ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.01.0021

Research Article

Nephroprotective potential effect of U-50488H in renal ischemia reperfusion injury in adults Males rats' model: Role of NRF2 pathway

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Received: 07.11.19, Revised: 19.12.19, Accepted: 23.01.20

ABSTRACT

Renal ischemia reperfusion injury promotes tissue damage through inducing uncontrolled inflammation, oxidative stress and excessive renal tubular epithelial cell death. Renal ischemia reperfusion injury is one of the main causes of acute kidney injury, it occurs in various clinical situations such as partial nephrectomy, renal transplantation, cardiopulmonary resuscitation, sepsis and shock. Nuclear factor Erythroid 2-related factor 2 pathways are essential in protecting the kidney against renal ischemia reperfusion injury. U-50488H is a highly selective Kappa-opioid receptor agonist. It has anti-inflammatory effect. The present study aims to study the nephro-protective potential effect of U-50488H in renal ischemia reperfusion injury in rats' model via modulation of Nrf2 pathway. At the end of the experiment, in Control group, as compared with Sham group, the renal tissue levels of Nrf2, HO-1, P-Akt, NF-kB, IL-1β, F2-isoprostane, NGAL and levels of blood Urea and serum Creatinine were significantly ($p<0.05$) increased, in addition, Control group causes a significant histopathological tissue damage ($p<0.05$). Pretreatment with U-50488H exert protective role in the kidney against injury by a significant ($p<0.05$) more increase in the tissue level of Nrf2, HO-1, P-Akt and decrease in the tissue levels of IL-1β, NF-kB, F2-isoprostane, NGAL and levels of blood Urea and serum Creatinine. Furthermore, U-50488H a significantly ($p<0.05$) reverse the severity score of renal tissue damage. From the overall results, we conclude that U-50488H a significantly decrease renal ischemia reperfusion injury in adult male rats model through Nrf2 pathway via it is pleiotropic effects as anti-oxidant, anti-inflammatory and anti-apoptotic.

Keywords: U-50488H in Renal Ischemia/Reperfusion Injury

INTRODUCTION

Renal ischemia/ reperfusion injury is characterized by limitation of blood equipping to kidney followed by re-conquest of blood flow and re-oxygenation. This disorder indicated elevation of intracellular reactive oxygen species that led to cause apoptosis of renal tubular cells [1]. In addition, pro-inflammatory mediators play a crucial role in development of renal IRI; it is prevalent cause of acute kidney injury. The kidneys are particularly susceptible to ischemic injury and represent a challenge in various clinical disorders such as renal transplantation, renal artery angioplasty, sepsis, cardiopulmonary bypass and aortic bypass surgery, certain hypertensive states and by the action of vasoconstrictor drugs [2], hemorrhagic shock and partial nephrectomy. **Nuclear factor kappa B (NF-KB)** is a reproduction gene that controls many cellular processes including cell proliferation, immune and inflammatory responses, apoptosis, migration and differentiation, nuclear factor-kappa B most frequently activated by a wide range of stimuli relevant to renal injury such as cytokines, metabolic stress, pathogen-associated damage and growth factor. This pathway is implicated in the genesis of renal IRI and has a complex role in kidney functions; therefore, NF-KB activation is very

essential for inflammatory and oxidative stress signaling in renal IRI. Furthermore, NF-KB-mediated inflammatory signaling including TNF-α, IL-1β and Monocyte chemo-attractant protein-1(MCP-1) by elevating immune cell attraction in different tissue types, target genes of Nrf2 such as HO-1 can also affect the efficacy of NF-KB, this suggest that there is a crosslink between Nrf2, NF-KB and inflammation. **Nuclear factor Erythroid 2-related factor 2 (Nrf2)**is a stimulated transcription factor that controls numerous cellular antioxidant systems that restrict oxidative stress through kidney damage generated by ischemia [3] and it is accountable for the phase II enzymes, the fundamental function of which is to decrease redox stress. Furthermore, Nrf2 has anti-inflammatory characteristic. Consistence with its function, substantially expression of Nrf2 in the tissue that is orderly risky for metabolic and environmental stresses including intestine, lungs, liver and kidneys, where stress-protection mechanisms are necessary to the permanence of singles cells and complete organ action. The veritable stress-sensor is Kelch-like ECH-associated protein 1 (Keap1), which is monitoring Nrf2 protein level based on cellular stress events. In normal states, Nrf2 is degenerated by Keap1-dependent pathways. Keap1 connects together Nrf2 and cullin (cul3) - based ubiquity E3 ligase complex, causing stable ubiquitination and proteasome degradation of Nrf2. Electrophilic modification of specific Keap1, for instance, dietary photochemical and ROS, stimulates a conformational modification in Keap1 inhibiting Nrf2 ubiquitination. As a consequence, Nrf2 protein will accumulate, translocates to the nucleus and bind to antioxidant response elements (ARE) in the promoter of target genes involve Heme oxygen-1(HO-1) and superoxide dismutase [4-8]. Furthermore, Nrf2 has a defensive effect by preventing NF-KB signaling pathway. Nuclear factor Erythroid 2-related factor 2 has been suggested to the pivot protection versus oxidative stress in the pathophysiology of renal IRI [9]. **U-50488H** is selective kappa opioid receptor agonist (KOR) [trans-(±)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]-cyclohexyl)-benzeacetamide]. It is a new synthetic opioid non-fentanyl analog of Ace amide family [10-15]. U-50448H increased superoxide dismutase activity and nitric oxide levels, reduced the malondialdehyde and elevated the phosphorylation of Akt and phosphoinositol-3-kinase in renal tissue subjected to ischemia reperfusion injury in rat model. Therefore, U-50448H has a protective effect against renal IRI [16-20]. U-50488H produced anti-apoptotic effect during ischemia / reperfusion injury [21]. It is also exerting cardio-protective effect by reducing the myocardial infarct size caused by IR [22-26]. In myocardial ischemia / reperfusion injury, U-50488H lowered myocardial infarction area, creatinine kinase level, myeloperoxidase level and myocardial TNFα production. Kappa opioid receptor stimulation by U-50488H produces both anti-inflammatory and cardio-protective effects. These effects may be related to high nitric oxide production and inhibition of TNFα induction and Neutrophils accumulation [27].

MATERIALS AND METHOD

Preparation of Animals

In this study, adult male rats of Westar albino type with 18-28 weeks in age and with weighing 300-350 g were purchased from Animal Resource Center in Faculty of Veterinary medicine -Duhok University. The Animal Care and Research Committee of the University of Kufa approved all experiments. The animals were healthy, they were kept in the animal house of Faculty of Science in University of Kufa in temperature controlled (24°C ± 2°C) room with alternating 12-hr light/12-hr dark cycles and were allowed free access to water and diet. After 2 weeks of acclimatization in quarantine room, the experiment started.

Ethical Statement

This study was done according to the Guide for the Care and Use of Laboratory Animals Association for Laboratory Animals Science. The Animal Care Committee approved all animal considerations and conventions. All rats sacrifice was performed under ketamine and xylazine mixture anesthesia.

Design of the Study

In our experiment study, the rats were randomized into equal four groups (6 rats in each group). They were subjected to bilateral renal ischemia for 30 minutes by clamping and reperfusion for 2 hours [28-29] as follows:

1. **Sham group**: Rats underwent the same anesthetic and surgical procedure except the ischemia induction.
2. **Control group**: Rats underwent the bilateral renal ischemia for 30 min and reperfusion for 2 hours.
3. **Vehicle group for U-50488H**: Rats were pretreated with dimethyl sulphoxide (DMSO) by intra-peritoneal injection 30 min before ischemia reperfusion injury and undergo bilateral renal ischemia for 30 min and reperfusion for 2 hours.

4. **U- 50488H treated group**: Rats were pretreated with U-50488H(1mg/kg/rat) by intra-peritoneal injection 15 min before ischemia reperfusion injury [30] and undergo bilateral renal ischemia for 30 min and reperfusion for 2 hours.

Preparation of U-50488H

Pure U-50488H powder was purchased from Sigma Aldrich, Germany Company

Chemical Names

Trans-(±)-3,4–Dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide hydrochloride

Molecular Formula: $C_{19}H_{26}Cl_2N_2O \cdot HCl$

Molecular weight: 465.4

This product soluble in DMSO=40.58 mg/ml, Water 40.58 mg /ml according to Sigma Aldrich package insert.

Ischemia Reperfusion Model

Rats were weighed, anesthetized using an intra-peritoneal injection of ketamine in dose of 100 mg/kg and xylazine in dose of 10 mg/kg. Under sedation (5-10 min), rats were placed on their back, fixed their limbs and tail with stickers to ensure their stability during surgery. Hair in the chest area was shaved and the skin disinfected. The reflexes were checked through pinching the tail and hind feet to be sure that the rats were sufficiently anesthetized. By making midline laparotomy incision to expose the abdomen and to expose both renal pedicles, the intestine was retracted [31]. Renal ischemia/reperfusion model was induced by clamping of kidney arteries using non-traumatic micro vascular clamps, which were positioned around the left and right renal pedicles [32]; occlusion was confirmed by observing patched blanching of the entire kidney surface and changes the color of a kidney from red to dark purple after several minutes. The total time of clamp was 30 minutes and during this procedure, 1ml normal saline was administered into abdomen, then the abdomen was covered using warm and moist gauze to keep animals well hydrated. After 30 minutes, the clamps were removed from pedicles permitting renal blood flow restoration which represent the beginning of the reperfusion phase. The kidneys were returned back to their positions, and then the abdominal cavity incision was sutured in two layers using 3interrupted sutures (36). Postoperatively, both left kidney tissue and blood samples we recollected for analysis. Rats were euthanized by deep anesthesia [33].

Collection and Preparation of Samples

Preparation of Blood Samples for Measurement of Renal Function

At the end of the experiment, rats were anesthetized about and (1-2ml) of blood was directly gathered from the heart. The sample of blood was placed in a plane tube at 37°C without anticoagulant, then it was centrifuged at 3000 rpm for 10 min, then the serum obtained was used for the determination of Urea and Creatinine.

Preparation of Tissue for Measurement Nrf2, HO-1, P-Akt, IL-1 β, NF-kB, NGAL and F2-Isoprostane

Left kidney section was taken and homogenized with a high intensity ultrasonic liquid processor in 1:10 (w/v) phosphate buffered saline that contained 1% Triton X-100 and a protease inhibitor cocktail. The homogenate was centrifuged at 3000 rpm for 20 min at 4°C [34-35]. The supernatant was collected for determination of Nrf2, HO-1, P-Akt, IL-1β, NF-kB, NGAL and F2-Isoprostane levels by ELISA technique.

Tissue Sampling for Histopathology Analysis and Damage Scores

The left kidney tissue sample was fixed in 10% formalin, dehydrated in alcohol series, cleared in xylene and embedded in paraffin block. The tissue slide sections were cut about 5- μm thick horizontal and stained with H and E then sent to histopathology's for histological examination. After fixation, an investigator who was blinded to the experimental treatment groups performed an evaluation of scores. The tissue sections were examined by light microscopy and graded for degeneration/necrosis, using quantitative measurements for assessing scoring system of tissue damage. The damage of tubule is characterized as tubular epithelial swelling , loss of brush border, vacuolar degeneration, necrotic tubules, cast development; the degree of kidney injury was estimated at X40 magnification, the score of histological changes in the kidney was evaluated as previously described by the following Criteria:

Score 0, represents normal

Score1, represents < 25% of damage tubules

Score2 represents 25%-50% of damage tubules

Score3 represents 50% -75% of damage tubules

Score4, represents >75% of damage tubules

RESULTS

Effects of U-50488H on kidney markers following renal IRI

1. Effect on NF-KB

There was a statistically insignificant effect between Control and Vehicle groups P value 0.907. Control group causes a significant increase in the mean ± SEM of tissue level of NF-KB (1591.17 ± 8.98

pg/ml, p value 0.001) when compared with Sham group (205.29 ± 33.08 pg/ml), U-50488H causes a significant decrease in mean ± SEM of tissue level of NF-KB (978.08 ± 5.91 pg/ml, p value 0.001) when compared with Control group as shown in Figure 1.

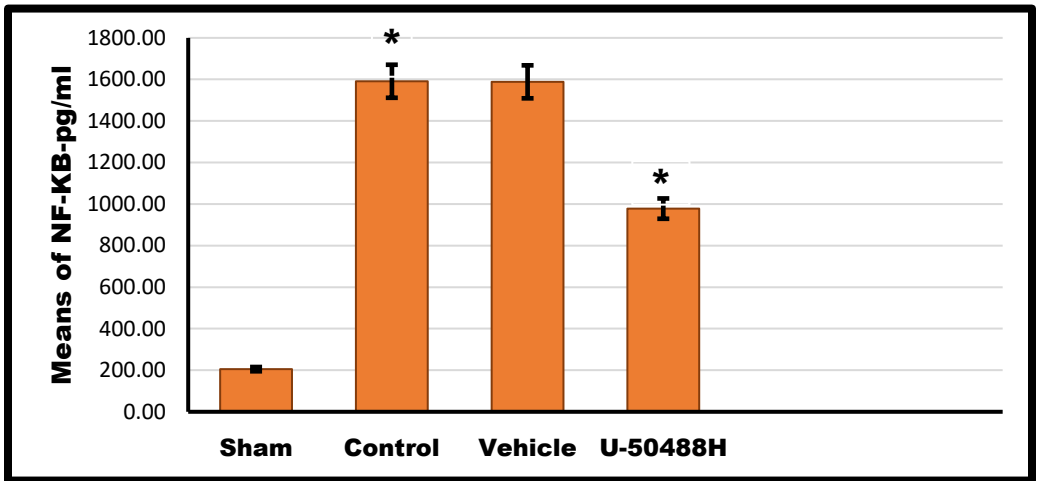

Fig.1: Error bar chart is showing the effect of U-50488H on tissue level of NF-kB following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

2. Effect on IL-1β

There was a statistically insignificant effect between Control and Vehicle groups P value 0.765. Control group causes a significant increase in the mean ± SEM of tissue level of IL-1β (954.30 ± 4.86 pg/ml, p value 0.001) when compared with Sham group (126 ± 6.49 pg/ml), U-50488H causes a significant decrease in mean ± SEM of tissue level of IL-1β (655.86 ± 5.36 pg/ml, p value 0.001) when compared with Control group as shown in Figure 2.

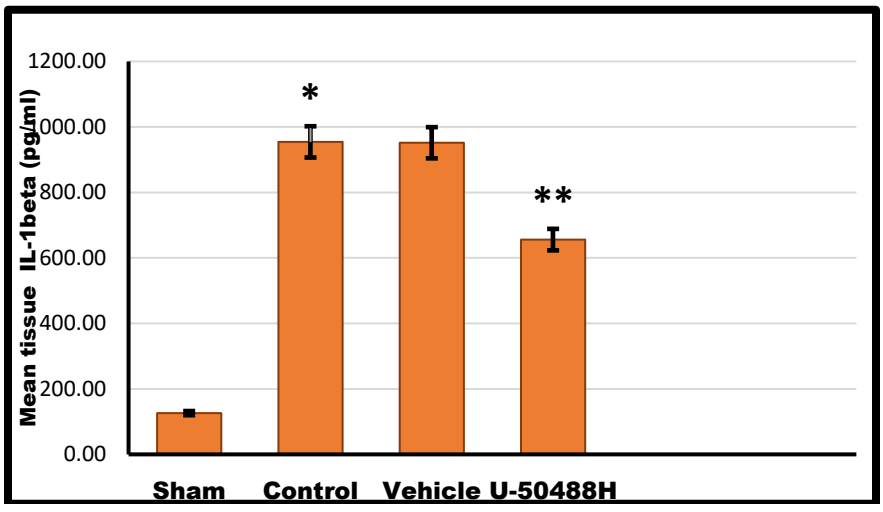

Fig.2: Error bar chart is showing the effect of U-50488H on tissue level of IL-1β following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

3. Effect on F2-Isoprostane

There was a statistically insignificant difference between Control and Vehicle groups P value 0.782. Control group causes a significant increase in the mean ± SEM of tissue level of F2-isoprostane (607.65 ± 5.01 pg/ml, p value 0.001) when compared with Sham group (78.92± 3.46 pg/ml), U-50488H causes a significant decrease in mean ± SEM of tissue level of F2-isoprostane (358.85± 4.41pg/ml, p value 0.001) when compared with Control group as shown in Figure 3.

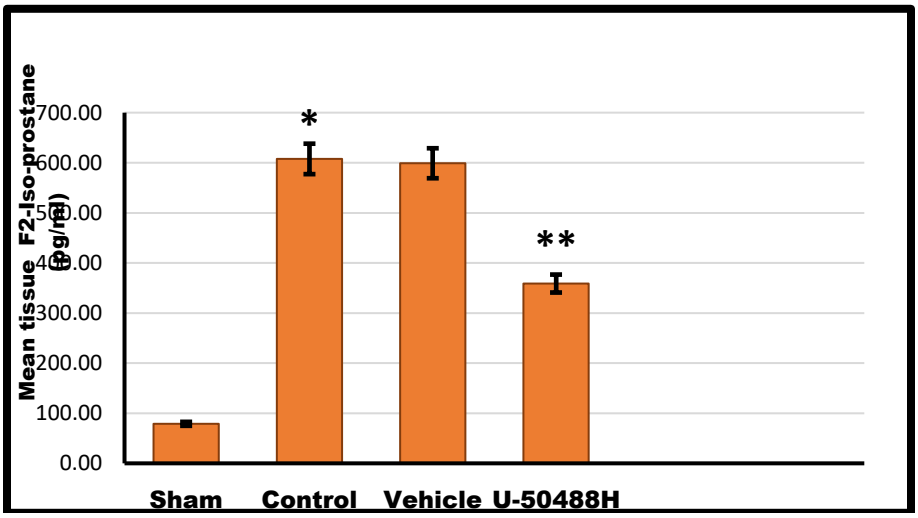

Fig.3: Error bar chart is showing the effect of U-50488H on tissue level of F2-Isoprostane following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

4. Effect on Nrf2

There was a statistically insignificant effect between Control and Vehicle groups P value 0.570. Control group causes a significant increase in the mean ± SEM of tissue level of Nrf2 (434 ± 6.22 pg/ml, p value 0.001) when compared with Sham group (94.26±8.26 pg/ml), U-50488H causes a significant increase in mean ± SEM of tissue level of Nrf2 (686.74 ± 5.14 pg/ml, p value 0.001) when compared with Control group as shown in Figure 4.

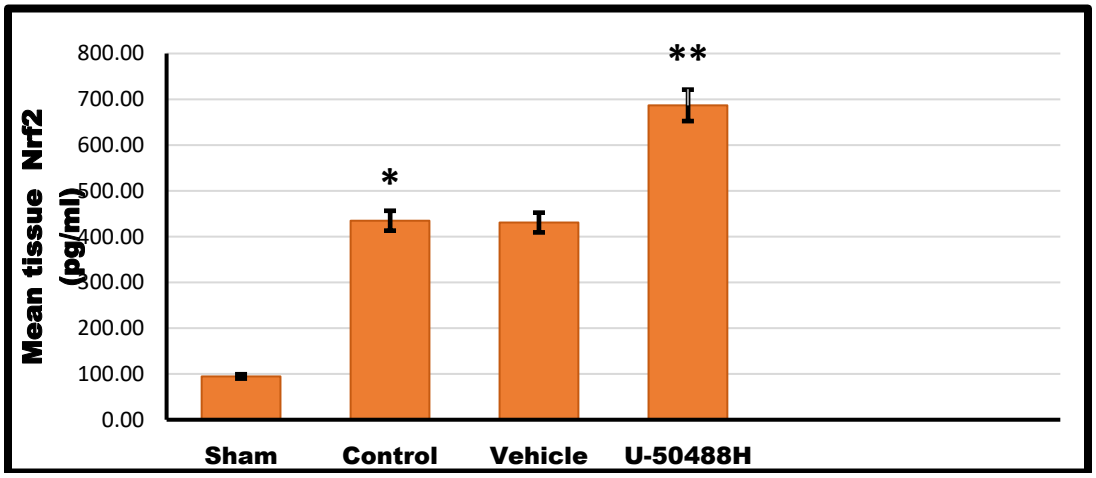

Fig.4: Error bar chart is showing the effect of U-50488H on tissue level of Nrf2 following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

5. Effect on HO-1

There was a statistically insignificant effect between Control and Vehicle groups P value 0.956. Control group causes a significant increase in the mean ± SEM of tissue level of HO-1 (4.00 ± 0.016 pg/ml, p value 0.001) when compared with Sham group (0.53 ± 0.01 pg/ml), U-50488H causes a significant increase in mean ± SEM of tissue level of HO-1(6.60 ± 0.07 pg/ml, p value 0.001) when compared with control group as shown in Figure 5.

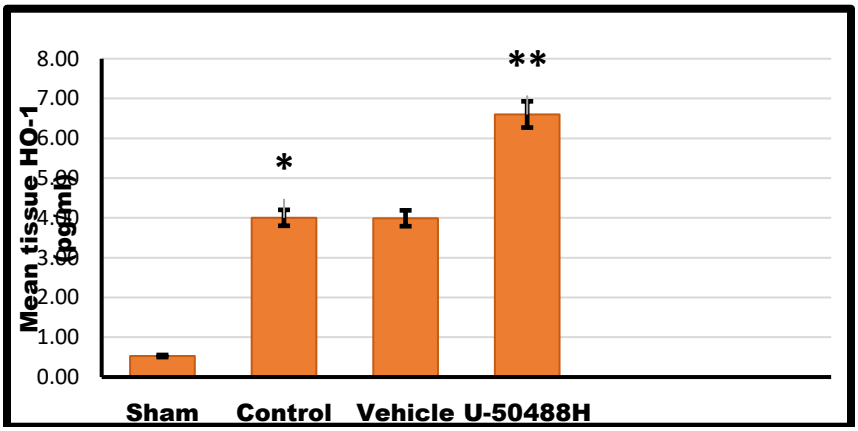

Fig.5: Error bar chart is showing the effect of U-50488H on tissue level of HO-1 following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

6. Effect on P-Akt

There was a statistically insignificant effect between Control and Vehicle groups P value 0.987. Control group causes a significant increase in the mean ± SEM of tissue level of P-Akt (296.23 ± 6.40 pg/ml, p value 0.001) when compared with Sham group (108.42 ± 3.42 pg/ml), U-50488H causes a significant increase in mean ± SEM of tissue level of P-Akt (553.63 ± 6.75 pg/ml, p value 0.001) when compared with Control group as shown in Figure 6.

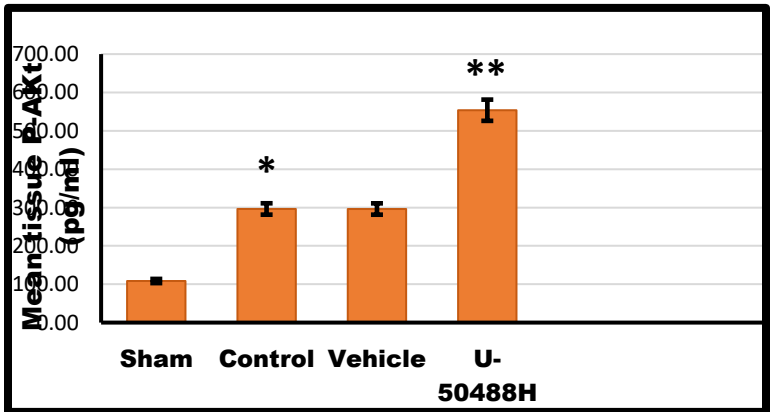

Fig.6: Error bar chart is showing the effect of U-50488H on tissue level of P-Akt following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

7. Effect on NGAL

There was a statistically insignificant effect between Control and Vehicle groups P value 0.951. Control group causes a significant increase in the mean ± SEM of tissue level of NGAL (1477.32 ± 7.97 pg/ml, p value 0.001) when compared with Sham group (182.84 ± 23.86 pg/ml), U-50488H causes a significant decrease in mean ± SEM of tissue level of NGAL (1142.35± 4.20 pg/ml, p value 0.001) when compared with Control group as shown in Figure 7.

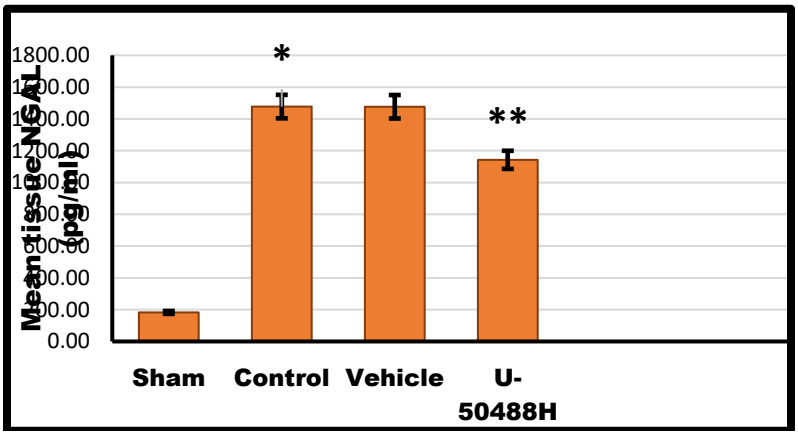

Fig.7: Error bar chart is showing the effect of U-50488H on tissue level of NGAL following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

8. Effect on Blood Urea

There was a statistically insignificant difference between Control and Vehicle groups P value 0.565. Control group causes a significant increase in the mean ± SEM of blood Urea level (91.1 ± 1.579 mg/dl, p value 0.001) when compared with Sham group (22.3± 0.714 mg/dl). U-50488H causes a significant decrease in mean ± SEM of serum level of urea (64.1 ± 1.137mg/dl, p value 0.001) when compared with Control group as shown in Figure 8.

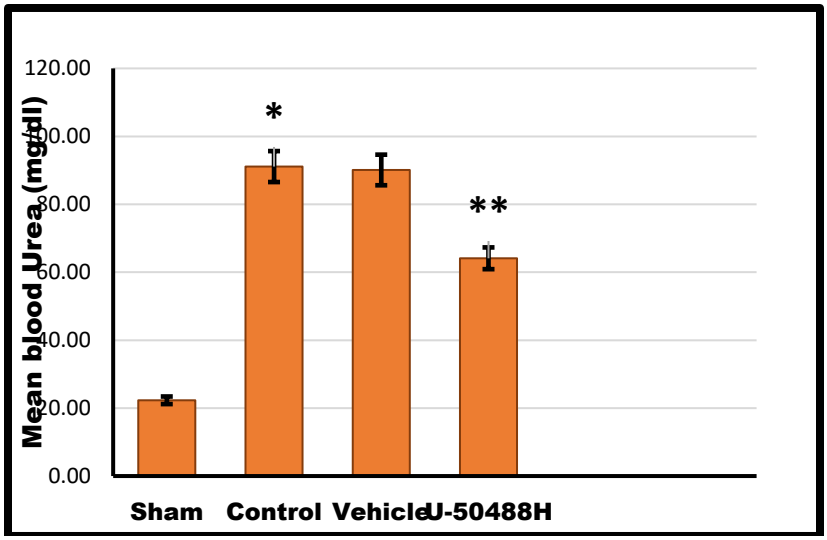

Fig.8: Error bar chart is showing the effect of U-50488H on level of blood Urea following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

9. Effect on Serum Creatinine

There was a statistically insignificant effect between Control and Vehicle groups P value 0.09. Control group causes a significant increase in the mean ± SEM of serum level of Creatinine (2.1 ± 0.047 mg/dl, p value 0.001) when compared with Sham group (0.6 ± 0.003 mg/dl). U-50488H causes a significant decrease in mean ± SEM of serum level of creatinine (1.5 ± 0.021 mg/dl, p value 0.001) when compared with Control group as shown in figure 3.9.

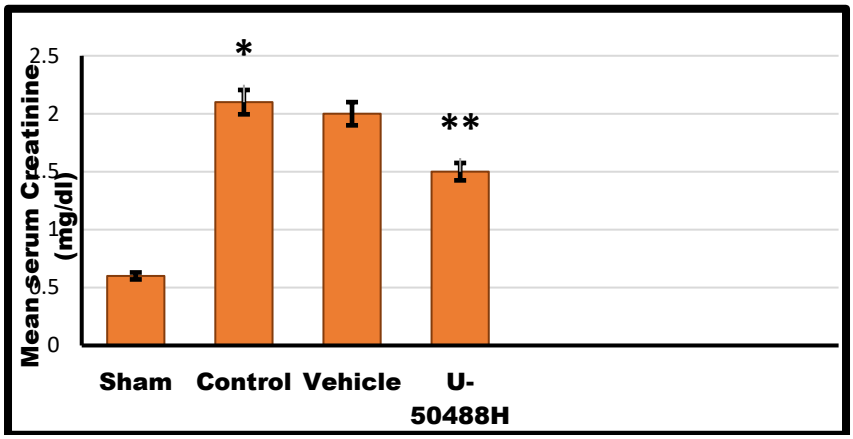

Fig.9: Error bar chart is showing the effect of U-50488H on level of serum Creatinine following renal IRI, expressed as mean ± SEM pg/ml in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group

** P value < 0.05 Control when compared with U-50488H.

10. Histopathology finding

There was a statistically insignificant effect between Control and Vehicle P value> 0.05. Figure 10, showed the histopathological score in four experiment groups, Renal IRI causing a significant tissue damage (p value 0.001) when compared with Sham group. Pretreatment with U-50488H cause a significant amelioration of tissue damage caused by IRI (p value 0.002). Figure (11) showed normal renal tubule without inducing IRI. Figures (12)(13) showed significant tissue damage caused by IRI, Figure (14) showed how pretreatment with U-50488H caused a significant reduction renal tissue injury.

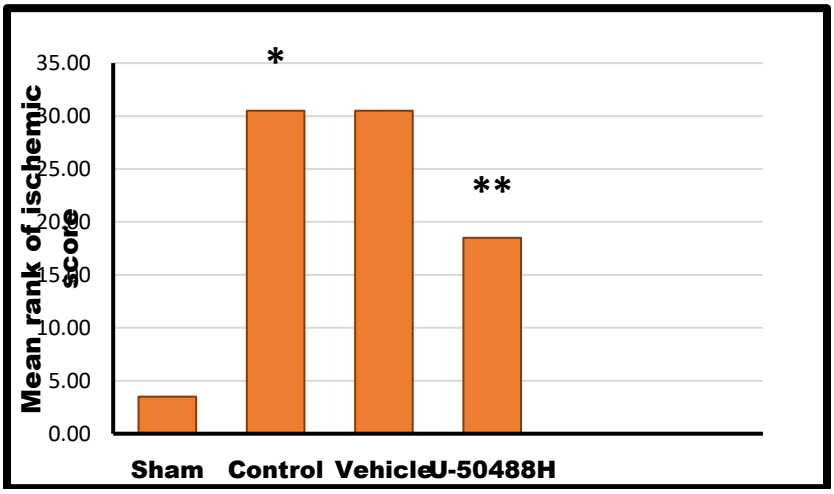

Fig.10: Error bar chart is showing the effect of U-50488H on histopathological score of the kidney injury following renal IRI, expressed as mean rank in four experimental groups (number of animals=6 in each group)

* P value < 0.05 Control when compared with the Sham group
** P value < 0.05 Control when compared with U-50488H.

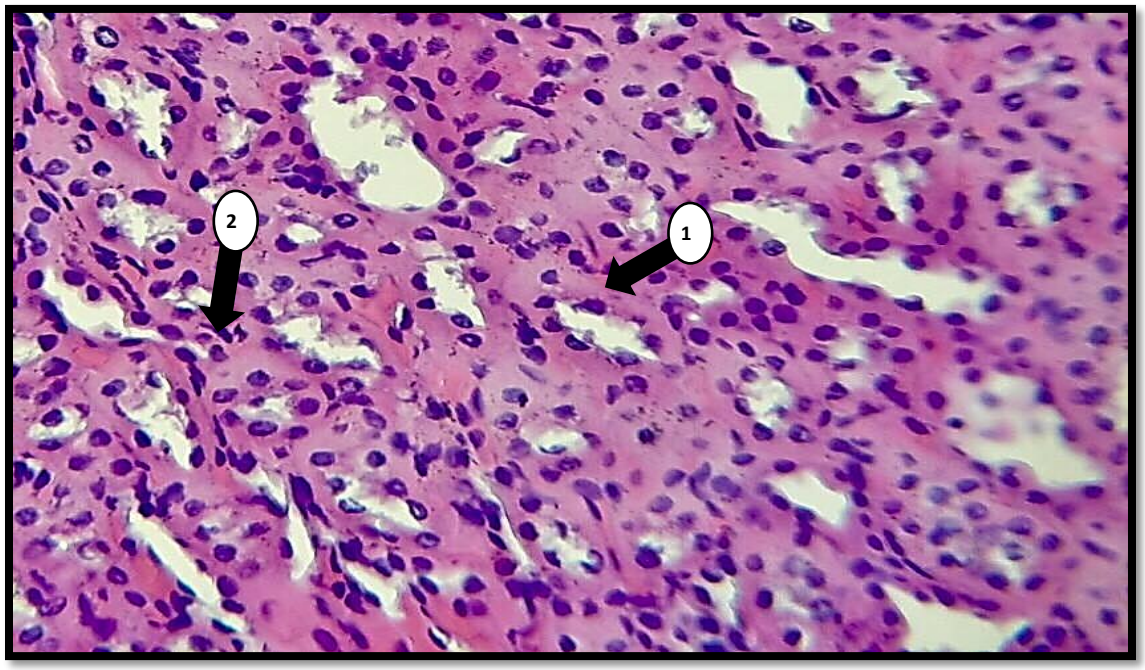

Fig.11: Photomicrograph of the renal section for the Sham groups shows the normal renal tubules The section stained H and E and magnification at (X40), illustrating severity score1 as marked (1) and (2) normal renal tubules.

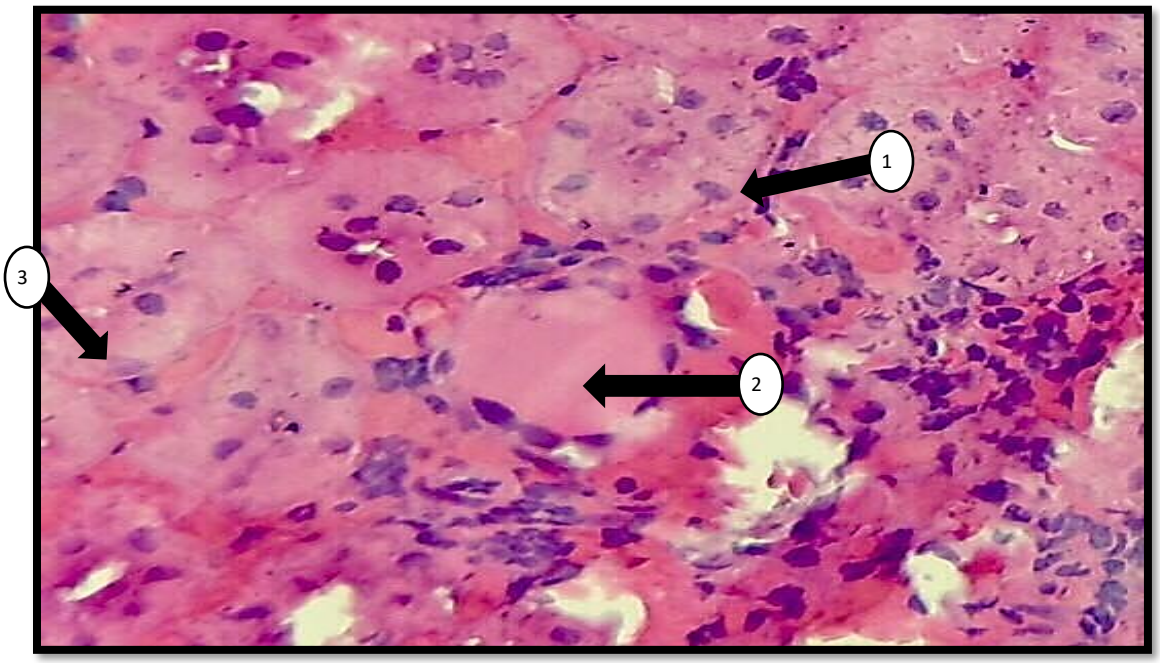

Fig.12: Photomicrograph of the renal section for the Control group showed ischemic changes including scattered individual cells with cellular swelling, loss of brush border, eosinophilic cast and karryolysis

The section stained with H and E and magnification at (X40), illustrating severity score 3 as marked (1) tubular cellular swelling (2) eosinophilic cast formation and (3) karryolysis.

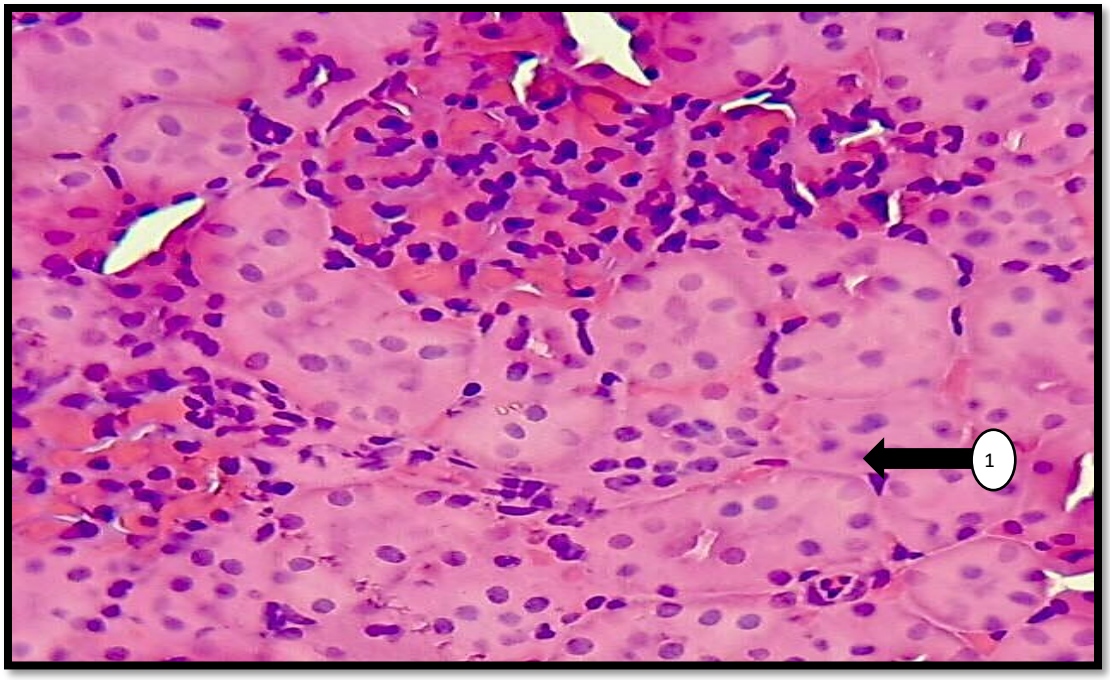

Fig.13: Photomicrograph of the renal section for the Vehicle group showed ischemic changes including scattered individual cells with cellular vacuolization, loss of brush border, eosinophilic cast formation and pyknotic nuclei

The section stained with H and E and magnification at (X40), illustrating severity score3 as marked (1) tubular cellular swelling.

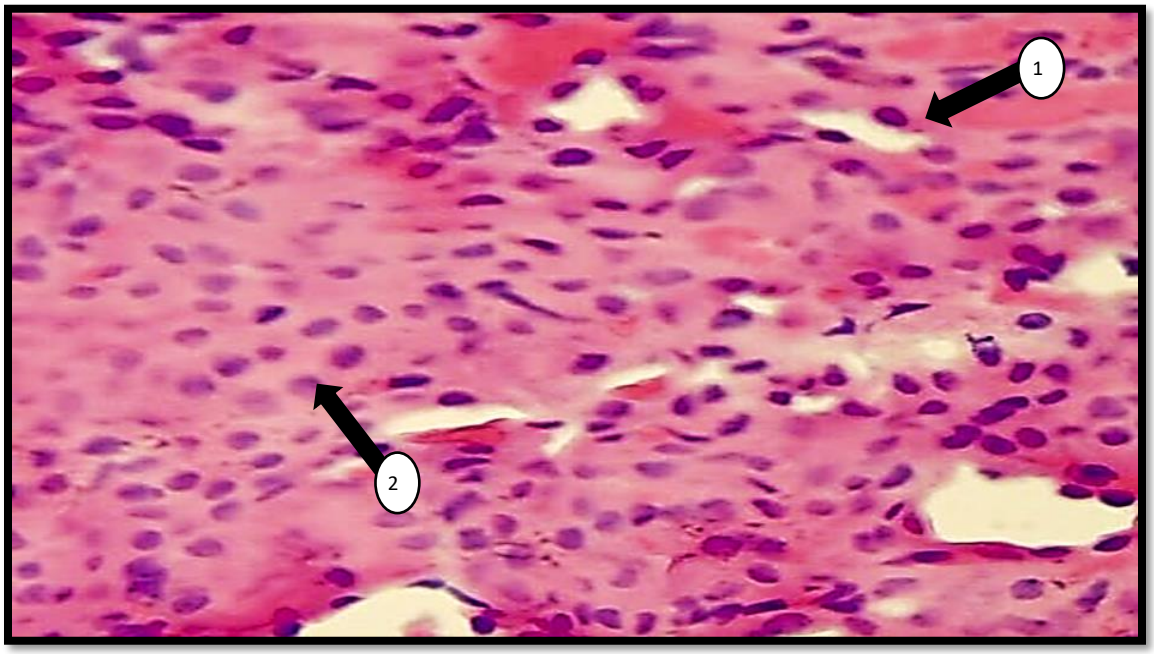

Fig.14: Photomicrograph of the renal section for U-50488H group showed less scattered individual cells with less cellular swelling, weak eosinophilic cytoplasm without pyknotic nuclei

The section stained with H and E and magnification at (X40), illustrating severity score1 as marked (1) and (2) normal renal tubules.

DISCUSSION

The renal ischemia/reperfusion injury is a major cause of AKI. It is characterized by reduced blood flow and organ oxygenation. The ischemia/reperfusion injury causes several morphological and biochemical alterations including high calcium and intracellular sodium, inflammation, oxidative stress, intracellular ATP depletion, fibrosis, tubular and glomerular damage and cell death by apoptosis or necrosis. The renal IRI induced apoptosis and necrosis of tubular epithelial cells are the main causes that result in acute renal failure. Reperfusion may be even more harmful than ischemic injury. Through the reperfusion, ROS causes endothelial damage, high micro vascular permeability, produces tissue edema, activates adhesion molecules, releases cytokines and leads to systemic inflammatory response [36]. We estimated the protective effect of U-50488H against experimental renal IRI.

Effect of Renal IRI on Inflammatory Mediators (IL-1β and NF-KB)

This research approved that there is a significant increase in renal tissue levels of inflammatory mediatorsIL-1β and NF-KB ($P<0.05$) in Control group when compared with Sham group after IRI. Mounting study has suggested that inflammation is a hallmark of AKI. The concentrations of inflammatory markers in kidney tissues of the ischemia/reperfusion group were considerably higher due to increasing Neutrophils infiltration and oxidative stress. Interluekine-1β is essential pro-inflammatory mediators in kidney ischemia, which produce a number of injurious changes in proximal tubular epithelial cells. Elevation level of NF-KB in ischemia/reperfusion group, when mice are subjected to 30 min ischemia and reperfusion for 2 hours and NF-KB possesses a critical role in the pathogenesis of renal ischemia/reperfusion injury. Nuclear factor-Kappa B is a transcription factor activated by cytokines and chemokine after acute kidney injury [37].

Effect of U-50488H on Inflammatory Mediators (IL-1β and NF-KB)

In this experimental study, we showed that there is a significant decrease in renal tissue levels of IL-1β and NF-KB, ($p<0.05$) forU-50488Hpretreated group as compared to Control group.

The role of the k-opioid receptor in inflammation is not well understood. The accumulating evidence suggests that U-50488H has therapeutic potential in protection during an anti-inflammatory response, which exhibits abroad inhibitory influence on cytokines and chemokine. U-50488H administration could inhibit Neutrophils, TNF-α induction in myocardial ischemia/reperfusion injury. Lin and followers reported that k-opioid receptor stimulation by U-50488H inhibits NF-KB signaling induced in ischemia/reperfusion, attenuation myocardial ischemia by U-50488H involved in down-regulation NF-KB signaling in rats model. To the best of our knowledge, there are no data available about the effect of U-50488H on NF-KB and IL-1β in renal ischemia/reperfusion injury.

Effect of Renal IRI on Oxidative Stress Marker F2-Isoprostane

In this study, the renal tissue level of f2-isoprostane was significantly increased ($p<0.05$) in Control group as compared with Sham group. Renal dysfunction is frequently associated with oxidative stress; as levels of different markers including F2-isoprostane and malonyldialdehyde are increased in patients with vary degree of kidney function including patients with ESRD. Elevated level of synthesis F2-isoprostane is inversely associated with GFR. Therefore, F2-isoprostane level is a marker of oxidative stress, increased significantly early through the progression of CKD. The outcome within the study is associated with other studies which indicated that the level of F2-isoprostane has been increased in renal IRI group.

Effect ofU-50488H on Oxidative Stress Marker F2-Isoprostane

In the present study, U-50488H pretreated group showed a significantly decreased tissue level of F2-isoprostane ($p<0.05$) when compared with Control group. According to Liu and followers reported that U-50488H administration caused suppression in level of other reactive oxygen species markers such as malonyldialdehyde in the ischemic renal tissues of rats model. To the best of our knowledge, there are no data available about the effect of U-50488H on oxidative stress marker (F2-isoprostane) in renal ischemia/reperfusion injury. The results of our study about the effect of U-50488H on F2-isoprostane are probably due to the anti-inflammatory and anti-oxidant effect.

Effect of Nrf2 and HO-1 on Renal IRI

In this study, the renal tissue level of Nrf2 and HO-1 was significantly increased ($p<0.05$) in Control group as compared with Sham group. This outcome is consistent with a previous study suggested that the Nrf2 pathway is important in protection against IRI. The deficiency of Nrf2 caused worsened ischemic kidney injury in animal model. The protective role of Nrf2 has been shown in lowering renal and possibly even systemic inflammation among hemodialysis patients. Heme

oxygenase-1 is induced via several stimuli and considered as a sensitive indicator of cellular stress. Up-regulation of HO-1 is adaptive mechanism that protects cells from stress such as hypoxia, ischemia and inflammation. Heme oxygenase-1 protects kidney structure and function from IRI.

Effect of U-50488H on Nrf2 and HO-1

The current study reveals that the pretreatment with U-50488H that is significantly increasing the tissue level of Nrf2 and HO-1 ($p<0.05$) when compared with Control group. Another research indicated that U-50488H amplified and prolonged the increase in HO-1 transcription and expression in post-ischemia/reperfusion, HO-1 activation is a key mediator of U-50488H induced protection and increases Nrf2 in nuclei, suggesting Nrf2 nuclear translocation in cardiac ischemia. To the best of our knowledge, there is no previous study to investigate the effect of U-50488H on Nrf2 and HO-1 in renal ischemia/reperfusion injury in animal model. The results of our study about the effect of U-50488H on Nrf2 and HO-1 are probably due to the anti-inflammatory and anti-oxidant effects.

Effect of P-Akt on Renal IRI

In this study, the renal tissue level of P-Akt was significantly increased ($P<0.05$) in Control group as compared with Sham group after IRI. A Previous research demonstrated that the phosphorylated protein kinase B which was a cell survival regulation pathway could protect the kidney of rat from apoptosis via enhancing antioxidant capacity and reducing apoptotic protein content. Phosphorylation of Akt was increased after ischemia/reperfusion of kidney, renal cell proliferation, which is high after IRI, suggesting that P-Akt maintains cell viability after ischemia, thus playing an essential role in the regulation of renal repair after IRI.

Effect of U-50488H on p-Akt

In this study, we demonstrated that U-50488H significantly increased tissue level of P-Akt in renal IRI ($p<0.05$) when compared with Control group. Our result is consistent with another study that reported U-50448H protects against renal IRI in rat model by activation of Akt signaling pathway.

Effect of Renal IRI on Renal Injury Marker NGAL

The new marker that has entered recently is NGAL; it is shown to be the most expressed protein in the kidney for a short time before Creatinine after ischemic injury. Therefore, renal function can be monitored using NGAL biomarker. After acute kidney injury NGAL, the protein is early identified as one of the earliest markers of renal damage after ischemic injury in animal models. In this study, the renal tissue level of NGAL was significantly increased ($p<0.05$) in Control group as compared with Sham group after IRI. This result is consistent with other studies that predicated that NGAL has been introduced as a biomarker of AKI. Neutrophils gelatinase- associated lipocalinis is produced by injured tubular epithelial cells. Elevated NGAL level was already done by 10 min ischemia and was further elevated significantly from 10 min to more severe 20 min or 30 min renal ischemia. There is evidence that NGAL detects renal injury before kidney function impairment in clinical trials.

Effect of U-50488H on NGAL

Our study showed that there was a significant lower in the renal level of NGAL in U-50488H treated group ($P<0.05$) as compared with Control group.

The best of our knowledge, there may be no data available about the effect of U-50488H on renal injury marker NGAL in regional renal ischemia/reperfusion injury. The results of our study have a look at the impact of U-50488H on NGAL that can be due to antioxidant effect.

Effect of Renal IRI on Renal function Parameters (Urea and Creatinine)

The experimental study confirmed that Control group causes a significant increase ($P<0.05$) in blood Urea and serum Creatinine levels when compared with Sham group. These observations were in agreement with others studies. Blood Urea and serum creatinine levels are the most commonly used markers in evaluating renal function. Significantly increased Urea and Creatinine levels are typical indicators of acute kidney injury. A swift change in serum Creatinine is largely frequent sign of AKI. Due to the excess of Creatinine, the acute inflammatory edema and tubular necrosis formation are accompanied by significant changes in the incidence of cellular proliferation. There are ample reports concerning reduce in GFR of ischemia/reperfusion in rats model because of remarkable increase in blood Urea and serum Creatinine levels which are in accordance with the earlier findings.

Effect of U-50488H on Renal Function Parameters (Urea and Creatinine)

In this study, U-50488Hsignificantly lowers the levels of blood Urea and serum Creatinine ($P<0.05$) when compared with Control group. Those finding indicated improvement of biochemical renal function markers via decreasing the degree of renal function injury, Those results are consistent with previous study.

Effect of Renal IRI on Renal Parenchyma

The total severity scores of the sections of Control group were significantly higher than that of Sham

group (P< 0.05). After renal IRI histological examination of sections from Control group exhibit more tissue injuries including disruption of normal kidney architecture with marked glomerular damage and congestion, inflammatory cell infiltration, epithelial atrophy and cell desquamation in the tubules, loss of brush border, hyaline cast formation, interstitial expansion, tubular dilation, vacuolar degeneration and edema of the tubules.

Effect of U-50488H on Renal Parenchyma

The treatment of rat with U-50488H improved renal damage significantly compared with Control group (P<0.05) and overall severity score mean of this group confirmed a moderate kidney injury. This study established that U-50488H, which was administered before renal IRI, caused attenuation of renal injury through histopathological parameters, these results are in agreement with those said.

CONCLUSION

From the overall results, we conclude that U-50488H significantly decrease in renal ischemia reperfusion injury in rat model through Nrf2 pathway via it is pleiotropic effects as anti-oxidant, anti-inflammatory and anti-apoptotic.

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