The Potential Renoprotective Effect of Fedratinib in Renal Ischemia Reperfusion Injury in Male Rat Model

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RIRI. JAK/STAT, Fedratinib

ABSTRACT
Renal Ischemia Reperfusion Injury (RIRI) is a serious condition that arises following kidney transplantation or other circumstances that result in decreased blood supply to the kidneys, these conditions cause a harm to the renal tissue. Gaining insight into the fundamental mechanisms of RIRI, including inflammation, cell death pathways, is essential for the development of successful therapeutic approaches.
Objective: This study aimed to assess the effects of Fedratinib, a JAK2 inhibitor, in reducing RIRI in a rat model.
The study involved rats divided into four groups: Sham group, control group, vehicle group, and Fedratinib group. Rats underwent anaesthesia and midline incision to expose renal pedicles, with blood flow halted using microvascular clamps. The wound was stitched, and the animals were left for 90 minutes to allow reperfusion. Blood samples were collected from the heart apex to assess serum urea and creatinine, and renal slices were used for PCR to detect the jak2/stat3 pathway. The Elisa was used to evaluate TNF, IL-6, NF-κB, Bax, Bcl2, and HMGB levels.
Results: IL-6 and TNF-α, and HMGB1 were identified to have a role in the development of RIRI, inhibition of JAK2/STAT3 pathway by Fedratinib significantly decrease their concentration and minimizing RIRI. Suppression of JAK2/STAT3 pathway led to a significant inhibition in NF-κB and apoptosis.
Conclusion: Fedratinib has a potential preventive effect against RIRI through their activity as JAK2 inhibitor and inhibition of the following downstream inflammatory pathways.

1. Introduction

Renal ischemia-reperfusion injury (RIRI) is a serious condition that occurs when there is an interruption in the blood flow to the kidneys, which is then followed by its restoration. Such occurrences can arise following kidney transplants, cardiac surgical interventions, or other situations that lead to diminished blood circulation to the kidneys. Ischemia, which is defined as a condition of inadequate oxygen and nutrients, can lead to the demise of renal cells[1], while reestablishing the flow of blood might subsequently trigger inflammation and worsen damage to the kidneys [2]

Pro-inflammatory cytokines, including TNF-α, IL-6, and HMG-box, play a crucial role in initiating and worsening RIRI. These cytokines might potentially exacerbate the severity of injury by facilitating tissue damage, oxidative stress, and inflammation. Despite pro-inflammatory cytokines are important for tissue regeneration and wound healing, an excessive amount of these molecules might hinder the recovery process of RIRI [3], [4]

NF-κB governs gene expression in several biological processes such as inflammation, immunity, apoptosis, cell proliferation, and differentiation. It activates pro-inflammatory cytokines and toxic chemicals during RIRI, resulting in elevated levels of inflammatory mediators and apoptosis [5]

Apoptosis may be categorised into two primary pathways: extrinsic and intrinsic. Both routes have a reciprocal influence on each other. The death-signalling complex activates caspase-8, which in turn cleaves caspase-3. This cleavage process leads to the proteolysis of cells that are injured, causing them to undergo self-destruction. Hypoxia, radiation, or cellular toxins trigger the intrinsic pathway (mitochondrial pathway) and disturb the integrity of the mitochondrial membrane, leading to the activation of pro-apoptotic proteins. Ischemia-reperfusion damage results in a rise in cytoplasmic Bad, which then binds to Bcl-2 and Bcl-XL[5]. When Bax and Bak are digested and incorporated into the mitochondrial membrane, Cytochrome c, apoptosis-inducing factor, and endonuclease G are released. Cytochrome c stimulates procaspase-9 by attaching to APAF-1, resulting in the formation of the apoptosome. The apoptosome activates caspase-9 to initiate apoptosis [6] .

JAK-STAT signalling system has a vital role in the onset and advancement of several biological processes and illnesses, including RIRI. Research suggests that RIRI activates the JAK-STAT signalling pathway, resulting in harmful consequences [7]. induction of the JAK-STAT pathway leads to the generation of pro-inflammatory cytokines, including as IL-6 and TNFα, chemokines, and adhesion molecules. These chemicals elicit the migration of immune cells to the site of damage. Suppression of the JAK-STAT pathway reduces inflammation, oxidative stress, and programmed cell death, resulting in a decreased risk of RIRI. Therefore, focusing on the JAK-STAT signalling pathway might be a potentially effective strategy for treating RIRI [8].

JAK/STAT signalling pathway includes JAKs and STATs. The JAK family includes JAK1, JAK2, JAK3, and TYK2. Stat1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 comprise the STAT family[9].

Fedratinib, an orally administered JAK2 inhibitor, received approval in August 2019 for the treatment of myelofibrosis in adult patients [10]. It selectively binds to the kinase domain of the JAK2 protein, therefore decreasing its activity and impeding the proliferation of cancer cells. The recommended dosage is 400mg daily. Fedratinib has efficient absorption, moderate plasma protein binding, moderate hepatic metabolism, and a half-life of elimination ranging from 36 to 48 hours [11].

The potent inflammatory cascade of RIRI affects the delicate functioning of the kidneys, posing a significant medical challenge. Comprehending the intricate process of pro-inflammatory cytokine signalling, NF-κB activation, and apoptotic pathways is essential for efficient control. Recent research has discovered promising therapy approaches that primarily focus on the JAK-STAT signalling system, despite the detrimental consequences of RIRI. By investigating the intricate correlation between inflammatory mediators and therapeutic targets, we can advance the development of novel RIRI medicines that effectively mitigate kidney injury and improve patient outcomes[12]

2. Methodology

Design of The Study

Male Sprague-Dawley rats were present from Kufa University's Animal Centre and accommodated in a well-maintained facility, which was sanctioned by the Ethical Committee. The study has stratified rats into four groups: sham, control, vehicle(DMSO), and fedratinib treated group.

Rats were split into the following 4 groups (n = 10) following their one-week acclimatisation period:

1. Sham group: Rats underwent surgical incision and anaesthesia for the same amount of time for RIR with the exception of RIRI induction.
2. Control group, which did not receive any treatment, acted as a model for ischemia reperfusion injury.
3. Vehicle group {given reference standard vehicle DMSO I.P+ RIR).
4. The Fedratinib group (RIR + Fedratinib).

Preparation of Drugs

Fedratinib, a white powder, was requested from Chem scene company (Cat.NO.CS-052) dissolved in (DMSO) vehicle (100mg/ml)[11].To make stock solution and used immediately, and given in doses(30mg/kg) i.p [13]. 1hr before ischemia. It was administered at a dosage of 30mg/kg, one hour prior to the onset of ischemia[13].

Experimental Procedures:

The rats were administered an anaesthesia intraperitoneally using ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg)[14]A midline laparotomy incision was performed to expose the renal pedicles in rats. The blood flow to the kidney was halted using a microvascular clamp. After a duration of 30 minutes, the clamp was then removed to reinstate the blood flow. Upon releasing the

clamp, visual examination confirmed effective reperfusion as evidenced by a transformation in the colour of the kidneys from a dark blue to a bright red shade. The surgical wound was stitched, and the animals were placed back in their cages and left undisturbed for a period of 90 minutes to allow for reperfusion. Following reperfusion, Blood samples were collected from the apex of the heart to assess the levels of serum urea and creatinine. The renal slices were utilised for PCR to detect the activation of the jak/stat pathway and for ELISA analysis to measure cytokine levels . part of renal tissues was preserved in a 10% formalin solution and then underwent haematoxylin-eosin staining. The histopathological damage was assessed based on morphological criteria [15]

Preparation of Samples

Blood Sampling

Following the experiment's completion, a gel tube was used to collect around 1 ml of blood from the heart. The collected blood samples underwent centrifugation at a rate of 4000 revolutions per minute (rpm) for 10 minutes. Subsequently, the serum component was isolated and subjected to analysis for levels of urea and creatinine.

Tissue homogenization

The renal slice was effectively conserved by the utilization of a tissue stabilizer, which maintained its structural integrity and cellular components. The homogenization method involved the utilization of a high intensity ultrasonic liquid processor in a solution of phosphate buffered saline with a concentration ratio of 1:10 (w/v). The solution also contained 1% Triton X-100 and a protease inhibitor cocktail. The homogenate was subjected to centrifugation at 4°C for 20 minutes, with rotational speeds of 14000 rpm and 4000 rpm. The Elisa technique was employed to evaluate the levels of TNF, IL-6, NF-B, Bax/Bcl2, and HMGB1 in the supernatant.

Tissue for PCR

A renal slice was acquired and subsequently rinsed with a solution of normal saline. The slice was then divided into smaller fragments and subsequently immersed in a tissue stabilizer.

Histopathological Analysis

Following standard histological procedures, the kidney tissues were stored in a 10% formalin solution and then set in paraffin blocks. Segments with a thickness of 5μm were prepared and subjected to staining using the hematoxylin-eosin (H&E) technique with the purpose of conducting histological analysis at a later time (5). Following the implementation of the changes that were needed, the assessment of scores was conducted by an examiner who remained blind to the experimental treatment groups during the evaluation process. The histopathological damage was evaluated based on the morphological standards outlined in the methodology of reference [15]

1. score 0: normal.
2. score 1:area of damage <25% of tubules.
3. score 2: damage involve 25-50% of tubules.
4. score 3 : damage involving 50-75% 0f tubules.
5. score 4 :75-100% of the area being involved.

Ethical approval

The experimental protocol was approved in accordance with Order (3/12/915 on February 18, 2023) and issued by the Ethical Committee for the Care and Use of Laboratory Animals in the Department of Pharmacology and Therapeutics, College of Medicine, University of Kufa

3. Results and discussion

Serum urea showed a major rise ($p < 0.01$) in both the control and control vehicle groups compared to the sham group. A little difference was seen between the control group and the control vehicle group.

There was a significant decrease in renal serum urea level observed in the groups treated with Fedratinib, in comparison to the control groups. The figure (1)provides a summary of the effect observed in renal levels of serum urea

Serum creatinine showed a highly significant rise ($p < 0.01$) in both the control and control vehicle groups compared to the sham group. A nonsignificant difference was seen between the control group and the control vehicle group.

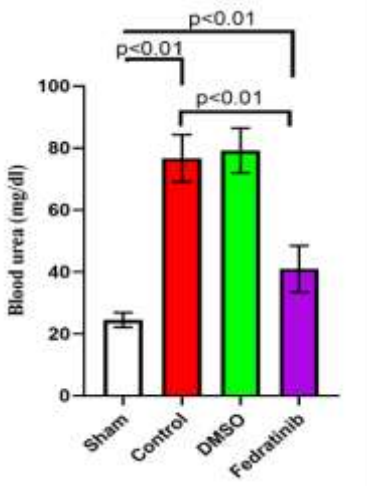


Figure (1) Mean level of serum urea (mg/dl) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

There was significant elevation in serum creatinine levels observed in the groups treated with Fedratinib, in comparison to the sham group and significant decrease from control groups.

The figure (2) provides a summary of the effect observed in levels of serum creatinine.

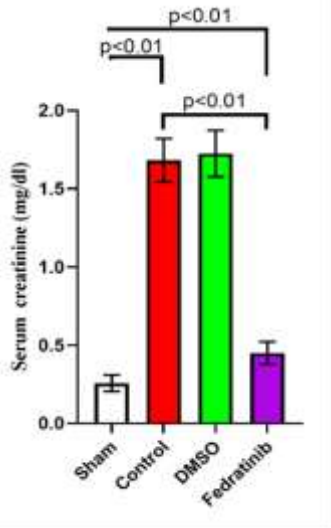


Figure (2) Mean level of serum creatinine (mg/dl) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Effect of Fedratinib on inflammatory mediators in different study groups

Effects on renal TNF-α level

The renal concentration of TNF-α showed a statistically significant rise ($p < 0.01$) in both the control and control vehicle groups compared to the sham group. A non-significant change was seen between the control group and the control vehicle group.

There was a significant decrease in renal TNF-α levels observed in the groups treated with Fedratinib in comparison to control groups . The figure (3) provides a summary of the effect observed in renal levels of TNF-α.

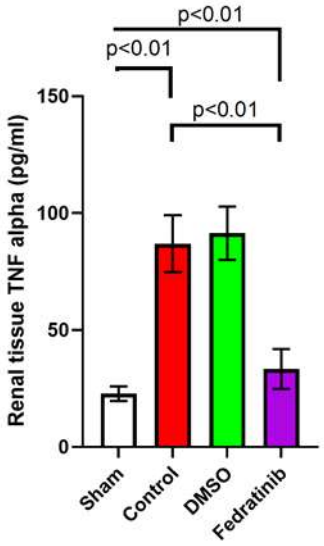


Figure (3) Mean level of TNF-α (pg./ml) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Effect on renal IL-6 level

The renal level of IL-6 exhibited a statistically significant increase ($p < 0.01$) in both the control and control vehicle groups as compared to the sham group. A non-significant difference was seen between the control group and the control vehicle group.

A significant decrease in renal IL-6 levels was detected in the groups treated with Fedratinib compared the control groups. The figure (4) provides a summary of the variations observed in renal levels of IL-6.

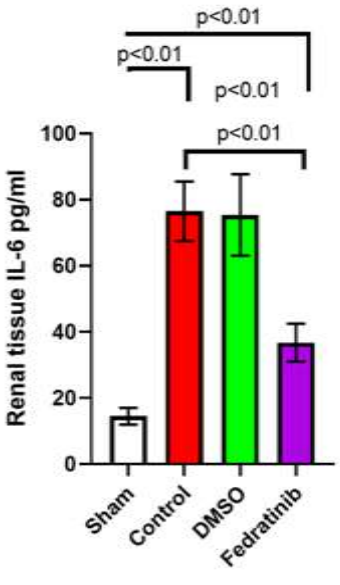


Figure (4) Renal mean level of IL6 (pg./ml) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Effect on renal HMGB-1 levels

The renal level of HMGB-1 exhibited a statistically significant increase ($p < 0.01$) in both the control and control vehicle groups as compared to the sham group. A non-significant difference was seen between the control group and the control vehicle group.

A statistically significant decrease in renal HMGB-1 levels was detected in the group treated with Fedratinib compared to the control groups. The figure (5) provides a summary of the variations observed in renal levels of HMGB-1.

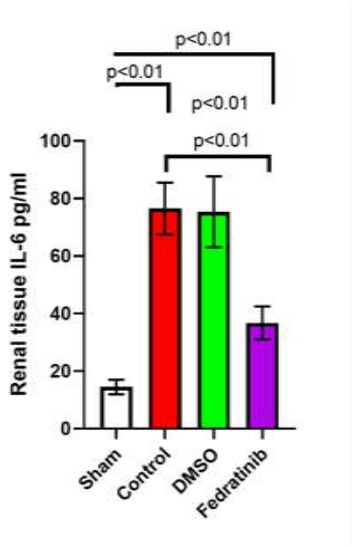


Figure (5) Renal mean level of HMGB-1 (pg./ml) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Impact on renal NF-kB level

The renal level of NF-kB exhibited a statistically significant increase ($p < 0.01$) in both the control and control vehicle groups as compared to the sham group. A non-significant difference was seen between the control group and the control vehicle group.

There was a significant decrease in renal NF-kB levels observed in the groups treated with Fedratinib, in comparison to the control groups. The figure (6) provides a summary of the variations observed in renal levels of NF-kB.

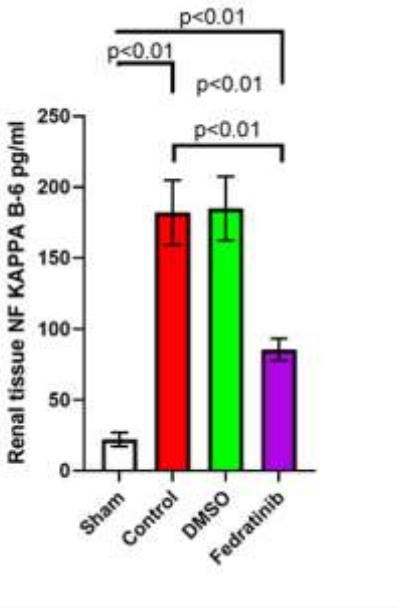


Figure (6) Renal mean level of NF-kB (pg./ml) of the four experimental groups at the end of the experiment (No of animals = 10 in each group)

Effect of RIRI on apoptosis parameters in study groups.

Effect on pro-apoptotic Mediator (BAX).

The renal expression level of Bax significantly increased ($p < 0.01$) in both the control and control vehicle groups compared to the sham group. A non-significant difference was seen between the control group and the control vehicle group.

The renal Bax levels in the Fedratinib group exhibited a significant decrease ($p<0.01$) in comparison to the control vehicle group. The figure (7) provides a summary of the variations observed in renal levels of BAX.

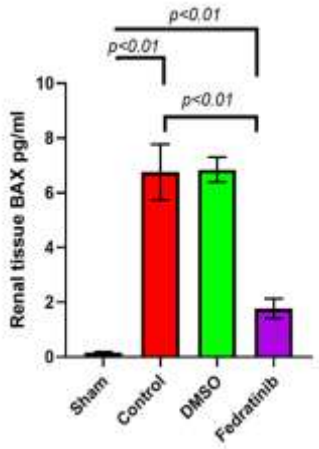


Figure (7) Renal mean level of BAX (pg./ml) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Effect on pro-apoptotic Mediator (Bcl-2).

The renal expression level of BCL2 exhibited a statistically significant increase ($p < 0.01$) in both the control and control vehicle groups as compared to the sham group. A non-significant difference was seen between the control group and the control vehicle group.

The renal Bcl-2 level in the Fedratinib group was found to be significantly lower ($p<0.01$) compared to the control vehicle group. No significant differences were observed between the two treatment groups. Figure (8) provide a summary of the variations observed in renal levels of Bcl-2.

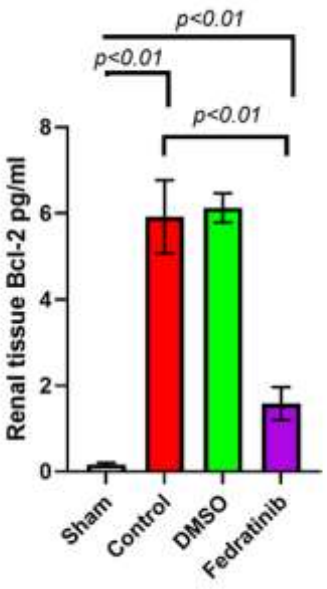


Figure (8) Renal mean level of BCL2 (pg./ml) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

JAK2 expression

The renal expression level of JAK2 exhibited a statistically significant increase ($p < 0.01$) in both the control and control vehicle groups as compared to the sham group.

A non-significant distinction was seen between the control group and the control vehicle group. In the Fedratinib group there were no significant difference from control groups. figure (10) provides a summary of the variations observed in renal JAK2 expression.

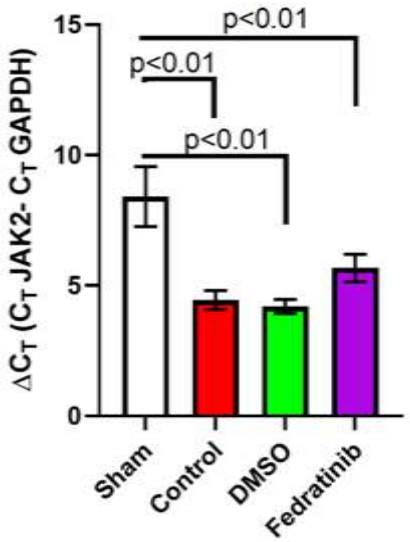


Figure (9) Renal mean JAK2 expression (pg./mg) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Stat 3 expression

The renal expression level of Stat3 exhibited a statistically significant increase ($p < 0.01$) in both the control and control vehicle groups as compared to the sham group. Non-significant distinction was seen between the control group and the control vehicle group.

The renal Stat3 expression with Fedratinib group was significantly lower than that of control groups ($p<0.01$).

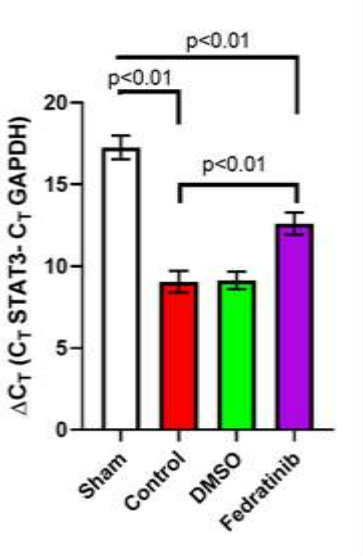


Figure (10) Renal mean Stat 3 expression (pg./mg) of the four experimental groups at the end of the experiment (No of animals = 10 in each group).

Histopathological finding

Acute kidney injury was assessed in the renal rats of the four experimental groups at the end of the study and the results are as follows:

Sham group

A cross-section of the sham renal rats showed normal renal structure. All rats in this group showed normal renal tissue 100% as shown in figure (11).

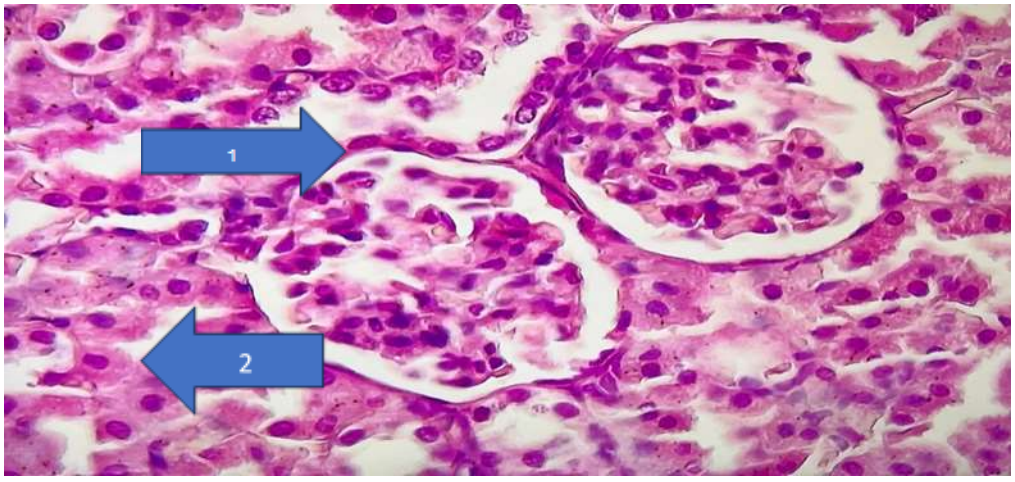


Figure 11: Section through kidney (sham group) showing almost normal glomerulus (1) and normal

renal tubule (2). H and E stain (40X)

Control group

There was a statistically significant difference between the control group and sham group ($P < 0.05$) and the total severity scores of the control group showed that 70% of this group had a severe renal injury, 30% had a moderate renal injury as shown in figure (12).

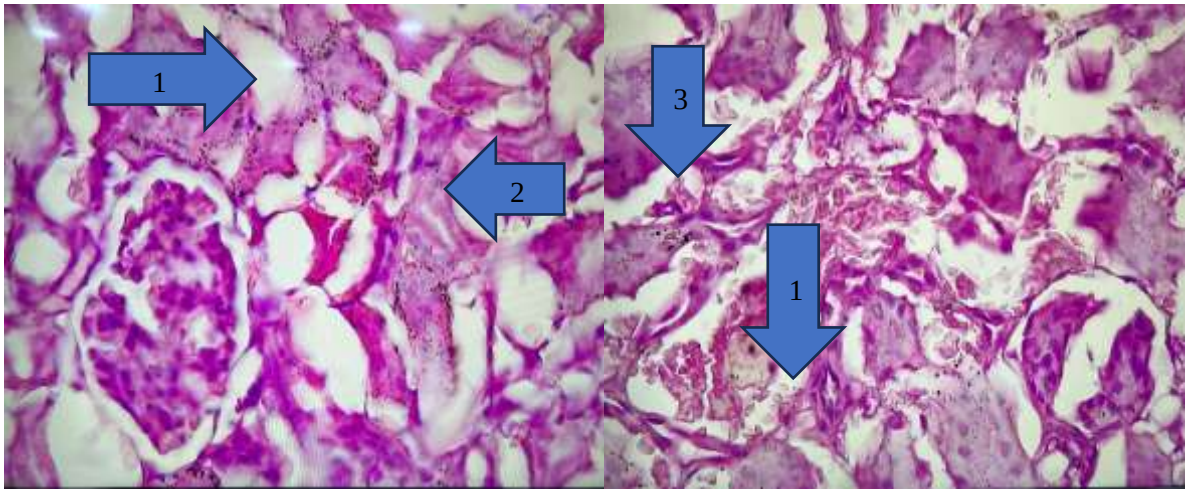
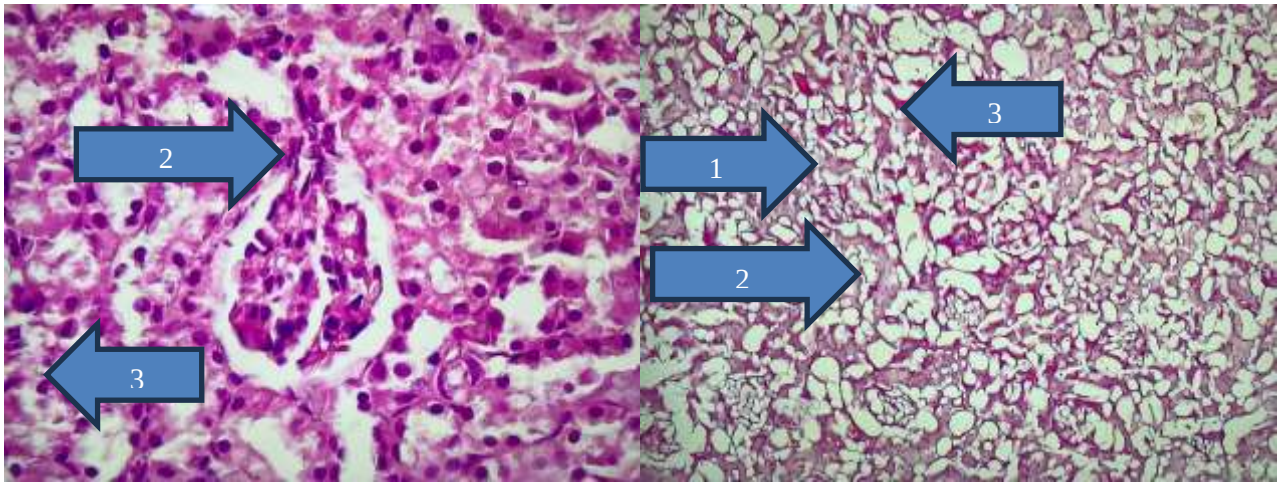


Figure 12 : Ischemic change to the tubule Loss of tubular architect and loss of the nucleus in comparison to glomeruli (1), increased cytoplasmic eosinophilia and fragmentation together with neutrophilic inflammatory infiltrate (2) and marked haemorrhage and congestion (3) . H and E stain (40X).

Control vehicle group

There was the statistically insignificant difference between control vehicle group and control group ($P>0.05$) and the total severity scores of the control vehicle group showed that 25% of the group had a moderate renal injury, 75% had a severe renal injury, As shown in figure (13).



Fedratinib treated group

Treatment of rats with Fedratinib improved renal injury significantly (P <0.05) as compared with the control vehicle group and the total severity score mean of this group showed that 60% had slight renal injury and 40% had moderate renal injury, As shown in figure (14).

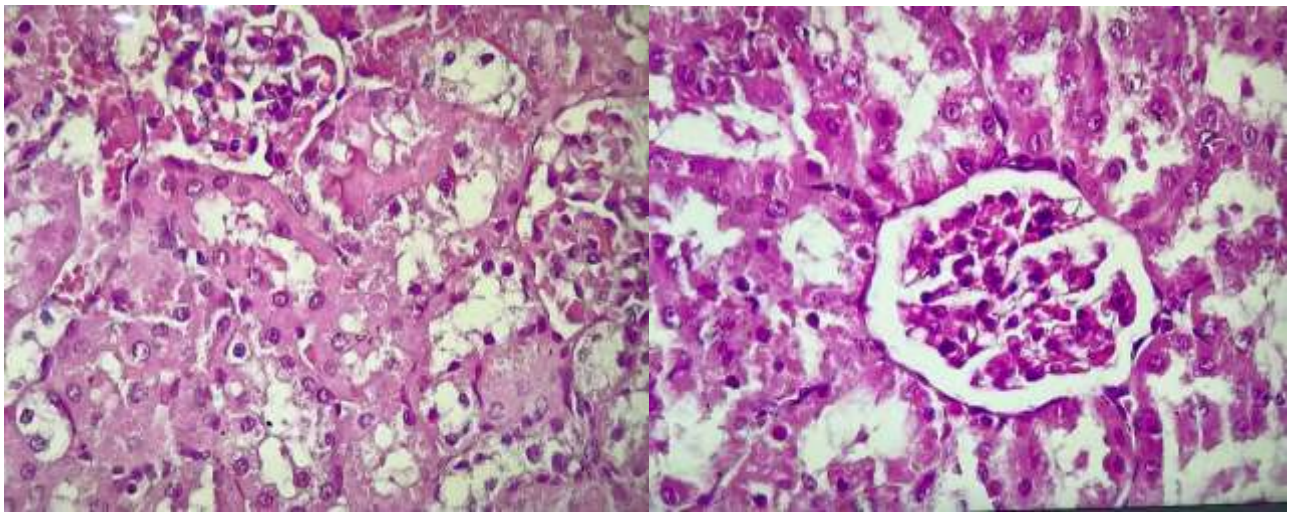


Figure 14: Mild congestion with a moderate ischemic change a lot of tubules kept their boundaries with a little Edematous change.

Discussion

Ischemic-reperfusion damage exerts acute stress on healthy tissue. This occurs as

Discussion

Inadequate oxygen and nutrition delivery, leading to a cascade of inflammatory responses that can result in a significant damage to both local and systemic tissues .AKI triggers a broad spectrum of inflammatory reactions, mostly induced by local oxidative stress and lipid peroxidation [16] .

This work aimed to evaluate the therapeutic effect of fedratinib, a jak2 inhibitor with anti-inflammatory properties, in a rat model RIRI . The study results demonstrate significant increases in serum urea and creatinine levels in both the control and control vehicle groups, when compared to the sham group. This finding is consistent with the acknowledged influence of ischemia-reperfusion injury (IRI) on kidney, which leads to decrease in glomerular filtration rate (GFR) and the simultaneous buildup of nitrogenous waste compounds like urea and creatinine in the blood[17], [18].

Jak inhibitors might offer some degree of protection against RIRI leading to less severe urea elevation compared to the control groups. This could be due to its anti-inflammatory and other renoprotective properties [18], [19]

The group treated with Fedratinib showed a significant reduction in kidney TNF-α levels compared to the control groups. These findings indicate that the medication may possess anti-inflammatory characteristics in the kidney, possibly by inhibiting the generation or function of TNF-α. Due to its nature as a JAK inhibitor, Fedratinib is capable of blocking the signalling pathways that are associated with inflammation and the regulation of TNF-α production through JAK-STAT signalling. Consequently, the inhibition of JAK can directly reduce the expression of TNF-α. In addition, JAK inhibition can have anti-fibrotic actions, so indirectly mitigating inflammation through the prevention of renal scar tissue development. This may result in a reduction in the generation of TNF-α by inhibiting the activation of inflammatory cells that contribute to fibrosis [20], [21]

Cytokines such as interleukin-6 (IL-6) can significantly impact the progression of cancer and

inflammation by triggering signal cascades. Bei Huang's research reveals that the excessive production of IL-6 stimulates the activation of the JAK/STAT pathway, resulting in the development of an inflammatory environment. This process is responsible for triggering epithelial-mesenchymal transition (EMT). Increased phosphorylation of STAT3 and JAK2 is observed in glioma cells. Blocking the IL-6/JAK2/STAT3 pathway has a significant effect on decreasing cell growth, cytokine generation, and stimulating cell death. Similarly, it has been noted that liver cancer cells have elevated levels of IL-6, phosphorylated JAK2, and phosphorylated STAT3. Application of a JAK2 inhibitor and IL-6 neutralising antibody induces cellular senescence and significantly inhibits cellular proliferation [22],And this is come in consistence with our findings that showed a significant decrease in IL6 concentration when we administered Fedratinib which is a JAK2 inhibitor.

According to Andrassy M and Volz H, HMGB1 has a significant impact on the initial stages of I/R injury by binding to RAGE, which leads to the activation of proinflammatory pathways and increased damage to the heart muscle[23]. Liu Q and Xie W's research establishes a correlation between HMGB1 and the STAT1 pathway, revealing their collaborative role in inducing lung inflammation and initiating cellular apoptosis in VILI. Suppressing the expression of HMGB1 provides cellular defence against damage caused by the combination of LPS and CS in a laboratory setting. AZD1480, a JAK2 inhibitor, effectively decreases lung damage, inflammation, and cellular apoptosis in a mouse model of ventilator-induced lung injury (VILI).AZD1480 hinders the process of phosphorylation of JAK2/STAT1, hence impeding the movement of HMGB1 from the nucleus to the cytoplasm[24].Our investigation revealed that Fedratinib-induced JAK2 inhibition resulted in a drop in HMGB1 levels, which is consistent with the above researches.

NF-κB is a crucial transcription factor that controls the expression of genes involved in inflammation. During the process of inflammation and repair, it is triggered by a range of stimuli such as cytokines and chemokines [5]. As a result, the creation of pro-inflammatory cytokines, chemokines, and adhesion molecules occurs, which contributes to the occurrence of tissue damage. Inhibiting upstream signalling pathways: JAKs participate in signalling pathways of cytokines will lead to reduce NF-κB in a direct and indirect manner[25]

JAKs have the ability to directly suppress the activation of NF-κB by interacting with and phosphorylating its subunits. JAK inhibitors can impede this phosphorylation process and the resulting activation. Ying et al. determined that sodium butyrate alleviated lung ischemia-reperfusion injury via suppressing the NF-κB and JAK2/STAT3 signalling pathways[25] And this is supported by our findings.

Bax, a member of the Bcl-2 protein family, promotes apoptosis by inducing mitochondrial membrane permeabilization and initiating caspase activation. Fan S and He J et al .demonstrated that IMD effectively alleviated lung injury and inflammatory response in VILI by primarily reducing reactive oxygen species (ROS) levels, enhancing superoxide dismutase (SOD) content, decreasing malondialdehyde (MDA) content, and suppressing the expression of Bax and caspase-3. In addition, they found that IMD exerted its antioxidant and anti-apoptotic effects through the JAK2/STAT3 signalling Pathway[26]

Karjalainen R and Pemovska T showed that different signalling pathways that are activated by chemicals secreted by bone marrow stromal cells are responsible for the resistance shown in bone marrow stroma conditions. This resistance entails a change in cell survival from BCL2-dependent to BCLXL-dependent. The BCL2 inhibitor venetoclax's resistance was remarkably reduced by the JAK1/2 inhibitor ruxolitinib, suggesting that JAK inhibitors may be able to overcome resistance caused by BM stroma in AML [27]. Furthermore, activation of AK enhances cell survival, potentially influencing BCL2 expression or activity. However, leukemia cells' BCL2-mediated survival can be indirectly decreased by decreasing JAK activity with JAK inhibitors, which explains why leukaemia cells are susceptible to BCL2 inhibitors. This idea is expressed by [28]

JAK2/STAT pathway is implicated in both cell protection and cell injury, and its role in nephrotoxicity

has not been addressed. Static therapy reduces tubular damage, inflammatory cytokines/chemokines, macrophage infiltration, and fibrosis in mice models of unilateral ischemia-reperfusion injury (IRI) and unilateral ureteral blockage. In vitro inhibition of STAT3 downregulates fibrosis and death in human tubular epithelial cells and reduces inflammation mediated by pSTAT3α. The advancement of IRI is linked to the activation of STAT3, and STAT3α may play a crucial role in this process. This suggests that STAT3 may impact the transition from acute kidney injury (AKI) to chronic kidney disease (CKD), suggesting a new approach to managing AKI using STAT3 inhibitors [29]. Inhibition of STAT3 signaling with S3I-201 may hold therapeutic potential for fibrotic kidney diseases.[30]

A study investigates the role of STAT3 in diabetic nephropathy (DN) development. It found that inhibiting STAT3 pharmacologically can restrict DN progression. A 16-week treatment with a specific inhibitor, S3I-201, reduced kidney damage in diabetic mice caused by streptozotocin. This improvement was accompanied by a decrease in TGF-β1, ACE/AT1, and VEGF expression in the kidneys. The findings were validated by genetic knockdown of STAT3 in mice using AAV2 containing STAT3 shRNA. STAT3 was found to be predominantly expressed in tubular epithelial cells. Pretreatment with S3I-201 and a specific siRNA sequence reduced STAT3 levels in renal tubular epithelial NRK-52E cells, preventing overexpression of TGF-β1, ACE/AT1, and VEGF generated by high glucose. This study suggests that renal tubular epithelial cells contribute to DN progression through STAT3 signaling and provides initial evidence that inhibiting STAT3 can reduce DN severity [31].The aforementioned findings are consistent with our results, which show that inhibiting JAK2-STAT3 might lead to a reduction in inflammatory mediators.

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