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Nephroprotective Potential Effect of Sacubitrilate in Renal Ischemia Reperfusion Injury Role of NF-Kbpathway in Rat

Alaa Kadhum Mosa [1] , Basem I. Mohammad Al-Shibani [2] , Mohammed A. Abdulhussein [1] , Najah R. Hadi [1] , Hyder Qassam [1] , Yasmeen Ali Hussin [3]

[1] Kufa University/Faculty of Medicine, Najaf, Iraq

[2] Qadisiyah, College of Pharmacy, Qadisiyah, Iraq

[3] Al-Kafeel University, College of Pharmacy Najaf, Iraq,

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ABSTRACT

Acute kidney injury (AKI) with all advances in nursing measures and therapeutic strategies, such as kidney transplantation and dialysis, mortality rate of patients with AKI is very high in past 30 years. Despite the path physiology of IRI is not totally understood, several critical mechanisms leading in kidney failure have been demonstrated. In ischemic kidney and successive of generation of reactive oxygen species (ROS), re-oxygenation at reperfusion phase activate a cascade of destroying cellular responses causing to cell death, inflammation, and acute kidney failure. Sacubitrilate is neprilysininhibitor attenuated renal injury possibly through its anti-inflammatory and antioxidant effects. The main objective of the present paper is to study the Nephroprotective potential effect of Sacubitrilate in RIRI. Renal IRI causes significant ($p<0.05$) increase in tissue level of IL-1β, NGAL, f2- isoprostane, TLR-4 and NF-KB p65 and serum urea, creatinine. Pretreatment with, Sacubitrilate cause significant ($p<0.05$) decrease in tissue level of IL-1β, NF-KB p65, TLR-4, NGAL, f2- isoprostane, and serum urea, creatinine. Also cause significant reversal of tissue damage when compared with IRI group. Pretreatment with Sacubitrilate significantly decreases renal ischemia reperfusion injury in the rat via their pleiotropic effects as anti-oxidant, anti-inflammatory and anti- apoptotic activity.

Keywords: Renal Ischemia/Reperfusion Injury (RIRI), Sacubitrilate.

Correspondance:
Alaa Kadhum Mosa
Kufa University
Faculty of Medicine, Najaf, Iraq

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INTRODUCTION

Acute renal injury (AKI), also known as acute renal failure, is a global public health problem that affects millions of people, and it has become more prevalent in recent years. Several risk factors like age, race, genetic factor, hypertension, and diabetes are associated with AKI (1). Acute kidney injury has been largely studied in clinical and experimental animal model. The mechanisms of the pathogenesis and etiology of AKI are complicate and involve ROS, mitochondrial dysfunction, inflammation, Autophagy, apoptosis and necrosis. Inflammatory responses are other critical part in the induction and exacerbation of the AKI. Despite inflammation is a crucial part of the body's immune system, extra stimulation of cytokine secretion and inflammatory cells cause serious injury to the renal parenchyma cells. Ischemia activates a great delivery of substance from the injured tissue (DAMPs), like heat shock proteins, hyaluronan acid, fibronectin and DNA that activate Toll-like receptors (TLR2, TLR4, and TLR5) the evolutionary maintain family of trans-membrane receptors that are a kind of (PRR) (2). Evocation of (PRR) maybe activates production of pro-inflammatory cytokines and the death signaling pathway (3). When engaged TLRs activate the formation of a chemokine, such as TNF-α, IL-1β, IL-6 and chemokine formed by keratinocytes, further accompanied by macrophage and Neutrophils infiltration and pro-inflammatory cytokines (4). Transduction of the signals after TLR evocation related to many adaptive proteins, such as MyD88 which is the most critical one, leading in evocation of transcriptional factor NF-κB, that lead to subsequent formation of chemokine, pro-inflammatory cytokines (5)(4). Also TLR-2 and TLR-4 expressed by dendrite cells, macrophages and tubular epithelial cells. This expression is raised at the time of kidney IRI (6). TLR-4 lack on kidney parenchyma cells inhibited the raise of pro-inflammatory cytokines and chemokine formation and macrophages and Neutrophils aggregation in experimental RIRI models (4). Nuclear Factor Kappa B p65 (NF-KB p65)Until know, detected data suggest one of the critical players in the pathogenesis of ischemia-reperfusion injury is the NF-κB pathway (7). Considerable evidence suggests NF-κB in formation of chemokine, cytokines, (ROS) also in regulation of anti and pro-apoptotic signaling, seemingly important in the pathogenesis of IRI (8-9). NF-κB act as transcription factor in inflammatory cells and tubular epithelial cell, associating the cell dying signaling pathway and coordinated inflammatory proposed in conception of necro-inflammation. It has been demonstrate NF-κB evocation in kidney tubular epithelial cell irritated tubular damage and aggravated inflammation in animal models of RIRI (10). Sacubitrilate, which selectively inhibits neprilysin, a neutral end peptidase that cleaves natriuretic peptides causing natriuretic and diaursis as well as certain vasoconstrictions peptides including as angiotensin I and II, and endothelin-1, additionally, Sacubitrilate may inhibit neprilysin-mediated catabolism of certain peptide-based agents, thereby improving their in vivo stability and increasing tumor cell exposure (11).

MATERIALS AND METHODS

Preparation of animals

24 adult male of Swiss Albino rat (weighting 250 - 350 g, aged 10-12 weeks) were purchased from animal resource center, College of Science University of Duhok (UOK) . All experiments were approved by animal care and research committee of the University of Kufa. Animals were housed in animal house of University of Kufa, in a temperature controlled (24°C ± 2°C) room with alternative 12-hr light and 12-hr dark cycles and were allowed free access to water and diet until the start of experiment.

Ethical Statement

This study was accordance in exacting understanding with the suggestions in the Guide and Use of Laboratory animals association for Laboratory animal science. All animals' considerations and conventions were approved by animal care committee. All rats are sacrifice was performed under xylazine and ketamine mixture anesthesia.

Design of the study

The rats were randomized into 6 groups (6 rats each) as following:

Rats will be subjected to bilateral renal ischemia for 30 min; followed Sacubitrilate administrated 25mg/kg dissolved in DMSO solution (11), administrated ip. 30 minute before RIRI. Selected biochemical and morphological parameters will be followed in the sham-operated animals and rat subjected to RIRI and pretreated with DMSO, Sacubitrilate.

1-IRI (control) group (n=6), rats underwent 30 min bilateral renal ischemia (and 2 hr reperfusion (12)

2-Sham group (n=6), rats underwent same anesthetic and surgical procedures except for ischemia.

3-Vehicle group (n=6), rats pretreated with DMSO Sacubitrilate was given intraperitoneal injection at 30 minutes before bilateral renal artery clamping and reperfusion for 2 hr.

4- Sacubitrilatetreated group (n=6), rats underwent IRI and pretreated with 25mg/kg (11) by intraperitoneal injection 30 minutes before bilateral renal artery clamping and reperfusion for 2 hr.

Preparation of Sacubitrilate

Sacubitrilate, This product is soluble in DMSO 2 mg/mL (clear solution) according to medchemexpress package insert.

Experimental study model

Rat was anesthetized using an intraperitoneal injection of ketamine in a dose of 100 mg/kg and xylazine in a dose of 10 mg/kg (13). Under sedation (5–10 min), rat were placed on its back, fixed their limbs, and tail with stickers to ensure their stability during surgery. Hair in the abdomen area was shaved and the skin disinfected. The reflexes were checked through pinching the tail and the hind feet to be sure that the rat were sufficiently anesthetized By making midline laparotomy incision to expose the abdomen and in order to expose renal pedicles, the intestines were retracted, Right and left renal nephrectomy using the bilateral model of ischemia, the micro vascular clamp was positioned around the right and left renal pedicles (14).The total time of clamp was 30 min and through this procedure, the abdomen covered using warm and moist gauze. During the procedure, rat were kept well hydrated with warm sterile saline at the constant temperature (37°C) (15). After ischemia, atraumaticmicrovascular clamps were removed for reperfusion (16). The incision line in the abdominal region was closed using 3/0 silk suture. After 2 hr, anesthesia was administered to all animals including the control group, and bilateral nephrectomy was performed via laparotomy (17), the animals are euthanized and both blood as well as tissue samples were collected for analysis. All blood was taken through cardiac puncture. For all groups, one of the kidneys removed was stored in 10% formalin for histopathological evaluation, and the other kidney was freeze-stored at –80-°C for tissue enzyme and receptor analysis (18).

Collection and Preparation of blood samples

Serum sample

At the end of procedure (reperfusion time 2 hr), the rats re-anesthetized with xylazine 10 mg/kg and ketamine 100mg /kg. About (2.5-3ml) of blood was collected directly from the heart after reperfusion time (at the end of procedure) (19). For establishment of baseline cytokine values, blood was collected from rat and for ensuring uniformity, all samples were processed identically. Blood collected and then centrifuged at 3000 RPM for 10 minute. Serum was collected and centrifuged for a second time at 3000 RPM for 1 minute to ensure elimination of any red blood cells (20). At the end of experiment, the animals were sacrificed by deep anesthesia (21)

Preparation of tissue for measurement of TLR4, NF-KBp65, IL1β, NGAL, F2-isoprostane

The kidney tissue was rinsed with ice-cold saline to remove any red blood cells or clots and then homogenized with a high-intensity ultrasonic liquid processor in 1:10 (w/v) PBS that contained 1%Triton X-100 and a protease inhibitor cocktail (22). The homogenate was centrifuged at 15000xg for 30 min at 4°C (23, 12). The supernatant was collected for measurement of the above analyses

Tissue sampling for histopathological analysis and damage scoring

Left kidney tissue obtained after the sacrifice of rat were fixed immediately in 10 % formaldehyde and were processed in paraffin tissue blocks and macroscopic sections were taken to include the renal cortex and pelvis. Sections of 5 µ thickness cut from formalin fixed paraffin-embedded block were stained with hematoxylin eosin dye As described

previously (24) histopathological changes, including loss of brush border, tubular dilation, cast formation, and cell lyses, were evaluated. Tissue damage was examined in a blind manner and scored according to the percentage of damaged tubules: 0, no damage; 1, <25%; 2, 25 to 50%; 3, 50 to 75%, 4, >75% (25-26). Histopathological examination was performed in 40-100 original magnification with light microscopy were used (27-28). Statistical analyses were performed using SPSS 24.0 for window. Inc. Data were expressed as mean ± SEM analysis of variance (ANOVA)was used for the multiple comparisons among all groups followed by post –hoc tests using Bonferroni method. For the histopathological renal changes, the Mann-Whitney U was used to assess the statistical significance of difference between two groups, the Kruskal -Wallis test was used to assess the statistical significance of difference across multiple groups in total severity score in all tests P<0.05 was considered statistically significant.

Effect of Sacubitrilate on kidney markers following renal IRI

Effect on IL1

Renal IRI causes significant increase in mean ± SEM of tissue level of IL1β (3890.69±72.24pg/ml, p value 0.001) when compared with sham group (198.18±8.49 pg/ml, p value 0.001), Sacubitrilate cause significant decrease in mean ± SEM of tissue level of IL1β (1019.57±11.51pg/ml, p value 0.001) when compared with IRI group.

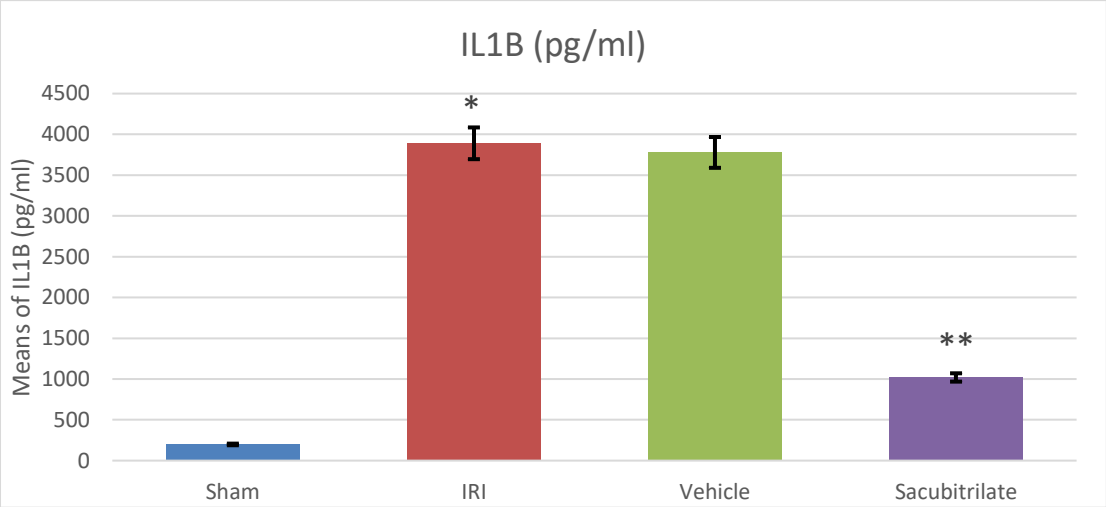

Figure3.1 Bar chart error show the effect of Sacubitrilate on tissue IL1β level following renal IRI , expressed as mean ± SEMng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

Effect on NF-KB p65

Renal IRI causes significant increase in mean ± SEM of tissue level of NF-KBp65 (4682.06±114.02pg/ml, p value 0.001) when compared with sham group (120.45±19.01pg/ml, p value 0.001), and Sacubitrilate group, Sacubitrilate cause significant decrease in mean ± SEM of tissue level of NF-KBp65 (1685.65±189.31pg/ml, p value 0.001) when compared with IRI group.

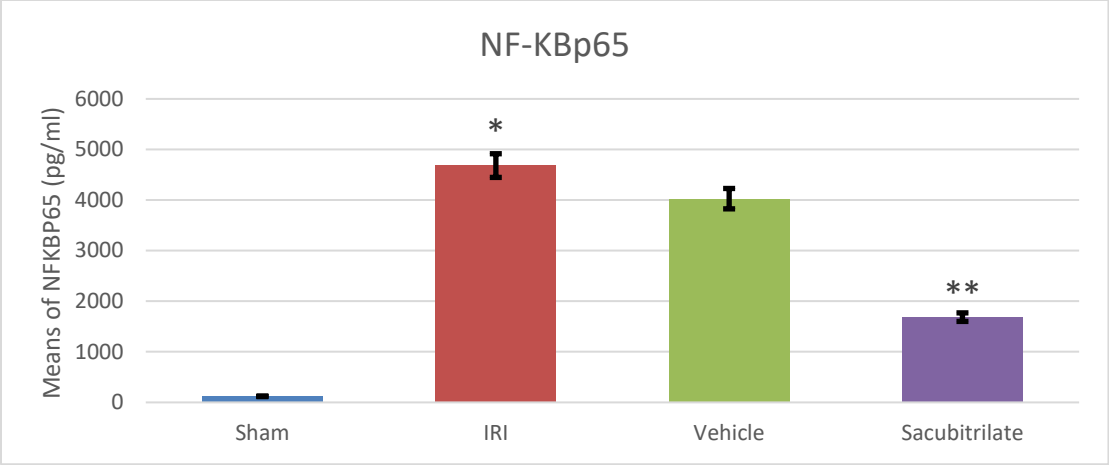

Figure3.2 Bar chart error show the effect of Sacubitrilate , valsartan and their combination on tissue NF-KBp65 level following renal IRI , expressed as mean ±SEM ng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

Effect on TLR4

Renal IRI causes significant increase in mean ± SEM of tissue level of TLR4 (18.67±0.40pg/ml, p value 0.001) when compared with sham group (1.06±0.20pg/ml, p value 0.001). Sacubitrilate cause significant decrease in mean ± SEM of tissue level of TLR4 (12.51±0.21pg/ml, p value 0.001) when compared with IRI group.

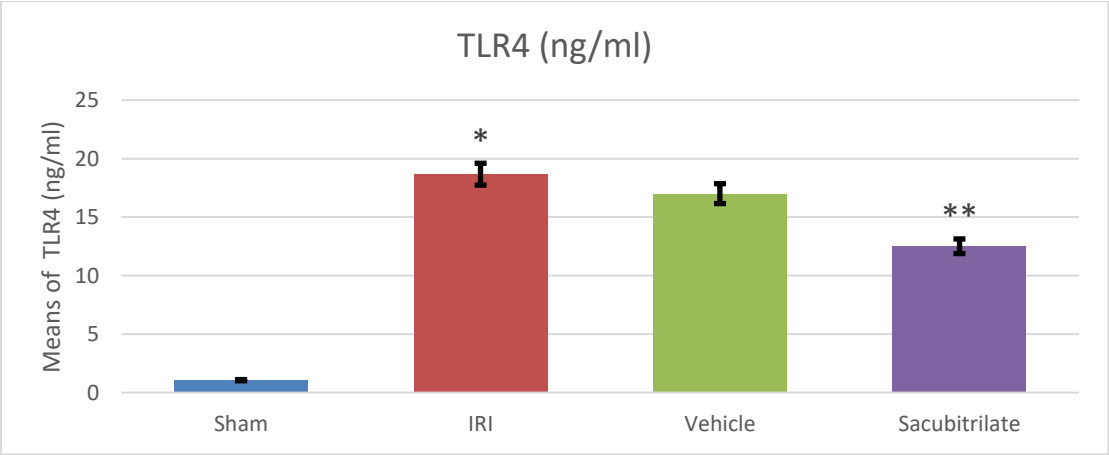

Figure3.3 Bar chart error show the effect of Sacubitrilate, valsartan and their combination on tissue TLR4level following renal IRI , expressed as mean ± SEMng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

Effect on NGAL

Renal IRI causes significant increase in mean ± SEM of tissue level of NGAL (3459.39±59.42pg/ml, p value 0.001) when compared with sham group (192.55±16.15 pg/ml, p value 0.001), Sacubitrilate cause significant decrease in mean ± SEM of tissue level of NGAL (1578.52±144.87 pg/ml, p value 0.001) when compared with IRI group.

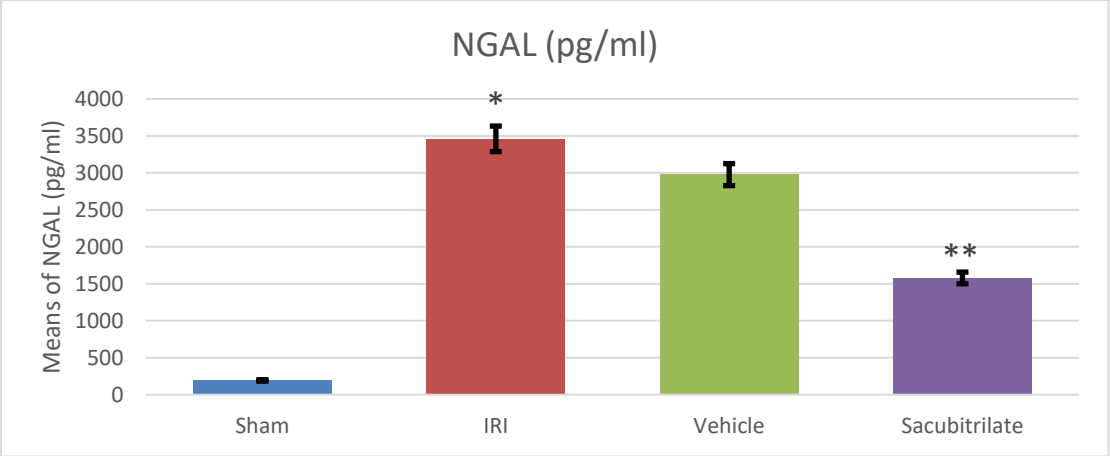

Figure 3.4: Bar chart error show the effect of Sacubitrilate, valsartan and their combination on tissue TLR4level following renal IRI , expressed as mean ± SEMng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

Effect on F2-isoprostane

Renal IRI causes significant increase in mean ± SEM of tissue level of F2- isoprostane (885.25±5.68pg/ml, p value 0.001) when compared with sham group (78.41±31.51pg/ml, p value 0.001). Sacubitrilate cause significant decrease in mean ± SEM of tissue level of F2-isoprostane (212.45±6.75pg/ml, p value 0.001) when compared with IRI group.

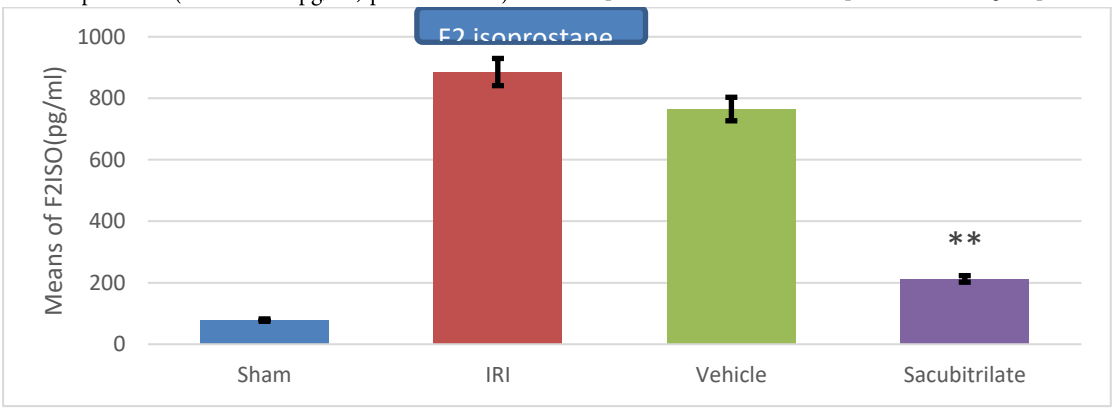

Figure 3.5: Bar chart error show the effect of Sacubitrilate , valsartan and their combination on tissue 8-epi-PGFα level following renal IRI , expressed as mean ± SEMng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

Effect of Sacubitrilate on blood urea and serum creatinine following renal IRI

Renal IRI causes significant increase in mean ± SEM of both blood urea and serum creatinine (101.17±2.29, 0.90±0.02 mg/dl, p value 0.001, 0.001 respectively) when it compared with sham group (56.50±0.56, 0.30±0.03 mg/dl, p value 0.001, 0.001 respectively). Sacubitrilate cause significant decrease in mean ± SEM of both blood urea and serum creatinine (68.33±2.23, 0.52±0.01 mg/dl, p value 0.001, 0.001 respectively) when it compared with IRI group.

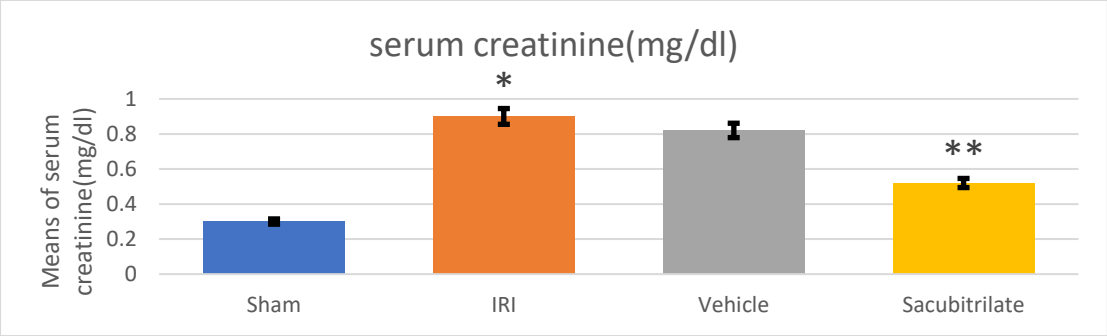

Figure3.6: Bar chart error show the effect of sacubitrilateon blood urea following renal IRI, expressed as mean ± SEMng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

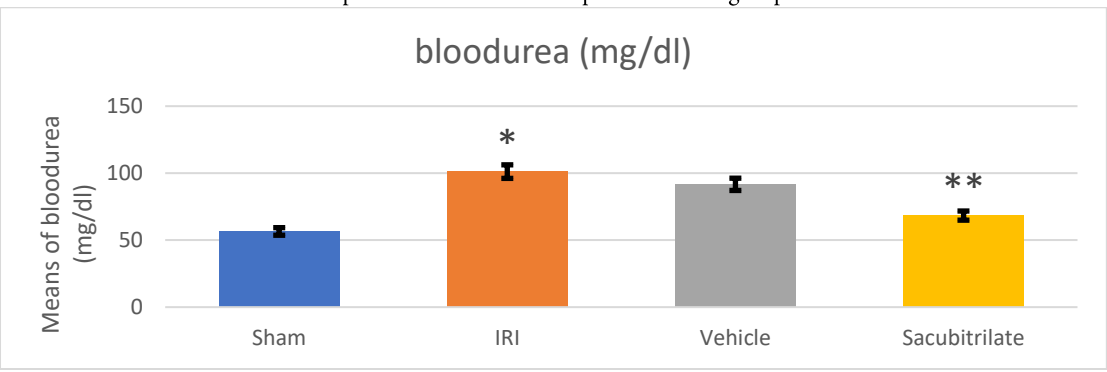

Figure 3.7: Bar chart error show the effect of Sacubitrilate , valsartan and their combination on serum creatinine following renal IRI , expressed as mean ± SEMng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

Histopathological findings

Figure 3.8shown the Histopathological score in the four experimental groups, IRI cause significant tissue damage (p value 0.001), and pretreatment with Sacubitrilate cause reducing of tissue damage (p value 0.002,). Figure (3.9) showed normal renal tubules without inducing IRI .figures (3.10, 3.12) showed significant tissue damage caused by IRI mainly apoptic/necrotic renal tubules, figure (3.11) shown how pretreatment with Sacubitrilate caused significant reduction in necrosis of rtubules that caused by RIRI.

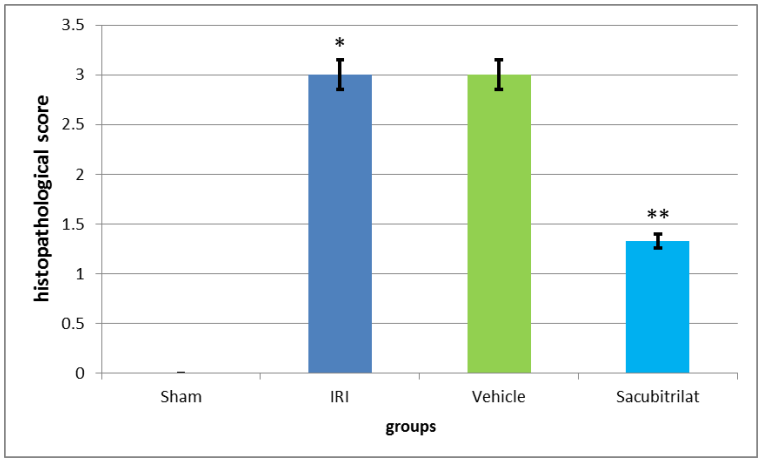

Figure3.8.Bar chart error show the effect of sacubitrilateon Histopathological score of kidney injury following renal IRI, expressed as mean ± SEM ng/ml, n=6 in each group

*p value ≤ 0.05 when compared with the sham group

**p value ≤ 0.05 when compared with IRI group

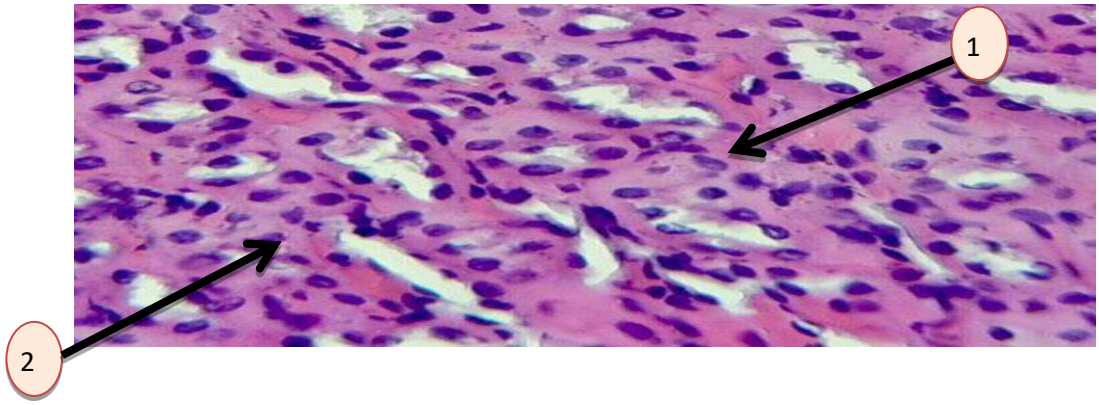

Figure 3.9: Photomicrograph of adult male rat kidney with H and E staining at x40 magnification for the sham-operated group (without induced IRI), in which it did not show any morphological changes (illustrating severity score zero as it shows normal renal tubules) as marked (1), (2) normal renal tubules with intact glomeruli (red raw) and normal Baumann capsule (blue raw)

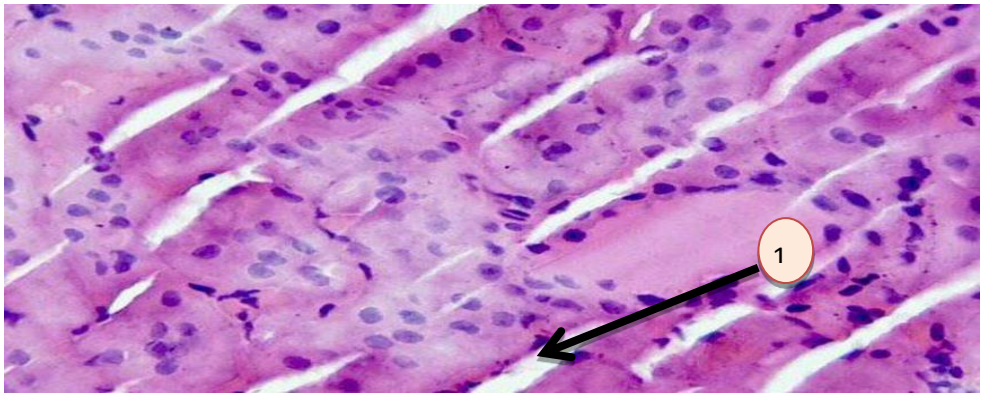

Figure 3.10: Photomicrograph of un treated ischemic adult male rat kidney with H and E staining at x40 magnification for the control group (with induced IRI) in which it showed morphological changes as cellular vacuolization, nuclear shading karryolysis and moderate to severe necrosis (illustrating severity score three as it shows tubular cellular swelling, loss of brush borders and eosinophilic cast) as marked (1) for eosinophilic cast.

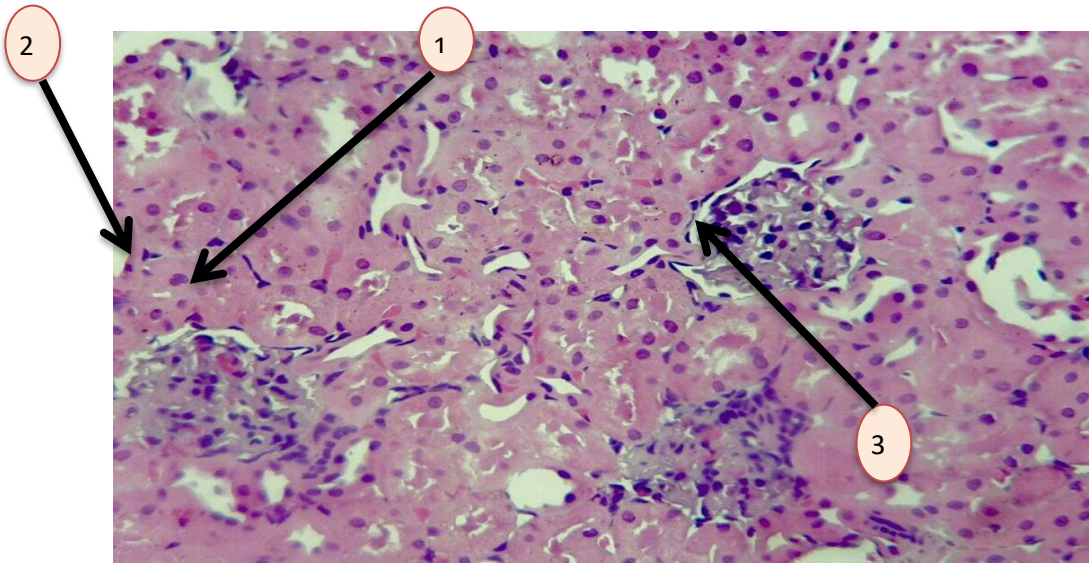

Figure 3.11: Photomicrograph of adult male rat kidney with H and E staining at x100 magnification for the Sacubitrilate group (with induced IRI) and pretreated with it in which morphological changes have not showed mostly as cellular vacuolization, nuclear degeneration and moderate to severe necrosis (illustrating severity score one ischemia cellular swelling in <25% of tissue as it shows normal renal tubules , less scattered apoptotic/necrotic cells with weak eosinophilic cytoplasm and without pyknotic nuclei) as marked (1), (2) normal renal tubules and (3) normal glomerulus. The entire field showed mild necrotic renal tubules.

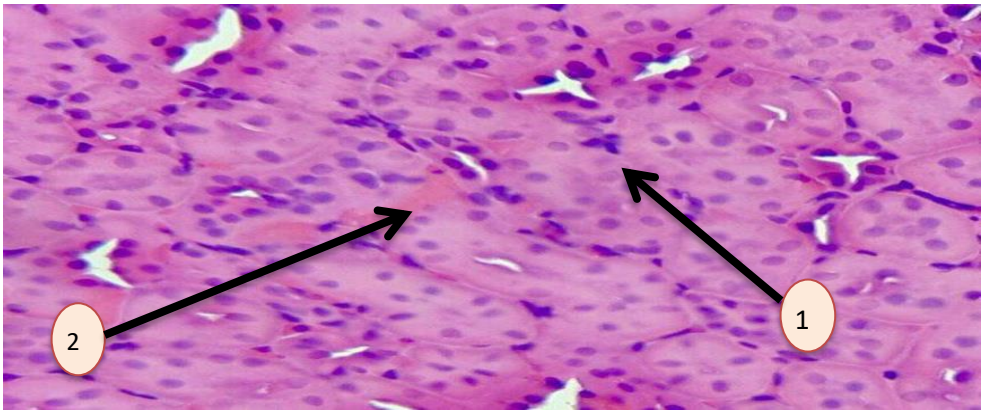

Figure 3.12: Photomicrograph of adult male rat kidney with H and E staining at x40 magnification for the vehicle group(with induced IRI) that were pretreated with DMSO and in which it showed morphological changes as cellular vacuolization , nuclear degeneration, and moderate to severe necrosis (illustrating severity score three as it shows tubular cellular swelling ,loss of brush borders and eosinophilic cast formation)as marked (1)and (2)for tubular cellular swelling. The entire field showed mostly necrotic renal tubules.

Discussion

Ischemia-reperfusion injury (IRI) is induced by a rapid temporary deterioration of the blood flow to the specific organ. IRI mostly is occurred with a powerful oxidative stress and inflammatory responses to the hypoxia and reperfusion that disrupts the organ function. RIR caused acute kidney injury (AKI) present with high morbidity and mortality rate in a broad range of injuries (29).

Effect of Renal IR on Inflammatory Mediators (IL-1β and NF-κB p65)

This research proved that there is a significant increase in inflammatory mediators (IL-1β and NF-κB p65) were found in the control and control vehicle group as compare with the sham group, additionally the present study mounted that the Inflammatory Mediators had been proven to response key roles within the path physiology of ischemia/reperfusion injury (30). IL-1β is considered as chemo-attractant that recruits leukocytes to the area of the renal inflammation which causes eventually to the kidney damage. During the early phase of IRI, Large amounts of reactive oxygen species and nitrogen intermediates and pro-inflammatory cytokine such as (IL-1β and TNFα) are produced by M1machrophage and drive a polarized Th1 immune response which contributes to injury (31), (23) showed that levels of (NF-κB p65) was increased in I/R group when the rats subjected to 30 min ischemia, 2 hours reperfusion

Effect of Sacubitrilate on Inflammatory mediators (IL-1β and NF-κB p65)

The present study was found that there is a significant decrease in renal level of (IL-1β and NF-κB p65) for Sacubitrilate treated group in comparison to control vehicle group. A few of the therapeutic actions of neprilysin are mediate by their preventive brunt on the RAAS pathway, there is sufficient evidence that neprilysin (especially ANP and BNP) have anti-inflammatory, antioxidant and anti-fibrotic activity which may significantly related to their. There is sufficient evidence that NPs (especially ANP and BNP) have anti-inflammatory, anti-oxidant and anti-fibrotic activity which may significantly related to their renoprotective effects (32). For example, it has been identified that ANP blocks the activation of the nuclear factor NF-κB and iNOS the remnant kidney indicated a significant raise in the nuclear translocation of p65, denoting to activation of NF-κB, the general transcription factor for various pro-inflammatory molecules including IL-1β and MCP-1 that were significantly increased, so reduction in the formation of halogen, reactive nitrogen and oxygen species and decreases release and generation of chemokine and cytokine(33). Also ANP has been found to have a preventive action on the formation of inflammatory mediator in macrophages, cardiomyocytes cells and endothelial (34)

Effect on Renal TLR-4 level

In the present study, a significant increase in tissue TLR-4 level was found in the control and control vehicle group as compared with the sham group, Moreover, the present study established that the TLR-4 signaling pathway plays an important role in the renal IRI-associated inflammation and apoptosis. This result is in agreement with those reported by

(35). Their results suggest that activation of TLR-4 signaling contributes to the pathogenesis of renal I/R injury, whereas TLR-9 may be redundant for the development of this injury (36) Showed that the TLR-4 is markedly up-regulated in both experimental and human acute renal allograft rejection.

Effect of Sacubitrilate on TLR-4 level

The present study found that there is a significant lower level of TLR-4 in Sacubitrilate treated group compared to the control vehicle group. To the best of our knowledge, there is no data available about the effect of Sacubitrilate on TLR-4 in renal ischemia/reperfusion injury. The results of our study about the effect of Sacubitrilate TLR-4 are probably due to the anti-inflammatory and apoptotic effect and through NF-KB pathway.

Effect of Renal IR on Renal Injury marker NGAL

In the present observe, a considerable increase in tissue level of NGAL found in the induced un treated group when compared with sham group. In this study we observe that in settlement with (37) who confirmed that there has been a higher plasma concentration of NGAL following 6 hours renal IRI and 24 hours reperfusion. Mouse unilateral IRI suggested that tissue NGAL and MCP1 expression, in addition to M2 macrophage markers, are promising markers as screening to determine severity of fibrosis after AKI. Those consequences in the present study are in settlement with those said by (38) who confirmed that following a forty five min period of intestinal ischemia, 2hours reperfusion drastically extended serum NGAL level on the RIR group as compared with the Sham group in mice (39). These effects recommend that the level of NGAL is sensitive and particular markers of acute kidney injury in animals.

Effect of Sacubitrilate on Renal Injury marker NGAL

The study showed that there was a significant decrease in renal level of (NGAL) in Sacubitrilate treated group compared to control vehicle group. This result are in agreement with those pronounced with the aid of (38), confirmed that treatment with Sacubitrilate significantly reduced the renal injury and apoptosis compared with IR group.

Effect of Renal I/R injury on oxidative stress marker F2-isoprostane

In this study it has been observed that it is significantly increased in F2-isoprostane level in the control group as compared to the sham group .Increased oxidative stress and production of reactive oxygen species play a key role in triggering and retaining the inflammatory response.

Measurement of F2-isoprostanes has been proven to be one of the maximum consistent processes to measure oxidative stress condition (40). The outcomes within the present study are in settlement with the ones stated by means of (41) who exhibited that Plasma F2-isoprostane level accelerated substantially extra all through surgery among topics in the end recognized with AKI than amongst non-AKI risk matched control subjected.

Effect of Sacubitrilate on F2-isoprostane

Sacubitrilate treated group showed a decrease in the renal level of F2- isoprostane as compared with control vehicle group. To the best of our knowledge, there is no data available about the effect of Sacubitrilate on oxidative stress markers (F2-isoprostane) in renal ischemia/reperfusion injury. The results of the study about the effect of Sacubitrilate on F2-isoprostane are probably due to the anti-inflammatory and antioxidant effect (42).

Effect of Renal I/R on renal function parameters (urea and creatinine)

In this study, I/R appeared a higher level of urea and creatinine in control and control vehicle group as compare with sham group. This result is agreement with those reported by (43) revealed that I/R caused a critical rise in serum levels of creatinine and alanine aminotransferase after 30 min ischemia 4hour reperfusion (44). Appeared that the untreated IRI showed a higher level of urea and creatinine in control group as compared with the sham group (45) showed that the ischemia reperfusion group showed significantly higher concentration of creatinine and urea after 45 minute ischemia, 24 hour reperfusion in a rat model.

Effect of Sacubitrilate on renal function parameters (urea and creatinine)

In this study, Sacubitrilate significantly lower the levels of urea and creatinine when we compared with that of control group representing preservation of the renal function. This result is in agreement with those reported by (46) indicated that the treatment of CRF rats with Sacubitrilate appearing that significant diminish in the levels of urea and creatinine. In an animal model of CKD, Cao et al demonstrated that treatment with, a Combination neprilysin and Valsartan was associated with significant reduction in urea and creatinine.

Effect of renal IR injury on renal parenchyma

After IRI histological examination showed more tissue injuries, including, dilatation of the Bowman's capsule lack of brush borders, necrotic areas, vacuolization dilatation of renal tubule and glomerular modifications. These changes had been in agreement with some other studies with the aid of their outcomes (23), showed that there was significant swelling vacuolar degeneration in mitochondria, shrinkage of microvilli, the disappearance of brush borders, segmental foot method fusion, and glomerular basement membrane thickening in kidneys.

Effect of Sacubitrilate on Renal Parenchyma

Treatment of rat with Sacubitrilate improved renal damage significantly as compared with control vehicle group and the overall severity scores mean of this group confirmed a slight renal injury. The existing have a look at established that Sacubitrilate, which was administered previous to renal I/R injury, prevented renal injury over histopathological parameters. The consequences in the present observe are in agreement with those said (47).

CONCLUSIONS

From the overall results, we conclude that Sacubitrilate significantly decrease RIRI in rat model through TLR4/NF-Kb p65 pathways via it is pleiotropic effects as anti-inflammatory, anti-oxidant and anti-apoptotic.

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