ISSN: 1343-4292
Volume 140, Issue 07, October, 2022

Toll-like receptors 4 antagonist, Ibudilast, ameliorates acute renal impairment induced by sepsis in an experimental model.

Shaymaa Nasser Hussein[1], Sahar A. Majeed[1], Fadhaa Abdulameer Ghafil[1], Ekhlas Sabah Hassan[1*], Najah R. Hadi[1]

Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Iraq[1]

Corresponding author: 1*

ABSTRACT— In critically ill patients, acute kidney damage (AKI) is a frequent consequence that is linked to higher morbidity and mortality. The most typical cause of AKI is sepsis. Ibudilast is a strong inhibitor of phosphodiesterase (PDE)-4 and other PDE subtypes as well as a TLR-4 antagonist. To examine the nephroprotective effect of Ibudilast by their anti-inflammatory, antioxidant effect and modulation of apoptosis through their effect on TLR- 4/NF- kB signaling pathway in experimental model of sepsis. 24 adult male mice divided randomly in to 4 groups (Sham group, cecal ligation and puncture (CLP) group, Vehicle group, Ibudilast (10 mg/kg) treatment group). We sacrificed the animals after 24 hours, and each mouse's blood sample was drawn. To identify markers (tumor necrosis factor alpha (TNF-α), interleukin- 6 (IL-6), macrophage migration inhibitory factor (MIF), interleukin- 10 (IL-10), F2-isoprostane, caspae-3, B-cell lymphoma-2 (Bcl-2), toll like receptor- 4 (TLR-4) in kidney homogenate. Part of the kidney was studied for histopathology. Ibudilast significantly reduced TNF-α, IL-6, MIF, induced IL-10, lowered F2-isoprostane, dropped caspae-3, rose Bcl-2, lowered toll like receptor- 4 protein expression, reduced blood urea and reduced serum creatinine. Ibudilast lessens kidney damage induced by sepsis through blocking TLR-4/NF-kB downstream signal transduction pathways, oxidative stress and modulation of apoptosis.

KEYWORDS: sepsis, TLR- 4/ NF- kB signaling pathway, apoptosis, ibudilast.

1. INTRODUCTION

Fundamentally, sepsis is an inflammatory condition that is regulated by the immune system of the host. The pattern recognition receptors (PRR) are activated during the early stages of sepsis, which aids the innate immune response [1]. Pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs), such as mitochondria released from damaged tissues, can both evoke the receptor response, which is very dynamic [2]. Sepsis caused by infection is still one of the major health issues in the world. It is difficult to determine the exact global impact of the illness, although thirty million individuals are thought to be affected annually [3], [4]. In hospitalized and critically ill patients, sepsis-associated acute kidney damage (S-AKI) is a frequent consequence that raises the likelihood of developing chronic comorbidities and is linked to a very high fatality rate [5]. The most popular method for creating an animal model of sepsis in mice is cecal ligation and puncture (CLP). Sepsis's innate inflammatory response starts with abnormalities in normal mitochondrial activity, which may harm organs. A complicated network of interconnected antioxidant defenses works normally to fight oxidative stress and stop mitochondrial damage. It is commonly acknowledged that organ failure is largely caused by oxidative stress-mediated damage [6]. Numerous cytokines are released during sepsis and are classified as either pro-inflammatory (like IL-6, TNFα and MIF) or anti-inflammatory (IL-10) [7]. Oxidants and antioxidants, as well as

mitochondrial dysfunction in cells, are critical players in the pathophysiological pathway. The oxidant and antioxidant systems in the body are typically in balance; oxidative stress happens when oxidant levels are higher than antioxidant levels, which contributes to the sepsis process and may result in organ damage [8]. The depletion of immune cells through apoptosis, the upregulation of regulatory T (Treg) cells, the expression of programmed cell death on CD4+ T cells, and cellular fatigue are some of the mechanisms that cause sepsis to cause immunosuppression. The pathogenesis of septic complications is becoming understood to be largely influenced by immune cell death [9]. Toll-like receptor 4 (TLR4), a crucial member of the TLR family, is a pattern recognition receptor that is expressed on the cell surface and responds to both endogenous and external stimuli as well as to foreign microorganisms, playing a crucial role in the generation of pro-inflammatory responses [10]. In addition, TLR4 normally communicates primarily through the nuclear factor kappa B (NF-B) and mitogen-activated protein kinase (MAPK) signaling pathways [11], [12]. A nuclear transcription factor called NF-B becomes active when it moves into the nucleus [13]. Ibudilast has reportedly been shown to protect against neuroinflammation in conditions like Parkinson's disease, post-stroke delirium, and dizziness [14]. By inhibiting the TLR4/NF-B pathway [15], ibudilast reduces the inflammatory response induced by the human immunodeficiency virus-1 in microglial cells [16].

Aim: To examine the nephroprotective effect of Ibudilast by their anti-inflammatory, antioxidant effect and modulation of apoptosis through their effect on TLR- 4/NF- kB signaling pathway in experimental model of sepsis.

2. Methods

2.1 Reagents and animals

1. Ibudilast powder has been purchased from Solarbio company, diluted with (vehicle) mixture of (DMSO 5%, 40% PEG300,5% Tween,50% normal saline), Both complete protease inhibitor (Cocktail inhibitor liquid) and dimethyl sulfoxide (DMSO) were purchased from MedchemExpress in the USA. The TNF-, IL-6, IL-10, MIF, TNF, F2- isoprostane, Caspase-3, Bcl-2, and TLR-4 ELISA kits were bought from (BT-LAB /China). The blood kits for urea and creatinine were bought from Beckman Coulter in the USA.
2. 24 adult male mice divided randomly in to 4 groups (six animals in each group).

A. Sham group: Without CLP, anesthesia was administered to each animal in this group, and a surgical intervention was performed. As surgical control groups, sham groups are used.

B. Sepsis (CLP) group: The cecum of each mouse in this group were tied off and pierced. These CLP-controlled mice serve as an illustration of septic shock.

C. Vehicle group: all mice inside this group were given an equal volume of vehicle (DMSO 5% + 50% NS + 40% peg300 + 5% Tween) intraperitoneal 2 hour after surgery [17].

D. Ibudilast treatment group: After the CLP procedure, all mice in this group received treatment with (10 mg/kg) intraperitoneally Ibudilast [17].

blood had been immediately gutted from each mouse's heart. Kidney tissue were homogenated then used in the ELISA procedure to identify markers (TNF-α, IL-6, IL-10, MIF, TNFα, F2- isoprostane, Caspase- 3, Bcl- 2 and TLR- 4) [1]. Part of the kidney was dried and cleaned before being fixed in paraffin and sliced in to 5Mm thick slices with a rotary microtome. After fixing, the kidney segment on slides, staining it with Hematoxylin &Eosin staining dye, and securing it with a covered slide, it is ready for study under a microscope [18]. The scoring system that was employed consisted of five scores (score 0 refers to None or Normal, score 1 refers to <25% of damage, score 2 refers to 25-50% of damage, score 3 refers to 50-75% of damage, score 4 refers to >75% of damage [19].

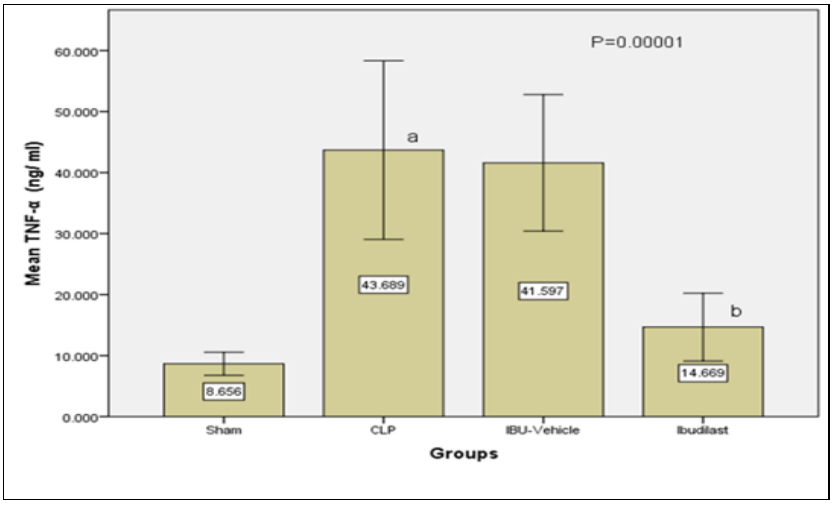
ISSN: 1343-4292
Volume 140, Issue 07, October, 2022

statistical analysis:

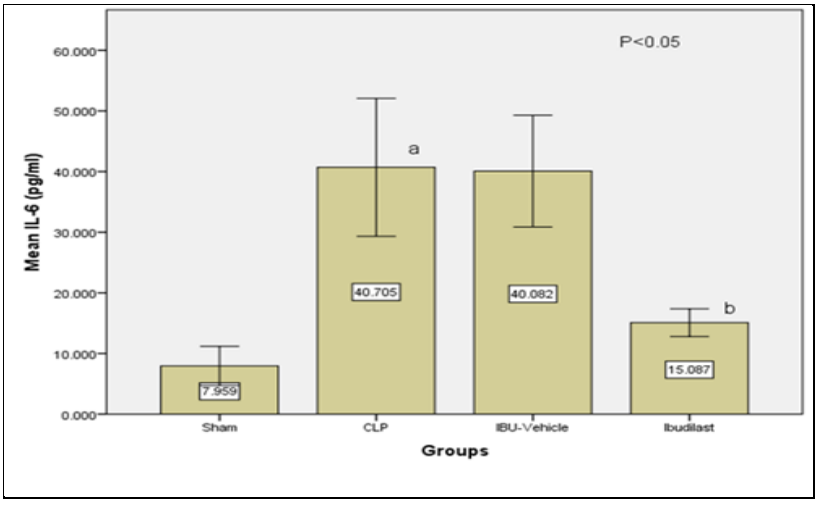
After ensuring that the data were normal using the Kolmogorov-Smirnov and Schapiro tests, an ANOVA was conducted using SPSS version 26 at a significance level of 0.05 to determine the mean difference of parametric numerical variables among study groups and their bar graph.

3. Results

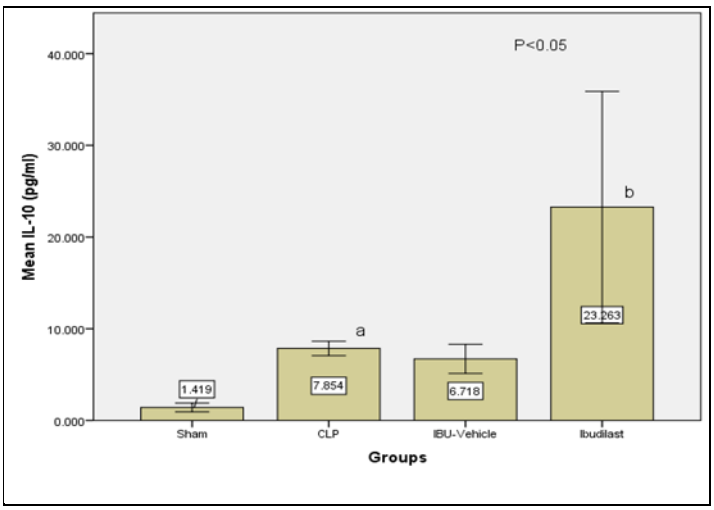
The results of this experimental study revealed that after induction of sepsis by CLP, the levels of TNFα, IL-6, IL-10, MIF, F2-isoprostane, caspase-3 and TLR-4 in their renal tissue had significantly higher than those in the sham groups except Bcl-2 marker were reduced significantly in the sepsis renal tissue than those in the sham groups. Ibudilast significantly reduced the proinflammatory markers (tumor necrosis factor alpha (TNFα), interleukin- 6 (IL-6), macrophage migration inhibitory factor (MIF)) but induced the anti-inflammatory marker, interleukin- 10 (IL-10). it significantly lowered oxidative stress (as evidenced by the decline in F2-isoprostane. It significantly lowered apoptosis because it significantly dropped caspae-3 and significantly rose B-cell lymphoma-2 (Bcl-2). ibudilast significantly lowered toll like receptor- 4 protein expression. Also it reduced levels of blood urea and levels of serum creatinine significantly. As showed in (figures 1,2,3,4,5,6,7,8,9,10) below respectively.



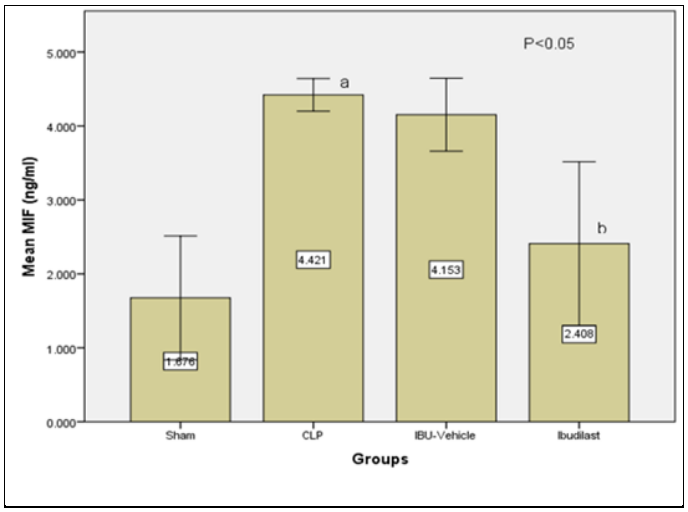
(Figure 1) Difference of mean TNFα



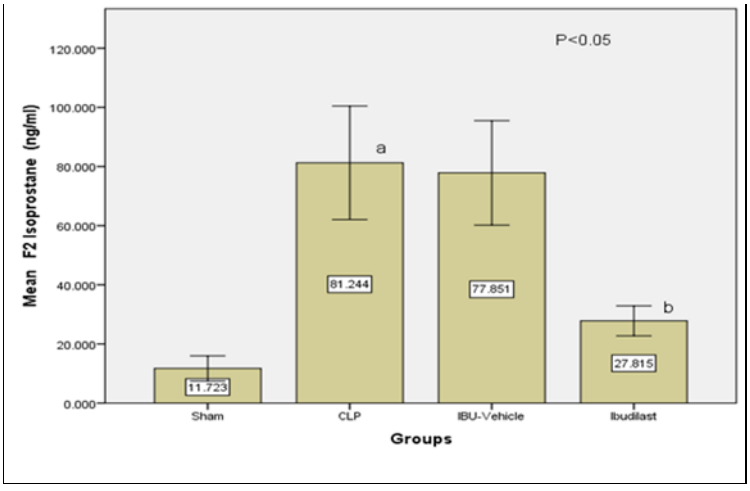
(Figure 2) Difference of mean IL- 6



(Figure 3) Difference of mean IL- 10



(Figure 4) Difference of mean MIF



(Figure 5) Difference of mean F2- isoprostane

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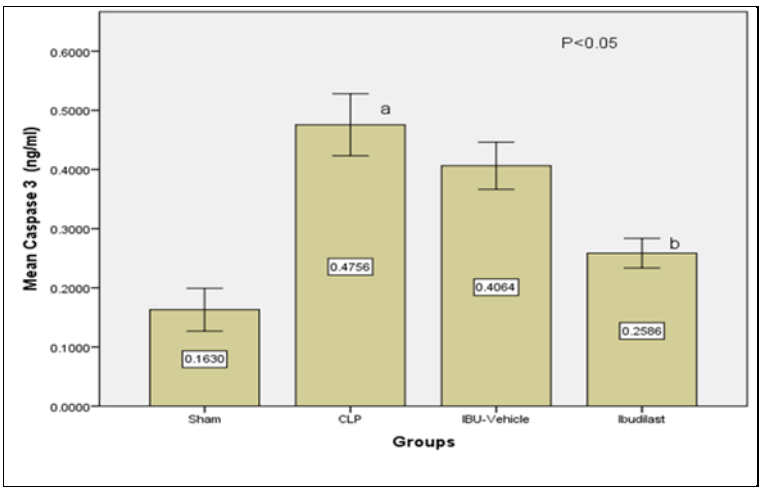
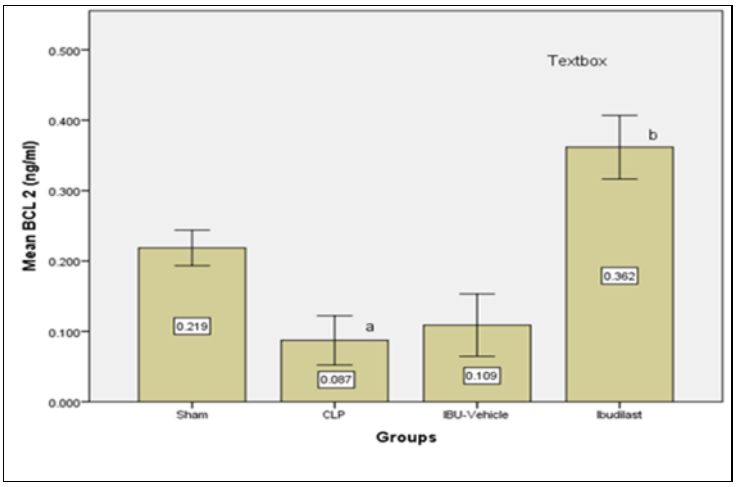
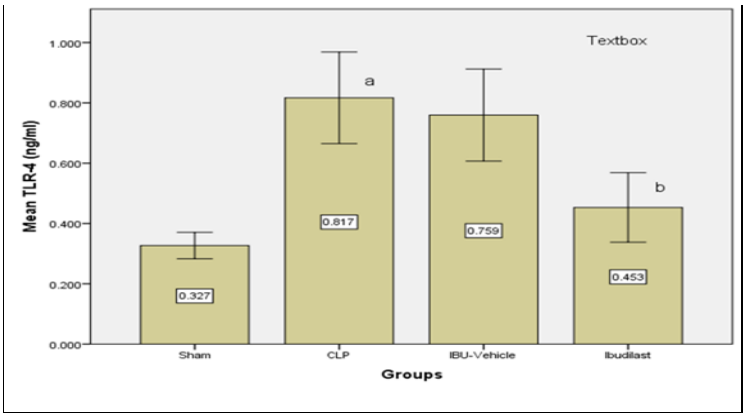
Volume 140, Issue 07, October, 2022



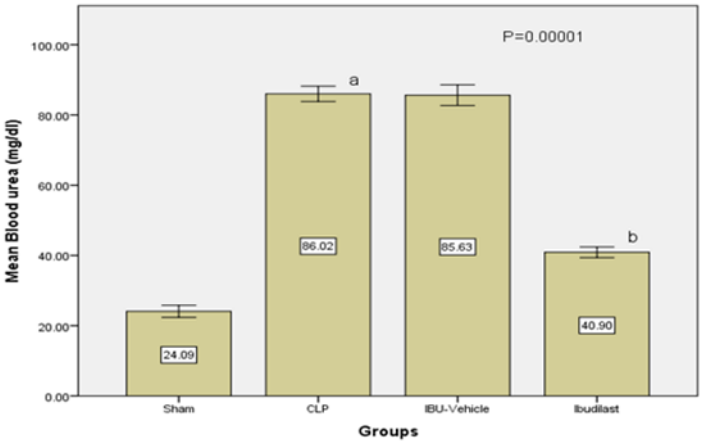
(Figure 6) Difference of mean caspase- 3



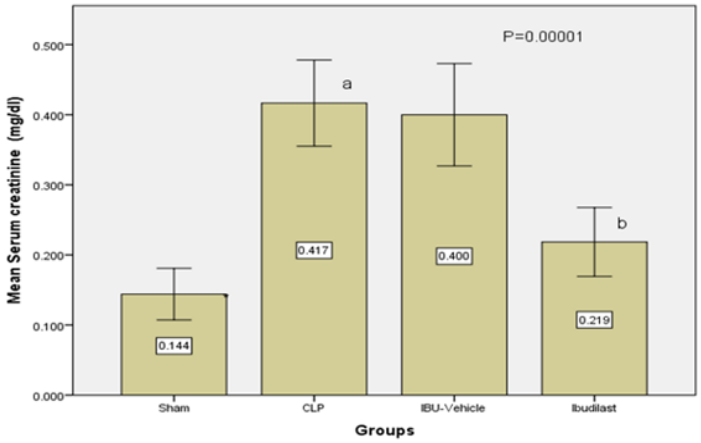
(Figure 7) Difference of mean Bcl- 2



(Figure 8) Difference of mean TLR- 4



(Figure 9) Difference of mean blood urea,



(Figure 10) Difference of mean serum creatinine

a/ Differences is significant between sepsis (CLP) and Sham groups, P value < 0.05
b/ Differences is significant between ibudilast and sepsis (CLP) groups, P value < 0.05

Histological finding

Sham group: healthy tissue (Fig. 11), CLP group: renal tubules with a score of 4 damaged renal tubules due to sepsis as evidenced by cellular swelling with enhanced cytoplasmic eosinophilia, vascular congestion, and extravasation of RBCs (Fig. 12). Vehicle group: score 4 damaged renal tubules (Fig. 13). Celastrol group: score 2 damaged renal tubules, yet have normal glomeruli (Fig. 14).

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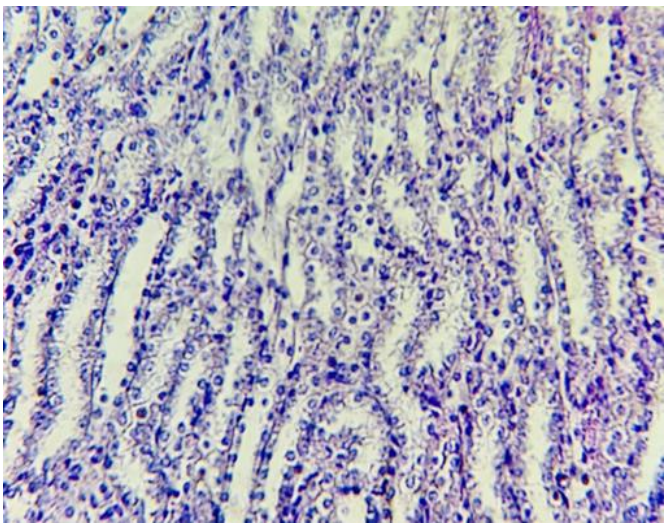
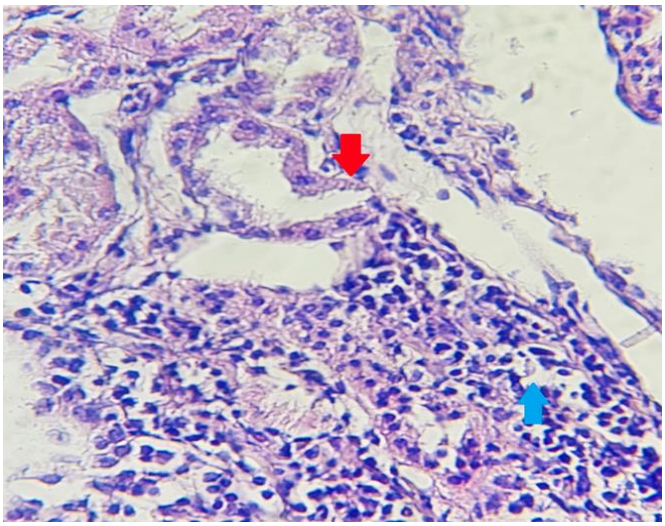
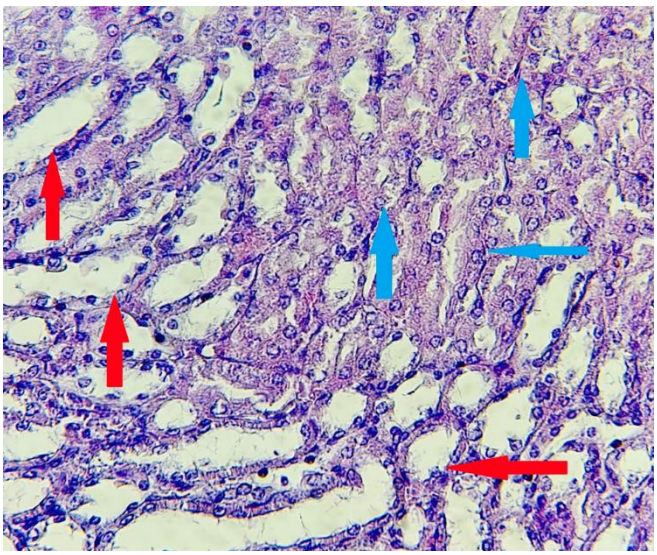
Volume 140, Issue 07, October, 2022



(Fig. 11) Sham group: normal renal tubules H.&E. X400

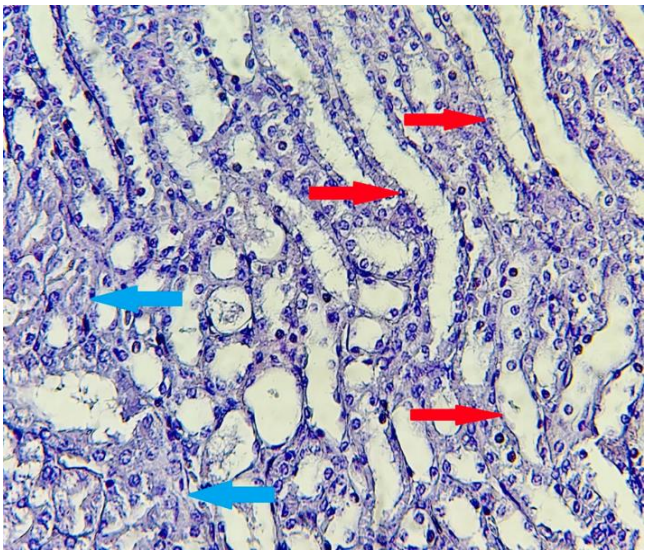


(Fig. 12) sepsis group: Score 4 damaged renal tubules H.&E. X400. Damaged renal tubules (red arrow), interstitial inflammation (blue arrow).



(Fig. 13) Vehicle group: Score 4 damaged renal tubules H&E x 400, damaged renal tubules (blue arrow),

area of normal tubules (red arrow).



(Fig. 14) ibudilast group: Score 2 damaged renal tubules H&E x 400, Damaged renal tubules (blue arrows). normal tubules (red arrows).

4. Discussion

Even though sepsis is one of the main causes of death worldwide, there is only critical care unit care available [20]. The body's pro- and anti-inflammatory responses interact extensively to determine the patient's fate [21]. Depending on which organ systems are affected by sepsis, different signs and symptoms may occur. One of the organ dysfunctions is renal dysfunction (oliguria and/or increased creatinine concentration) [22]. Renal failure can be brought on by a variety of causes, such as oxidative stress, complement system activation, and inflammation [23].

Isoprostanes are produced by lipid peroxidation, which occurs when polyunsaturated fatty acid free radicals, such as linoleic acid or arachidonic acid, are oxidized. This process is thought to be related to sepsis due to the oxidation products produced during it [24].

Lipopolysaccharide (LPS), an endotoxin found on bacterial cell walls that directly triggers apoptosis in renal tubular cells by releasing systemic cytokines, is the main cause of sepsis [25]. The main mediator of apoptosis, or programmed cell death, is caspase 3 (pro apoptotic) [26]. Although it is believed that the anti-apoptotic BCL-2 protein plays a key role in both the apoptosis process and the permeabilization of the mitochondrial outer membrane [27].

Sepsis-induced kidney damage has been shown to be influenced by the NF-kB signaling pathway. Additionally, the toll-like receptor 4 (TLR4) and p65 play important roles in sepsis-induced KI [22]. TLR-4 is the receptor that is sensitive to LPS and activates intracellular signaling pathways, including NF- kB [16]. Our findings showed that ibudilast treatment after sepsis induction significantly decreased urea and creatinine levels, enhancing renal function may be due to its stopping of the activation of TLR4 [13].

Comparing the levels of IL-6, MIF, F2-isoprostane, caspase-3, TLR-4, and TNFα in renal tissue in sepsis group and ibudilast treatment group, ibudilast may significantly ($P < 0.05$) reduce those levels while increasing those of IL-10 and Bcl-2 those lead to reduce oxidative stress and inflammation.

These ibudilast's anti-inflammatory, anti-apoptotic and anti-oxidative stress may be Due to ibudilast's TLR-4/NF-kB blocking effect and its inhibition of the binding of lipopolysaccharides (LPS) to innate immune signaling myeloid differentiation factor 2 (MD2), TLR-4 activation in primary macrophages is decreased. These findings suggest that ibudilast's potential anti-inflammatory properties, which are mediated through lowering NF-kB and TLR-4 expression in the kidney, may be the basis for the drug's protective benefits.

By preventing the activation of the TLR4/NF-kB-mediated pathway and the NLRP3 infammasome, ibudilast treatment may successfully suppress the pro-inflammatory response [13].

Ibudilast allosterically inhibits macrophage migration inhibitory factor, according to yet another study by Cho. Ibudilast might stop MIF from catalyzing as a result. These compounds block MIF tautomerase activity noncompetitively, allowing inhibitors to attach to enzymes that are bound to substrates, products, or free enzyme [28].

This ibudilast action probably due to NF-kB inhibition, which results in a decrease in IL-8 expression and a suppression of caspase-dependent apoptosis, protecting cells against oxidative stress-related cell damage [13]. NF-kB is a crucial transcription factor that has been connected to apoptosis, oxidative damage, and inflammation. TLR4 is one of the receptors whose activation triggers the NF- kB transcription factor [29]. In order to explain the effects of ibudilast on study inflammatory, apoptotic, and oxidative stress markers, we may say that any medication that inhibits or antagonizes TLR4 can be thought of as counteracting apoptosis, oxidative stress, and inflammation.

5. Conclusion

Ibudilast alleviate kidney damage induced by sepsis by inhibiting the TLR-4/NF-kB signaling pathway, which reduces the inflammatory process, oxidative stress, and modulate apoptotic process.

6. Ethical Clearence

The current study has been approved by the animal ethical committee in Faculty of Medicine, University of Kufa (no. 2935 in 2/2/2022).

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