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The effects of dexmedetomidine on early acute kidney injury in severely burned rats

A. OZDEMIR[1], A. TOPÇU[2], T. MERCANTEPE[3], M. ARPA[4], S. MATARACI KARAKAŞ[4], AS. OZDEMIR[5], L. TÜMKAYA[3], F. MERCANTEPE[6]

[1]Department of Anesthesiology and Reanimation, [2]Department of Pharmacology, [3]Department of Histology and Embryology, [4]Department of Medical Biochemistry, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[5]Department of Anesthesiology and Intensive Care, Ministry of Health, Education and Research Hospital, Rize, Turkey
[6]Department of Endocrinology and Metabolic Diseases, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey

Abstract. – OBJECTIVE: Burns are a global medical and economic problem. In addition to high costs, the lengthy therapeutic process and the emotional trauma experienced by patients and their families indirectly worsen the socioeconomic damage caused. Kidney failure observed after burns is highly correlated with mortality.

MATERIALS AND METHODS: Twenty-eight male Sprague-Dawley rats (age four months, weight 250-350 g) were included in the study. They were randomly assigned into four groups consisting of seven rats each with similar mean weights. Group 1 (n=7) represented the healthy control group (C), Group 2 (n=7) the Sham+dexmedetomidine (DEX) 100 mcg/kg (three doses) (S+DEX100) group, Group 3 (n=7) the 30% Burn (B), and Group 4 (n=7) the 30% Burn+-DEX 100 mcg/kg/day group (B+DEX100) (three doses). Thiobarbituric acid reactive substances (TBARS), total thiol (TT), interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) values in kidney tissues were investigated biochemically, and histopathological analyses were also performed. Nuclear factor κB (NF-κB)/p65 was measured using immunohistochemistry, and the TUNEL assay was applied to indicate apoptotic tubular epithelial cells.

RESULTS: TBARS, IL-1, and TNF-α in kidney tissues decreased in the B+DEX100 group compared to the 30% burn group, while total thiol values increased. Histopathologically, atypical glomeruli, particularly necrotic tubules, and inflammation in peritubular areas decreased in the B+DEX100 group compared to the 30% burn group. In addition, apoptotic tubular epithelial cells exhibiting TUNEL positivity and tubular epithelial cells exhibiting NF-κβ/p65 positivity also decreased in the B+DEX100 group compared to the 30% burn group.

CONCLUSIONS: Dexmedetomidine reduced apoptotic activity in rats and exhibited anti-inflammatory antioxidant effects in the burn model in this study.

Key Words:
Burn, Dexmedetomidine, Inflammation, Kidney, Oxidative stress, Rat.

Introduction

Burns are a medical problem resulting in approximately 180,000 deaths annually worldwide, as well as representing an economic problem entailing high treatment costs. According to the 10-year American Burn Association report[1] published in 2019, the total cost for surviving patients with burns exceeding 10% of the total body surface is approximately 260,000 US dollars ($), rising to approximately $350,000 for non-surviving patients. The ages of burns cases also exhibit bimodal distribution. The highest prevalences are between 1 and 15.9 years in the pediatric age group, constituting 22.5% of total burns, and between 20 and 59.9 years in the adult age group, representing 56% of burns. Male and female gender distributions between 1 and 15.9 years are very similar to one another, while male gender predominates in the 20-59.9 age range[1].

In addition to high costs, the lengthy therapeutic process and the emotional trauma experienced by patients and their families indirectly exacerbate the socioeconomic damage resulting from burns.

Corresponding Author: Abdullah Ozdemir, MD; e-mail: abdullah.ozdemir@erdogan.edu.tr

Burns occur in association with various causes. Millions of individuals may experience these due to thermal, chemical, electrical, inhaler, and other etiological causes. In terms of the pathophysiology, burns are characterized by systemic inflammation. Due to their local and systemic effects, burns produce effects in both the immediate region and in distant organs. Although the mechanism is unclear, proinflammatory mediators and reactive oxygen radicals emerging after burns have been implicated[2,3]. Activation of the proinflammatory cascade commencing after large body surface burns has been implicated in the development of immune and multiorgan dysfunctions. Hyperactivation of neutrophils and macrophages following inflammation causes oxidative damage in distant organs. It has also been concluded that other proinflammatory cytokines, particularly interleukin-1 (IL-1), play an important role in distant organ complications[4,5].

The manifestation of kidney failure emerging following burns appears in two forms, depending on the time of onset. One is the form that occurs in the first few days and is characterized by low cardiac output, decreased renal blood flow, impaired tubular function, and increased catecholamine levels. The second entails a more complex pathogenesis particularly involving sepsis and multiorgan failure. Increased proinflammatory cytokines, oxygen radicals, and lipid and protein oxidation, and decreased antioxidant levels [particularly glutathione (GSH)] play an important role in the pathogeneses[4]. Free radicals emerging as a result of oxidative stress and inflammation attack the cell membrane. The production of hydroperoxide [thiobarbituric acid reactive substances (TBARS)] and subsequently malondialdehyde (MDA) increases as a result[6]. Increasing free radical activity and lipid peroxidation are eliminated by GSH, one of the body's natural antioxidants. The amount of change in GSH levels depends on the number of free radicals. The levels of oxidative stress products naturally increase after burns, while those of endogenous antioxidants may be expected to decrease[7].

The incidence of kidney failure following burns among adult patients ranges between 26% and 53%. Various factors affect this wide range[8]. Effective post-burn treatment depends on several factors, such as fluid balance, nephrotoxic drug use, and the presence of sepsis. Kidney failure seen after burns is closely correlated with mortality[9].

Dexmedetomidine (DEX) is an alpha-2 adrenoreceptor agonist frequently employed in intensive care units due to its analgesic, anesthetic, and sedative effects. In addition to these effects, several studies[10,11] have also reported organ-protective activity due to its antioxidant, apoptotic, and anti-inflammatory properties. DEX has been shown[12] to provide protection against oxidative stress and cell apoptosis in multiple organs, including the kidney, lung, and intestine. Studies[13] have shown that it exhibits its anti-inflammatory effects by reducing levels of IL-1β, tumor necrosis factor-α (TNF-α), and IL-6 in particular.

The purpose of the present study was to shed new light on the treatment of burns in the near future by examining the regulatory effects of alpha-2 adrenergic receptors in the early period of kidney damage in an experimental model of full thickness burns.

Materials and Methods

Experimental Animals

After obtaining the local Ethical Committee approval (No. 2022/02- 28.01.2022), 28 male Sprague-Dawley rats (age four months, weight 250-350 g) were housed in standard cages under normal laboratory conditions (20-26°C, 50-70% humidity, fresh air change every 12 h, and a 12-h light-dark cycle) with *ad libitum* access to food and water. All animals were cared for in line with the principles for the Care and Use of Laboratory Animals set out in the National Research Council guideline.

Chemicals

DEX (Sedadomid 200 mcg/2 mL, sterile vial for perfusion) was provided by Koçak Farma Pharmaceutical Industry Inc. (Istanbul, Turkey). Anesthesia was administered using ketamine hydrochloride (Ketalar, 100 mg/kg, Pfizer İlaçları Ltd. Şti., Istanbul, Turkey) and xylazine hydrochloride (Rompun, 10 mg/kg, Bayer, USA). Analgesia was administered using fentanyl citrate (Talinat, 0.5 mcg/10 mL, Vem Pharmaceutical Industry Inc., Ankara, Turkey). All chemicals used in the laboratory experiments were provided by Sigma Chemical Co. (Saint Louis, MO, USA) and Merck (Darmstadt, Germany).

Experimental Protocol

The rats were randomly assigned into four groups with similar mean weights, each consisting of seven animals.

Group 1 (n=7): The healthy control group (C), received only intraperitoneal (i.p.) 0.9% NaCl (saline).

Group 2 (n=7): The Sham+DEX 100 mcg/kg/day (S+DEX100) group. Following anesthetization, these animals were exposed to 25°C water

for 17 s. DEX 100 mcg /kg/day was then administered i.p. three times[14].

Group 3 (n=7): The 30% burn group (B). A hollow metal 2 cm-diameter cylinder was placed on the shaved area on the dorsal skin. Boiling water (94±1°C) was then poured into the cylinder, which was held in place for 17 s.

Group 4 (n=7): The 30% burn+DEX 100 mcg/kg/day group (B+DEX100). A hollow metal 2 cm-diameter cylinder was placed on the shaved area on the dorsal skin. Boiling water (94±1°C) was then poured into the cylinder, which was held in place for 17 s. Three doses of DEX 100 mcg / kg/day were subsequently administered i.p.[11].

All surgical procedures were performed under sterile conditions. The burn model was established, and the surgical procedures were conducted under 100/10 mg/kg ketamine/xylazine anesthesia. Burn wounds were induced using the model previously described by Alemdaroğlu et al[15] and Vorauer-Uhl et al[16], with minor modifications. The dorsal skin was exposed by shaving the fur with an electric razor. A hollow metal 2-cm diameter cylinder was next placed on the shaved area. This was then filled with boiled water (94±1°C) and left *in situ* for 17 s. The groups exposed to burns received a single dose of fentanil 1.5 mcg/kg i.p. for analgesia. At the end of the experiment the rats were euthanized by high-dose anesthesia. One of the renal biopsies was stored at -80ºC until the day of the laboratory tests, on condition that the cold chain was not disturbed for biochemical investigations, while the other was placed into 10% neutral formalin for histopathological analyses.

Biochemical Procedure

Tissue sampling and homogenization

For tissue homogenization, a mixture of 20 mM 1 L of sodium phosphate + 140 mM of potassium chloride (pH 7.4) was first prepared[17]. Next, 1 mL of homogenization solution was added to 100 mg of tissue sample, which was then homogenized [tissue specimens were homogenized for 5 min at 30 Hz using a Tissuelyser-II device (Qiagen, Germany)]. The homogenates were finally centrifuged at 800 g at 4°C for 10 min.

Thiobarbituric Acid Reactive Substances (TBARS) Analysis

TBARS in tissue was measured manually (using chemicals). The results were determined using the modified Draper and Hadley method[18]. Briefly, to 200 uL tissue supernatant were added 50 uL 8.1% sodium dodecyl sulfate, 375 uL 20% acetic acid (h/h) (pH 3.5), and 375 uL 0.8% thiobarbituric acid (TBA). The mixture was then vortexed and left to incubate for 1 h in a boiling water bath. Following the incubation period, the reaction mixtures were cooled for 5 min in iced water and centrifuged at 750 g for 1 min. The resulting pink color was analyzed at 532 nm on a spectrophotometer. The results were expressed as nmol/g.

Total Thiol Analysis

Ellman's reactive method[19] was employed to measure total thiol levels in kidney tissues. Ellman's reagent and spectrophotometry were used to determine the color of the sulfhydryl groups in the tissue supernatants. Briefly, 200 uL 3M Na_2HP0_4 and 50 uL, 5',5'-(2-dithiobis nitro benzoic acid – DTNB) (4 mg DTNB was prepared in 1% 10 mL sodium citrate solution) were added to 50 uL supernatant. The resulting mixture was vortexed, and the absorbance was measured at 412 nm. The results were calculated using standard charts for 1,000 μM-62.5 μM reduced GSH and were expressed as mmol/g tissue.

IL-1 and TNF-α Analyses

IL-1 and TNF-α levels in rat kidney tissue were determined using rat IL-1 and TNF-α ELISA kits (Catalog No. E0107Ra and E0764Ra, respectively, Bioassay Technology Laboratory, Shanghai, China). Sensitivity values were 0.51 pg/mL and 2.51 ng/L, and standard curve intervals were 1-300 pg/L and 5-1,000 ng/L, respectively. Tissue homogenates were produced in a laboratory environment in accordance with the manufacturer's instructions. IL-1 and TNF-α concentrations in each tissue specimen were measured in triplicate.

Histopathological Analysis

Kidney tissue specimens were trimmed to a volume of 1.5 cm. In accordance with routine histological preparation procedures, these were fixed by being kept in 10% neutral formalin solution (Sigma Aldrich, St. Louis, MO, USA) for 24 h. Following fixation, the kidney tissue specimens were dehydrated by being passed through increasing alcohol series (50%, 70%, 80%, 90%, 96%, 100%, and 100%; Merck GmbH, Darmstadt, Germany) using a tissue processing device (Shendon Citadel 2000, Thermo Scientific Inc., Waltham, MA, USA). They were then cleared in two xylol solution series (Merck, Darmstadt,

Table I. Biochemical analysis results (mean±standard deviation).

Group	TBARS (nmol/g tissue)	Total Thiol (µmol/g tissue)	IL-1 (pg/g tissue)	TNF-α (pg/g tissue)
Control	54.25±33.06	7.33±1.07	540.49±10.63	2,677±380.07
DEX100	67.76±7.42	8.73±0.57	579.5±15.06	2,623.75±418.25
Burn (B)	168.43±31.26[a,b]	5.19±0.65[a,b]	730.29±33.80[a,b]	3,477.19±315.93[d,b]
B+DEX100	76.14±13.38[c]	7.67±1.04[c]	616.13±79.32[c]	2,617.63±371.88[c]

[a]p=0.001: *vs.* the control group, [b]p=0.001: *vs.* the DEX 100 group, [c]p=0.001: *vs.* the B+DEX100 group, [d]p=0.018: *vs.* the control group. One-Way ANOVA-Tukey HSD test.

Germany). The tissues were placed into soft and hard paraffin (Merck GmbH, Darmstadt, Germany) and embedded in paraffin blocks. Sections of 5 µm in thickness were taken using a rotary microtome (Leica RM2525, Leica Biosystems, Wetzlar, Germany). The sections were finally stained with Harris hematoxylin and Eosin G (Merck, Darmstadt, Germany) using a histological staining device (Leica Biosystems, 5020ST, Wetzlar, Germany).

Immunohistochemical (IHC) Analysis

Kidney tissue sections were examined using a TUNEL assay kit (ab206386, Abcam, Cambridge, UK) and primary antibody and NF-κβ/p65 primary antibody (ab194726, Abcam, Cambridge, UK) kits. A secondary antibody was also used with the primary antibody (Goat Anti-Rabbit IgG H&L HRP, ab205718, Abcam, Cambridge, UK). Following deparaffinization, the 5 µm-thick kidney tissue sections were treated with 3% H_2O_2 for 15 min in order to block endogenous peroxidase activity using a Bond MAX IHC/ISH (Leica Biosystems, Wetzlar, Germany) device. A secondary blocking solution was applied for 20 min to prevent background staining, after which the tissues were incubated with primary antibody for 60 min. After the application of the primary antibody, the tissue specimens were incubated with secondary antibody. Diaminobenzidine chromogen (DAB Chromogen, Abcam, Cambridge, UK) solution was then dropped onto the tissues, and an image signal was obtained on the light microscope. The tissues were finally counterstained with Harris hematoxylin (Merck, Darmstadt, Germany) and covered with an appropriate solution.

Semi-Quantitative Analysis

Kidney Damage Pathology Scores (KDPS) in the kidney tissue sections were calculated on the basis of tubular necrosis, atypical glomeruli, inflammation, and tubular dilatation in line with previous burn-application acute kidney injury studies[20-24] (Table I). Thirty different areas in each section were evaluated by two histologists blinded to the study groups.

Apoptotic cells, found to exhibit TUNEL and NF-κβ/p65 positivity using immunohistochemical methods, were scored as shown in Table II and III. Thirty different areas from each section were evaluated by two histologists blinded to the study groups.

Statistical Analysis

Renal histopathological damage scoring, scoring of immune positive cells, semi-quantitative

Table II. Kidney histopathological damage score (KDPS) values.

Grade	Findings
Tubular Necrosis Score (with necrotic tubular cells causing major cytoplasmic vacuolization)	
0	<5%
1	6-25%
2	26-50%
3	≥50%
Proximal Tubule Brush Border Loss	
0	<5%
1	6-25%
2	26-50%
3	≥50%
Inflammation	
0	<5%
1	6-25%
2	26-50%
3	≥50%
Atypical Glomeruli	
0	<5%
1	6-25%
2	26-50%
3	≥50%

Depicts the participants' academic performance in terms of the semester and cumulative scores. The median semester score was 4.47 (range 1.1 -5.8) with two outliers while the median cumulative score was 4.44 (range 2.8-5.0).

Table III. Immunopositivity scoring method.

Grade	Findings
Tubular Cell Immune Positivity Score	
0	≤5%
1	6-25%
2	26-50%
3	≥50%

Depicts the participants' academic performance in terms of the semester and cumulative scores. The median semester score was 4.47 (range 1.1 -5.8) with two outliers while the median cumulative score was 4.44 (range 2.8-5.0).

analyses (non-parametric), and analysis of biochemical (parametric) data were performed on SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Parametric data yielded by the analyses were expressed as mean±standard deviation, and non-parametric data as mean and 25th-75th percentiles. Differences between groups were analyzed using One-Way ANOVA and Tukey's HSD test for parametric data. Non-parametric data were analyzed using the Kruskal-Wallis' test followed by the Mann-Whitney U test with Bonferroni correction. p-values <0.05 were regarded as statistically significant.

Results

Biochemical Analysis Results

Analysis of TBARS values, measured to determine lipid peroxidation, showed that levels in kidney tissue were significantly higher in the burn group compared to the control and DEX only (S+DEX100) groups (Table I; p=0.001 and p=0.001 for both). In contrast, TBARS levels in kidney tissue were lower in the B+DEX100 group than in the burn group (Table I; p=0.001).

Total thiol levels, measured in kidney tissue in order to determine antioxidant activity, were significantly lower in the burn group compared to the control and DEX only (S+DEX100) groups (Table I; p=0.001 for both). In contrast, total thiol levels increased in the B+DEX100 group compared to the burn group (Table I; p=0.001).

Examination of IL-1 levels in kidney tissue between the groups revealed a significant increase in the burn group compared to the control and DEX only (S+DEX100) groups (Table I; p=0.001 for both). In contrast, IL-1 levels were lower in the B+DEX100 group than in the burn group (Table I; p=0.001).

Examination of TNF-α levels in kidney tissues revealed that these were higher in the burn group compared to the control and DEX only (S+DEX100) groups (Table I; p=0.018 and p=0.001, respectively). In contrast, TNF-α levels were lower in the B+DEX100 group than in the burn group (Table I; p=0.001).

These data indicate that DEX reduces apoptotic activity and inflammatory and oxidant effects in burn-related kidney tissue damage.

Histopathological Analysis Results

Examination of kidney tissues under light microscopy revealed normal glomeruli and proximal and distal tubules in the control group [Figure 1A-B: Table IV; KDPS: 1 (0-1)]. Similarly, typical glomeruli and tubular epithelial cells were observed in sections from the DEX only group [Figure 1C-D: Table IV; KDPS: 1 (0-1)]. In contrast, numerous necrotic tubular cells, accompanying wide vacuolization in the cytoplasm of tubular epithelial cells, were observed in sections from the burn group. In addition, tubular dilatations and loss of the brush border structure were present in the proximal tubules. Atypical glom-

Table IV. Kidney Histopathological Damage Score (KDPS) results [median-(25-75% interquartile range)].

Group	Tubular Necrosis Score	Proximal Tubule Brush Border Empty	Tubular Dilatation	Inflammation	Atypical Glomeruli	KDPS
Control	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-1)
DEX100	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-1)
Burn (B)	3 (2-3)[a,b]	3 (2-3)[a,b]	2 (1-2)[e,b]	2 (2-2)[a,b]	2 (2-2)[a,b]	11 (10.5-12)[a,b]
B+DEX100	1 (0-1)[c]	1 (1-1)[d]	1 (0-1)[f]	1 (1-1)[g]	1 (0-1)[g]	5 (4-5)[h,i]

[a]p=0.001; between the control and burn groups, [b]p=0.001; between the DEX100 and burn groups, [c]p=0.002; between the DEX100 group and burn groups, [d]p=0.016; between the burn and B+DEX100 groups, [e]p=0.01; between the control group and burn groups, [f]p=0.023; between the burn and B+DEX100 groups, [g]p=0.001; between the burn and B+DEX100 groups, [h]p=0.001; between the control and B+DEX100 groups, [i]p=0.044; between the burn and B+DEX100 groups. Kruskal-Wallis/ Mann-Whitney U with Bonferroni confirmation.

Table V. Semi-Quantitative Analysis [median-(25%-75% interquartile range)].

Group	TUNEL	NF-κβ/p65
Control	0 (0-0)	0 (0-1)
DEX100	0 (0-0)	0 (0-1)
Burn (B)	2 (2-2)[a,b]	2 (2-2)[a,b]
B+DEX100	1 (0-1)[c]	1 (0-1)[c]

[a]p=0.001: Between the control group and burn groups, [b]p=0.001: Between the Dex 100 and burn groups, [c]p=0.001: Between the burn and B+DEX100 groups. Kruskal-Wallis/ Mann-Whitney U with Bonferroni corrections.

eruli and inflammation in peritubular areas were also observed [Figure 1E-F: Table IV; KDPS: 11 (10.5-12)]. On the other hand, a decrease was observed in atypical glomeruli, and particularly necrotic tubules, and inflammation in peritubular areas was detected in the B+DEX100 group. A distinct brush border structure in the proximal tubules was an especially notable finding [Figure 1E-F: Table IV; KDPS: 5 (4-5)].

Immunohistochemical Analysis

Examination of the TUNEL assay findings, performed to identify apoptotic cells in tubular epithelial cells, revealed an increase in tubular epithelial cells exhibiting intensive TUNEL positivity in the burn group compared to the control and DEX100 groups [Figure 2A-C: Table V; p=0.001, TUNEL positivity scores: 2 (2-2); 0 (0-0); and 0 (0-0), respectively]. In contrast, we observed a decrease in apoptotic tubular epithelial cells exhibiting TUNEL positivity in the B+DEX100 group compared to the burn group [Figure 2C-D: Table V; p=0.001, TUNEL positivity scores: 2 (2-2) and 1 (0-1), respectively].

Light microscopic examination of kidney tissues, incubated with NF-κβ/p65 primary antibody, revealed that the normal tubular epithelial cells in the control group were NF-κβ/p65-negative [Figure 3A: Table V; NF-κβ/p65 positivity score: 0 (0-1)]. Similarly, the typical epithelial cells in kidney tissues from the DEX100 group were also immune-negative [Figure 3A: Table V; NF-κβ/p65 positivity score: 0 (0-1)]. In contrast, tubular epithelial cells exhibiting intensive NF-κβ/p65 positivity increased in the burn group compared to the control and DEX100 groups [Figure 3A-C: Table V; p=0.001 for both: NF-κβ/p65 positivity score: 2 (2-2); 0 (0-1); 0 (0-1)]. On the other hand, a decrease was observed in tubular epithelial cells exhibiting NF-κβ/p65 positivity in the B+DEX100 group compared to the burn group [Figure 2C-D: Table V; p=0.001: NF-κβ/p65 positivity score: 2 (2-2); 1 (0-1)].

Discussion

The results of this study revealed a decrease in atypical glomeruli and inflammation in the peritubular area in the DEX treatment group, while in terms of immunohistochemistry, apoptotic activity decreased in the DEX treatment group compared to the burn group. DEX suppressed inflammation in the treatment group compared to the burn group, while increasing antioxidant total thiol levels. DEX reduced apoptotic activity and exhibited an anti-inflammatory antioxidant effect in the rat burn model in this study.

In their 2021 burn model study, Guo et al[25] reported significant decreases in tubular damage scores and MDA levels measured in the renal tissues in the groups administered with oxygenase-1 and TAK242 after burn induction. In addition, renal mRNA expression of TNF-α, IL-1β, IL-6, and ICAM-1 was lower in the treatment groups than in the burn groups. Similarly in the present study, KDPS scores, oxidative stress, and inflammation levels were lower in the B+DEX100 group than in the burn group[25].

In their study involving a rat ischemia/reperfusion model, Tang et al[26] reported that DEX reduced renal cell pro-apoptotic protein p53, Bax and active caspase-3, following myocardial ischemia/reperfusion injury. In another study[26,27], decreases were observed in TUNEL scores in renal tubule cells together with apoptosis after DEX administration following extreme stress in rats. In agreement with the previous literature, TUNEL scores and NF-κβ/p65 positivity in this study were lower in the B+DEX100 group compared to the burn group.

Studies[28] have shown that a manifestation of shock accompanied by hypovolemic and cardiogenic findings predominates in the first 48 hours after burns, with electrolyte imbalances due to protein and fluid losses occurring in the following hours. Studies[29] have also shown that early high-dose treatment with the antioxidant vitamin C reduces mortality. In the present research we administered DEX, identified as an antioxidant in previous study[12]. This was found to lower TBARS levels in the B+DEX100 group compared to the burn only group, while raising total thiol levels.

Endogenous antioxidant levels rise following burns as a result of lipid peroxidation. One endog-

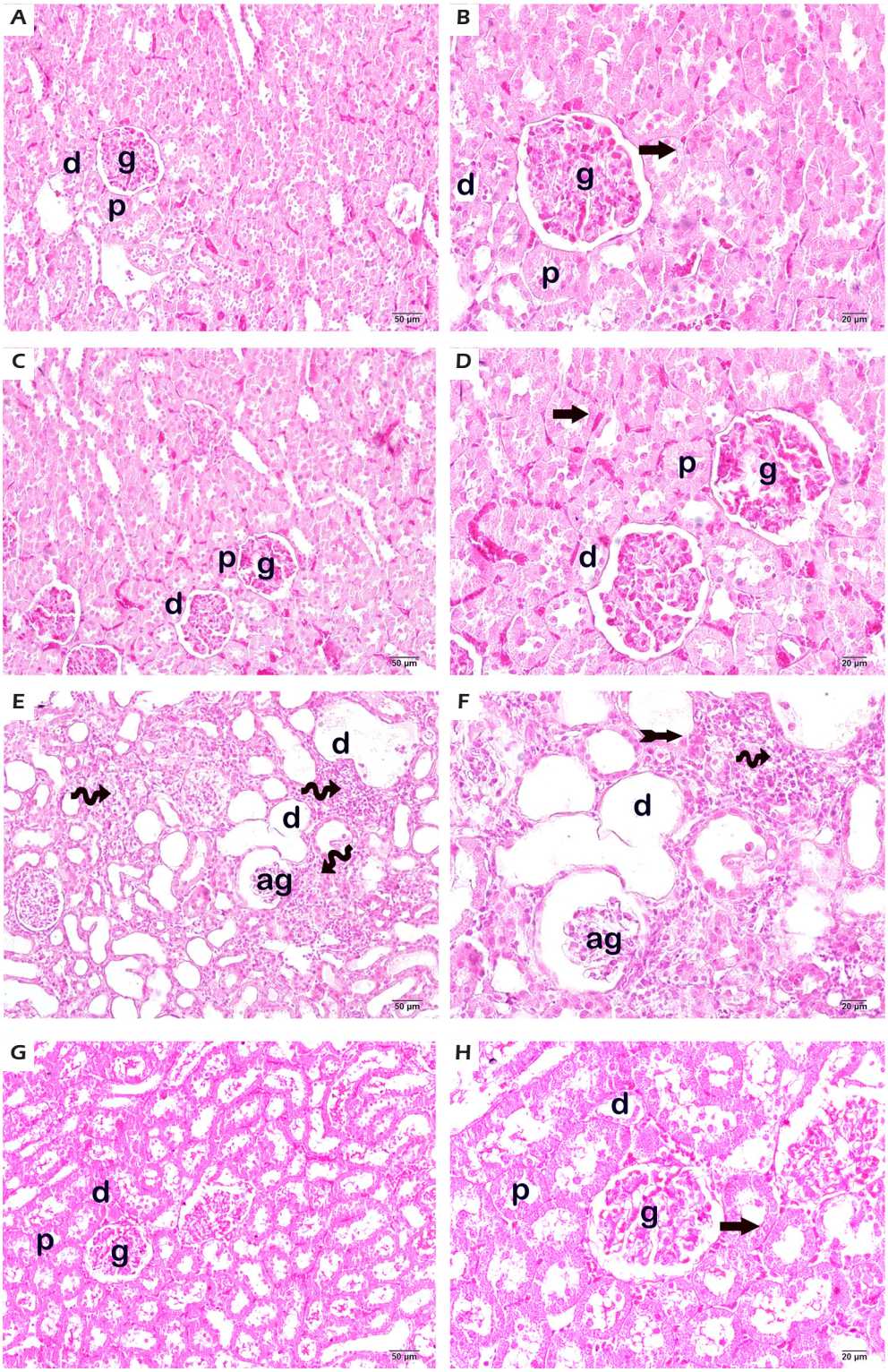

Figure 1. Light microscopic screen image of kidney tissue stained with H+E. **A**, (x20)-**B**, (x40) Control group. **C**, (x20)-**D**, (x40) DEX100 group. **E**, (x20)-**F**, (x40) Burn (B) group. **G**, (x20)-**H**, (x40) B+DEX100 group. g: glomerulus, p: proximal tubule, d: distal tubule, ag: atrophic glomeruli.

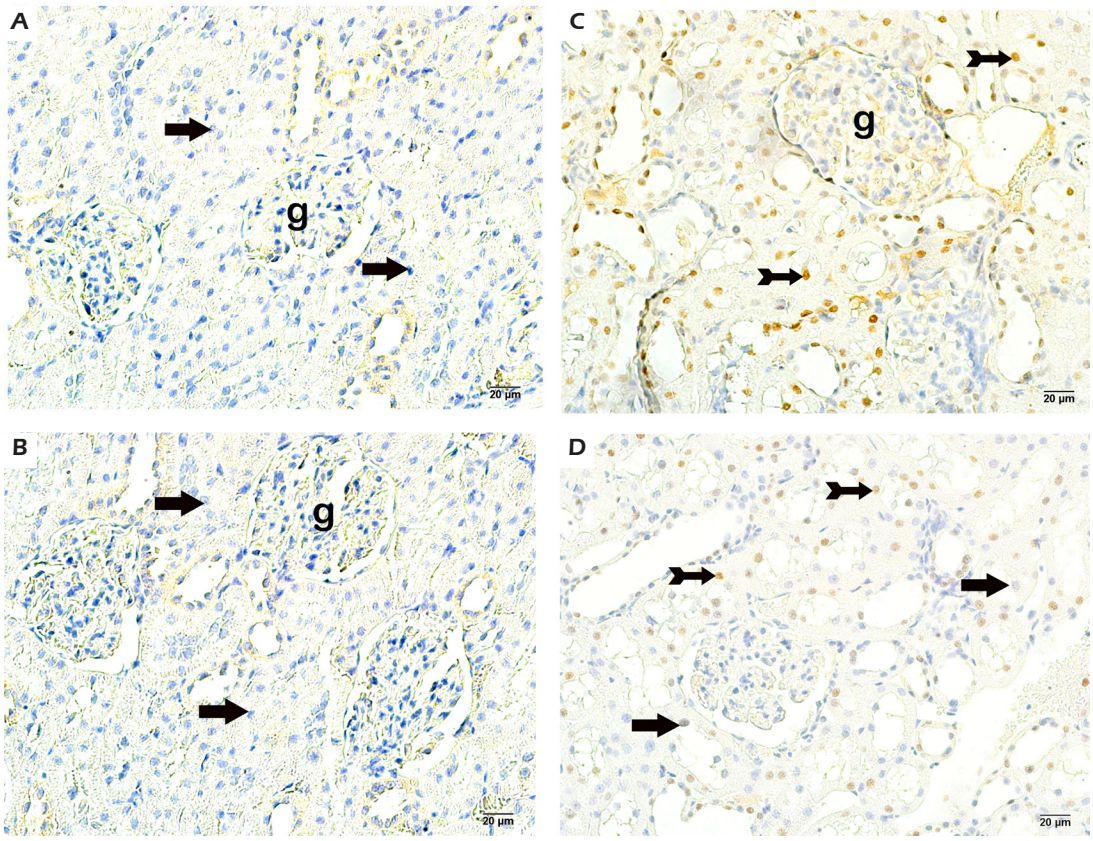

Figure 2. Light microscopic screen image of kidney tissue exposed to the TUNEL method to determine apoptotic cells. **A**, (x40) Control group. **B**, (x40**)** DEX100 group. **C**, (x40) Burn (B) group. **D**, (x40) B+DEX100 group. g: glomerulus.

enous antioxidant is melatonin, which is also used as an antioxidant agent in replacement therapies. A previous burn model study[30] reported decreases in MDA and GSH values in lung, liver, and gastrointestinal system tissues and an increase in GSH levels following 10 mg/kg i.p. melatonin administration 3 and 24 h after burns; MDA levels in lung, liver, and gastrointestinal system tissues decreased in the melatonin group compared to the burn group, while GSH levels were significantly higher in the melatonin group than in the burn group. In the present study, TBARS values decreased, while total thiol levels increased in the B+DEX100 group compared to the burn group.

The most common causes of mortality in burn patients are multiorgan failure and infections. Several studies have been performed using DEX, with its proven anti-inflammatory and antioxidant effects[10,11]. Liu et al[31] reported a renoprotective effect of DEX in patients who underwent renal transplantation. Yao et al[32] showed that DEX reduced MDA and hydrogen peroxide contents in a septic animal model and reversed oxidative stress by increasing GSH levels and catalase activity.

Systemic inflammation in the acute period, followed by an anti-inflammatory period, occur in major thermal injuries. Numerous complex cellular products play a role during this period. In their rat burn model study, Shen et al[33] observed decreased IL-1β, TNF-α, and HMGB1 protein expression levels in groups treated with glycyrrhizin (Gly) compared to a burn group. In agreement with that research, IL-1 and TNF-α levels in the present study decreased significantly in the B+-DEX100 group compared to the burn group.

Various factors, including fluid therapy, the use of nephrotoxic agents, the burn surface area, and the presence of sepsis are involved in the development of post-burn kidney failure. In the present study, rats were permitted oral water intake in line with the routine feeding model once the burn model had been established. However, some burn model rat studies[8] have reported results that may be dependent on the variety of fluid applied. For example, in one study[8], Ringer lactate (RL) and

hypertonic saline (HS) were applied 2, 8, and 24 h after induction of a burn model. Renal tubular scores, kidney MDA levels, and IL-1β and TNF-α levels were lower in the HS group than in the RL group, although kidney superoxide dismutase (SOD) levels increased. In the present study, TBARS levels decreased, and total thiol levels increased in the B+DEX100 group compared to the burn group.

An earlier animal study[34], involving a burn model with lycopene with its known antioxidant and antiapoptotic effects, showed that lycopene administration significantly ameliorated neutrophil-related pulmonary and renal injury developing as a response to thermal injury. Significant decreases have also been observed[34-36] in tissue MDA levels and myeloperoxidase (MPO) and caspase-3 activities, together with increases in GSH levels and SOD and catalase activities. Serum TNF-α and IL-β also decreased with lycopene treatment. The present study investigated the antiapoptotic and anti-inflammatory effects of DEX and obtained similar results to those of the previous literature[34-36].

Limitations

The present study investigated oxidative stress, inflammation, and apoptotic processes as a pilot study of the early effects on the kidney of DEX applied in a burn model in rats. However, it also has a number of limitations. In particular, the study budget was limited, and our research now needs to be supported by further studies including other antioxidant-oxidants and cytokines and renal functions.

Conclusions

DEX reduced apoptotic activity in renal tissue in a burn model in rats, and also exhibited systemic inflammatory and antioxidant effects.

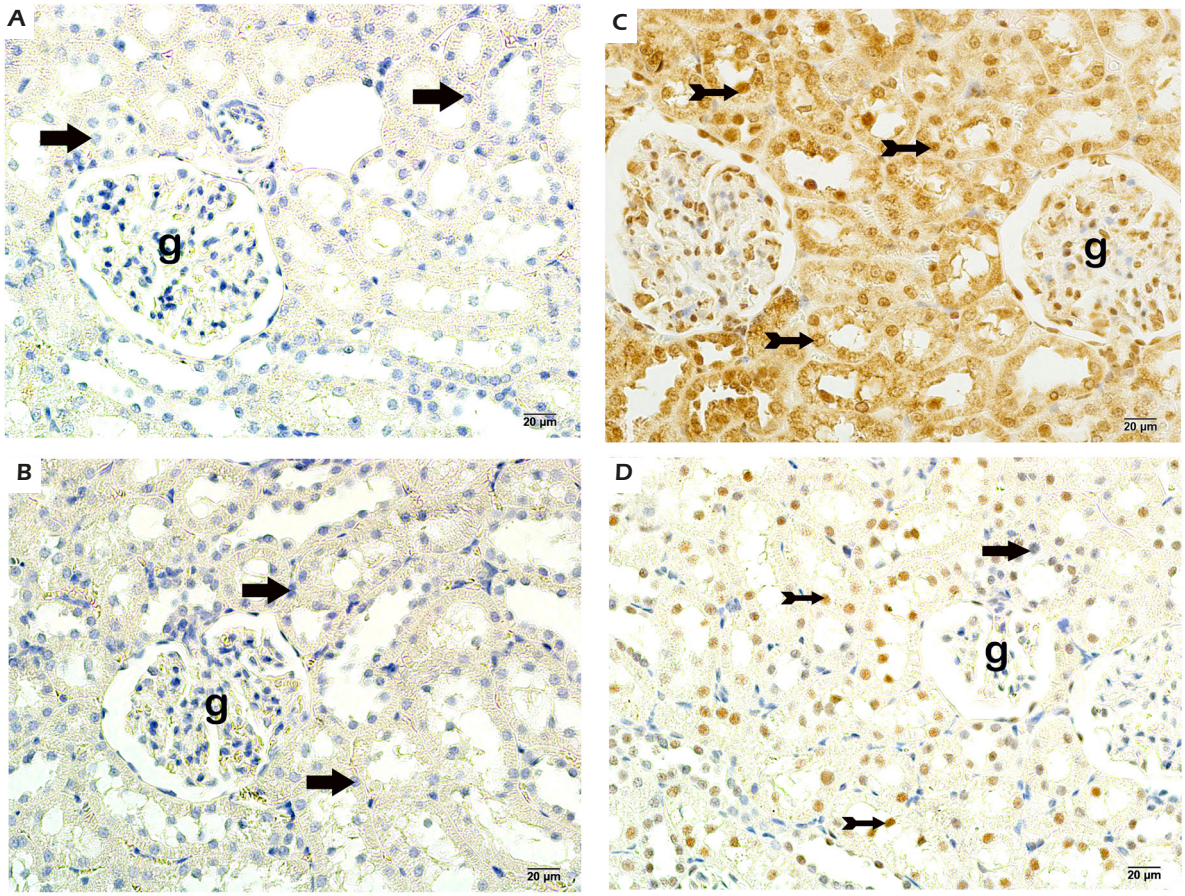

Figure 3. Light microscopic screen image of kidney tissue incubated with NF-κβ/p65 primary antibody. **A**, (x40) Control group. **B**, (x40) DEX100 group. **C**, (x40) Burn (B) group. **D**, (x40) B+DEX100 group. g: glomerulus.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Authors' Contributions
AO, AT, and TM designed the research and conducted the experiments. AO, TM, MA, SMK, AsO and LT contributed to the analysis and interpretation of data. AO and AsO wrote the manuscript. All authors read and approved the final manuscript.

ORCID ID
Abdullah Ozdemir: 0000-0002-4778-9622.
Atilla Topcu: 0000-0003-4730-5015.
Tolga Mercantepe: 0000-0002-8506-1755.
Medeni Arpa: 0000-0001-8321-4829.
Sibel Mataraci Karakas: 0000-0001-7147-5087.
Asiye Ozdemir: 0000-0002-7233-8380.
Levent Tumkaya: 0000-0001-5387-4666.
Filiz Mercantepe: 0000-0002-4325-1534.

Ethics Approval
The study protocol was approved by the Recep Tayyip Erdogan University Animal Ethics Committee (Approved No.: 2022/2, approval date: 28.01.22).

Informed Consent
Not applicable.

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