ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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IRBESARTAN REDUCES LIVER DAMAGE INDUCED BY LIPOPOLYSACCHARIDE VIA INHIBITION OF TOTAL OXIDANT STATUS, INTERLEUKIN-1B AND CASPASE-3 LEVELS

İRBESARTAN LİPOPOLİSAKARİT TARAFINDAN İNDÜKLENEN KARACİĞER HASARINI, TOPLAM OKSİDAN DURUMU, İNTERLÖKİN-1B VE KASPAZ-3 SEVİYELERİNİN İNHİBİSYONU YOLUYLA AZALTIR

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Öz

Amaç

Septik koşullarda hiperinflamatuar yanıta ve hepatotoksisiteye; oksidatif stres, inflamasyon ve apoptoz neden olur. Bir adrenerjik reseptör blokeri olan irbesartan (IB), antiinflamatuar ve antioksidan özelliklere sahiptir. Bu çalışmada, IB'nin lipopolisakkarit (LPS) kaynaklı akut hepatotoksisite üzerindeki koruyucu etkisinin araştırılması amaçlandı.

Gereç ve Yöntem

Üç grupta toplam sekiz sıçan kullanıldı; kontrol grubu, LPS grubu [5 mg/kg, intraperitoneal (IP)]; ve LPS + IB grubu [5 mg/kg LPS (IP) + 50 mg/kg IB (oral)]. Sakrifikasyondan sonra interlökin-1 beta (IL-1β), kaspaz-3 (Cas-3) alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), oksidatif stres indeksi (OSI), toplam oksidan durumu (TOS) ve toplam antioksidan durumu (TAS) gibi immünohistokimyasal ve biyokimyasal değerlendirmeler için karaciğer ve kandan dokular alındı.

Bulgular

Kontrol grubuyla karşılaştırıldığında kanda AST ve ALT düzeylerinde artış, biyokimyasal olarak dokuda TOS ve OSI düzeyinde artış ve TAS düzeyinde azalma, immünohistokimyasal olarak IL-1β, Cas-3 düzeyinde artış tespit edildi. Ayrıca LPS grubunda karaciğer dokusunda histopatolojik olarak hiperemi, kanama, vakuolizasyon ve belirgin nötrofil infiltrasyonu saptandı. IB tüm bu bulguları tersine çevirdi. IB uygulaması ile TAS seviyeleri yükselirken, TOS ve OSI seviyeleri azaldı ($p = 0.001$). IB ayrıca AST ve ALT değerlerini de düşürdü ($p = 0.001$). IB grubunda, Cas-3 ve IL-1β seviyeleri, IB uygulamasıyla önemli ölçüde azaldı ($p = 0.001$). Ek olarak, IB, artmış hiperemi, kanama, vakuolizasyon ve önemli nötrofilik lökosit infiltrasyon gibi histopatolojik bulguları iyileştirdi ($p = 0.001$). IB tedavisi, antioksidan, antienflamatuar ve antiapoptotik özellikleriyle LPS'nin neden olduğu hepatotoksisiteyi zayıflattı.

Sonuç

Karaciğer hasarını hafifletmek ve karaciğer fonksiyonunu eski haline getirmek sepsisli hastalarda morbi-

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dite ve mortalite oranlarını düşürür. IB, antioksidan, antiinflamatuar ve antiapoptotik özellikleri sayesinde karaciğer dokusunu LPS'nin neden olduğu hepatotoksisiteden korur. Karaciğerin sepsisteki rolünün daha fazla araştırılması, yeni terapötik hedeflerin ve stratejilerin geliştirilmesine yol açabilir. IB, sepsis sırasında akut hepatotoksisitenin önlenmesi için alternatif bir terapötik ajan olabilir.

Anahtar Kelimeler: Apoptoz, Hepatotoksisite, İnflamasyon, İrbesartan, Oksidatif stres, Sepsis

Abstract

Objective
In septic conditions, hyperinflammatory response and hepatotoxicity are caused by oxidative stress, inflammation, and apoptosis. Irbesartan (IB), an adrenergic receptor blocker, has anti-inflammatory and antioxidant properties. This study aimed to investigate the protective effect of IB on lipopolysaccharide (LPS)-induced acute hepatotoxicity.

Material and Method
A total of eight rats were used in three groups; a control group; LPS group [5 mg/kg, intraperitoneally (IP)]; and LPS + IB group [5 mg/kg LPS (IP) + 50 mg/kg IB (orally)]. After sacrification, tissues from the liver and blood were obtained for immunohistochemical and biochemical evaluations, such as interleukin-1 beta (IL-1β), caspase-3 (Cas-3) alanine aminotransferase (ALT), aspartate aminotransferase (AST), oxidative stress index (OSI), total oxidant status (TOS), and total antioxidant status (TAS).

Results
Compared with the control group, increased AST and ALT levels in the blood, biochemically increased TOS and OSI and decreased TAS levels in the tissue, immunohistochemically increased IL-1β, Cas-3, detected. Also, in liver tissue, histopathologically hyperemia, hemorrhage, vacuolization, and significant neutrophilia infiltration were found in the LPS group. IB administration significantly reversed all these parameters. TAS levels were increased by IB administration, whereas TOS and OSI levels were decreased ($p = 0.001$). IB also decreased AST and ALT values ($p = 0.001$). In the IB group, Cas-3 and IL-1β levels were significantly decreased by IB administration ($p = 0.001$). In addition, the IB ameliorated histopathological findings showed enhanced hyperaemia, haemorrhages, vacuolisation and significant neutrophilic leukocyte infiltration ($p = 0.001$). IB treatment attenuated LPS-induced hepatotoxicity by its antioxidant, anti-inflammatory and antiapoptotic properties.

Conclusion
Attenuating liver injury and restoring liver function lowers morbidity and mortality rates in patients with sepsis. IB protects liver tissue from hepatotoxicity caused by LPS thanks to its antioxidant, anti-inflammatory, and anti-apoptotic properties. Further investigation of the liver's role in sepsis may lead to the development of new therapeutic targets and strategies. IB may be an alternative therapeutic agent for the prevention of acute hepatotoxicity during sepsis.

Keywords: Apoptosis, Hepatotoxicity, Irbesartan, Inflammation, Oxidative stress, Sepsis

Introduction

Sepsis is a complex disease that is responsible for many deaths worldwide. A dysregulated inflammatory response plays a role in its pathophysiology, depending on the production of various mediators. The production and release of these substances into systemic circulation initiates various cellular and metabolic changes (1). Because it triggers a hyperinflammatory state, sepsis can cause a multisystemic condition that damages many organs, especially the lungs, liver, and kidneys (2). In clinical practice, only a few drugs prevent this inflammatory stage, besides to antibiotics (3). As in diseases with multi-organ involvement, such as COVID-19, the treatment of this condition is insufficient. Despite the anti-inflammatory properties of steroids, their immunosuppressive effects lead to opportunistic infections and increased mortality (4). To reduce the use of steroids, researchers are seeking new drugs with anti-inflammatory properties (5). Damage mechanisms, such as oxidative stress, apoptosis, and inflammation, are frequent and play significant roles in pathological processes at the tissue level. Previous studies have focused on oxidative stress indicator total antioxidant species (TAS), total oxidant species (TOS), oxidative stress index (OSI), acute inflammatory parameters interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and apoptosis marker Cas-3 (6, 7).

Lipopolysaccharides, used in experimental animals to mimic systemic inflammation and create septic conditions, can cause the synthesis of the factors described above by binding to their receptors in

cells, such as Toll-like receptor-4 (TLR-4), activating intracellular pathways. The cytokines released from damaged cells affect healthy cells, which causes the damage to increase cumulatively (Figure 1) (8, 9).

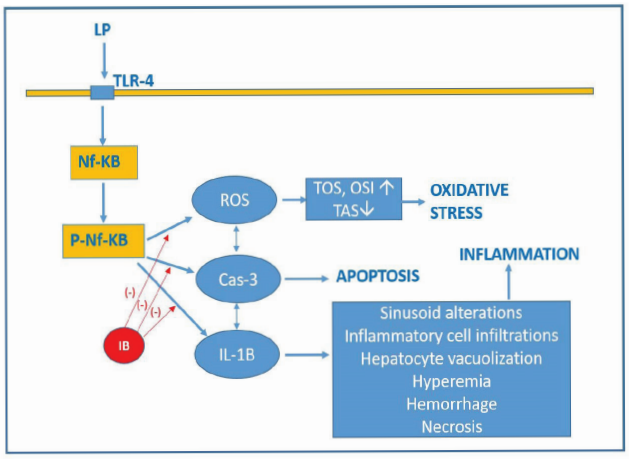

Figure 1

Effect of IB on LPS-induced liver toxicity

IB: Irbesartan, TLR-4: Toll-like-receptor-4, LPS: Lipopolysaccharide, Nf-KB: Nuclear factor kappa B, p-Nf-KB: phospho-nuclear factor kappa B, ROS: Reactive oxygen molecules, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, IL-1β: Interleukin 1 beta, Cas-3: Caspase 3

Irbesartan, an angiotensin receptor antagonist, is used to treat hypertension (10). Moreover, the drug's anti-inflammatory and antioxidant effects have been investigated (11, 12). Based on this information, we aimed to evaluate the IB's efficacy of IB in treating acute hepatotoxicity, which is common in clinical practice, complicating the management of sepsis.

Material and Method

Groups

Suleyman Demirel University's Local Animal Ethics Commission approved this research (Decision Number 17.10.2019/07). The experiment was conducted according to ARRIVE (Animal Research: Reporting in Vivo Experiments) guidelines, Version 2.0 protocol. Twenty-four adult male Wistar albino rats (weight 300–350 g) were housed at 21–22 ◦C and 60 ± 5% humidity with a 12 h light:12 h dark cycle. A standard commercial chow (Korkuteli Yem, Antalya, Turkey) was administered ad libitum with water. Three experimental groups were formed as follows:

1) Control group (n = 8): 0.5 mL of normal saline (NS) was administered IP + one hour before this application, 0.5 ml NS was administered orally again.

2) LPS group (n = 8): 5 mg/kg (IP) LPS (048K4126, Sigma Aldrich, USA) + one hour before this application, 0.5 ml NS was administered orally.

3) IB group (n = 8): 5 mg/kg (IP) LPS + one hour before this application, 50 mg/kg IB (Karvea, Sanofi, Turkey) was administered orally. The reason for applying IB one hour before LPS administration is that the elimination half-life of the drug is > 10 hours, and maximum concentration in plasma is reached within 90-120 minutes (Figure 2) (13, 14).

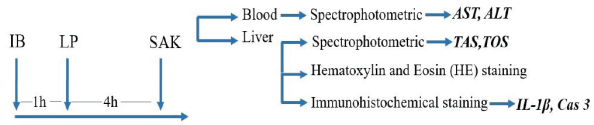

Figure 2

The schematic representation of the study design

IB: Irbesartan, LPS: Lipopolysaccharide, SAC: Sacrification, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, IL-1β: Interleukin 1 beta, Cas-3: Caspase 3, AST: Aspartate transaminase, ALT: Alanine transaminase

As described previously (15), animals were sacrificed using a solution of 100 mg/kg ketamine (Alfamin, Alfasan IBV) and 10 mg/kg xylazine bio 2% (Bioveta, Czech Republic) anesthesia five hours following IB treatment. Following an abdominal incision, blood was obtained from the vena cava inferior for biochemical evaluation, and liver tissues were extracted. One-half of the liver tissues were collected and kept at -20 °C to assess TAS, TOS, and OSI levels. The remainder of the tissues were preserved in 10% buffered formaldehyde for histological examination and immunohistochemical analysis of IL-1 and Cas-3 expression.

Histopathological Examination

Liver specimens were taken by necropsy at the end of the trial and preserved in a formalin solution with 10% buffered. The samples of tissue were obtained immediately after a two-day fixation period according to a standard tissue processing method using automated tissue processing equipment (Leica ASP300S, Wetzlar, Germany) and then buried in waxy paraffin. After chilling the paraffin blocks in the refrigerator, 5 m slices were cut with a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Hematoxylin-eosin (H&E) was used for staining, followed by mounting with a

coverslip and examination under light microscope. A semi-quantitative scoring technique modified by Fang et al. was utilised for the histopathological analysis. The scoring criteria are shown in Table 1 (16).

Immunohistochemical Examination

Immunohistochemical analyses were performed as described previously (17). Two set specimens were obtained using blocks and then drawn on slides covered with poly-L-lysine. They were marked immunohistochemically for Cas-3 (Cas-3 (E-8): sc-7272) and IL-1β (IL-1β (11E5):sc-52012, 1/100 dilution) (Santa Cruz Texas, USA). The streptavidin-biotin method was used for expression depending on the manufacturer's guides. After 60 minutes of incubation with primary antibodies, the sections were immunohistochemically stained by biotinylated secondary antibodies and a streptavidin-alkaline phosphatase compound. For the second group of antibodies, the EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) (Abcam, Cambridge, UK) was utilised. Diaminobenzidine (DAB) was utilized as chromogen. An antigen dilution compound was utilized instead of a major antibody for the negative controls. A pathologist from another institution completed all the evaluations of blind specimens. In immunohistochemical analyses, separate sections were examined for every antibody. A semi-quantitative analysis was used to assess the immunostaining degree of cells using a ranging from 0 to 3: 0 = no staining, 1 = poor focal staining, 2 = poor diffuse staining, and 3 = strong diffuse staining (18). During the assessment, ten separate fields were examined in each section under 40X objective magnification. Morphometric analyses and microphotography were conducted using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan). The outcomes were documented and statistically evaluated.

Measurement of Blood Parameters

Blood samples from the rates were collected in tubes containing gel. After 10 minutes of spinning at 3,000 rpm, the serum samples were separated into three parts. Until they were examined, the samples were maintained at -80 °C. Serum aspartate aminotransferase (AST), alanine transaminase (ALT), and total bilirubin levels were quantified by the spectrophotometric method using a Beckman Coulter AU5800 auto-analyser (Beckman Coulter, USA) and a kit compatible with the instrument.

Measurement of Oxidative Stress Parameters

For the oxidant–antioxidant analysis, liver tissue samples were homogenised by an Ultra Turrax Janke & Kunkel T-25 homogeniser (IKA® Werke, Germany). TAS and TOS were assessed using industrial equipment (Rel Assay Diagnostics, Gaziantep, Turkey) and spectrophotometry (Beckman Coulter, USA). The OSI was determined using the following calculation: TOS/TAS = OSI (19).

Briefly, TAS and TOS analyses have already been described. Antioxidants in the specimen converted the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) reactive to a colourless reduced ABTS form. The sample's total antioxidant concentration was associated with a difference in absorbance at 660 nm. This approach was used to assess the anti-oxidative impact of the specimen versus the powerful free radical actions activity due to hydroxyl radicals produced. The findings were shown in millimolar Trolox equivalents per liter (mmol Trolox Eqv/L) (20).

Table 1 Parameters of semi-quantitative system for histopathological evaluation

	1	2	3	4
Sinusoid alterations	None	Mild dilatation	Moderate dilatation	Severe dilatation
Inflammatory cell infiltrations	None	Mild neutrophil infiltration	Moderate neutrophil infiltration	Severe neutrophil infiltration
Hepatocyte vacuolization	None	1-10% of all hepatocytes	11-30% of all hepatocytes	>31 of all hepatocytes
Hyperemia	None	Mild	Moderate	Severe
Hemorrhage	None	Mild	Moderate	Severe
Necrosis	None	1-10 in 5 HPF (40X)	11-30 in 5 HPF	>31 in HPF

HPF: High-power field

Throughout TOS analysis, oxidants available in the specimen oxidized the ferrous ion-dianisidine formation into ferric ions. Glycerol molecules drove the oxidation reactions in the reaction media. Under an acidic environment, ferric ions formed a colourful compound with xylenol orange. The colour intensity, evaluated spectrophotometrically, was associated with the quantity of oxidant molecules in the specimen. The findings were referred a micromolar hydrogen peroxide equivalent per liter (µmol H2O2 Eqv/L) after the test was validated using hydrogen peroxide (21).

Statistical Analyses

The immunohistochemical scores among the groups were compared using a one-way ANOVA and post-hoc Duncan test for histopathological findings and an LSD test for biochemical findings were applied using the SPSS v.22 package programme (SPSS Inc., Chicago, IL, USA). A P value less than 0.05 was considered significant.

Results

Biochemical Findings

In the LPS group, significantly decreased TAS levels and increased TOS and OSI levels were detected compared with the control (p = 0.001 all of them, respectively). The IB reversed all of these parameters significantly (p = 0.001 all of them, respectively) (Figure 3).

In the LPS group, ALT and AST levels were significantly higher than the control groups (p = 0.001 for all). The IB therapy dramatically decreased the levels of these markers compared to the LPS group (p = 0.001, all of them) (Figure 4).

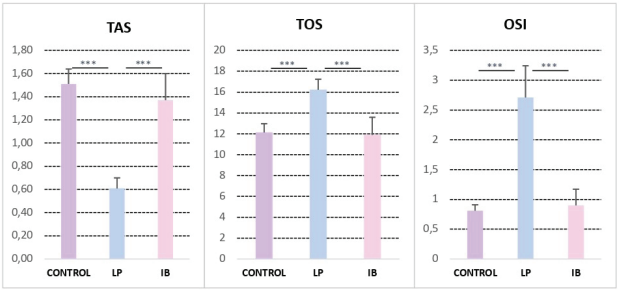

Figure 3

Statistical comparison of the groups' oxidative stress parameters.

IB: Irbesartan, LPS: Lipopolysaccharide, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index. Data expressed mean ± standard deviation (SD). One-way ANOVA LSD test. ***: p≤0,001.

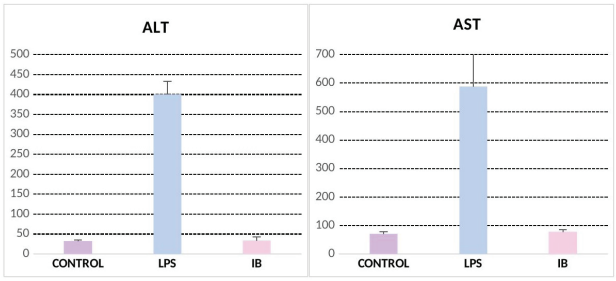

Figure 4

Hepatic injury indicators in the blood

IB: Irbesartan, LPS: Lipopolysaccharide, AST: Aspartate transaminase, ALT: Alanine transaminase. Data expressed mean ± standard deviation (SD). One-way anova LSD test. ***: p≤0,001.

Table 2 Statistical analysis result of histopathological scores between the groups.

	Control	LP	IB	P value
Sinusoid alterations	0.00±0.00	2.37±0.74*	0.62±0.51*,#	≤0.001
Inflammatory cell infiltrations	0.12±0.12	2.00±0.75*	0.37±0.18#	≤0.001
Hepatocyte vacuolization	0.12±0.12	2.62±0.51*	0.50±0.18#	≤0.001
Hyperemia	0.12±0.12	2.12±0.83*	0.50±0.18#	≤0.001
Hemorrhage	0.00±0.00	1.75±0.70*	0.25±0.16#	≤0.001
Necrosis	0.00±0.00	1.62±0.74*	0.25±0.16#	≤0.001

IB: Irbesartan, LP: Lipopolysaccharide. Statistical analysis of immunohistochemical scores.
*: compared with control and, #: compared with IB group, P≤0.001. Data standard deviation (SD). One-way anova Duncan test.

Histopathological Findings

The results of the microscopic analysis revealed normal liver histology in the control group. Marked hyperaemia, moderate to marked hyperaemia, slight to moderate haemorrhages and vacuolisation were identified, and numerous inflammatory cell infiltrations mainly composed of neutrophil leukocytes were observed in the LPS group. The IB treatment significantly ameliorated the histopathological findings in the IB group (Figure 5). The comparisons of histopathological scores are shown in Table 2.

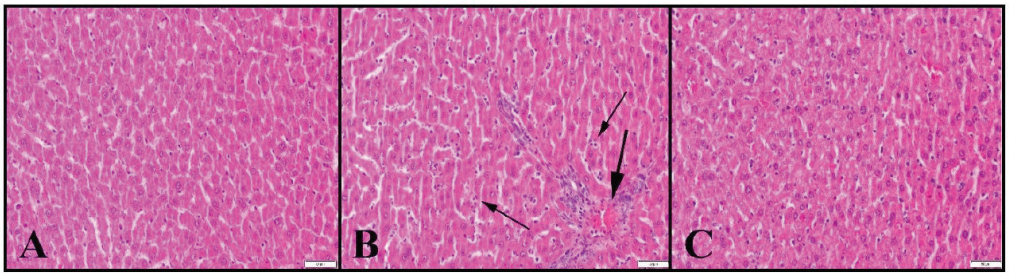

Figure 5

Microscopical findings in liver between the groups.

(A) Normal tissue architecture in control group. (B) Marked hyperemia (thick arrow) and neutrophil infiltrations (thin arrows) in LPS group. (C) Decrease in histopathological findings in LPS+IB group, H&E, scale bars=50μm.

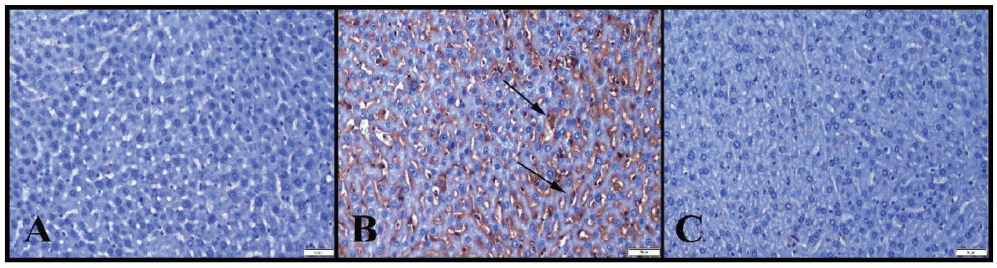

Figure 6

Caspase-3 immunohistochemistry findings between the groups.

(A) Negative expression in control group. (B) Marked increase (arrows) in liver in LPS group. (C) Decreased expression in LPS+IB group, Streptavidin biotin peroxidase method, scale bars=50μm.

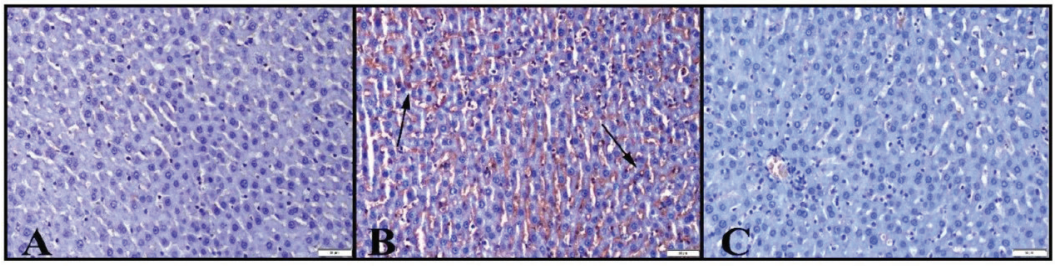

Figure 7

IL-1β expressions between the groups.

(A) No expression in control group. (B) Marked expression in liver in LPS group.
(C) Decreased expression in liver in LPS+IB group, Streptavidin biotin peroxidase method, scale bars=50μm.

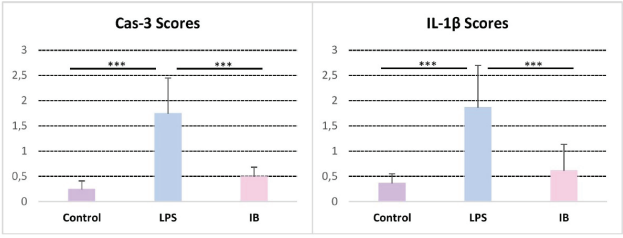

Figure 8

Statistical analysis result of immunohistochemical scores between the groups.

IB: Irbesartan, LPS: Lipopolysaccharide, IL-1β: Interleukin-1beta, Cas-3: Caspase-3. Data expressed mean ± standard deviation (SD). One-way ANOVA Duncan test. ***: p≤0,001.

Immunohistochemical Results

The immunohistochemistry analyses demonstrated negative to slight expressions in Cas-3 and IL-1β in the control group. The LPS group showed significant expression in hepatocytes and sinusoid cells. The IB therapy resulted in a significant reduction in the IB group (Figures 6 and 7).

Figure 8 shows the statistical evaluation of the immunohistochemistry scores. The findings indicate that LPS caused hepatic damage in rats. The IB treatment ameliorated the pathological findings.

Discussion

The liver is the most significant modulatory organ in the human system and is vital for metabolic and immune balance (22). It has more than 200 critical functions, primarily detoxification, coagulation, hormonal balance, energy production, nutrient conversion and storage (23, 24). Because of these crucial functions, the liver is a vital organ in the host's survival in severe pathological conditions, such as sepsis (25). Clinical and experimental studies reveal that liver dysfunction is an early manifestation of sepsis and a specific and independent risk factor for severe complications in sepsis patients. Serious complications can occur in cases of sepsis in the liver, leading to the progression of the disease and even death (26). The mechanism of acute liver injury has not been extensively investigated, and further research is needed to develop successful treatment methods. Based on the literature, the current study purposed to examine the acute effects of systematically applied LPS on liver tissue (27). Attacks by oxidants and inflammatory substances are the primary causes of damage to organs, which was also found in the present study.

Oxidative stress is associated with increased cellular reactive oxygen species (ROS), particularly when the antioxidant system is unable to balance them (28). Therefore, increased ROS in sepsis results in oxidative stress and low antioxidant potential. Lipopolysaccharide binding to inflammatory cells stimulates several intracellular signaling pathways, including the nuclear factor kappa-B (NFkB) and mitogen-activated protein kinase networks. These processes induce transcription factors, including the NFkB/Rel proteins, the activator protein 1 (AP-1), and the nuclear factor-interleukin 6. In healthy cells, antioxidants and ROS have conflicting effects, with antioxidants decreasing NFkB activation by increasing AP-1 activation while ROS stimulates NFkB. Thus, oxidative stress may be participating in direct or indirect pathways that result in cellular harm during sepsis (29). Disruption of defence mechanisms can damage cellular elements such as DNA, proteins and lipids (30, 31). Irbesartan has been shown to have substantial effects on the production and activity of intracellular antioxidant enzymes, hence improving the negative effects of oxidative stress on many bodily tissues (32). The TOS, OSI increment and TAS decrement, which are markers of oxidative stress in the liver tissue, showed that this stress had developed, and the reversal of the results on the IB side supported the antioxidant properties of IB (33). In Helal et al.'s study, liver damage caused by paracetamol toxicity was reduced by IB's antioxidant and anti-inflammatory effects, so the liver function decreased (34). A study by Kabel et al. found similar results, confirming IB's hepatoprotective properties in a mice model of hepatotoxicity caused by doxorubicin (35). The findings of this study showed that, as in the above studies, IB administration significantly reversed the oxidative stress parameters that cause damage to hepatocytes due to sepsis.

Serum aminotransferase levels (ALT and AST) are frequently used to evaluate various liver diseases, including metabolic conditions such as fatty liver and infectious etiologies such as viral hepatitis (36, 37). A high level of ALT and AST enzymes in the serum is thought to be a specific indicator of liver dysfunction and damage to the structural integrity of hepatocytes (38). In their study, Beheshti et al. examined the damage caused by LPS application at the liver level. Their findings showed that the AST and ALT values increased, and the oxidant substance increment, the antioxidant substance decrement (39). Similarly, in this study, AST and ALT serum activities were significantly higher, indicating acute damage after LPSS. However, administration of IB significantly reduced the levels of these markers.

These considerable decreases were consistent with several rare studies supporting IB's hepatoprotective impact (34, 35). AST and ALT values were similar to those in the oxidative stress situation described above, indicating that acute damage occurred, which was reduced by the administration of IB. Interestingly, some drugs that act primarily at the renal level, such as angiotensin receptor blockers, have hepatoprotective effects and should be investigated in future studies. The findings of the current study support this.

In addition to oxidative harm, activation of the inflammatory cascade also plays a critical role in the pathological process of sepsis-induced liver injury (25). The signaling pathway NF- B is a crucial regulator of inflammatory cytokines, including IL-1 , and TNF- and essential in inflammation responses (40). In addition, some studies demonstrate that IB may inhibit the inflammatory response by suppressing the NF- B pathway. Increased IL-1β levels, which are an essential indicator of acute inflammatory damage, were shown to be reversed by IB treatment (41, 42). The present preliminary investigation has also revealed that IB showed anti-inflammatory effects in the early period.

In the inflammatory process, ROS are known to be critical signaling molecules (43). It has been proven that this oxidant state in the tissue triggers inflammation and initiates apoptosis, which is also known as programmed cell death (44, 45). Previous studies on these intracellular mechanisms have shown that they are driven by apoptosis using the same or different pathways (46, 47). In this study, the decrement in increased Cas-3 levels in the LPS group showed that IB can be effective in protecting liver tissue. It can also contribute to the cell's survival by decreasing Cas-3 levels through its antiapoptotic features. Consistent with our results, Kabel et al. and Helal et al. verified that IB significantly reduces active caspase 3 expression in liver tissue (34, 35). These consequences may be attributable to irbesartan's capacity to reduce caspase 3 activity and inhibit the production of proapoptotic proteins (48, 49). Also, angiotensin II receptor blockers like irbesartan have been demonstrated to reduce the count of apoptotic cells while increasing the activity of antiapoptotic Bcl-2 proteins (50). However, IB's intracellular mechanisms should be examined in more detailed molecular studies. Furthermore, liver-specific damage indicators, such as sinusoid alterations and hepatocyte vacuolization, have been observed histopathologically in the present study. The fact that vascular damage states, such as hyperemia, hemorrhage, and necrosis, are parallel with increased Cas-3 levels indicates that the liver is highly affected by the hypoxia-induced inflammatory scene. A possible cause of liver failure could be hypoxic hepatitis. LPSS and inflammatory cytokines are two further risk factors for hypoxic hepatitis. In the early phases of sepsis, neutrophils are attracted to the liver in response to the release of TNF- and leukotriene B4 by KCs. Hepatocyte injury is facilitated by cytokines that neutrophils release (51). Liver sinusoidal endothelial cells are also implicated in the release of cytokines in response to LPSS stimulation, and they are the primary hepatic source of endothelin-1 (ET-1), a potent vasoconstrictor (51). Histopathologically, portal inflammation, centrilobular necrosis, lobular inflammation, and hepatocellular apoptosis were detected in most sepsis patients in postmortem studies (52). Ibrahim MA et al.'s findings showed that IB was protective and reduced Cas-3 levels in liver damage secondary to metabolic syndrome in fructose-fed experimental animals (53). These findings indicated that in addition to the cardiovascular vasodilator effects of IB, it has protective properties on tissues. However, the results of our study showed that IB can prevent inflammation, oxidative stress, and even apoptosis when applied in acute situations. Moreover, the increased expression of IL-1β accompanied by neutrophilic infiltration reversed with IB indicates that it can be used as a tissue protector in many pathologies.

The rate of sepsis-associated liver failure is difficult to determine, but it is indisputable that liver failure as a consequence of sepsis significantly impacts the patients' prognosis. Our results showed that acute damage to the liver tissue occurred with LPS and was regressed by IB administration through the drug's anti-inflammatory, antioxidant, and anti-apoptotic properties. Therefore, there is a need to apply IB to various disease models at different doses and durations to investigate detailed intracellular mechanisms.

Conclusion

Our findings indicate that IB may be an alternative therapeutic agent for the prevention of acute hepatotoxicity during sepsis. However, more detailed research on this medication's dose-dependent effects is required. Current research findings showed that IB treatment attenuated LPS-induced hepatotoxicity through its antioxidative, anti-inflammatory, and antiapoptotic properties. The challenges in sepsis management are associated with mixed pathogenesis and high mortality rates. Furthermore, the incidence of multi-resistant bacteria is increasing. Therefore, treatments to support antibiotics should be developed in future research.

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Conflict of Interest Statement
The author has no conflicts of interest to declare.

Ethical Approval
Suleyman Demirel University's Local Animal Ethics Commission approved this research (Decision Number 17.10.2019/07).

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Availability of Data and Materials
The underlying data supporting the results of our study were available on request. The corresponding author (ENT) is contacted to request the data.

Authors Contributions
ENT: Conceptualization; Investigation; Methodology; Writing-original draft

SAT: Formal analysis; Visualization

İİ: Data curation; Validation

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