Original Article

Irbesartan Attenuates Sepsis-Induced Renal Injury In Mice Models.

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Abstract

Sepsis is the main cause of death following infection and is regarded as a worldwide health problem. Sepsis is a systemic inflammatory side consequence of microbial infection. To investigate any possible reno-protective effects of irbesartan during sepsis-induced renal damage. Forty male albino Swiss mice, weighing 25–30 grams and aged 8–12 weeks, were used in the current investigation. Both food and water were freely available to these animals. Mice were separated into the following four groups after two weeks of adaption. (n = 10): (1) Normal group: apparently healthy mice. (2) CLP group: mice underwent CLP operation. (3) Vehicle group: mice received DMSO (4) irbesartan group: mice received irbesartan 3 mg/kg/day intraperitoneally for 5 consecutive days.) irbesartan group demonstrated a significant ($p<0.05$) decrease in the renal levels of NGAL as compared to the CLP group. Furthermore, the irbesartan group demonstrated a significant ($p<0.05$) decrease in the serum level of inflammatory cytokines (TNF-α, IL-6, & IL-1β) as compared to the CLP group. Additionally, the irbesartan group showed a significant ($p<0.05$) elevation in the renal SOD activity and reduction of MDA level as compared to the CLP group. Histologically, All mice in the CLP group showed a significant ($p<0.05$) renal tissue injury, while the irbesartan group showed a significant ($p<0.05$) reduced level of renal tissue injury. The anti-inflammatory effect through their ability to decrease serum levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6). Also the anti-oxidant effect through their ability to decrease renal levels of MDA and increase the renal activity of SOD.

Keywords: CLP, Polymicrobial sepsis, renal injury, NGAL, oxidative stress

1. INTRODUCTION

Severe public health problems associated with sepsis include an elevated mortality rate. The data for the Intensive Care Over Nations (ICON) study was gathered globally. Epidemiologic research found that 2,973 (29.5%) of the 10,069 patients in the intensive care unit (ICU) had sepsis when they arrived or developed it while they were there. Sepsis patients experienced ICU mortality rates of 25.8%, while hospital mortality rates were 35.3%, which was significantly higher than the overall ICU mortality rate. As the Surviving Sepsis Program shown during the previous 10 years [1], proper evidence-based sepsis care is required to reduce mortality. Septic shock, severe sepsis, and systemic inflammatory response syndrome [SIRS] with suspected infection were the early descriptions of sepsis. [2]. Sepsis, which is characterized by irreversible organ failure, is the result of an unbalanced host response to infection. The clinical criteria for sepsis include suspected or confirmed infection, as well as a sudden increase in two or more sequential organ failure assessments' (SOFA) values used as a proxy for true organ failure. When the underlying metabolic and circulatory issues are so serious that the danger of death is significantly elevated, a kind of sepsis known as septic shock takes place. Septic shock is defined by the sepsis clinical criteria despite adequate fluid resuscitation. Moreover, a vasopressor is required to raise the mean arterial pressure to 65 mm Hg and raise lactate levels to 2 mmol/L (18 mg/dl). The updated septic shock criteria are linked to a high fatality rate (40%) when compared to the death rate associated with the prior definition of sepsis, which was 10%. [3]. AKI appears in roughly 19% of patients with mild sepsis, 23% of patients with severe sepsis, and 51% of patients with septic shock when blood cultures are positive. Sepsis was discovered to be the most frequent cause of AKI in critically sick patients by the beginning and ending supportive therapy (BEST) renal investigators after studying a broad community in 54 hospitals situated in 23 different countries (47.5 percent). They came to the conclusion that septic AKI was associated with increased levels of aberrant hemodynamic and biochemical markers, worse disease severity, and a greater need for mechanical ventilation and vasopressor therapy. This investigation unearthed some further information. Oliguria was shown to be more common in septic AKI (67 percent vs. 57 percent; P 0.001). Septic AKI had a higher rate of in-hospital mortality than nonseptic AKI (70.2 vs. 51.8 percent; p 0.001). The median length of ICU and hospital stays for survivors of septic AKI were longer (37 vs. 21d; P 0.0001) [4]. As a result, separating bacterial from non-septic AKI may be more than just a matter of academic interest for medical professionals. The pathogenesis of septic AKI may be unique, according to certain publications. Patients with septic AKI can therefore react to therapies differently and see different outcomes from those with non-septic AKI [5]. Sepsis is the most common cause of AKI in severely unwell patients. The pathophysiological mechanisms underlying S-AKI are still unclear. As a result, there are no available prophylactic

therapies, and treatment is reactive and nebulous [6]. The primary causes of AKI (such as sepsis, surgical intervention, heart failure, and hypovolemia) are connected with hypoperfusion and shock, and ischemia damage can cause considerable cell damage (e.g., severe tubular necrosis). Although it is now clear that ischemia-reperfusion injury is not the main mechanism causing S-AKI, additional mechanisms must also be at play [7]. Irbesartan, a non-peptide angiotensin II receptor antagonist, is frequently used to patients with hypertension, diabetes type 2, increased blood creatinine, and proteinuria to treat their hypertension and diabetic nephropathy [8]. Irbesartan suppresses vasoconstriction and aldosterone-mediated Na+ retention by selectively binding to the angiotensin II type 1 receptor [9]. This inhibits the effects of angiotensin II. According to several studies, angiotensin II receptor blockers (ARBs) have a beneficial effect on CVD that cannot be fully explained by their ability to lower blood pressure. Instead, this beneficial effect appears to result from ARBs' capacity to reduce arterial stiffening, likely through inhibition of fibrosis and inflammation[10].

1.8.1 Pharmacology Of Irbesartan

Irbesartan is an orally active medication that has no metabolic requirements to become active. Following oral administration, it is quickly and thoroughly absorbed, with a bioavailability of between 60% and 80% that is unaffected by meals. 1.5 to 2 hours following oral administration is when Cmax (maximum) plasma drug concentration (Cmax) is reached[11]. More than 90% of irbesartan's plasma protein binding occurs, while its presence in the other blood cell streams is negligible. Irbesartan is a lipophilic drug with the volume of distribution in healthy male volunteers ranging from 53 to 93 L. The liver actively breaks down irbesartan by oxidative metabolism and glucuronic conjugation. Irbesartan glucuronide, inactive form, is its main metabolite (about 6 % of the total)[12].

Cytochrome P450 (CYP) isoenzyme 2C9 is primarily responsible for oxidizing irbesartan, with CYP3A4 playing a minor role. No interactions with drugs metabolized by CYP3A4, 1A1, 1A2, 2A6, 2B6, 2D6, or other CYP isoenzymes are anticipated, according to in vitro research. Because isoenzymes frequently associated with drug metabolism do not inhibit or increase the effects of irbesartan, there is little probability of a pharmacokinetic drug-to-drug interaction[13].

2. MATERIALS AND METHODS

2.1 Drugs And Chemical

Irbesartan powder was obtained from *Hebei Lingding biological, China,* prepared in a diluted DMSO vehicle [14]. Irbesartan was given intraperitoneally a once-daily dose of 3 mg/kg/day [15, 16].

2.2 Animals Of Study

Forty male albino Swiss mice, weighing 25–30 grams and aged 8–12 weeks, were used in the current investigation. These animals were kept in cages inside an animal house with 12:12 light: dark cycles, a room temperature of 25 °C, 60–65% humidity, and unrestricted access to water and food.

2.3 STUDY DESIGN

The forty mice were sorted into the four major groups after being let to adjust for two weeks. (n = 10):
1. Normal group: Apperantly healthy mice.
2. CLP group: Mice in this group underwent CLP operation.
3. DMSO group: Mice in this group received an equivalent volume of DMSO
4. Irbesartan group: Mice in this group received Irbesartan 3 mg/kg/day intraperitoneally for five consecutive days; CLP surgery on the fourth day; animal sacrifice on the fifth day [15, 16].

2.4 The Experimental Model Of Induced Sepsis (CLP)

Mice were used in the current study to induce polymicrobial sepsis. Based on previous research, sepsis was induced using the cecal ligation and puncture (CLP) model [16, 17]. In brief, an 18-g needle was used in conjunction with the twofold puncture technique to cause organ (renal) dysfunction within the first 24 hours of sepsis. The Mice were anaesthetized with a mixture of anesthetic solution that involved of ketamine (100mg/ml) and xylazine (20mg/ml) in a 2:1 combination ratio [18]. Then around a 2 cm midline incision with done in the mouse abdomen, the cecum was exposed. After ligating and puncturing the cecum right below the ileocecal valve, the cecum was relocated anatomically. A small amount of stool was extracted to ensure the patency of the puncture sites. After that, the abdomen was sutured. Then the animals have received a subcutaneous resuscitative dose of normal saline (20 mL/kg body weight).

2.5 Sample Collection

Animals were anesthetized on day five before being slaughtered, and tissue and blood samples were then collected as follows:

2.6 Blood Samples

Following the scarification procedure, blood was obtained utilizing the direct heart puncture technique. Withdrawn blood was placed in a gel tube for about 20 minutes to allow for the clotting process, after which it was centrifuged at 10,000 rpm for about 10 minutes, and the supernatant was stored at -20 °C for further analysis.

2.7 Tissue Preparation.

Renal was removed and divided into two halves at the conclusion of the experiment. One portion of the renal tissue was washed with saline to eliminate any red blood cells or clots, and then homogenized in a solution of 1:10 (w/v) phosphate-buffered saline (pH 7.4) that contains 1% triton X-100 and a protease inhibitor cocktail [19]. Utilizing a liquid processor with high intensity. Samples were homogenized and then centrifugation at 10,000 rpm for 20 minutes at 4 °C [20]. The supernatant was then gathered and utilized to quantify NGAL, MDA, and SOD. The other renal portion was examined histopathologically.

2.8 Outcome Measurement

2.8.1 Inflammatory Markers (Tnf-A, Il-1 B, And Il-6)

Following the manufacturer's instructions, an Enzyme-Linked Immunosorbent Assay (ELISA) was used to evaluate the serum levels of TNF-, IL-1, and IL-6. Mouse TNF-, IL-1, and IL-6 antibodies had been pre-coated on the ELISA kit plate, accordingly. The samples' TNF-, IL-1, and IL-6 levels were increased, then bound to antibodies and coated on the wells. Then, Mouse TNF-, IL-1, and IL-6 antibodies that had been biotinylated were introduced and bound to the samples' TNF-, IL-1, and IL-6 levels. The biotinylated TNF-, IL-1, and IL-6 antibodies were then combined with streptavidin-HRP and bound. After incubation, unbound Streptavidin-HRP washed away through a washing process. Subsequently, the substrate solution was added, and the amount of Mouse TNF-, IL-1, and IL-6 determined how the color evolved. At 450 nm, absorbance was measured after the reaction was stopped by the addition of an acidic stop solution.

2.8.2 ELISA Measurement Of Serum NGAL

100 l of the appropriate standard and sample should be added to each well. Cover firmly, gently shake, and incubate for 90 minutes at room temperature or overnight at 4°C. After removing the lid and discarding the solution, wash the plate three times with the Wash Buffer Working Solution, letting the solution sit in the wells between washes for a few minutes. To blot the plate, put some paper towels or another absorbent item nearby. Never allow the wells to completely dry out. The plate should then be incubated for 60 minutes at 37 °C after being filled with 100 l of the Biotin-Labeled Identification Antibody Working Solution in each well. Wash the plate three times with the buffer working solution, allowing one to two minutes between applications. After washing the dish with a buffer solution and discarding it, wipe it with a towel or another insulating material. The streptavidin-HRP working solution was added to each well, and the plate was incubated at 37 °C for 45 minutes. Rinse the plate five times with Elution Buffer Working Solution, letting the wash buffer sit in each well for one to two minutes each time. Elution buffer should be discarded, and the dish should be dried with a cloth or other insulating material. Each well should receive 100 l of TMB Substrate Solution before the plate is incubated at 37 °C in the dark for 30 minutes.

2.9. Oxidative Stress Markers

2.9.1 Renal Superoxide Dismutase (SOD)

The superoxide dismutase activity was assessed by colorimetry and a uv-vis spectrophotometer [21]. This process is based on the formation of pyrogallol-quinone through the sod, a reactive intermediate, and the ability of the semiquinone radical to suppress this reaction by radical dismutation. This brown material absorbs visible light at wavelengths below 420 nm.

2.9.2 Renal Malondialdehyde (Mda)

This process is merely being offered as a guide. The product manual could differ somewhat. The product manual that was packaged and sent with the item should be followed before using it. Add 50 µl of the standard or sample to each well, followed by 50 µl of the working solution for the biotinylated detection antibody. Aspirate and rinse the plate three times. Include a 100µL solution of the HRP conjugate. Include 90µL of the substrate agent. Include 50µL Stop Solution. Immediately begin reading the plate at 450nm.

2.10 Histopathological Analysis

The kidney tissues were treated using standard histological techniques and fixed in paraffin blocks after being fixed in 10% formalin. Hematoxylin-eosin (H&E) was used to stain 5-m thick slices for further histological analysis [22]. An investigator who was blind to the experimental treatment groups evaluated the scores after fixation. The histopathological damage was evaluated using the following morphological parameters in accordance with the methodology of [23].

Score 0: normal.
Score 1: Damaged area< 25% of tubules.
Score 2: Injuries affect 25–50% of the tubules.
Score 3: damage affecting 50% to 75% of the tubules.
Score 4: 75–100% of the region is affected.

The term "tubular injury" was used to describe tubular epithelial enlargement, loss of brush boundary, capillary degeneration, tubular necrosis, cast formation, and desquamation

2.10 Statistical Analysis

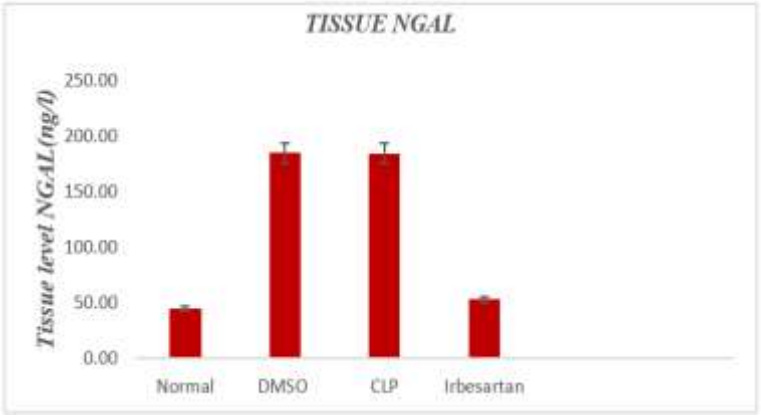
Statistical analysis was done using SPSS version 26. Student t-test and Analysis of variance (ANOVA) with LSD post hoc test was used to investigate differences between groups, and histological differences were confirmed using Kruskal-Wallis with Mann-Whitney U-test. Statistically, the present data significance was defined as $p \leq 0.05$ [24].

3. RESULTS

3.1 Effects Of Irbesartan On Levels Of Tissue And Serum NGAL

For more documented evident results both renal and serum levels of the specific renal injury marker NGAL 24 hours after polymicrobial sepsis induced by CLP, in all experimental groups were carried out with the aid of ELISA assay protocol. ELISA outcomes demonstrated that the serum and tissue levels of NGAL were significantly higher ($p < 0.05$) in the CLP and DMSO groups as compared to apparently healthy group. While, the Irbesartan group exhibited significantly lower levels ($p < 0.05$) of NGAL if compared with the CLP group. Figure 1

Figure 1: Mean tissue level of NGAL (ng/l) in the experimental groups.



*P: significant difference ($p < 0.05$) when compared with the apparently healthy group; #P: significant difference ($p < 0.05$) when compared with the CLP group.

3.2 Effects Of Irbesartan On The Levels Inflammatory Cytokines

To find out the impact of Irbesartan on the inflammatory response that occurs during CLP-induced polymicrobial sepsis the serum levels of inflammatory cytokines including (TNF-α, IL-6, and IL-1β) were measured using the ELISA technique 24 after CLP-induced polymicrobial sepsis. The current study demonstrated that the serum level of inflammatory cytokines (TNF-α, IL-6, and IL-1β) was significantly higher ($p < 0.05$) in the CLP group as compared with the healthy group. While, the Irbesartan group showed significantly lower ($p < 0.05$) levels of inflammatory cytokines (TNF-α, IL-6, and IL-1β) if compared with the CLP group, figure 2.

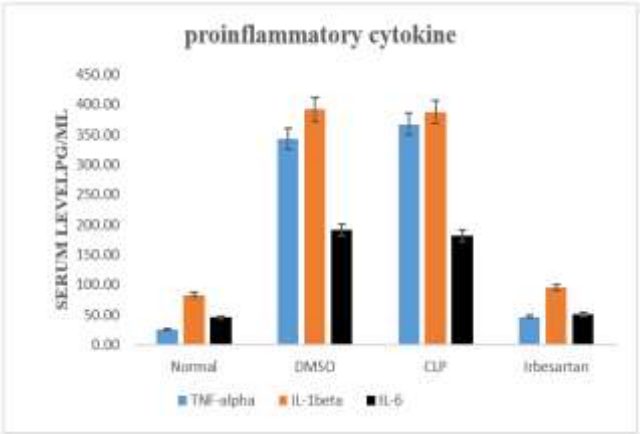


Figure 2: Mean serum level of inflammatory cytokines (TNF-α, IL-6, and IL-1β)

Data are expressed as mean ± SD; *P <0.05 versus the healthy group; #P <0.05 versus the CLP group.

3.3 Effects Of Irbesartan Oxidative Stress Biomarker

3.3.1 Effects Of Irbesartan On The Renal Tissue SOD Activity (U/Ml) In The Experimental Groups.

The current study outcomes showed that the degree of renal tissue SOD activity was significantly lower ($p < 0.05$) in the CLP and DMSO groups when compared with the apparently healthy group. While the irbesartan group showed a significantly higher degree ($p < 0.05$) of SOD activity as compared with the untreated CLP group. Figure 3

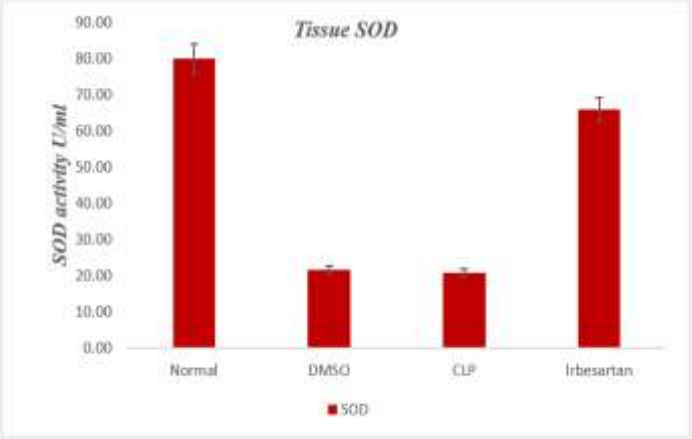


Figure 3: Mean tissue level of SOD activity (U/ml)

Data are expressed as mean ± SD; *P <0.05 versus the healthy group; #P <0.05 versus the CLP group.

3.3.2 Effects Of Irbesartan On The Renal Tissue Level Of MDA In The Experimental Groups.

Current study outcomes showed that the levels of renal tissue MDA were significantly higher ($p < 0.05$) in the CLP and DMSO groups as compared to the apparently healthy group.
On the other hand, irbesartan group showed significantly lower levels ($p < 0.05$) of MDA as compared to the CLP group.

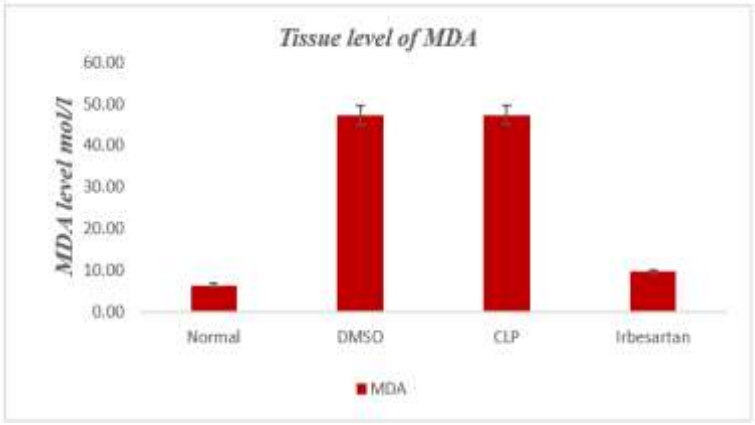


Figure 4: Mean tissue level of MDA activity (mol/l)

Data are expressed as mean ± SD; *P <0.05 versus the healthy group; #P <0.05 versus the CLP group.

3.4 The Effects Of Cilostazol On Sepsis-Induced Histopathological Changes

A histological investigation of renal tissue was conducted to provide more evidence on the effects of irbesartan on renal injury that occur during polymicrobial sepsis.

In this histopathological analysis serial sections of renal tissue were obtained after 24 hours of CLP-induced polymicrobial sepsis and subjected to Hematoxylin and Eosin staining (H&E). The histopathological findings were summarized according to each group of mice as follows. CLP renal tissue demonstrated a substantial significant ($P < 0.05$) renal damage as compared to the apparently healthy group, showing degenerated renal tubules. Hemorrhage in the interstitial tissue enlarged sub-capsular space and glomerular tuft degeneration and necrosis of the epithelial cells in the renal tubules. This group had 10% mild, 50% moderate, and 40% severe histological grading from normal renal tissue, and the final score was severe. Additionally, the irbesartan group showed a significant ($P<0.05$) reduction in renal injury as compared to the CLP group. Irbesartan group histological changes arranged from no change to moderate changes with a different number of mice.

In terms of histopathological grading from normal renal tissue, the Irbesartan group showed 10% no change, 60% mild, and 30% moderate and the final score was mild damage as shown in table 3-1.
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Table (3-1): Differences in the histopathological grading of abnormal renal changes in the four experimental groups

Score/Groups	Healthy		CLP		DMSO		Irbesartan	
	N	%	N	%	N	%	N	%
Normal (0)	10	100%	0	0	0	0	1	10%
Mild (1)	0	0	1	10%	1	10%	6	60%
Moderate (2)	0	0	5	50%	4	40%	3	30%
Severe (3)	0	0	4	40%	5	50%	0	0
Total	10	100%	10	100%	10	100%	10	100%
Final Score (Severity)	Normal		Severe		Severe		Mild	

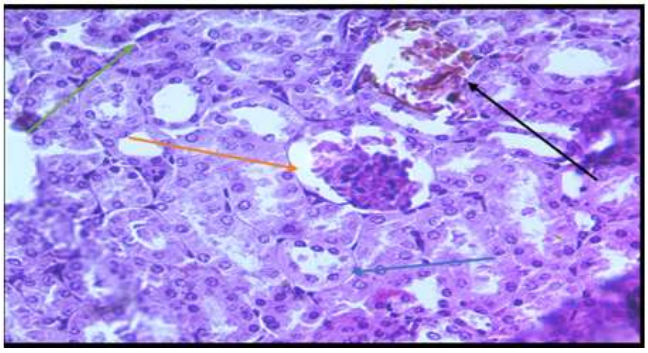

Figure 3.5: Cross section of the renal mice, CLP, blue arrow show degenerated renal tubules, black arrow show hemorrhage in the interstitial tissue, orange arrow showed enlarged sub-capsular space and glomerular tuft degeneration and the green arrow indicate necrosis of the epithelial cells in the renal tubules. (H and E)(10X&40X).

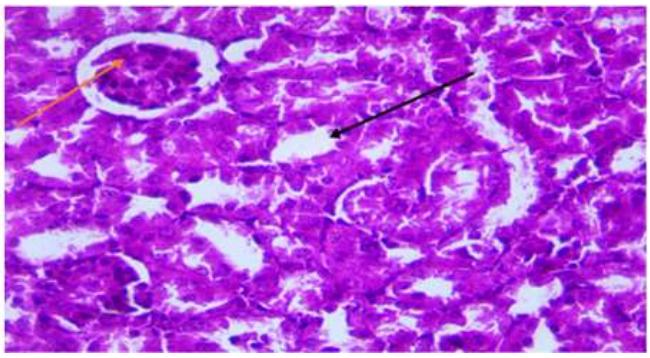

Figure (3.6): Cross section of the kidney of mice in healthy group shows normal all architecture of tissue (40x), (H & E). Orange arrows show normal glomeruli, black arrows show normal renal tubules and no neutrophil infiltration.

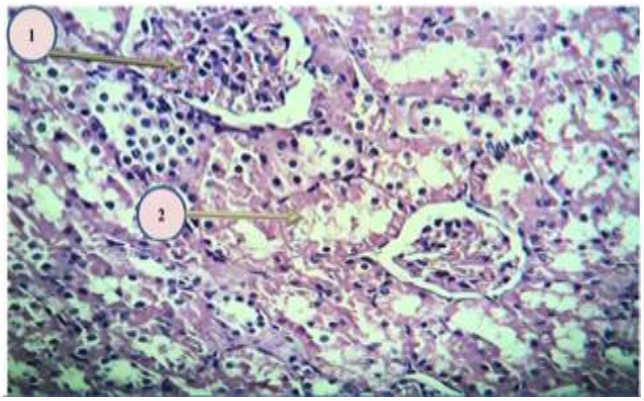

Figure3-7: Section through kidney (Irbesartan group) show in Almost normal glomerulus (1) and increased cytoplasmic eosinophilia With the fragmentation of renal tubule cells (2). H and E stain (40X).

4. DISCUSSION

Sepsis is a potentially fatal organ failure initiated by an uncontrolled immunological and inflammatory response within the body [25]. Acute kidney injury (AKI), a frequent complication of severe sepsis, is on the rise and is associated with a high rate of morbidity and mortality. Numerous pathways can result in septic AKI [26]

The current study declared that the serum and tissue levels of NGAL were significantly higher in the CLP group as compared with the apparently healthy group, these results agreed with the previous work done by [27], who found serum NGAL increased with sepsis severity, even much higher in septic patients with AKI than those without AKI. NGAL was for several years considered the 'troponin' of the kidney [28]. On the other hand when compared to the CLP group, the tissue and serum levels of NGAL were significantly lower in the irbesartan group. Previous study was demonstrated that human subjects who received short-term therapy of ARBs had considerably lower urine NGAL levels [29]. Consistently, we have recently observed that ARBs suppressed urinary excretion of NGAL in diabetic mice induced by streptozotocin (STZ) [30].

On the other hand when compared to the CLP group, the Irbesartan group's serum level of the pro inflammatory cytokine (TNF-α, IL-1β and IL-6) was significantly lower, these results were consistent with previous studies that shown treatment with irbesartan reduce the serum level of inflammatory cytokines [31] Furthermore, previous study exerted that treatment with irbesartan decrease the level of proinflammatory cytokines IL-6, IL-1β, and TNF-α patients with chronic glomerulonephritis [32]. The current investigation showed that the tissue level of MDA in the irbesartan group was much lower than in the CLP group. Irbesartan can reduce oxidative stress in AKI, as evidenced by the fact that MDA activity was decreased in the therapy group. Irbesartan has been found to reduce oxidative stress, inflammation, and metabolic syndrome. Most people agree that blocking the renin-angiotensin-aldosterone pathway lowers MDA [33]. Irbesartan has been shown by Kuwabara et al. to have antioxidant properties and to decrease NADPH oxidase activation [34]. When compared to the CLP group in the current investigation, the irbesartan group showed a considerably higher level of SOD activity.

Irbesartan is conventionally used for the prevention and management of kidney diseases [35].

Irbesartan has a considerable nephron-protective effect through improving podocyte function and decreasing renal inflammation and effect on acute nephrotoxicity reduction via control of antioxidant and oxidative stress ability [36].

During sepsis-induced renal damage there was several histological alterations have been observed during sepsis-induced kidney injury, including the CLP procedure caused significant induced kidney injury in rats by inducing sepsis because the CLP group displayed increased inflammatory infiltration, obvious edema, significant hemorrhagic spots in the interstitium, narrower renal tubules, enlarged glomerular, and degenerated epithelial cells[37]. The mice in the apparently healthy group showed normal architecture and without neutrophil infiltration and normal glomerulus and renal tubules; comparing the CLP group to the apparently healthy group in the current investigation, a significant histopathological alteration was seen in the CLP group. Showing degenerated renal tubules, hemorrhage in the interstitial tissue enlarged sub-capsular space, and glomerular tuft degeneration and necrosis of the epithelial cells in the renal tubules, these findings are consistent with a previous study that was done on mice to study the protective effects of sirtuin 3 in a murine model of sepsis-induced acute kidney injury [38]. Regarding the irbesartan group in the current study, this group showed a significant reduction in the histopathological changes that arranged from no change to moderate changes as compared to the CLP group. The irbesartan treatment resulted in a marked reduction in renal injury as compared to the CLP group. Irbesartan group histological changes were arranged from no change to moderate changes with a different number of mice. Show in the almost normal glomerulus and increased cytoplasmic eosinophilia with the fragmentation of renal tubule cells. These findings are agreed with a previous study that was done on rats to assessment of nephron-protective role of Irbesartan against gentamicin induced nephrotoxicity in rats [39].These reno-protective effects of irbesartan may be attributed to its anti-inflammatory and antioxidant effect [40].

5. CONCLUSION

Irbesartan demonstrate itṡ renoprotective effects possibly through itṡ anti-inflammatory and antioxidant effects. Irbesartan, also exhibit renoprotective properties by suppressing the expression of a renal-specific marker (NGAL). In addition, this medications have a nephroprotective impact by reducing the histological alterations that result from polymicrobial sepsis.

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