Histopathological and biochemical effects of 18β-glycyrrhetinic acid application on lipopolysaccharide-induced kidney toxicity in rats

ABSTRACT

Lipopolysaccharide (LPS) is an endotoxin found in the wall of gram-negative bacteria and causes acute inflammation when it enters the tissues. 18β-glycyrrhetinic acid (18β-GA) is a substance found in licorice root and is responsible for this plant's antiallergic, antioxidant, and anti-inflammatory activity. This study aimed to examine the possible effects of 18β-glycyrrhetinic acid on the damage caused by LPS in kidney tissue. The study was divided into six equal groups containing 48 Sprague Dawley adult male rats (n = 8). The groups were created as follows; the Control group; the group that received 1cc physiological saline throughout the experiment was the DMSO group; DMSO, an intraperitoneal carrier substance, was given. LPS group; A single dose of 7.5 mg/kg intraperitoneal (i.p) LPS was administered. 18β-GA50+LPS group; 18β-glycyrrhetinic acid was given by gavage at 50 mg/kg daily for 10 days, followed by a single dose of 7.5 mg/kg i.p. LPS was administered. 18β-GA100+LPS group; 18β-glycyrrhetinic acid was administered by gavage at 100 mg/kg daily for 10 days, followed by a single dose of 7.5 mg/kg i.p. LPS was administered. 18β-GA100 group; 18β-glycyrrhetinic was given by gavage at 100 mg/kg daily for 10 days. 24 hours after LPS application to all groups, the kidney tissues of the rats were removed under anesthesia and placed in 10% formaldehyde. Histopathological and oxidative stress parameters analyses were performed in kidney tissue. These findings raised the possibility that 18β-GA could be an adjuvant therapy that protects kidney tissue from LPS-induced oxidative and tissue damage effects and reduces its side effects.

Keywords: Histopathology, kidney, lipopolysaccharide, oxidative stress, rat

INTRODUCTION

Sepsis physiopathology is a whole of complex mechanisms that begins with an excessive cellular immunological response against the infection focus that initiates the sepsis process and then damages the host at the level of organs and systems (Uchino et al., 2005). Mediators and cytokines that play a role in intercellular signaling play an important role in the sepsis formation process (Neveu et al., 1996; Silvester et al., 2001). Bacterial products called pathogen-associated molecular structures (PAMPs) can be detected and recognized by the body's natural immunity (Lopes et al., 2009; Oppert et al., 2008). Lipopolysaccharides (LPS) located in the cell walls of gram-negative bacteria are one of the most important PAMPs and play a very important role in triggering the septic process (Cunningham et al., 2002; Knotcke et al., 2001). The event that initiates septic shock is the passage of LPS or toxic cell wall components into the organism's circulatory system as a result of the lysis of bacteria (Morelli et al., 2013). LPS stimulates signaling pathways that lead to the synthesis and

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Research Article

Elif Erbaş[1a]
Volkan Gelen[2b]
Seda Yakut[3c]
Kevser Albayrak[4d]

[1]Atatürk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Erzurum, Türkiye

[2]Kafkas University, Faculty of Veterinary Medicine, Department of Physiology, Kars, Türkiye

[3]Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Burdur, Türkiye

[4]Erzurum Technical University, Molecular Biology and Genetics, Erzurum, Türkiye

ORCİD-

[a]0000-0003-1750-3889

[b]0000-0002-5091-1262

[c]0000-0003-1673-5661

[d]0009-0003-1014-485X

Correspondence
Elif Erbaş
eliferbas4154@gmail.com

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release of cytokines and other mediators. Thus, TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) are released from monocytes. IL-1 and IL-6 activate T cells and ensure the secretion of interferon gamma (IFN-γ), interleukin-2 (IL-2) and interleukin-4 (IL-4) (Hagiwara et al., 2009). Mediators such as TNF-α and IL-1 are released within 30 minutes after the appearance of LPS and cause the release of secondary cytokines, lipid mediators, and reactive oxygen metabolites, as well as initiating the release and synthesis of arachidonic acid metabolites, which are extremely important in sepsis (Mori et al., 2011). Since the events occurring in sepsis can affect the entire organism, this situation may extend to multiple organ failure. The most common organ failures in sepsis are lung, kidney, liver, and heart failure (Ogura et al., 2014). Various recent studies have shown that the use of agents with antioxidant and anti-inflammatory effects prevents organ damage in kidney damage occurring in the LPS-induced sepsis model (Gomez et al., 2014). Various studies have reported that Glycyrrhiza glabra L. (licorice root) has antioxidant and anti-inflammatory effects (Eisenbrand, 2006; Kang et al., 2014). The reason why this plant exhibits the mentioned properties is due to the many biological compounds found in its structure (Hasan et al., 2015; Mahmoud and Al Dera**,** 2015; Wu et al., 2015). Its main component is glycyrrhizin, which makes up approximately 10% of the dry weight of licorice root. Glycyrrhizin is a glycyrrhetinic acid glycoside containing two glucuronic acid residues. After oral administration, glycyrrhizin is rapidly and almost completely metabolized to glycyrrhetinic acid by intestinal bacteria (Ishii et al., 2000; Ma et al., 2016). Glycyrrhetinic acid, specifically 18β-Glycyrrhetinic acid, is the main active metabolite of glycyrrhizin and is responsible for most pharmacological properties. Studies have demonstrated the pharmacological and health-promoting effects of 18β-Glycyrrhetinic acid, including antioxidant, anti-inflammation, anticancer, and metabolic regulation (Itoh et al., 1999; Kalaiarasi and Pugalendi, 2009; Young, 1995; Zeller et al., 1984). In line with all this information, present study aims to introduce the possible protective effects of 18β-GA in the LPS-induced acute kidney toxicity model in rats, which has not yet been reported in the literature, and to contribute to filling the gap in this field.

MATERIALS AND METHODS

In the present research, we were studied the renal toxicity model induced by LPS (*O55*:*B5*, Sigma-Aldrich) (7.5 mg/kg, i.p., single dose) in rats, and 18β-GA (Cayman Chemical Company-11845) (50 mg/kg) and 18β-GA (100 mg/kg, dose i.g., 10 days) was applied. Experimental animals were obtained from Atatürk University Medical Experimental Research and Application Center. Rats were fed ad-libitum until the time of study and kept in a ventilated environment with a 12-hour light-dark cycle and a room temperature of approximately 25°C. To provide sufficient kidney tissue samples in each group, 8 rats were used, and 6 groups were formed. A total of 48 12-week-old adult Sprague Dawley male rats weighing 220-250 g were used. The experimental groups were formed as presented in Table 1 and the experimental procedure was applied as written.

All animals were subjected to standard care and feeding conditions. At the end of the experimental applications, after the live weight of the rats was weighed, kidney tissues were taken following intracardiac blood collection and cervical dislocation under sevoflurane anesthesia. After weighing these tissues, a portion of the kidney tissue of 8 rats from each group was immediately placed in 10% formaldehyde after washing with physiological saline for histopathological examinations. The remaining part of the kidney tissue of the rats was washed with physiological saline and then immediately placed in liquid nitrogen and frozen until biochemical analysis.

Table 1: Experimental groups and experimental procedure.

Number of groups	Number of animals	Application
Group 1 (n=8)	Control	i.p saline 10 days
Group 2 (n=8)	DMSO	0.1 ml i.p DMSO injection
Group 3 (n=8)	LPS	7.5mg/kg i.p LPS single dose
Group 4 (n=8)	18β-GA50+LPS	18β-GA at 50 mg/kg i.g dose for 10 days and 7.5mg/kg i.p LPS as a single dose for 10 days
Group 5 (n=8)	18β-GA100+LPS	18β-GA at 100 mg/kg i.g dose for 10 days and 7.5mg/kg i.p LPS as a single dose for 10 days
Group 6 (n=8)	18β-GA100	18β-GA at 100 mg/kg i.g dose for 10 days

Biochemical analyzes

At the end of the experiment, 50 mg of kidney tissue obtained from rats was weighted and homogenized with tissue homogenate buffer at 30 hz for 3 minutes in tissue liser (Qiagen TissueLyser II). It was then centrifuged at 12000 rpm at 4°C for 15 minutes. The supernatant obtained was taken and GSH analysis was performed according to Sedlak et al., 1968. For MDA analysis, 50 mg of kidney tissue obtained from rats at the end of the experiment was weighted and homogenized with tissue homogenate buffer at 30 hz for 3 minutes in tissue liser (Qiagen TissueLyser II). It was then centrifuged at 4000 rpm at 4°C for 15 minutes. The supernatants obtained were analyzed according to the method of Ohkawa et al., (1979).

Histopathological analysis

Rat kidney tissues obtained at the end of the experiment were placed in 10% neutral formaldehyde solution and fixed for 72 hours. Then, they were passed through graded alcohol and xylol series and embedded in paraffin blocks and 5µ thick sections were taken with a microtome device (Leica RM2125 RTS) for histopathologic evaluations. For histopathologic examination, tissue damage was evaluated by staining the sections with Mallory's Triple Staining method modified by Crossman. Each section was scored from 0 to 4 to evaluate histopathologic damage in the kidney tissue. 0 indicates no tissue damage, 1 indicates mild damage, 2 indicates moderate damage, 3 indicates severe damage and 4 indicates very severe damage (Niu et al., 2019). A trinocular microscope (Zeiss AXIO Scope.A1, German) with computer and camera attachment was used for microscopic examination.

RESULTS

Biochemical results

When the MDA level was compared between the groups, we observed that the kidney tissue MDA level of the LPS-treated groups increased significantly compared to the control and other groups. On the other hand, we determined that the application of 18β-GA prevented this LPS-induced increase. When the GSH level was compared between the groups, the kidney tissue GSH level of the LPS-treated groups increased significantly and decreased compared to the control and other groups. On the other hand, we determined that 18β-GA application prevented this LPS-induced decrease. Biochemical results of all groups are presented in Figure 1.

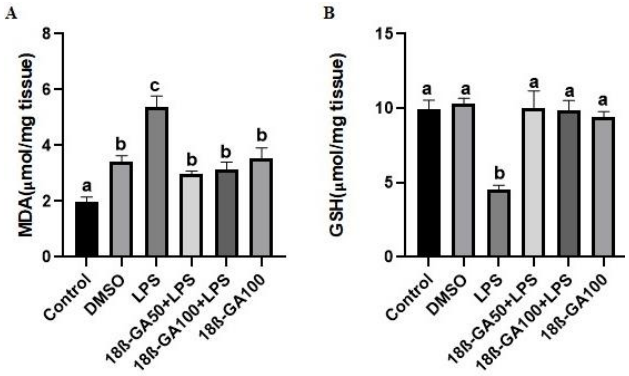

Figure 1. The effects of LPS and 18β-GA administration on MDA (A), and GSH (B) levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group.

Histopathological results

In the current study, kidney tissue was observed to be normal in the Control, DMSO, and 18β-GA100 groups, and glomerular and tubules in the cortex and medulla tubules were observed to be healthy. In the LPS group, it is observed that

the normal structure of the kidney is completely disrupted and the glomerul and tubules lose their normal structure. It is observed that the Bowman space in the glomerul is widened and the glomerular tangle shrinks and exhibits a degenerative appearance. Widespread hemorrhagic areas are noted in the cortex and medulla.

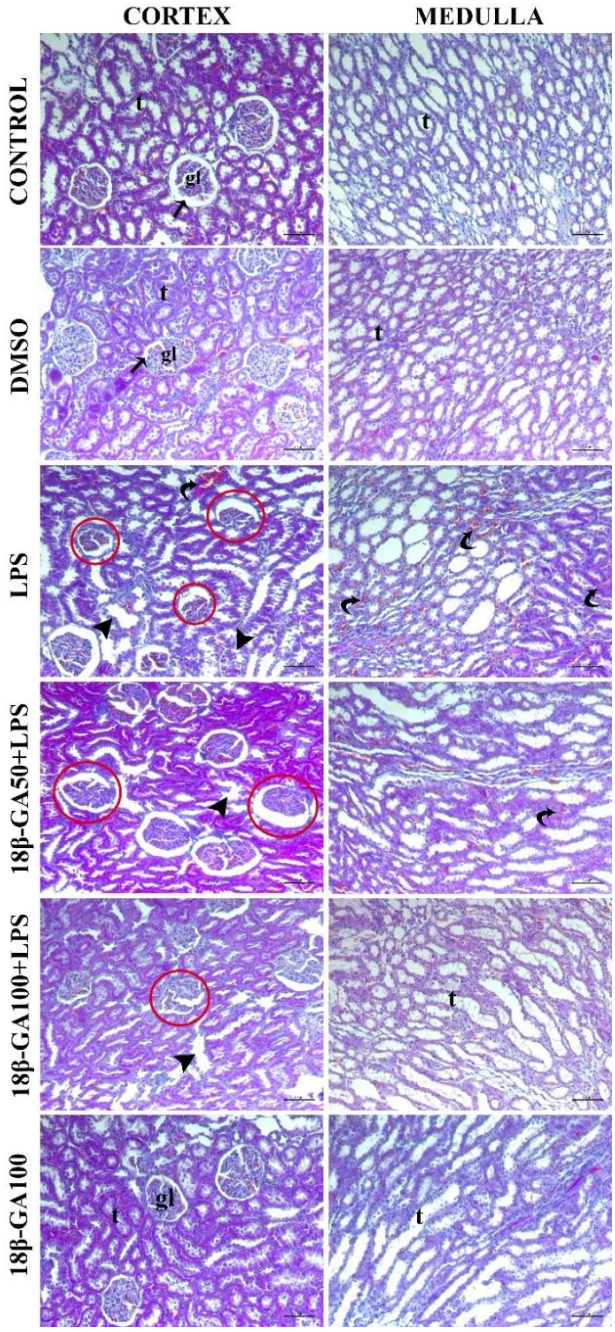

Figure 2. Kidney tissues stained with Mallory's Triple Stain Modified by Crossman. gl: Renal glomerulus, t: Renal tubules, Arrow: Bowman's space, Red circle: Degenerative glomerulus, Arrowhead: Degenerative tubule, Curved arrow: Hemorrhagic area.

While the recovery is better, especially in the 18β-GA100+LPS group, a near-normal appearance is observed in the glomerular and tubules in the 18β-GA50+LPS group. It is noteworthy that the Bowman space is normal and the degeneration in the tubules is reduced. Hemorrhagic areas have decreased considerably. Histopathological evaluation results of all groups are presented in Figure 2 and 3.

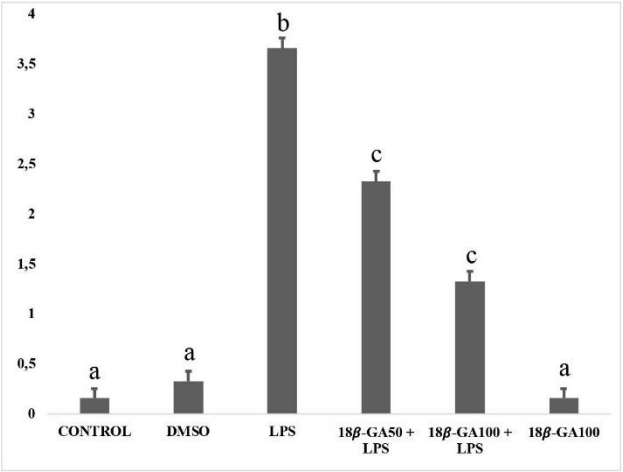

Figure 3. Assessment of renal histopathology.

DISCUSSION

LPS is the structure found in the cell wall of gram-negative bacteria and is responsible for the inflammation and apoptosis caused by these bacteria in the tissue (Hayashi et al., 2001; Tsao et al 2004). LPS administration causes toxicity in the lung, brain, kidney, and testicular tissues as well as the liver (Boveris and Cadenas, 1997; Kadkhodaee and Osami, 2004; Tiwan et al., 2005). If toxicity develops in organs, disruptions in the physiological functions of the organ, loss of function, and organ failure occur (Gündoğdu et al., 2023; Iguchi et al., 1992; Kobayashi et al., 2015). The effects of 18β-GA, a flavonoid compound with antioxidant and anti-inflammatory effects, in experimental organ toxicity models have been reported in many studies (Kao et al., 2010). In the present study, the possible effects of 18β-GA on LPS-induced oxidative stress and tissue damage were investigated.

Imbalances in the typical cellular redox state cause perturbations in biological components such as lipids, proteins, and DNA (Gelen et al., 2023). The extent of ROS production determines the extent of cell membrane damage and leads to the occurrence of lipid peroxidation through oxidative modification of polyunsaturated fatty acids within the composition of the membrane (Alwazeer, 2023; Kara et al., 2016). In the present study, MDA, one of the lipid peroxidation indicators, increased, and 18β-GA application significantly reduced the MDA level. Oxidative stress can be defined as the disproportion between oxidant and antioxidant defense systems. MDA is a suitable lipid peroxidation biomarker (Kara et al., 2023). The elevation observed after LPS kidney injury was significantly attenuated by oral dosage of 18β-GA. Oxidative stress can be described as the disruption of the balance between the mechanisms that produce oxidants and the mechanisms that provide antioxidant protection. The production of reactive oxygen species (ROS) effectively counteracts both enzymatic (such as SOD, GSH-Px, and CAT) and non-enzymatic (such as GSH) antioxidant defenses (Gelen et al., 2021; Gelen et al., 2023). LPS significantly decreased GSH levels while increasing MDA levels in kidney tissue. In a study, it was determined that 18β-GA had an antioxidant role in nephrotoxicity in rats (Abd El-Twab et al., 2016). In the present study, we determined that LPS induces oxidative stress in kidney tissue and 18β-GA application prevents these changes.

In some previous studies, it was observed that LPS application caused congestion, interstitial edema, degeneration of cells, necrosis and calcification in rat kidney tissue (Ban et al., 2022). In the present study, LPS application caused the integrity of the kidney tissue to completely deteriorate and the glomerulus to lose its normal structure. In previous studies, it was determined that LPS administration caused damage to kidney tissue (Raghavan and Weisz, 2015). The data obtained in these studies are compatible with the data obtained in the present study. On the other hand, it was determined that 18β-GA application significantly prevented these changes. Various studies have shown that 18β-GA application prevents kidney tissue damage caused by some toxic agents. These data are compatible with the data we obtained.

CONCLUSION

In conclusion, the findings obtained in this study show that LPS administration triggers ROS production and causes kidney tissue damage. These findings raised the possibility that 18β-GA could be an adjuvant therapy that protects kidney tissue from LPS-induced oxidative and tissue damage effects and reduces its side effects.

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Author contributions: Research and technique and writing innovative validation software; EE, VG, SY, and KA. Writing: EE, VG, SY, and KA; preparing the initial draft Conceptualization: Every author has reviewed and approved the published version of the study.

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