immunofluorescence examinations were carried out.

Results: Melatonin significantly abated acrylamide-induced rise in serum urea and creatinine levels. Acrylamide caused oxidative stress, inflammation, apoptosis, as well as DNA and tissue damage in the kidneys. Melatonin treatment alleviated acrylamide-induced renal damage by exhibiting antioxidant, anti-inflammatory, and anti-apoptotic effects. Moreover, melatonin significantly ameliorated acrylamide-caused histopathological changes in kidney tissue.

Conclusions: Melatonin attenuates acrylamide-induced renal oxidative stress, inflammation, apoptosis, and DNA damage in rats.

KEYWORDS: Acrylamide; Apoptosis; Inflammation; Melatonin; Nephrotoxicity; Oxidative stress; Rat

1. Introduction

Acrylamide (ACR) is a compound used in various industrial fields, such as press, fabric production, construction materials production, molecular laboratories, paper, and cosmetics industry, especially in the purification of wastewater and cigarette filters[1]. It has been determined that employees are more or less exposed to ACR because it is widely used in many work areas. In Sweden, severe levels of ACR have been detected in the blood of non-smokers who have been exposed to ACR leakage from tunnel construction. ACR exposure can occur in humans not only in industrial areas and products but also in foods[2]. ACR formation has been reported due to exposure of carbohydrate-rich foods to 120 ℃ and higher temperatures[2–4]. Exposure to ACR can be by the respiratory, skin, and digestive tracts. It can be rapidly and highly absorbed in the stomach and intestines. ACR also passes into breast milk and may cause fetal anomalies by crossing the placental barrier[3]. ACR

Significance

Acrylamide can cause nephrotoxicity. Currently, there is no study on the effect of melatonin on acrylamide-induced renal damage in rats. The present study shows that melatonin has nephroprotective effects against acrylamide-induced nephrotoxicity in rats. Therefore, melatonin can be further explored as a potential agent for the treatment of acrylamide-induced nephrotoxicity.

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causes neurotoxicity[4] and lung toxicity[5], induces bladder, prostate, and kidney cancer[6], and has toxic effects on genital organs[7]. In addition, ACR exposure causes degenerative vacuolar changes in renal tubule cells, inflammatory cell infiltration, and periglomerular edema formation[8]. Renal dysfunctions, renal inflammation, and DNA damage have been reported in rats treated with ACR. To prevent or treat nephrotoxicity caused by ACR, a lot of research has been done and is being done[9].

The immunostimulatory and cytoprotective effects of melatonin have roles in regulating the sleep-wake cycle and stimulating the activation of natural killer cells, monocytes, and B/T lymphocytes. Studies have also reported that it induces thymocyte proliferation and cytokine release and has antioxidant, anti-inflammatory, anticancer, and anti-apoptotic effects[10,11]. Although the effects of melatonin have been reported in experimentally induced nephrotoxicity studies with different compounds[12], no studies have been conducted on the effects of melatonin on ACR-induced kidney damage in rats. The present study aimed to evaluate the effect of melatonin on kidney function, oxidative stress, inflammation, apoptosis, DNA damage, and tissue damage caused by ACR.

2. Materials and methods

2.1. Chemicals used in the study

ACR (≥ 99%) was supplied by Sigma Chemical Co. (St. Louis, MO), and Alfa Aesar supplemented melatonin (≥ 99%). Catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), heme oxygenase 1 (HO-1), interleukin-1beta (IL-1β), interferon-gamma (IFN-γ), interleukin-10 (IL-10), malondialdehyde (MDA), nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor kappa (NF-κB), superoxide dismutase (SOD), tumor necrosis factor-alpha (TNF-α), peroxisome proliferator-activated receptor gamma (PPAR-γ) and kidney injury molecule-1 (KIM-1) enzyme-linked immunoassay (ELISA) kits were purchased from Sunred (Shangai, China). Antibodies to caspase-3, 8-hydroxy-2'-deoxyguanosine (8-OHdG), and c-Jun *N*-terminal kinase (JNK) were provided from Santa Cruz (United Kingdom).

2.2. Animals

In the study, fifty adult male Sprague-Dawley rats (approximately 200-250 g) were obtained from the Experimental Research and Application Center, Atatürk University, Erzurum, Turkey. The rats were housed in a 12-hour light/dark environment with (55±5)% humidity at (21±2)℃ room temperature. They were fed *ad libitum* with water and pellet feed (Bayramoglu Feed and Flour Industry Trade I.C., Erzurum).

2.3. Experimental design

At the beginning of the experiment, all rats were weighed, their weights were standardized, and they were randomly divided into five groups. The control group was given *i.g.* 1 mL of distilled water for 11 d. The ACR group received ACR (50 mg/kg, *i.g.*)[13] for 11 d. In the treatment groups (MEL10+ACR and MEL20+ACR groups), melatonin was intraperitoneally injected with 10 and 20 mg/kg for 11 d, respectively[14]. ACR (50 mg/kg, *i.g.*) was given 1 h after each melatonin administration. In the MEL20 group, melatonin (20 mg/kg, *i.p.*) was injected for 11 d.

2.4. Determination of kidney and body weights and biochemical parameters

On the 12th day of the experiment, the rats were weighed, and blood samples were collected from their hearts while they were under ketamine-xylazine anesthesia. Afterward, the rats were decapitated. The kidney tissues were immediately removed, and their weights were recorded. The left kidney of each rat was washed with physiological saline and then fixed in 10% formaldehyde for histopathological, immunohistochemical, and immunofluorescent examinations. The right kidneys of the rats were taken for biochemical analysis. After that, the kidneys were washed with physiological saline and stored at −80℃ until used.

2.5. Analysis of renal function markers

The serum urea and creatinine levels were analyzed in an auto-analyzer (Randox IV Monaco Auto-Chemistry Analyzer) in the Central Diagnostic and Analysis Laboratory of the Veterinary Faculty at Atatürk University.

2.6. Analysis of renal oxidative stress markers

As described in a previous study[15], kidney tissue supernatants were obtained for the analysis of biochemical markers. The levels of MDA, GSH, Nrf2, and HO-1, as well as the activities of SOD, GPx, and CAT in the kidney tissue supernatants were measured using ELISA kits as per the manufacturer's instructions. The analysis was performed using an ELISA plate reader (Bio-Tek, Winooski, VT, USA), and the absorbance was read at a wavelength of 450 nm.

2.7. Determination of inflammation markers and KIM-1 levels

The levels of TNF-α, IL-1β, IFN-γ, NF-κB, IL-10, PPAR-γ, and KIM-1 in the kidney tissue supernatants were measured by ELISA kit following the manufacturer's instructions.

2.8. Histopathological examinations

The left kidney tissues from the rats were preserved by fixing them in a 10% formalin solution for 48 h. Routine tissue follow-up procedures were then carried out, and paraffin blocks were created. Sections of 4 μm thickness were obtained from each block, which

was then stained with hematoxylin-eosin (H&E) and examined under a light microscope (Olympus BX 51, Germany)[16]. The histopathological features of the sections were evaluated and graded as absent (-), mild (+), moderate (++), or severe (+++) based on the severity of the observed changes.

2.9. Immunohistochemical examinations

Once the sections were taken from the tissue-followed paraffin blocks, they have mounted on adhesive (poly-*L*-lysin) slides for immunoperoxidase examination. The sections were then passed through a series of xylene and alcohol, where they were deparaffinized and dehydrated to prepare them for further analysis. After being deparaffinized and dehydrated, the sections were washed with distilled water (for 5 min) and phosphate buffer solution (PBS) with a pH of 7.2. They were then placed in 3% H_2O_2 for 10 min to remove endogenous peroxidase. After washing with PBS for 5-10 min, the sections were incubated for 5 min with a protein block that is compatible with all primary and secondary antibodies[17]. Primary antibodies [8-OHdG (Cat No: sc-66036; Dilution ratio: 1:100) and caspase-3 (Cat No: sc -53066; Dilution ratio: 1:100)] were dropped without washing and incubated for 1 h at 25℃. Then, primary antibodies were washed twice using PBS and incubated with biotinized secondary antibodies for 10-30 min at 25℃. It was washed with PBS, then 3-3' diaminobenzidine was dropped on the sections, and chromogen was added. The sections were stained with Mayer's hematoxylin for 1-2 min and then washed in tap water. They were passed through alcohol and xylol series, covered with a coverslip, and examined with a light microscope (Leica DM 1000).

2.10. Immunofluorescence examinations

After deparaffinization and dehydration, sections taken at 5 µm on poly lyzed slides were washed with PBS. It was kept in a 3% H_2O_2 solution for 10 min to block the endogenous peroxidase activity. The sections were removed after they were kept in the antigen retrieval in a microwave oven for 5 min four times and allowed to cool at 25℃. After cooling outside without removing it from the solution, it was washed with PBS at the end of the period, and the protein block was dropped. Then, primary antibody JNK (Cat No: sc-514539; Dilution ratio: 1:100) was dropped on the tissues, incubated in a humidified cabinet for the recommended time, and then washed with PBS. The immunofluorescent antibody (FITC Cat No: ab6785; Diluent ratio: 1:500) was dripped onto the pre-preparations and kept in the dark for 45 min. DAPI (Cat No:D-1306; Dilution ratio: 1:200 US) was dripped onto the washed tissues and incubated in the dark for 5 min. Then, 1:9 mixture of glycerol and water was dripped onto the tissues, covered with a coverslip, and examined under a fluorescent microscope (ZEISS Germany)[18]. Sections were examined under a fluorescent microscope (ZEISS Germany) and were evaluated in the ZEISS Zen Imaging Software program according to the manufacturer's recommendations.

2.11. Statistical analysis

The quantitative values obtained at the end of the study were analyzed using one-way ANOVA analysis, followed by Tukey test. These analyses were performed using the GraphPad Prism 8.0.1 statistical data program. A value of $P<0.05$ was considered significant.

Statistical analysis of histopathological data was conducted using the SPSS 13.0 package program. Two nonparametric tests, the Kruskal-Wallis and Mann Whitney *U* tests, were used to compare the differences between groups.

2.12. Ethical statement

The Animal Experiments Local Ethics Committee of Atatürk University approved the protocol for this study (HADYEK: 2020/146).

3. Results

3.1. Effect of melatonin on kidney and body weights

At the beginning of the experiment, the body weights of the rats were similar across all groups. However, at the end of the experiment, the body weights of the rats in the ACR and MEL10+ACR groups were significantly lower than those in the other groups ($P<0.05$). The kidney weights of the rats did not differ between the groups ($P>0.05$, Table 1).

3.2. Effects of melatonin on urea and creatinine levels

Administration of ACR for 11 d significantly increased serum urea and creatinine levels ($P<0.05$). Treatment with a low dose of melatonin only prevented the increase in urea level, while a high dose of melatonin significantly reversed the increase in serum levels of urea and creatinine ($P<0.05$, Table 1).

3.3. Effects of melatonin on lipid peroxidation and antioxidant enzymes

ACR induced renal lipid peroxidation and caused a significant increase in MDA level ($P<0.05$). A high dose of melatonin significantly inhibited ACR-induced lipid peroxidation (Figure 1A). GPx (Figure 1C) and GSH (Figure 1D) levels, as well as SOD (Figure 1B) and CAT (Figure 1E) activities were significantly lower in the ACR and MEL+10ACR groups compared to the control ($P<0.05$). Treatment with a low dose of melatonin significantly increased GPx level and CAT activity ($P<0.05$), while a high dose of melatonin significantly prevented ACR-induced reduction in all antioxidant enzymes ($P<0.05$).

3.4. Effects of melatonin on renal Nrf2, HO-1, and KIM-1 levels

The Nrf2 and HO-1 levels were lower in the ACR and MEL10+ACR groups compared to the other groups (P<0.05), as shown in Figure 2. Melatonin at a high dose reversed ACR-induced reduction in HO-1 and Nrf2 levels (P<0.05). Moreover, the level of KIM-1 was significantly higher in the ACR and MEL10+ACR groups compared to the control group (P<0.05, Figure 2C). However, KIM-1 level was markedly reduced by treatment with a high dose of melatonin (P<0.05, Figure 2C).

3.5. Effects of melatonin on renal inflammation

TNF-α (Figure 3A), IL-1β (Figure 3B), NF-κB (Figure 3D), and IFN-γ (Figure 3E) levels were significantly increased in the ACR and MEL10+ACR groups compared with the control group (P<0.05). Melatonin treatment at a high dose pronouncedly abrogated ACR-induced increases in these parameters (P<0.05). Additionally, IL-10 (Figure 3C) and PPAR-γ (Figure 3F) levels in the ACR group were significantly decreased in comparison to the control group (P<0.05). Treatment with a high dose of melatonin significantly increased these parameters (P<0.05).

3.6. Histopathological findings

The kidney tissues of the control and MEL20 groups had typical histological structures. In the kidney tissues of the ACR group, severe degeneration and necrosis of the tubular epithelium, dilatation of the Bowman's capsule, atrophy in the glomerulus, severe dilatation in some tubular lumens and severe hyperemia in the vessels and glomeruli were observed. In the group treated with a low dose of melatonin, moderate degeneration and mild necrosis in the tubules, dilatation in some tubules, and severe hyperemia in the

Table 1. Effects of melatonin on body weights, kidney weights, as well as serum urea and creatinine levels in rats with acrylamide-induced nephrotoxicity.

Parameters	Control	ACR	MEL10+ACR	MEL20+ACR	MEL20
Initial body weights (g)	215.62±10.12^{a}	219.25±8.87^{a}	215.50±14.85^{a}	216.00±13.50^{a}	214.52±11.08^{a}
Final body weights (g)	257.62±8.65^{ac}	191.50±12.98^{b}	204.62±10.16^{b}	239.50±10.02^{a}	264.25±11.16^{c}
Kidney weights (g)	1.16±0.08^{a}	1.01±0.09^{a}	1.01±0.11^{a}	1.02±0.12^{a}	1.11±0.07^{a}
Urea (mg/dL)	35.40±3.13^{a}	107.40±8.20^{b}	54.20±6.01^{c}	44.00±8.63^{ac}	36.20±3.70^{a}
Creatinine (mg/dL)	0.17±0.01^{a}	0.32±0.09^{b}	0.21±0.02^{b}	0.19±0.03^{a}	0.17±0.02^{a}

The values are expressed as mean ± SEM and analyzed using one-way ANOVA analysis, followed by Tukey test. The letters (a-c) on the same line indicate a significant difference (P<0.05) among groups. ACR: acrylamide; MEL10: 10 mg/kg melatonin; MEL20: 20 mg/kg melatonin.

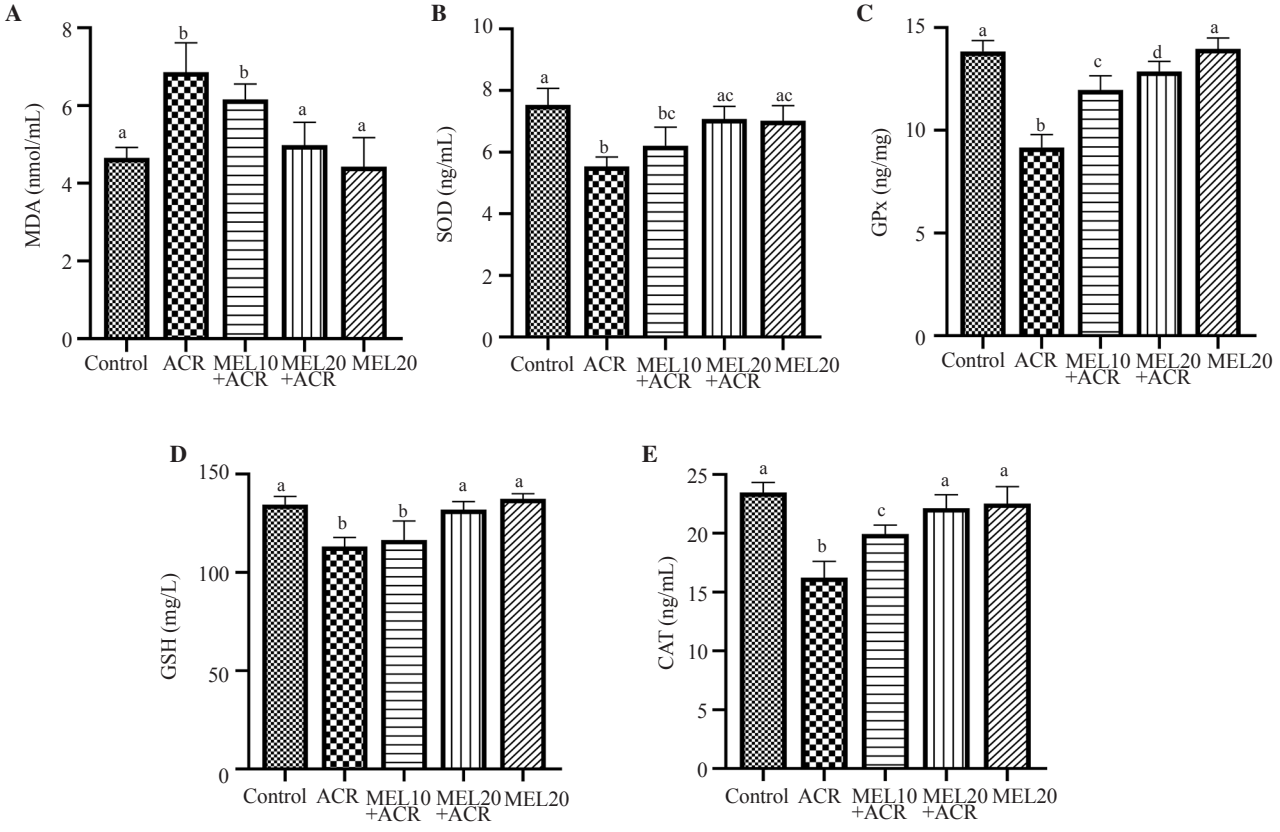

Figure 1. Effects of melatonin on lipid peroxidation and antioxidant enzymes in rats with acrylamide-induced nephrotoxicity. The values are expressed as mean ± SEM and analyzed using one-way ANOVA analysis, followed by Tukey test. The letters (a-d) indicate a significant difference at P<0.05. CAT: catalase; GPx: glutathione peroxidase; GSH: glutathione; MDA: malondialdehyde; SOD: superoxide dismutase.

Table 2. Scoring of renal histopathological structure after treatment with melatonin.

Results	Control	ACR	MEL10+ACR	MEL20+ACR	MEL20
Degeneration of renal tubular epithelium	-	+++	++	+	-
Necrosis of renal tubular epithelium	-	+++	+	-	-
Atrophy of the glomeruli	-	+++	++	-	-
Dilatation of tubules and Bowman's capsule	-	+++	++	+	-
Congestion in glomerular and interstitial vessels	-	+++	+++	+	-

Kidney tissues are evaluated as absent (-), mild (+), moderate (++), and severe (+++) according to their histopathological findings.

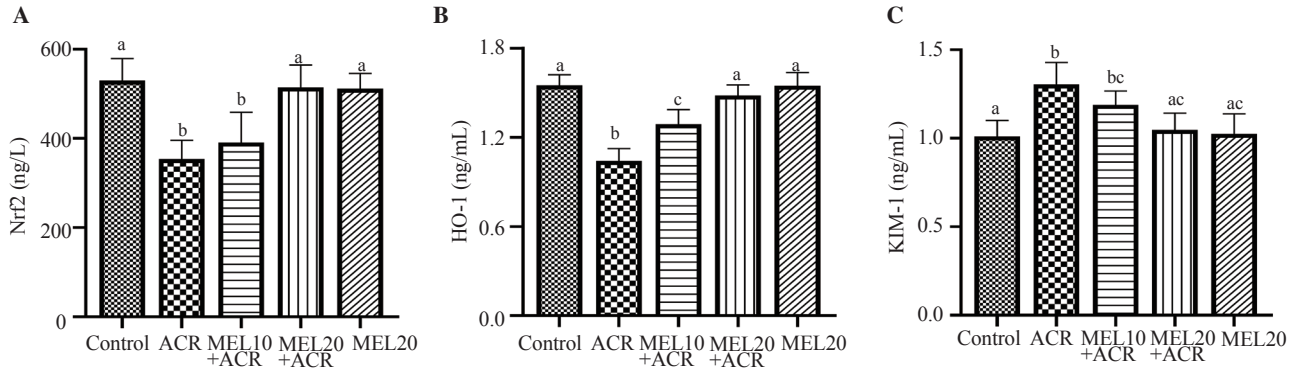

Figure 2. Effects of melatonin on renal Nrf2 (A), HO-1 (B), and KIM-1 (C) levels in rats with acrylamide-induced nephrotoxicity. The values are expressed as mean ± SEM and analyzed using one-way ANOVA analysis, followed by Tukey test. The letters (a-c) indicate a significant difference at $P<0.05$. HO-1: heme oxygenase 1; Nrf2: nuclear factor erythroid 2-related factor 2; KIM-1: kidney injury molecule-1.

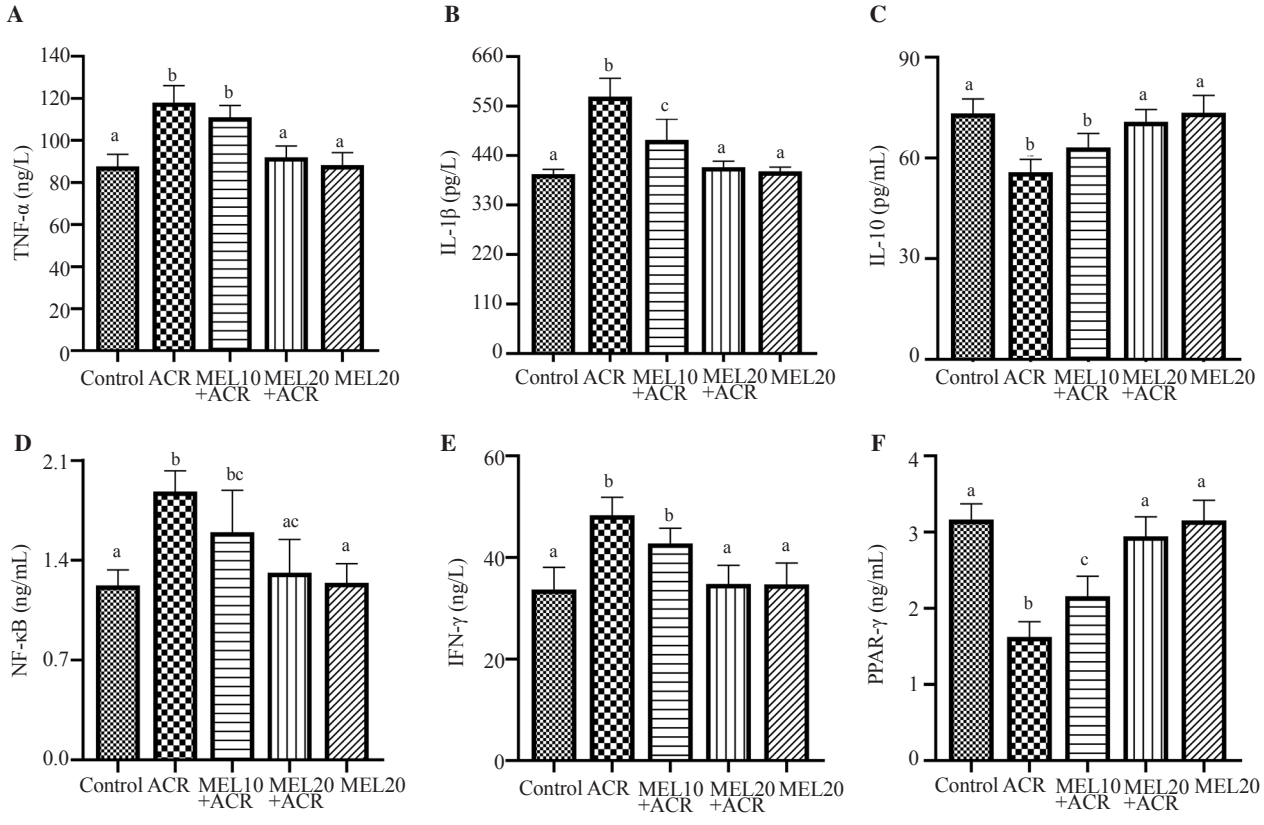

Figure 3. Effects of melatonin on inflammatory parameters in rats with acrylamide-induced nephrotoxicity. The values are expressed as mean ± SEM and analyzed using one-way ANOVA analysis, followed by Tukey test. The letters (a-c) indicate a significant difference at $P<0.05$. TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1beta; IL-10: interleukin-10; NF-κB: nuclear factor kappa; IFN-γ: interferon-gamma; PPAR-γ: peroxisome proliferator-activated receptor gamma.

Table 3. Effect of melatonin on the expressions of 8-OhdG, caspase-3, and JNK by immunohistochemical and immunofluorescent analyses.

Parameters	Control	ACR	MEL10+ACR	MEL20+ACR	MEL20
8-OHdG	19.25±2.50[a]	79.17±5.20[b]	60.65±3.10[c]	38.79±2.20[d]	19.96±3.50[a]
Caspase-3	19.84±1.90[a]	76.77±4.70[b]	59.41±3.40[c]	39.79±1.90[d]	20.17±3.20[a]
JNK	21.24±2.60[a]	80.47±4.10[b]	61.42±4.70[c]	40.14±3.50[d]	21.15±3.80[a]

Different letters (a-d) on the same line indicate a significant difference (P<0.05) among the groups.

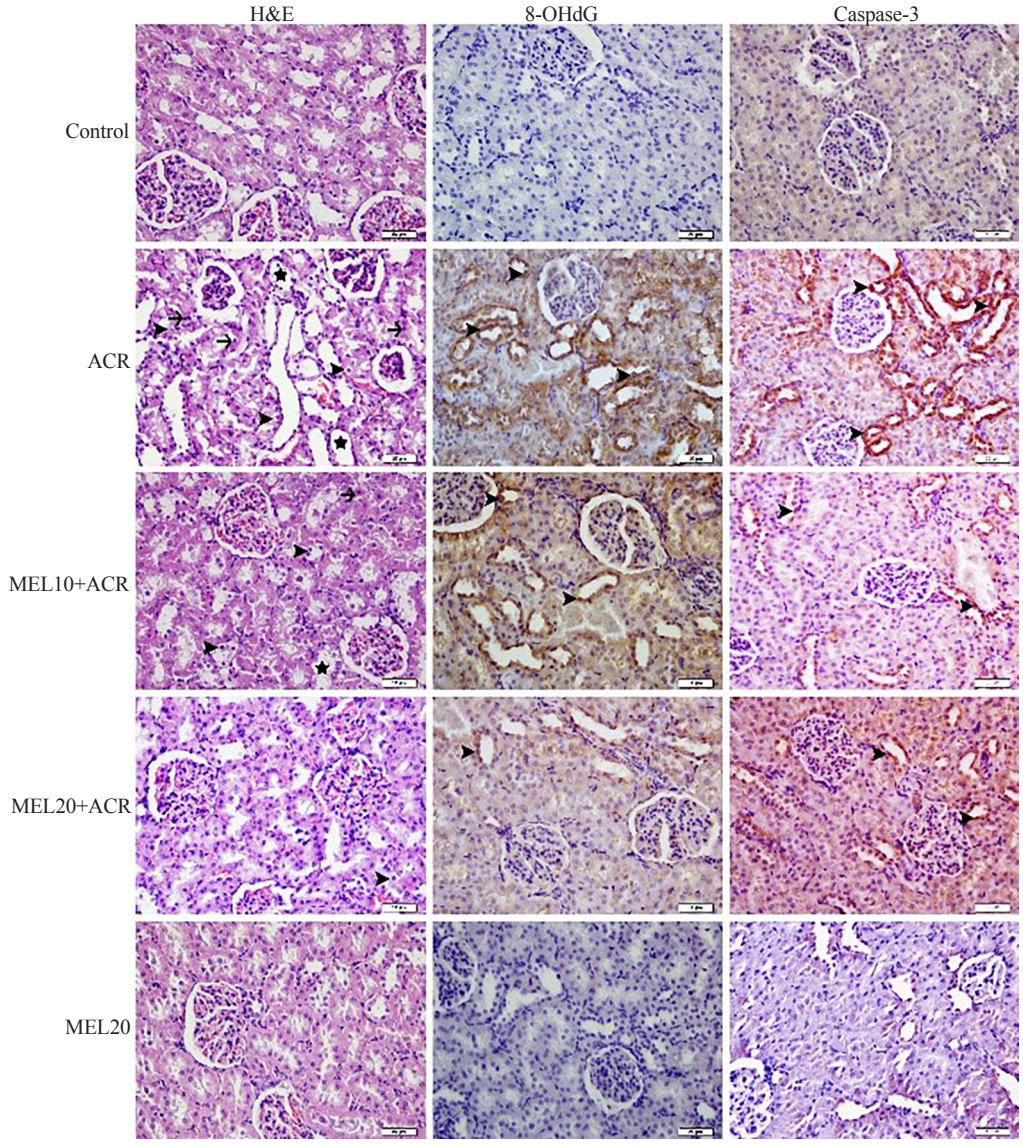

Figure 4. Effect of melatonin on 8-OHdG and caspase-3 levels in kidney tissues of rats. Degeneration (arrowheads), necrosis (arrows) of tubular epithelium, dilatation of tubules (stars), H&E, scale bar: 50 μm; cytoplasmic caspase-3 expressions (arrowheads) of tubular epithelium, tubular epithelium 8-OHdG expressions (arrowheads), IHC-P, scale bar: 50 μm.

vessels and glomeruli were detected. Mild degeneration of tubular epithelium and mild hyperemia of vessels were detected in the group treated with a high dose of melatonin. Histopathological findings are shown in Table 2 and shown in Figure 4.

3.7. Immunohistochemical findings

The levels of 8-OHdG and caspase-3 in kidney tissues of the control and MEL20 groups were evaluated as negative. In the ACR group, severe levels of cytoplasmic 8-OHdG and caspase-3 were detected in the renal tubular epithelium. The MEL10+ACR group showed moderate levels of cytoplasmic 8-OHdG and caspase-3, while the MEL20+ACR group showed mild levels of cytoplasmic 8-OHdG and caspase-3 in the renal tubular epithelium. Immunohistochemical findings are summarized in Table 3 and shown in Figure 4.

3.8. Immunofluorescent findings

The expression of JNK was negative in the control and MEL20 groups. In the ACR group, severe JNK expression was observed

in the renal tubular epithelium, while moderate and mild JNK expressions were detected in the MEL10+ACR group and the MEL20+ACR group, respectively. Immunofluorescent findings are summarized in Table 3 and shown in Figure 5.

4. Discussion

This study investigated the potential effects of melatonin on renal oxidative stress, inflammation, apoptosis, and DNA damage caused by ACR. Urea and creatinine levels are essential parameters frequently used in the clinical evaluation of kidney functions. An increase in serum urea level is reported as an indicator of acute renal failure, and an increase in creatinine level indicates loss of function in proximal tubule cells. Sengul *et al*.[15] and Ghorbel *et al*.[19] determined that the application of ACR in rats caused a significant increase in serum urea and creatinine levels. In some studies, it has been reported that melatonin application prevents the increase in urea and creatine levels in models of nephrotoxicity induced by different agents[20]. The findings of our study are compatible with the literature. ACR exposure caused a significant increase in serum urea and creatine levels, and a high dose of melatonin significantly prevented the increase in these parameters caused by ACR.

While important events continue in cells, chemical compounds called oxidants are constantly formed, and the level of oxidants in the cell is kept under constant control by antioxidants. When the cell's balance between oxidants and antioxidants is disturbed, oxidative stress occurs. It is known that oxidative stress plays an essential role in the pathogenesis of many diseases[21]. ACR causes the formation of large amounts of free oxygen radicals in hepatic and renal tissues.

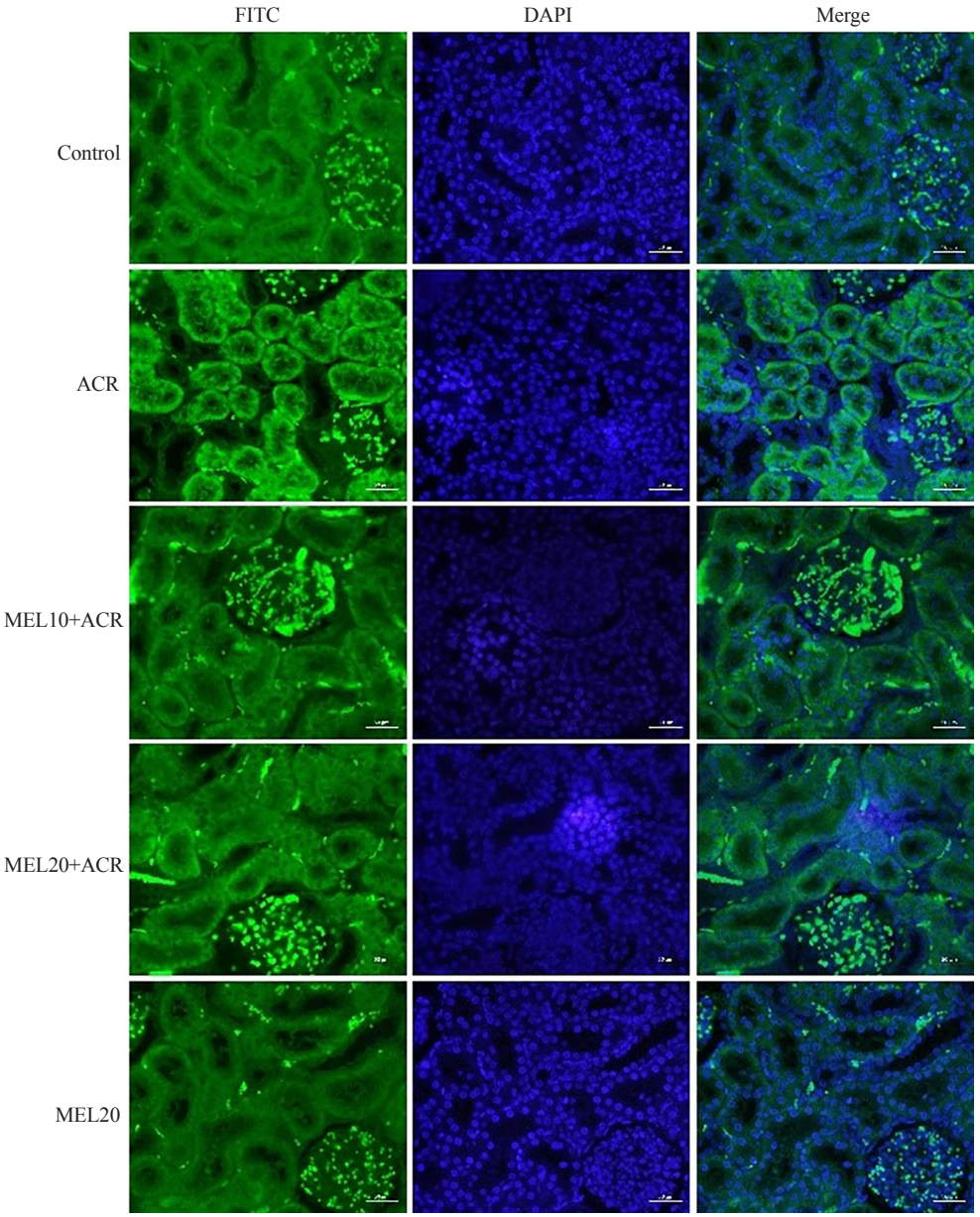

Figure 5. Effect of melatonin on JNK expression in rats. Scale bar: 50 μm.

Free oxygen radicals cause peroxidation of lipid membranes, breakdown of polyunsaturated fatty acids in cell membranes, and formation of MDA as a final product[15]. Catalgol *et al*.[22] reported that ACR increased MDA levels by inducing lipid peroxidation in erythrocytes. Antioxidant enzymes such as SOD, CAT, GSH, and GPx are essential in combating the harmful effects of free radicals[23]. GSH, which protects from the harmful effects of reactive oxygen species (ROS), plays an essential role in reducing ACR toxicity[15]. GPx can convert H_2O_2 to a water molecule and helps convert GSH into oxidized glutathione. The CAT enzyme converts H_2O_2 into a water molecule and renders it harmless. SOD combats the increased level of ROS by oxidizing one superoxide radical to O_2 and catalyzing the reduction of another superoxide radical to H_2O_2, a less reactive molecule[24]. ACR exposure causes oxidative stress by increasing ROS production in tissues, reducing the level of antioxidant enzymes (SOD, CAT, GSH, and GPx) in cells[10]. Tozan-Beceren *et al*.[25] stated that melatonin was protective against ACR-induced oxidative damage in rats. In the present study, ACR increased lipid peroxidation and caused decreases in antioxidant enzyme levels. In line with the findings of the literature[26], our findings show that melatonin, especially at a high dose, prevented the increase in MDA level, as well as decreases in GSH, SOD, GPx and CAT activities, thus alleviating ACR-induced renal oxidative stress.

The Nrf2/HO-1 signaling pathway regulates the expression of several antioxidant genes in response to various stimuli and protects the cell against oxidative stress and inflammation[27]. It is known to activate the expression of more than 250 antioxidant enzymes such as Nrf2, HO-1, CAT, GPx, and glutamate, which is a transcription factor that plays a vital role in the regulation of oxidative stress[28]. The HO-1 is one of the most critical cytoprotective mechanisms activated in conditions that induce cellular stress, such as inflammation, ischemia, hypoxia, hyperoxia, hyperthermia, or radiation, and it has an antioxidant role[27]. Nrf2 activation is suppressed by Kelch-like ECH-associated protein (Keap1) and when tissue damage occurs, the Keap1-Nrf2 interaction becomes unstable and causes Nrf2 activation. Nrf2 then translocates to the nucleus and upregulates genes, including HO-1. Nrf2 is a critical mechanism that mediates protection against acute kidney injury through HO-1 induction[29]. In our previous study, it was observed that Nrf2 and HO-1 levels were significantly decreased in nephrotoxicity induced by different agents in rats[30] and melatonin administration suppresses oxidative stress by activating the Nrf2/HO-1 pathway[19]. In the present study, ACR caused a decrease in Nrf2 and HO-1 levels, and a high dose of melatonin activates the Nrf2/HO-1 pathway and suppresses oxidative stress.

Excessive production of ROS in cells causes the release of inflammatory signaling molecules. In tissues exposed to oxidative stress, various transcription factors cause inflammation to become active. Of these inflammatory factors, IL-1β is released from hematopoietic cells such as macrophages and monocytes, and TNF-α is released by immune cells[31]. NF-κB, a transcription factor, regulates genes involved in different immune and inflammatory response processes, and IFN-γ causes NF-κB activation. NF-κB activates the release of cytokines such as IL-1β, IL-6, and TNF-α[32]. IL-10, an anti-inflammatory cytokine, inhibits NF-κB activation and plays a critical role in inhibiting inflammation[33]. In a previous study, there was a significant increase in NF-κB, TNF-α, and IL-1β levels in ACR-induced nephrotoxicity in rats[15]. Studies have shown that melatonin has an anti-inflammatory effect by preventing the increase in proinflammatory cytokine levels and the decrease in anti-inflammatory cytokine levels. Cano Barquilla *et al*.[34] reported that melatonin prevented the increase in serum TNF-α, IL-1β, IL-6, and IFN-γ levels and the decrease in IL-10 levels in a diet-induced metabolic syndrome model in rats. Another study determined that melatonin alleviated increased TNF-α, IL-1β, IL-6, and NF-κB levels in methotrexate-induced testicular toxicity in rats[35]. Our findings are consistent with the literature and show that ACR significantly increased TNF-α, IL-1β, IFN-γ, and NF-κB, and decreased IL-10. In addition, melatonin ameliorated ACR-induced renal inflammation. PPAR-γ, a nuclear receptor superfamily member, has critical roles in the immune system. PPAR-γ is expressed in many cells, including monocytes/macrophages, dendritic cells, T/B lymphocytes, and platelets, and exerts an anti-inflammatory effect by dose-dependently inhibiting the production of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6[36]. In the study by Badawy *et al*.[37], renal PPAR-γ level was decreased significantly in cisplatin-induced nephrotoxicity. It has been reported that melatonin treatment has an anti-inflammatory effect by increasing the level of PPAR-γ[38]. Consistent with the findings of Badawy *et al*., the present study shows that melatonin had an anti-inflammatory effect by preventing the decrease in ACR-induced PPAR-γ level in a dose-dependent manner.

KIM-1 is a type 1 transmembrane glycoprotein markedly increased in renal proximal tubule epithelial cells in the event of renal injury[39]. Sengul *et al*.[15] reported that the application of ACR in rats significantly increased the level of KIM-1 in the kidneys. In experimentally induced renal toxicity models, it has been determined that melatonin administration prevents increased KIM-1 levels[40]. In our study, KIM-1 was increased significantly in rats with ACR-induced nephrotoxicity, and a high dose of melatonin abrogated ACR-induced increase in KIM-1 level, which is consistent with the literature.

ACR also causes changes in the histological structures of kidney tissues. In the case of ACR toxicity, Bowman's capsule enlargement, tubular dilatation, leukocyte infiltration, and vascular occlusions develop between tubules and parts of the glomerulus[19]. It has been reported that melatonin administration prevents renal tissue damage in cisplatin-induced nephrotoxicity in rats[26]. In our study, ACR application caused tubular necrosis, cell swelling, mononuclear cell infiltration, glomerular obstruction, interstitial hemorrhage, severe degeneration, and necrosis in the tubular epithelium in the kidney. In addition, it was observed that it caused dilatation in Bowman's capsule, atrophy in the glomerulus, severe dilatation in some tubular lumens, and severe hyperemia in the vessels and glomeruli. Melatonin treatment significantly alleviated ACR-induced damage in the kidney tissue in a dose-dependent manner.

Apoptosis is programmed cell death and is influenced by internal and external factors. Caspase-8 and caspase-9 pathways control the intrinsic and extrinsic mechanisms of apoptosis. In the

mitochondria-dependent intrinsic pathway, Bcl-2 family proteins in the mitochondrial membrane activate procaspase-9, and activated caspase-9 plays a role in the activation of caspase-3. Renal caspase-3 levels are significantly increased in ACR-induced nephrotoxicity in rats[15]. Erdemli *et al*.[41] reported that melatonin administration suppressed increased caspase-3 expression in acetamiprid-induced nephrotoxicity in rats. Our study shows that ACR severely increased the cytoplasmic caspase-3 expression in kidney tubule epithelium, and melatonin dose-dependently prevented an increase in caspase-3 expression.

The 8-OHdG is the most critical indicator of DNA damage and its expression levels are evaluated to determine DNA damage in kidney tissue[15]. Li *et al*.[42] reported that 8-OHdG expression was concentrated in Bowman's capsules and renal tubule regions in mice administered arsenic trioxide. It was exceptionally high in epithelial cells of proximal tubules and podocytes. It was reported that renal 8-OHdG expression increased significantly in cisplatin-administered rats, and melatonin administration significantly prevented this increase[43]. Our findings were consistent with previous studies; ACR increased renal 8-OHdG expression, and melatonin dose-dependently alleviated this increase.

The JNK pathway is activated in cells in response to stress and plays a vital role in apoptosis and inflammation. The toxic agents cause renal JNK activation, and this activation induces inflammation and subsequent apoptosis[44]. Some compounds with antioxidant and anti-inflammatory effects reduce renal damage by preventing renal JNK activation[45]. The data we obtained are similar to these studies. ACR increased JNK expression in the kidney tissues of rats, whereas melatonin administration suppressed JNK expression in a dose-dependent manner.

Our study demonstrates that ACR caused renal oxidative stress, inflammation, apoptosis, and DNA and tissue damage in rats. Melatonin treatment has a nephroprotective effect against ACR-induced renal damage by exerting antioxidant, anti-inflammatory, and anti-apoptotic effects. However, there is a need for a more comprehensive study of the therapeutic effect of melatonin on ACR-induced damage in other organs.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

HA, EŞ, FI, and SY contributed to experimental design and experiment implementation. EŞ and FI performed biochemical analyses and evaluations. SY performed the histopathological, immunohistochemical, and immunofluorescent examination. HA, EŞ, SY, and FI contributed to the writing and editing of the article and read and approved the final text.

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