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RESEARCH ARTICLE

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Evaluation of the protective role of resveratrol against sepsis caused by LPS via TLR4/NF-κB/TNF-α signaling pathways: Experimental study

Aslı Okan[1] | Züleyha Doğanyiğit[1] | Seher Yilmaz[2] | Sümeyye Uçar[3] | Evrim Suna Arikan Söylemez[4] | Rukset Attar[5]

[1]Department of Histology and Embryology, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey

[2]Department of Anatomy, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey

[3]Department of Anatomy, Faculty of Medicine, Erciyes University, Kayseri, Turkey

[4]Department of Medical Biology, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyon, Turkey

[5]Department of Obstetrics and Gynecology, Yeditepe University, Istanbul, Turkey

Correspondence

Züleyha Doğanyiğit, Department of Histology and Embryology, Faculty of Medicine, Yozgat Bozok University, Yozgat 66100, Turkey.
Email: zuleyha.doganyigit@gmail.com

Abstract

The development and progression of sepsis are multifactorial and influence the immunological, endocrine, and cardiovascular systems of the body. Our knowledge of the key mechanisms involved in the pathogenesis of sepsis has expanded exponentially, yet this still needs to be translated into effective targeted therapeutic regimes. In the present study, we aimed to determine whether resveratrol has positive effects in the experimental sepsis rat model. Twenty-eight male Spraque–Dawley rats were randomly divided into four groups ($n = 7$) as follows: control, lipopolysaccharide (LPS) (30 mg/kg dose), resveratrol, and LPS and resveratrol. After the experiment, liver and kidney tissues were collected for histopathological evaluation, blood serums were collected to measure malondialdehyde levels with enyzme-linked immunosorbent assay, and Toll-like receptor-4 (TLR4), tumor necrosis factor-α (TNF-α), nuclear factor-κB (NF-κB) immunoreactivity density was evaluated immunohistochemically. In addition, messenger RNA expression levels for *TLR4*, *TNF-α*, *NF-κB*, *interleukin-1β*, and *interleukin 6* were measured. In addition, the damage observed in liver and kidney tissue was determined by AgNOR (argyrophilic nucleolar organizer regions) staining. LPS application caused severe tissue damage, oxidative stress, and increased the expressions of proinflammatory proteins and genes we evaluated, while resveratrol application eliminated these negativities. Resveratrol has been proven to suppress the TLR4/NF-κB/TNF-α pathway, a possible therapeutic signaling pathway that is important in initiating the inflammatory response in an animal model of sepsis.

KEYWORDS

NF-κB, oxidative stress markers, resveratrol, sepsis, TLR4 signaling, TNF-α

1 | INTRODUCTION

Sepsis is a pathology characterized by an immunological response in the body that leads to multiorgan failure.[1] In sepsis, which is the reaction of the host against the infection developing in the body, pro- and anti-inflammatory responses accompany this reaction.[2] In the pathology of sepsis, the intracellular signaling process leads to the expression of genes associated with adaptive immunity and inflammation. The dysregulated inflammatory response may lead to low levels of peripheral vasodilation associated with abnormal

platelet stimulation seen in the early stage of sepsis.[3] In this context, it is suggested that sepsis arises from endocrine disorder, coagulopathy, and dysregulated inflammation due to complement stimulation.

Lipopolysaccharide (LPS), a glycolipid of the outer membrane of Gram-negative bacteria, is a well-known pathogenic compound in septic shock that has been used in numerous experimental studies to induce acute inflammatory shock reactions.[4] LPS, an endotoxin, is distributed in various organs, including the brain, in part through the induction of inflammatory mediators.[5] It has detrimental effects on the body, which can lead to a state of oxidative stress. The latter is characterized by increased lipoperoxidation with depletion of intracellular stores of endogenous antioxidants or rapid alteration of antioxidant enzymes such as superoxide dismutase, CAT, and peroxidase.[5]

The use of natural products for the treatment of various diseases, including sepsis, continues to be popular today. One of the natural compounds used for this purpose is resveratrol. Resveratrol is a natural polyphenol compound, a sirtuin-1 (SIRT-1) activator with anti-inflammatory, antiviral, antibacterial, and antifungal inhibitory abilities, as well as cardiovascular and antitumor protective effects.[6] Resveratrol, which stands out with its strong antioxidant activity, is widely found in the roots, stems, leaves, and fruits of plants.[6]

The liver is one of the central organs for the onset, modulation, and termination of systemic toxicity produced by Gram-negative bacteria during sepsis. In the liver, LPS binds to Toll-like receptors (TLR) on Kupffer cells and causes the production of nitric oxide (NO) and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6. These molecules, together with the direct action of the accumulating leukocytes and LPS, cause a stress response of hepatocytes, which is then characterized by the generation of reactive oxygen species and the corresponding protein and lipid membrane failure.[7]

The kidneys mainly provide blood filtration and salt and water homeostasis,[8] as well as they also produce hormones such as renin, erythropoietin, and calcitriol/vitamin D3, which regulate blood pressure, help to control red blood cell production, and maintains bone metabolism and health.[8] Since the livers and kidneys are among the organs most affected by sepsis, we aimed to evaluate the effects of resveratrol on these two vital organs in our study.

Our current study is the first study in which the applied doses and TLR4/nuclear factor-κB (NF-κB)/TNF-α signaling pathway were evaluated with immunohistochemistry and real-time reverse transcription-PCR, and a comprehensive evaluation with the AgNOR (argyrophilic nucleolar organizer regions) staining method. These features are important for the originality of our study.

Significance Statement

Toll-like receptors (TLRs) are potent activators of the inflammatory response. TLR4 is a potential therapeutic target for sepsis. Resveratrol has a protective role on liver and kidney functions in sepsis via the TLR4/nuclear factor-κB/tumor necrosis factor-α signaling pathway. AgNOR (argyrophilic nucleolar organizer regions) staining intensity, which quantifies tissue damage, increased in liver tissues in a lipopolysaccharide-induced sepsis model.

2 | MATERIALS AND METHODS

2.1 | Chemicals

LPS (*Escherichia coli* LPS, serotype 0127: B8) and resveratrol (CAS number:501-36-0) were obtained from Sigma-Aldrich. The LPS and resveratrol doses to be applied to the rats were determined based on the information in the literature.[9,10]

2.2 | Animals and experimental protocols

The protocol of this study was approved by the Animal Experiments Local Ethics Committee of Erciyes University (21/24). The study used adult male Sprague–Dawley rats that were bred at Erciyes University DEKAM mouse facility. The water and food needs of the rats that were kept in cages were met through the normal course of the day at 21°C and with a 12-h light/dark cycle. The number and breed of the animals to be used in the study and the doses to be applied were determined based on the information in the literature.

For the study, 28 adult male Sprague–Dawley rats were randomly divided into four equal groups ($n = 7$):

- *Group 1 (Control)*: Only saline (SF) (0.9% NaCl sol.) 1 mL was administered intraperitoneally (ip) to the group (every day for 10 days) ($n = 7$).
- *Group 2 (LPS)*: 1 mL of a single dose of 30 mg/kg LPS was administered ip on the last day of the experiment[9] ($n = 7$).
- *Group 3* (resveratrol): 1 mL of resveratrol at a dose of 200 mg/kg was administered orally (every day for 10 days) [10] ($n = 7$).
- *Group 4* (resveratrol + *LPS*): 1 mL of resveratrol at a dose of 200 mg/kg was administered orally (every day for 10 days) and 1 mL of a single dose of 30 mg/kg LPS was administered ip on the last day of the experiment ($n = 7$).

LPS and resveratrol doses to be administered to rats were selected based on the literature.[9,10] Since it was a study to determine whether resveratrol has a protective effect, resveratrol was applied to the 3rd and 4th groups for 10 days. After resveratrol administration was completed, that is, at the end of 10 days, 1 h after the administration, LPS was given to group 4 ip. In the LPS applied groups, after the experimental protocol was completed 6 h after the application, ketamine hydrochloride (50 mg/kg intramuscular (im)/ip) and 2% xylazine hydrochloride (10 mg/kg im/ip) were given as an anesthetic to the rats, and the liver and kidney were taken. During this time, the animals' body weights were recorded. In addition, daily

changes were observed for each animal. All ip injection and gavage applications were performed at the same time of the day.

2.3 | Histological analysis

After collecting the liver and kidney tissues, they were placed in formaldehyde and fixated for 2 days in parallel to the size of the tissue, and then the tissues were kept under running water overnight following fixation.[11] The tissues were then embedded in paraffin blocks following the routine histological method. Five-micrometer-thick cross-sections of the samples were taken from these blocks onto slides, then deparaffinized for histochemical staining, and finally stained with hematoxylin and eosin. Hemorrhage, vacuolization, inflammation, and glomerular damage were evaluated in the kidney samples for each animal. Pycnotic hepatocytes, hemorrhage, sinusoidal enlargement, and inflammation were detected in the liver samples. The damage ratios for each category were scored as: 3, severe; 2, moderate; 1, mild; and 0, none.[11,12] The tissues were subjected to histochemical analyses under an Olympus BX53 light microscope.

2.4 | Immunohistochemical analysis

Immune reactivity of p-NF-κB (Bioassay Technology Laboratory; BT-MCA1291), TLR4 (Elabscience; E-AB-70375), and TNF-α (Elabscience; E-AB-22159) proteins in kidney and liver tissues were determined using the avidin–biotin peroxidase method.[11] In summary, deparaffinized sections of 5 μm thickness were heated in a microwave oven at 300 W in citrate buffer 2 × 4 times to open epitopes (pH: 6.0). The preparations were then taken into a solution of 3% hydrogen peroxide in methanol to inhibit endogenous peroxidase activity. Ultra V block solution was applied to prevent nonspecific staining. The sections were then incubated with primary antibodies at 4°C overnight. The biotinylated secondary antibody, streptavidin–horseradish peroxidase, and 3,3′-diaminobenzidine chromogens were applied, respectively, and then the sections were counterstained with Gill hematoxylin. It was dehydrated by passing through a series of increasing alcohol and covered with a sealant called entellan. Sections were examined with an Olympus BX53 light microscope. Evaluation of immunoreactivity levels was done with the Image J program.

2.5 | ELISA assay

Tissue samples, obtained from rats, were used for biochemical analysis. Malondialdehyde (MDA) levels were measured in liver and kidney tissues. The protocol, using the kit provided by the manufacturer, was conducted to specify the levels of rat MDA (cat. no.: 201-11-0157; Sunred Bio). The results were measured with an enzyme-linked immunosorbent assay (ELISA) reader device at 450 nm, and they were provided in nmol/mL for MDA.

2.6 | AgNOR detection

The obtained liver and kidney tissue of the animals was dissected (approximately 1 × 1 × 1 cm^3 in size). After routine histological follow-up, the liver tissue was cut into 4-mm-thick sections and deparaffinized in xylene, and then rehydrated in graded alcohol solutions before AgNOR staining. The slides were air-dried for 15 min at room temperature and fixed in a fixative (3:1 ratio of methanol and acetic acid solution) for 5 min. AgNOR staining was carried out according to the Benn and Perle and the Lindner protocols, with a slight modification to all slides of the groups.[13,14]

AgNOR-stained liver and kidney cells were viewed using a light microscope (Eclipse 80i; Nikon) and photographed via a digital camera (DigitalSight DSfi1; Nikon). The captured images were transferred to an image processing software (ImageJ version 1.47t; National Institutes of Health). Fifty nuclei per slide were evaluated and the total AgNOR area per nuclear area (TAA/NA) and mean AgNOR number were detected via the "freehand selections" tool for each nucleus.[15]

2.7 | Gene expression alteration of *TLR4*, *Nf-κB*, *TNF-α*, *IL-1β*, and *IL-6* in the rat liver and kidney

Total RNA from the liver and kidney tissues of the rats was extracted with a PureZole reagent (Bio-Rad; cat. no.: 732-6890) according to the manufacturer's protocol. Then, RNA amount and RNA purity were quantified for each RNA sample by Nanodrop ND-1000 spectrophotometer V3.7. RNA samples were stored at −80°C until use. All the RNA samples were reverse-transcribed into complementary DNA (cDNA) from 1 μg of total RNA (iScript Reverse Transcription Supermix; Bio-Rad; cat. no.: 170884) under the following conditions: one cycle at 25°C for 5 min, 46°C for 20 min, and 95°C for 1 min. *Nf-κB*, *TLR4*, *TNF*-α, *IL-1β*, and *IL-6* messenger RNA (mRNA) expression analysis was performed by Rotor Gene-Q (Qiagen). The reaction mix was prepared by related cDNAs, *Nf-κB*, *TLR4*, *TNF*-α, *IL-1β*, and *IL-6* (primer sequences were designed as mentioned in Iwashita et al.[16] for *TNF*-α, *IL-1β*, and *IL*-6, Le Mandat Schultz et al.[17] for *TLR4*, Kermanian et al.,[18] for *Nf-κB*), *GAPDH* primers, SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix (Bio-Rad; cat. no.: 172-50-16) and nuclease-free water according to the manufacturer's protocol. *GAPDH* is a reference gene for normalization. Oligonucleotide primers were designed by Oligomere based on the following primer sequences:

Rat-*TLR4* F: 5′-AATCCCTGCATAGAGGTACTTCCTAAT-3′.
Rat -*TLR4* R: 5′-CTCAGATCTAGGTTCTTGGTTGAATAAG-3′.
Rat- *Nf-κB* F: 5′-GCAAACCTGGGAATACTTCATGTGACTAAG-3′.
Rat- *Nf-κB* R: 5′-ATAGGCAAGGTCAGAATGCACCAGAAGTCC-3′.
Rat-*TNF-α* F: 5′-AAATGGGCTCCCTCTCATCAGTTC-3′.
Rat-*TNF-α* R: 5′-TCTGCTTGGTGGTTTGCTACGAC-3′.
Rat-*IL-1β* F: 5′-CACCTCTCAAGCAGAGCACAG-3′.
Rat-*IL-1β* R: 5′-GGGTTCCATGGTGAAGTCAAC-3′.

Rat-*IL-6* F: 5′-TCCTACCCCAACTTCCAATGCTC-3′.
Rat-*IL-6* R: 5′-TTGGATGGTCTTGGTCCTTAGCC-3′.
Rat-*GAPDH* F: 5′- GAGGACCAGGTTGTCTCCTG-3′.
Rat-*GAPDH* R: 5′-GGATGGAATTGTGAGGGAGA-3′.

We used the following RT-PCR protocol for *TNF*-α, *IL-1β*, and *IL-6* mRNA amplification: 98°C for 3 min initial denaturation, followed by 40 cycles of 98°C for 15 s and 61°C for 30 s; and for *Nf-κB*, *TLR4* mRNA amplification: 98°C for 30 s initial denaturation, followed by 40 cycles of 98°C for 5 s and 58°C for 30 s. Melting-curve analysis was performed for confirmation of single-product amplification at the end of the PCR. 65–95°C, 0.5°C increments at 5 s/step. Each run has been performed in triplicate.

2.8 | Statistical analysis

Genetical data analysis were performed using REST 2009 V2.0.13,[19] where $p < .05$ is deemed to represent a statistically significant result. GraphPad Prism 8 software program was used for the analysis of the data of the other methods. One-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison test was applied to evaluate Ag-NOR parameters. Two-way ANOVA and Tukey's multiple comparison test were used to evaluate the data obtained from the histopathological scoring of the liver and kidney tissues of the experimental groups. One-way ANOVA and Dunnet's multiple comparison test were used to evaluate the numerical data obtained from the immunohistochemical findings and ELISA analysis.

3 | RESULTS

3.1 | Histological findings

As a result of the histomorphological examinations, it was observed that hemorrhage, vacuolization, and inflammation were more intense in the tubulointerstitial areas in the kidney sections (Figure 1) in the LPS group compared to the control and resveratrol groups ($N = 6$, 20 sections were examined in each animal).

In particular, it was observed that vacuolization and inflammation decreased in LPS-resveratrol-administered groups. It was determined that glomerular damage was increased in the LPS and LPS-resveratrol groups, but the damage rate in the LPS-resveratrol group was much lower than that in the LPS group ($p < .05$; Table 1).

According to the findings we obtained from the histomorphological examinations, it was observed that there was an increase in the number of pycnotic hepatocytes in the liver sections of the LPS group ($N = 7$, at least 10 sections were examined in each animal), hemorrhage and sinusoidal enlargement were more frequent, and inflammation was more common than the other groups (Figure 2 and Table 2). Resveratrol application seems to alleviate these negative effects.

3.2 | Immunohistochemical findings

Compared with the control group, an increase in the intensities of TLR4, p-NF-κB, and TNF-α immunostaining was observed in both liver and kidney tissues in the LPS and LPS-resveratrol groups (Figures 3A and 4A). When compared with the LPS group, it was observed that the expression of these proteins in the kidney and liver tissues of the LPS-resveratrol group decreased (Figures 3B and 4B; $p < .05$).

3.3 | ELISA findings

While the MDA levels observed in the resveratrol and control groups were lower than those in the LPS-administered groups, a decrease in MDA levels was observed in the LPS-resveratrol group compared to LPS (Figure 5A,B).

3.4 | AgNOR staining results

The mean AgNOR number and TAA/NA values are shown in Figure 6. When compared with the control group, it is seen that there is an increase in AgNOR numbers and TAA/NA ratios in LPS and LPS + resveratrol groups in liver tissue. In kidney tissue, there was

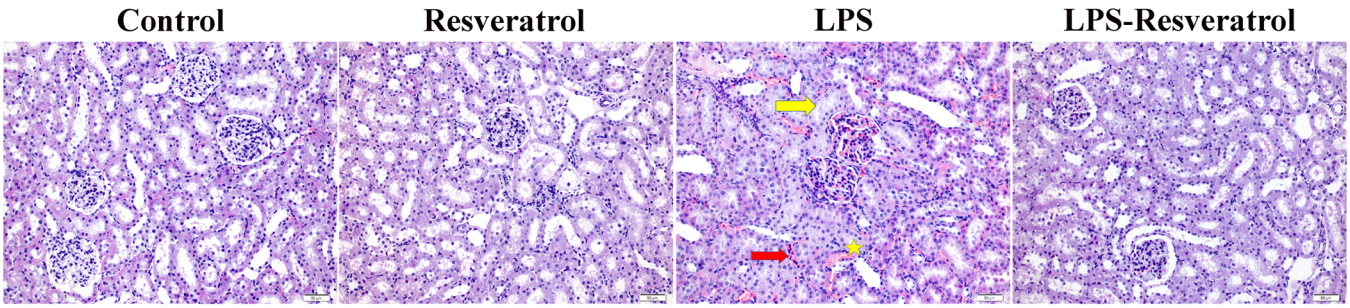

FIGURE 1 Hematoxylin and eosin staining images of kidney tissues of the experimental groups. The red arrow indicates inflammation, the yellow arrow indicates vacuolization, and the star indicates hemorrhage. Scale bar = 50 μm, magnification ×20. LPS, lipopolysaccharide.

TABLE 1 Damage rates were observed in the kidney tissues of the experimental groups.

	Control	Resveratrol	LPS	LPS-resveratrol
Hemorrhage	0.61 ± 0.06	0.52 ± 0.04	1.41 ± 0.23*	0.68 ± 0.05
Vacuolization	0.58 ± 0.06	0.63 ± 0.04	1.75 ± 0.10*	0.93 ± 0.08*
Inflammation	0.28 ± 0.07	0.32 ± 0.05	0.72 ± 0.07*	0.44 ± 0.03*
Glomerular damage	0.44 ± 0.03	0.42 ± 0.03	1.74 ± 0.19*	1.01 ± 0.12*

Note: Two-way analysis of variance and Tukey's multiple comparison test were applied and compared with the control group.

Abbreviation: LPS, lipopolysaccharide.

*$p < .05$ represents a statistically significant difference from the control group.

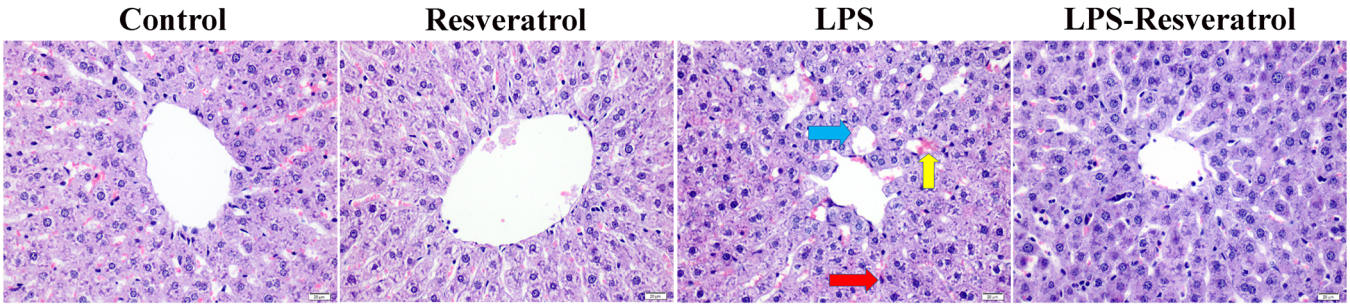

FIGURE 2 Hematoxylin and eosin staining images of liver tissues of the experimental groups. Red arrow indicates pycnotic hepatocytes, yellow arrow indicates bleeding, and blue arrow sinusoidal enlargement. Scale bar = 20 μm; magnification ×40. LPS, lipopolysaccharide.

TABLE 2 Rate of damage observed in the liver tissues of the experimental groups.

	Control	Resveratrol	LPS	LPS-resveratrol
Pycnotic hepatocytes	0.36 ± 0.07	0.34 ± 0.08	1.62 ± 0.26*	0.81 ± 0.18*
Hemorrhage	0.41 ± 0.15	0.49 ± 0.10	0.51 ± 0.13*	0.77 ± 0.11*
Sinusoidal enlargement	0.39 ± 0.08	0.49 ± 0.08	1.65 ± 0.22*	0.75 ± 0.11*
Inflammation	0.28 ± 0.09	0.41 ± 0.05	0.75 ± 0.09*	0.55 ± 0.08*

Note: Two-way analysis of variance and Tukey's multiple comparison test were applied and compared with the control group.

Abbreviation: LPS, lipopolysaccharide.

*$p < .05$ represents a statistically significant difference from the control group.

no significant difference between the groups in terms of AgNOR numbers and TAA/NA ratio (Figure 6A,B).

3.5 | mRNA analysis results of *Nf-κB*, *TLR4*, *TNF-α*, *IL-1β*, and *IL-6*

The mRNA levels of *Nf-κB*, *TLR4*, *TNF-α*, *IL-1β*, and *IL-6* genes expressed in liver and kidney tissues of control rats (only SF, 1 mL/day) and liver and kidney tissues of rats exposed to LPS (30 mg/kg), resveratrol (200 mg/kg), LPS + resveratrol (for 10 days 200 mg/kg dose resveratrol and on the 10th day 30 mg/kg LPS) were analyzed by real-time PCR. Alteration of *Nf-κB*, *TLR4*, *TNF-α*, *IL-1β*, and *IL-6* genes mRNA levels of each application group tissues was determined according to the mRNA levels of *Nf-κB*, *TLR4*, *TNF-α*, *IL-1β*, and *IL-6* genes expressed in control tissues. The *GAPDH* gene was used as a reference gene for normalization.

3.6 | Liver

The mRNA levels of the *Nf-κB* gene were altered in the livers of LPS, resveratrol, and LPS + resveratrol groups compared to the control group (1.79, 0.29, 3.25; fold regulation value, respectively) ($p > .05$). The mRNA levels of the *TLR4* gene were downregulated in the livers of LPS, resveratrol, and LPS + resveratrol groups compared to the control group (0.48, 0.32, 0.69; fold regulation value, respectively). This downregulation was

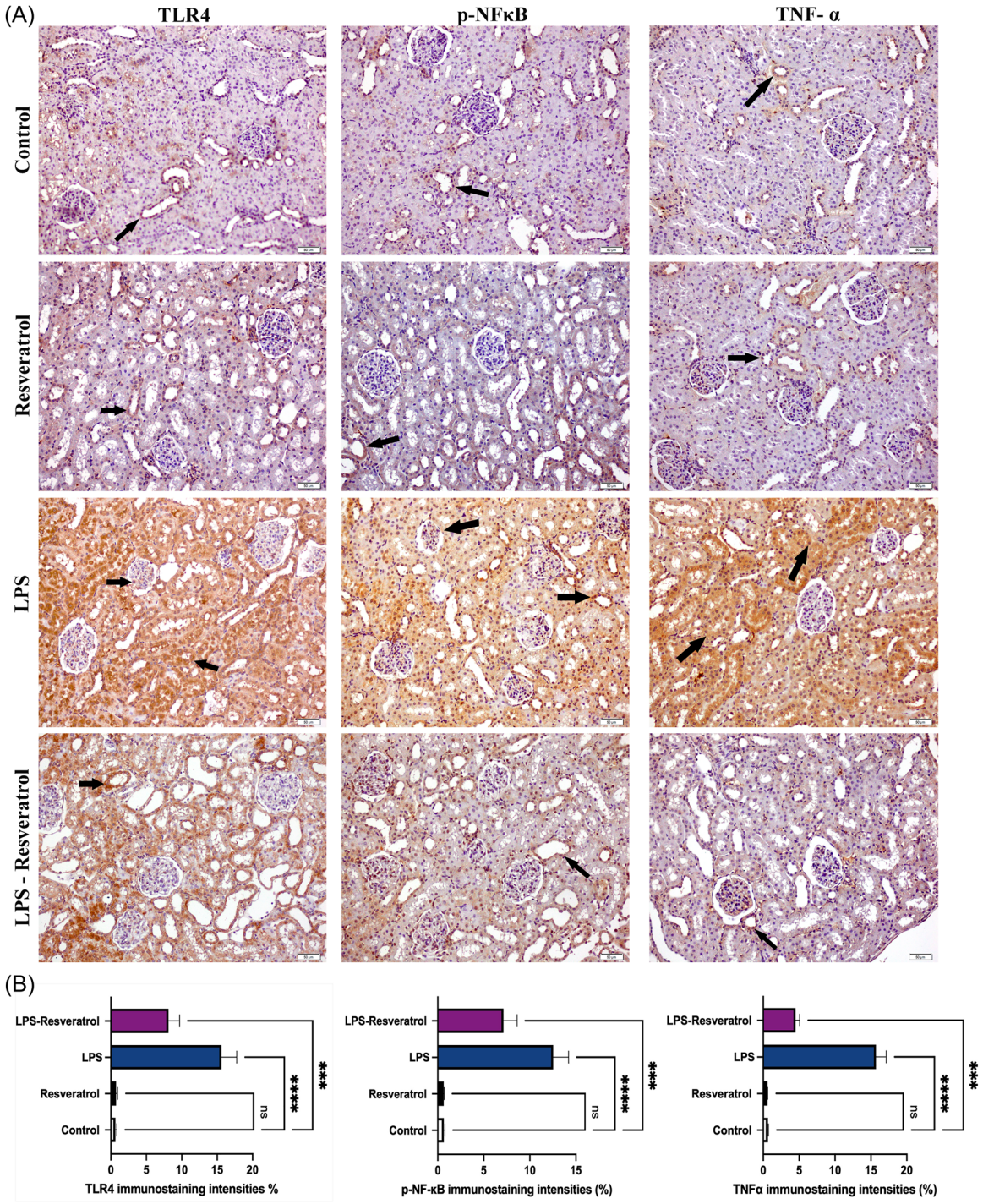

FIGURE 3 Toll-like receptor 4 (TLR4), nuclear factor-κB (NF-κB), and tumor necrosis factor-α (TNF-α) protein expression images in the kidney (A). The data presented in the bar graphs showing the immunostaining intensities of the studied proteins are expressed as mean ± SD (B). One-way analysis of variance (ANOVA) and Dunnet's multiple comparison test were applied to the control group (***$p = .0003$; ****$p < .0001$ indicates a statistically significant difference). LPS, lipopolysaccharide; ns, not significant.

statistically significant in the resveratrol group ($p < .001$). The mRNA levels of the *TNF-α* gene were upregulated in the livers of LPS, resveratrol, and LPS + resveratrol groups compared to the control group (17.73, 2.66, 15.01; fold regulation value, respectively) These upregulations were statistically significant in LPS and LPS + resveratrol groups ($p < .001$). The mRNA levels of the *IL-6* gene were upregulated in the livers of LPS, resveratrol, and LPS+ compared to the control group (15.76, 2.82, 20.51; fold

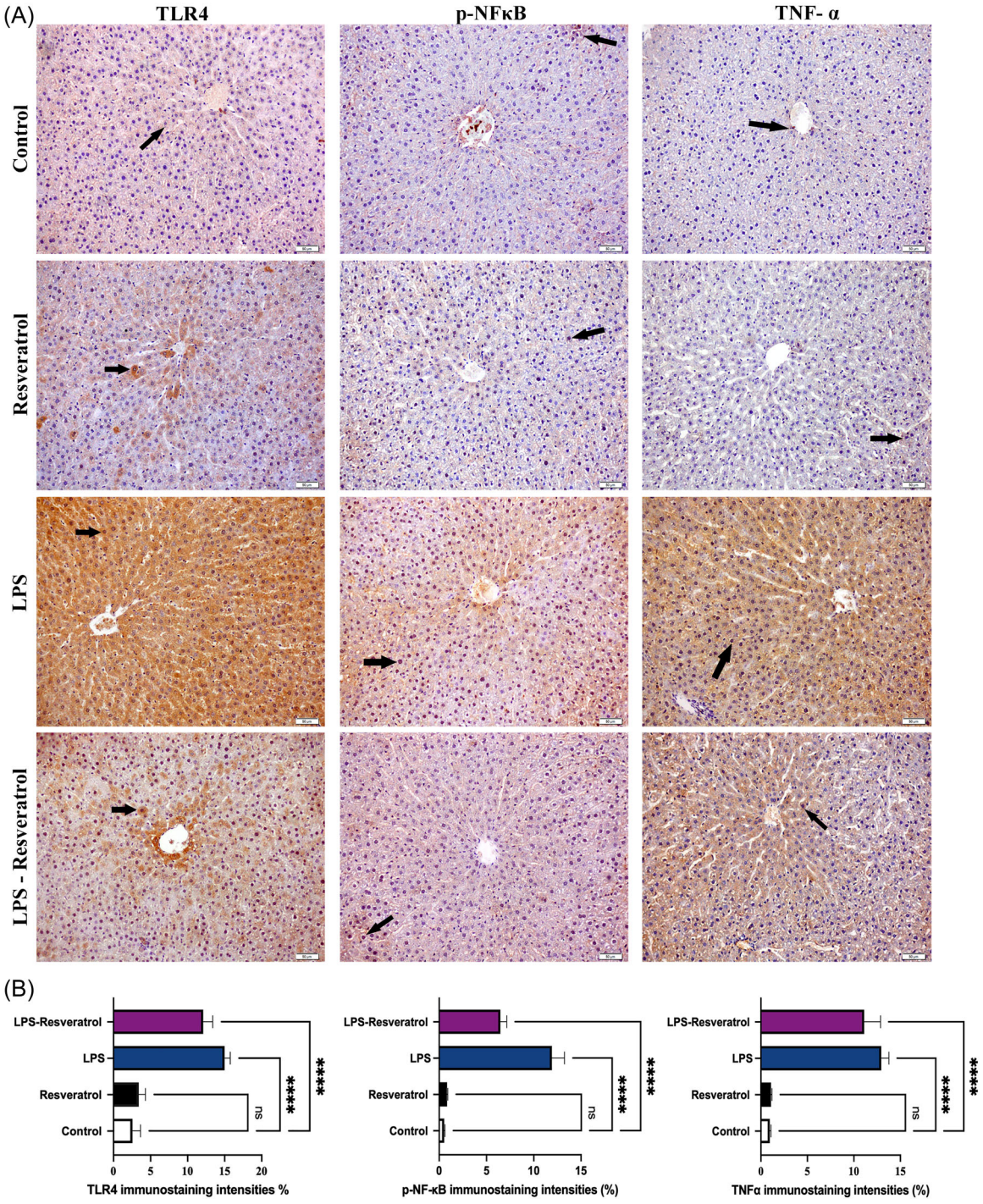

FIGURE 4 Toll-like receptor 4 (TLR4), nuclear factor-κB (NF-κB), and tumor necrosis factor-α (TNF-α) protein expression images in the liver (A). The data presented in the bar graphs showing the immunostaining intensities of the studied proteins are expressed as mean ± SD (B). One-way analysis of variance (ANOVA) analysis of variance and Dunnet's multiple comparison test were applied to the control group (****$p < .0001$ indicates a statistically significant difference).

regulation value, respectively) ($p < .001$). The mRNA levels of the *IL-1β* gene were upregulated in the livers of LPS, resveratrol, and LPS + resveratrol groups compared to the control group (6.10, 1.69, 4.82; fold regulation value, respectively). These upregulations were statistically significant in LPS and LPS + resveratrol groups ($p < .001$) (Figure 7).

3.7 | Kidney

The mRNA levels of the *Nf-κB* gene were altered in the kidneys of the LPS, resveratrol, and LPS + resveratrol groups compared to the control group (2.56, 0.64, 1.63; fold regulation value, respectively) ($p > .05$). The mRNA levels of the *TLR4* gene were downregulated in

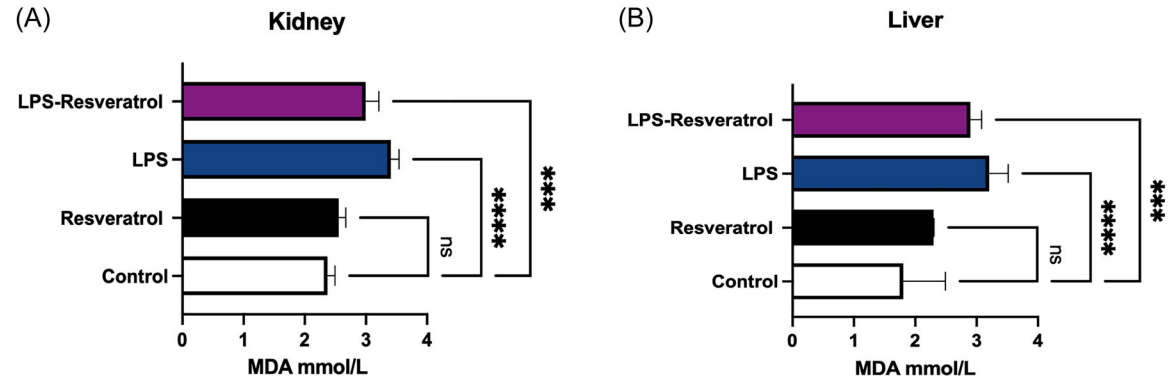

FIGURE 5 Malondialdehyde (MDA) levels in the kidney (A) and liver (B) tissues. Data presented in bar charts are expressed as mean ± SD. One-way analysis of variance (ANOVA) and Dunnet's multiple comparison test were applied to the control group (for kidney, ***p = .0004, ****p < .0001; for liver ***p = .0005, ****p < .0001 indicates a statistically significant difference). LPS, lipopolysaccharide; ns, not significant.

the kidneys of the LPS, resveratrol, and LPS + resveratrol groups compared to the control group (0.43, 0.26, 0.5; fold regulation value, respectively). These downregulations were statistically significant in the resveratrol group (p < .001). The mRNA levels of the *TNF-α* gene were upregulated in the kidneys of the LPS, resveratrol, and LPS + resveratrol groups compared to the control group (3.61, 22.16, 42.46; fold regulation value, respectively). These upregulations were statistically significant in RES, and LPS + resveratrol groups (p < .001). The mRNA levels of the *IL-6* gene were upregulated in the kidneys of the LPS, resveratrol, and LPS + resveratrol groups compared to the control group (12.13, 7.75, 16.54; fold regulation value, respectively) (p < .001). The mRNA levels of the *IL-1β* gene were upregulated in the kidneys of the LPS, resveratrol, and LPS + resveratrol groups compared to the control group (5.67, 5.50, 14.07; fold regulation value, respectively) (p < .001) (Figure 8).

4 | DISCUSSION

Monocytes, macrophages, dendritic cells (DCs), neutrophils, and natural killer (NK) cells play an important role in maintaining the innate immune system.[20] These immune cells can recognize and respond to invasion by providing effective protection by specific receptors such as TLRs.[20]

TLRs are potent activators of the inflammatory response. TLR activation can produce cytokines, chemokines, and interferons, and the transcription factor NF-κB. NF-κB pathways play an important role in various diseases by regulating immunity, proliferation, differentiation, and apoptosis. Due to its important role in the initiation of the inflammatory response, TLR4 is a potential therapeutic target for sepsis. Therefore, we targeted TLR4/NF-κB and TNF-α signaling pathways in our study.

In the devastating clinical picture of sepsis, inflammatory mediators can regulate the inflammatory response. In addition, various molecules such as neutrophils secreted by inflammatory mediators can increase inflammation in target organs. In this respect, pro- and anti-inflammatory mediators are also associated with various lethal conditions such as multiple organ failure in sepsis, vessel damage, and pathologies in the brain. New therapeutic approaches to these mediators may help reduce the high mortality rate of sepsis.

Resveratrol stands out as one of the substances that can be used for this purpose. Resveratrol is a natural polyphenol compound, a SIRT-1 activator with anti-inflammatory, antiviral, antibacterial, and antifungal inhibitory abilities, as well as cardiovascular and antitumor protective effects.[6] Administration of resveratrol in endotoxemic rats can reduce the occurrence of oxidative damage by inhibiting erythrocyte lipid peroxidation and catalase (CAT) activity, reducing NO release, downregulating MDA levels, and maintaining iron homeostasis.[5]

In addition, Luo et al.[21] showed that resveratrol can reduce kidney injury by inhibiting NF-κB activation and reducing endoplasmic reticulum stress in a septic rat model. In addition, in another animal experiment, it was found that resveratrol could decrease LPS-induced cytokine production, decrease IL-1β, IL-6, monocyte chemoattractant protein-1, and TNF-α concentrations in plasma and kidneys, and reduce renal tubular vacuole changes and pathological apoptosis.[22]

Nucleolar regulatory regions (NORs) are DNA loops located in cell nuclei where ribosomal RNA (rRNA) genes are located. Therefore, the number and appearance of NORs reflect the nuclear activity of the cells. Studies have shown that the number and size of NOR-related proteins reflect RNA activity. It has been determined that NOR numbers reflect cell activity increase in malignant events.[15,23] The NORs of metaphase chromosomes are associated with a group of acidic proteins called the argyrophilic NOR region (AgNOR) proteins. When viewed with a light microscope, silver-stained AgNOR areas appear as black dots that can be measured to quantify rRNA transcription activity.[23] The size and number of AgNOR correlate with the proliferation capacity of cells.[24,25] In our study, we used the AgNOR staining method to determine the amount of tissue damage in liver and kidney tissues in the LPS-induced sepsis model. Data from the total AgNOR number and TAA/core area (NA) ratio were used in the evaluation. In the data obtained, it was

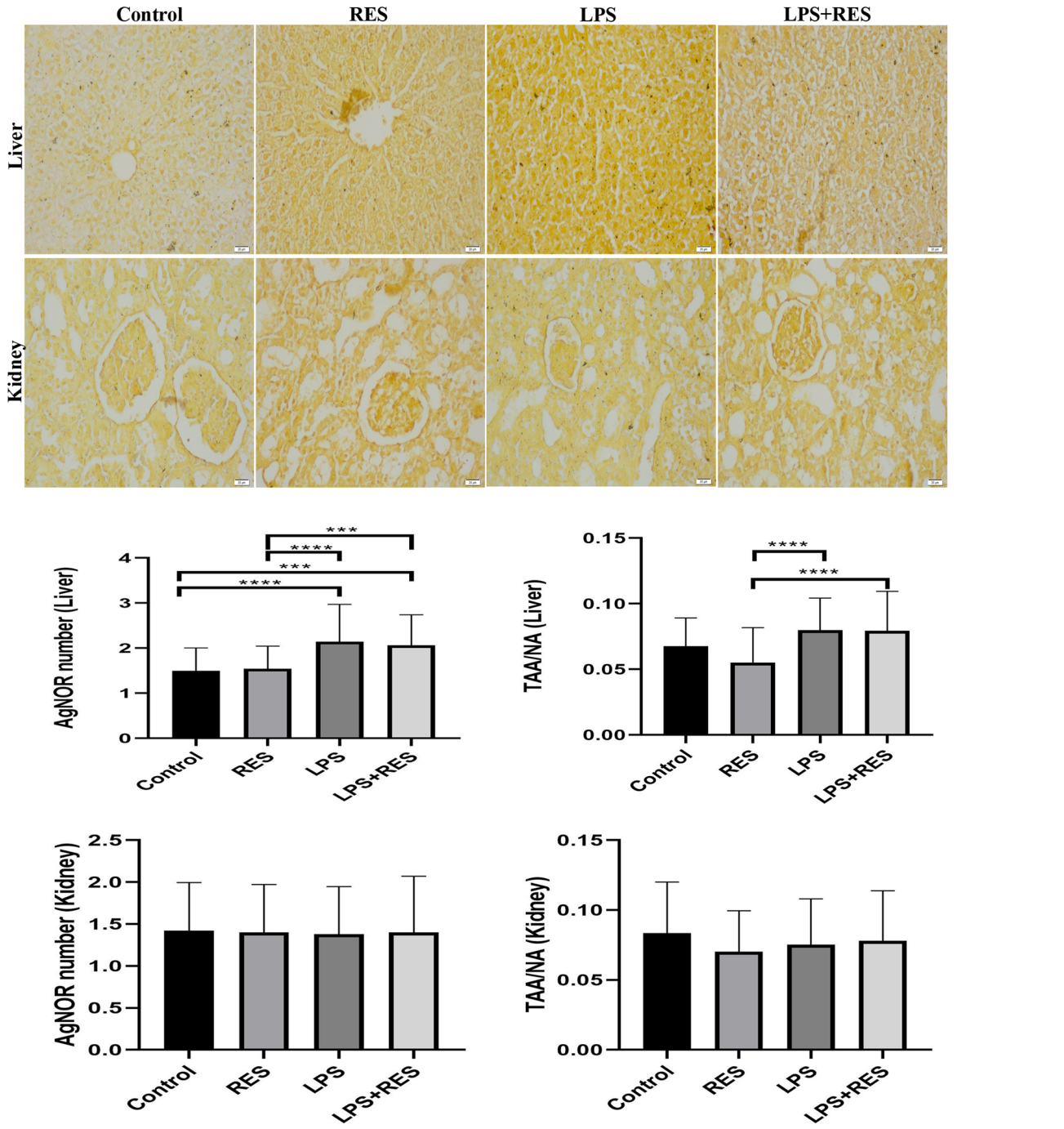

FIGURE 6 Preparations of liver and kidney tissues stained with AgNOR (argyrophilic nucleolar organizer regions) staining method (A). Data presented in bar charts are expressed as mean ± SD. One-way analysis of variance (ANOVA) and Dunnet's multiple comparison test were applied to the control group (B). ****$p < .0001$ and ***$p < .005$ indicates a statistically significant difference. LPS, lipopolysaccharide; RES, resveratrol; TAA/NA, total AgNOR area/nuclear area.

observed that there was a significant increase in the AgNOR numbers and TAA/NA ratio in the liver tissue of the LPS and LPS + RES groups when compared to the control group.

Sepsis negatively affects public health and poses a serious burden on the health sector and the country's economies. Therefore, it is vital to develop new strategies for the treatment of sepsis to reduce mortality and shorten the length of hospital stay. There are no Food and Drug Administration-approved specific therapeutic agents for the treatment of sepsis in the current literature. Accordingly, complications in experimental sepsis can be attenuated by therapeutic approaches that reduce the levels of proinflammatory mediators or modulate immune responses.[26] Evaluating the dynamic processes that create and regulate pro- and anti-inflammatory responses in sepsis may contribute to cytokine-based treatment approaches.

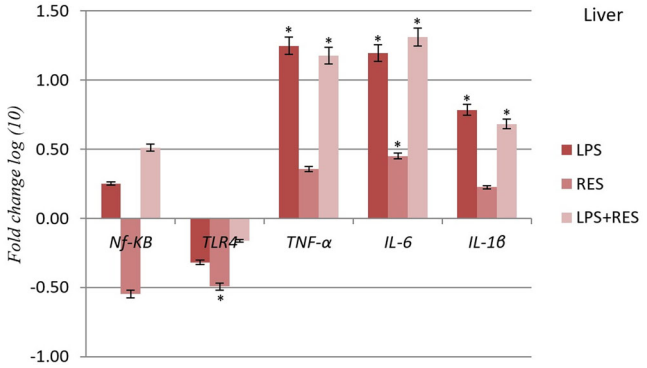

FIGURE 7 Results of real-time PCR analysis. Relative messenger RNA (mRNA) expression of *Nf-κB*, *TLR4*, *TNF-α*, *IL-1β*, and *IL-6* genes in livers exposed to lipopolysaccharide (LPS), resveratrol (RES), and LPS + RES were given as fold regulation levels, log(10). *Significance of $p < .001$ compared to the control. *GAPDH* is a reference gene for normalization.

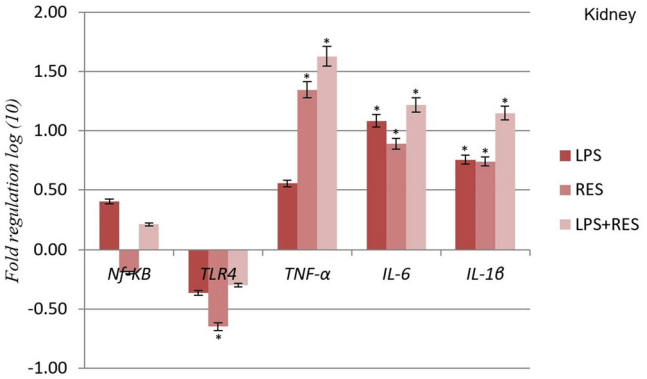

FIGURE 8 Results of real-time PCR analysis. Relative messenger RNA (mRNA) expression of *Nf-κB*, *TLR4*, *TNF-α*, *IL-1β*, and *IL-6* genes in kidneys exposed to lipopolysaccharide (LPS), resveratrol (RES), and LPS + RES were given as fold regulation level, log(10). *Significance of $p < .001$ compared to the control. *GAPDH* is a reference gene for normalization.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Züleyha Doğanyiğit http://orcid.org/0000-0002-6980-3384
Seher Yilmaz http://orcid.org/0000-0003-4551-995X

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