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Serelaxin alleviates ischemia reperfusion-induced kidney injury by modulating inflammatory response and inhibiting of notch-2/hes-1 signaling pathway

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Abstract: **Background**: Renal transplantation is the last standard option for the treatment of patients with end-stage renal failure. During renal transplantation, the renal damage occurs after a short time of warm ischemia after the harvest of the kidney from the donor, a long period of the cold ischemia through cryopreservation and a last phase of the warm ischemia through recipient implantation. Ischemia reperfusion (IR) complications are one of the challenges of transplantation. **Objectives**: The study aimed to diagnose the protective effect of serelaxin against kidney damage induced by IR and to understand the possible impact of the Notch-2/Hes-1 signaling pathway in this problem. **Methods**: Eighteen Sprague Dawley rats were divided into three groups: serelaxin group was treated with serelaxin (5µg/kg SC), while the control group was treated with distilled water and the sham group was not treated with serelaxin or D.W. The renoprotective effect of serelaxin was studied by assessing the kidney function by measuring of serum urea and creatinine. The anti-inflammatory impact, was evaluated by measurement of interleukin-1β. Also the effect of serelaxin on Notch-2/Hes-1 pathway in ischemia reperfusion injury was investigated. **Results**: The results disclosed that serelaxin pretreatment remarkably restored S.u, S.cr, and ameliorated kidney damage. Through IHC, the result showed that serelaxin produced a magic effect against IRI through downregulated the protein expression of cytoplasmic and nuclear cleaved Notch-2 in addition to nuclear Hes-1. Serelaxin also produced potent anti-inflammatory effect via the reduction in the protein expression of the IL-1β. **Conclusion**: These results highlight the nephroprotective effects of serelaxin against IRI by numerous pathways that also include Notch-2/Hes1. These novel findings uphold the use of serelaxin or serelaxin derived agents as a promising medication for those illnesses in which IRI is the main pathogen.

Keywords: Ischemia/reperfusion injury; Inflammation; Interleukin-1β; Notch-2/Hes-1; Serelaxin

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INTRODUCTION

Renal transplantation is the last standard option for the treatment of patients with end-stage renal failure. The kidney artery is clamped during operation, which can lead to so called ischemia-reperfusion injury (IRI). This injury is an inevitable adverse event that occurs following kidney transplantation (Fernandez *et al.,* 2020).

Ischemia/reperfusion injury is considered one of the fundamental clinical reasons of the early kidney dysfunction following renal transplantation. Because of the anatomical features of the kidney tissue, the epithelial cells of tubules are very sensitive to IRI. The affluence of clinical evidence has shown that the severity of renal IRI is positively linked with the frequency of the acute rejection episodes. Sharp IRI can result in delayed graft function and acute tubular cell necrosis as a major pathological manifestation, that can produce acute and/or chronic rejection immunoresponse and diminish graft survival rate (Wenbo *et al.,* 2023).

During renal transplantation, the renal damage occurs after a short time of the warm ischemia after the harvest of the kidney from donor, a long period of the cold ischemia through cryopreservation and a last phase of the warm ischemia through recipient implantation. The blood reperfusion that followed revascularization prompts a series of the events that may exacerbate renal damage, leading to inflammation and injury to tubular epithelial cells that associated with hemodynamic fluctuations. The pathogenesis of renal IR injury may be imputed to the structural and functional disturbance of the kidneys occurred by a variety of factors, like oxygen free radical over generation, excess of the intracellular of Ca^{+2} level, inflammatory elements and transmitters, cellular membrane lipid-peroxidation, change in nitric oxide level and the apoptosis (Zhe *et al.,* 2023).

Inadequate blood supply and oxygen transmission during ischemia and consequent mitochondrial dysfunction can result in oxidative burst and uncontrolled generation of reactive oxygen species during reperfusion stage. This overproduction of ROS may induce cell death by

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destruction of DNA, cell membrane, and also the protein structure (Ucar *et al.,* 2021).

The cells of renal tubules are more susceptible to renal IRI, and the renal tubule epithelial cells can release the cytokines besides chemokines in response to the pathogenic stimulators. At the last stage of the reperfusion, the inflammatory cytokines liberated from destroyed cells bind to certain receptors on the tubular cells. Binding of ligand-receptor produces a vicious circle of the inflammatory cascades, resulting in further injury for tubular cells. This inflammatory response is activated rapidly after the damage and continues, and can also be augmented during the whole period of the disease, next induction of the tubular cell apoptosis (Shao *et al.,* 2021).

Ischemia reperfusion (IR) complication is one of challenges of transplantation. Many of studies were done to alleviate the serious effects of IR. Thus, establishment and development of new therapeutic strategies are necessary to reduce tubular damage through inhibiting of inflammation and oxidative stress that induce apoptosis. This improves of life and reduces treatment costs (Hanieh *et al.,* 2020).

The Notch signaling pathway has been known as an evolutionarily conserved inter-cellular signaling system which has serious functions in tissue/organ formation throughout embryonic development, cell homeostasis and disease, and a wide number of cellular activities like the cell proliferation, programmed cell death (apoptosis), and cell fate decisions. The Notch signaling pathway participates in the maintenance and repair of the mature tissue (Yaser *et al.,* 2022).

Notch pathway also takes an important role in development of various organs, involving the liver, brain, and bone. In addition, this pathway serves a critical function in formation of the nephron and segmentation, and also has been informed to be contributed in kidney disease (Malini *et al.,* 2019).

Numerous of Notch related kidney diseases have been reported in human, such as diabetic nephropathy (diabetic kidney disease), Alagille syndrome (ALGS; MIM118450), congenital anomalies of the kidney and urinary tract (CAKUT), Kidney Cancers, and Hadju-Cheney syndrome (Malini *et al.,* 2019).

There is a cross talk between Notch signaling and both innate immunity and inflammatory cytokines, where Notch-2 has been presented to be active in inflammatory conditions, like in rheumatoid arthritis and some microbial infections as bacterial and viral infections. Notch pathway inhibitor agents have been noticed to control vasculature endothelial dysfunction in atherosclerosis (Yaser *et al.,* 2022).

The Notch system is composed of three elements: the first, Notch ligands (DSL-proteins), the second, Notch-receptors, and the third, DNA-binding proteins (CSLs) (Zhenbo *et al.,* 2023). There are four different transmembrane Notch receptors in mammals that are Notch1, Notch2, Notch3, and Notch4 and five of two classes of ligands [Delta-like-1, 3, 4 (Dll-1, Dll-3 and Dll-4) and Jagged-1, 2 (Jag-1 and Jag-2)] that expressed on surface of the neighboring cells (Zhou *et al*., 2022).

After binding of ligand and activation of Notch pathway, the intracellular domain part will undergo proteolytic cleavage by γ-secretase. The cleaved Notch intracellular domain (Notch ICD) is liberated into cytoplasm, next translocates to the nucleus, and forms an active complex with transcription cofactors like RBPj and Mastermind-like proteins and (CSL [CBF1/Su(H)/Lag-1]), this leading to expression of the Notch target genes, which include hairy-enhancer of split (Hes) and Hes-related-YRPW (Hey), these genes encode the basic helix-loop-helix transcription factors, resulting in expression of the downstream genes that normalize cell fate. Generation of the cleaved Notch protein and expression of the target genes (Hes and Hey) have been utilized as biomarkers of stimulation of the Notch pathway. The biological impacts result from activation of Notch pathway can be tissue and binding ligand specific, and based on which of the Notch receptor types is triggered, and also the kind of the target genes that expressed (Zhou *et al.,* 2022).

Serelaxin is a recombinant product or an analog of human relaxin-2 hormone. It was industrialized as a potentially beneficial therapeutic medication because of both its vasodilating impacts and its direct protective impacts on organs (Metra *et al.,* 2019).

Human relaxin-2 is a low-molecular-weight (6 KDa) polypeptide hormone that related to a superfamily that is called insulin-relaxin. It was originally found out as a polypeptide hormone primarily isolated from corpus luteum of the ovary, which involve in regulation of the normal reproductive function in addition to the adaptive alterations in gestation. Currently, relaxin-2 is also released by various tissues of the unpregnant women and men. Relaxin-2 now is labeled as a prominent pleiotropic polypeptide hormone found in human body and produces a wide range of actions further than reproduction (Alana *et al*., 2024).

The majority of endogenous relaxin hormone in primates is coded via the RLN2 gene, and it has a great tendency to the cognate receptor (G protein-coupled) relaxin family peptide receptors-1. These types of receptors are scattered in the body where they are found in reproductive system and in other organs like the brain, lungs, heart, kidneys, and liver, in addition to the arteries and blood cells (Jakubauskiene *et al.,* 2020).

Relaxin-2 and for a long period was considered severely as a pregnancy hormone, where it contributes to numerous of changes in the cardiovascular and kidney function observed throughout the pregnancy (Jakubauskiene *et al.,* 2020; Alana *et al.,* 2024). Relaxin plays an essential role in hemodynamic alteration, like reducing systemic vascular resistance, enhancing cardiac output, beside improving global arterial compliance (Verdino *et al.,* 2023).

Recently, several of studies displayed that it has anti-oxidant, anti-inflammatory, anti-fibrotic, anti-apoptotic properties and cytoprotective effects (Jakubauskiene *et al.,* 2020; Du*,* 2022). Other studies showed that the relaxin induces different protective impacts in the ischemic myocardium, involving diminished the inflammasome activity, fibrosis and alleviation of arrhythmogenesis (Devarakonda *et al.,* 2022). Number of studies have shown that serelaxin can markedly reduce the fibrosis of each of the cardiac, kidneys and lungs. Furthermore, serelaxin has been reported to ameliorate the inflammatory response in the LPS-induced fibrosis (Xueping *et al*., 2023).

Because of the IRI complications, especially in renal transplantation, in addition to the lack of effective therapy to avoid these problems, and the lately discovered impacts of serelaxin encouraged to investigate its effect against IRI. Most of previous research is focused on the role of serelaxin in the heart failure. However, this study aimed to diagnose the protective effect of serelaxin against kidney damage induced by IR and understand the possible impact of the Notch-2/Hes-1 signaling pathway in this problem.

MATERIALS AND METHODS

Rats grouping and establishment of renal ischemia reperfusion injury

The experimental animals (male rats, 12-14 weeks, of 240-255 g) were purchased from the Laboratory Animal of Faculty of Science/University of Kufa. The procedures were conformed with ethical criterion that were approved by the Animals Care Committee, and according to the directives of the University of Kufa/ Ethics Committee. All the animals were fostered in specific non-pathogenic cages for at least eight days for acclimation and under organized light conditions (12 h dark and light cycle) at 22 ± 2 ^{o}C and fifty percent humidity with free access to water and food. One hour before the model was started, the rats in the serelaxin +I/R group were given a single dose of serelaxin (5 µg/kg) subcutaneously (Juan *et al.,* 2017). The serelaxin was dissolved in D.W to prepare before injection.

The rats were divided into three groups, the serelaxin+I/R group (Serelaxin, n= 6) was treated with serelaxin (5 µg/kg SC) 1 h prior to I/R operation, while the I/R group (Control, n= 6) was treated with D.W. and the sham group (Sham, n= 6) was not treated with serelaxin or D.W.

To begin an in vivo model, rats were anesthetized with 100 mg/kg of ketamine hydrochloride and 10 mg/kg of xylazine hydrochloride intraperitoneally. An incision was done along the midline of the abdomen of rat, and both kidney vascular pedicles were fully uncovered. Non-traumatic vascular clips were utilized to clamp both kidney pedicles for 30 min, and then removed the clips from the pedicles, and the abdominal incision was sutured. Two hours later, the rats were sacrificed, and both renal tissue and blood were gathered directly for evaluating the specific biomarkers. The rats of sham group did not expose to ischemia and reperfusion (Thu-Alfeqar *et al.,* 2022).

Assessment of renal function

The serum urea and creatinine of rats for all groups were tested. At the end of operation, the blood samples were collected directly from heart in gel tubes and let for about 30 minutes at usual room temperature, after that centrifuged at 4^{o}C for 15 minutes to isolate the serum, which utilized for measurement of both biochemical markers by commercial analysis kits.

Histological assessment

To measure the histopathological changes, harvested kidneys washed rapidly with precooled PBS, cut sagittally, one half submerge in 10% neutral-buffered formalin, dehydrated, and then embedded in paraffin. After slicing process, hematoxylin and eosin (H and E) were utilized to stain the renal tissue samples through a standard staining procedure. Histologic assessment of kidney injury was performed via a semiquantitative analysis of damage including the occurrence of loss of the brush border, thickened basal membrane, endothelial swelling, disruption or loss, thickening of Bowman capsule, inflammation, hemorrhage and necrosis and graded as following: 0, normal kidney; 1, less than 25%; 2, (25-50) %; 3, (50-75) %; 4, more than 75% (Zhang *et al.,* 2023).

Assessment of interleukin-1β

For evaluating the effects of serelaxin on the inflammation, the tissue level of IL-1β was measured by ELISA kits according to the company's recommended protocol. Frozen kidney tissues were crushed by mortar and pestle after adding the homogenized solution (phosphate buffer saline (PBS) (pH 7.4), 1% Triton 100X, and 1% protease inhibitor cocktail) (Geurt *et al.,* 2010). Then, the suspension was homogenized by sonicator. The homogenate was centrifuged according to manufacturing process, then the supernatant was separated and stored at -80°C for using to determine the tissue concentration of IL-1β.

Immunohistochemical (IHC) assessment of notch-2 and hes-1

The kidney samples were cut into 5 μm thickness, and IHC staining was performed to determine the expression levels of cytoplasmic Notch-2, nuclear Notch-2, and nuclear Hes-1 as we reported previously (Thu-Alfeqar *et al.,* 2022). In brief, the tissue sections (5 μm thickness) were deparaffinized in xylene, followed by rehydration in graded ethanol, then stained using a polyclonal antibody against Notch-2 or against Hes-1. Bound antibodies were identified by horseradish peroxidase-conjugated corresponding IgG (subjected for 30 minutes), and 3,3'-diaminobenzidine tetrahydrochloride (for 8 minutes) was used to notice the presence and location of Notch-2 and Hes-1. Ultimately, using hematoxylin stain for counterstaining. Next, the stained slides were observed under the light microscope. Notch-2 and Hes-1 protein expression were expressed as H-score (0 to 300) that resulted from multiplying the stain intensity with percentage of the stained area. Where the intensity was graded as 0, for no stain; 1, for weak stain; 2, for moderate stain, and 3, for strong stain. While the percentage of staining area was scored (0 to 100) % (Rajarajan *et al.,* 2020).

Statistical analysis

The data were analyzed by SPSS version 21 and represented as Mean +/- SD. One-way ANOVA and Tukey post-hoc testing for multiple comparisons among the groups were utilized for most studied parameters (parametric). Kruskal–Wallis test followed by Mann-Whitney U-test post hoc was applied just for comparing histological scores (non-parametric). The p-values of less than 0.05 were considered statistical significance (*,**,*** mean $p < 0.05$, 0.01, and 0.001, respectively).

RESULTS

Effect of serelaxin on renal function

Ischemia reperfusion provoked a significant elevation in S. creatinine and S. urea compared with the sham group. In contrast, compared with control group, pre-treatment with serelaxin effectively attenuated the renal function abnormalities as evidenced by reducing S.cr and S.u (Fig. 1).

Effect of serelaxin on histopathological and structural alteration

Kidney tissue from the sham group showed a normal appearance of functional structure of nephron (Fig. 2A). On the contrary, ischemia reperfusion prompted prominent histopathological alterations in kidney tissue in the form of a (cellular swelling, loss of brush border, cytoplasmic eosinophil, eosinophilic cast, inflammatory cells (lymphocytes), vascular congestion and hemorrhage) (Fig. 2B). Interestingly, pre-treatment with serelaxin significantly decreased these histological abnormalities (Fig. 2C). Fig. 2D summarizes scoring kidney damage.

Effect of serelaxin on inflammatory marker in kidney tissue

The inflammatory responses induced by ischemia reperfusion were revealed by valuation of the inflammatory biomarker IL-1β. Tissue examination of IL-1β expression manifested a significant enhancement in the expression of this inflammatory element after ischemia and reperfusion (Fig. 3). Otherwise, co-treatment with serelaxin significantly downregulated IL-1β protein expression.

Effect of serelaxin on notch-2 activation

The cytoplasmic content of activated Notch-2 exhibited a strong rise in ischemic group as compared with sham group (Fig. 4A) as exposed by the intense brown staining (Fig. 4B). Fascinatingly, serelaxin treated group displayed a significant reduction in cytoplasmic level of activated Notch-2 (Fig. 4 D and E). In parallel, the nuclear level of activated Notch-2 showed a sufficient rise ($P < 0.05$) in control group (Fig. 4C) contrasted with sham group. On the other hand, serelaxin treated group appeared a significant reduction in nuclear expression of activated Notch-2 (Fig. 4 D and F) as compared with control group.

Effect of serelaxin on hes-1 expression

Immunohistochemical examination of renal tissue for Hes-1 expression displayed a significant upregulation in this protein following exposure to ischemia and reperfusion, and as shown by staining tissue with brown color (Fig. 5B) in comparison to sham (Fig. 5A). On the contrary, serelaxin pre-treated rats, the kidney tissues appeared significant downregulation in nuclear Hes-1 expression when compared with control group (Fig. 5C). All these were summarized in (Fig. 5D).

DISCUSSION

Ischemia reperfusion injury is one of important causes of renal graft dysfunction and rejection in kidney transplantation. During and after IR, numerous of key signaling pathways will be activated, where they have a role in pathogenesis of IRI, whether negatively or positively (Meng *et al.,* 2024). Notch signaling pathway is one of these pathways.

In experimental models, the Notch pathway is shown to be pathologic, leading to faulty differentiation of podocytes, programmed cell death, and finally causes renal failure. Stimulation of Notch pathway is participated in kidney damage and glomerular disease. Preventing the activation of Notch pathway has been demonstrated to be efficient in rescuing injury incurred in diabetes mellitus cases. One main finding is that activation of Notch pathway prompts cell death, which additionally harms tissue activity (Duan and Qin, 2020).

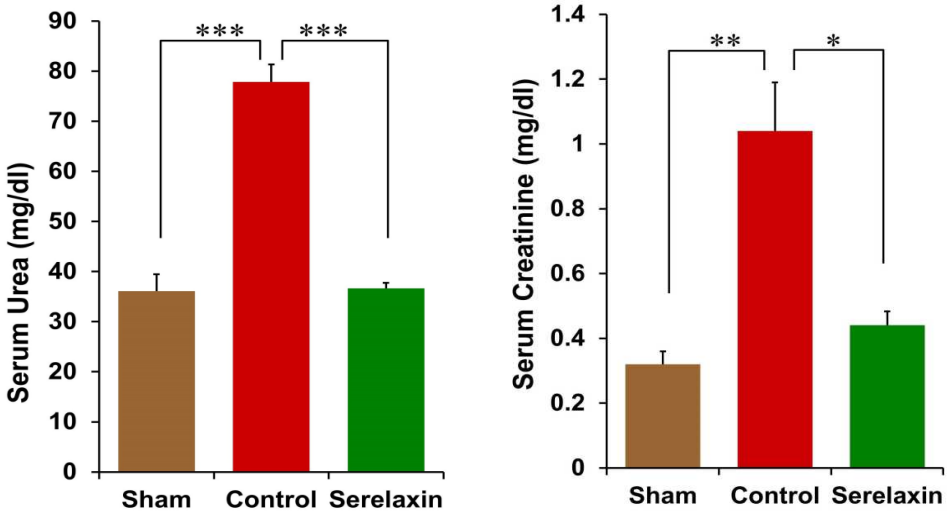


Fig. 1: Serum concentrations of urea and creatinine in the rats of different groups.
(*,**,*** mean $p < 0.05$, 0.01, and 0.001 respectively)

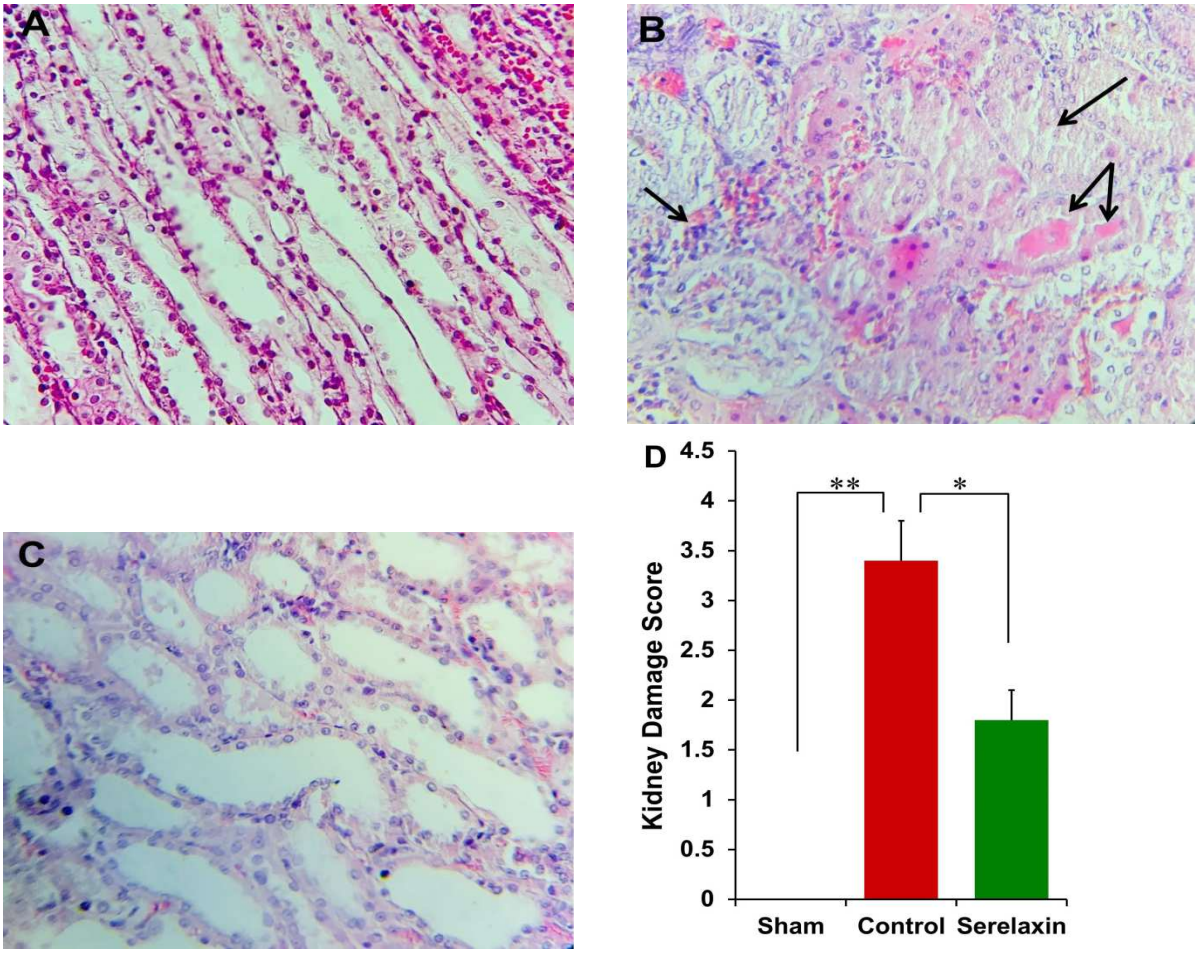


Fig. 2: Histopathological investigation of the kidneys in different groups via H and E staining (magnification × 400) and scoring of severity of renal damage. Ischemia reperfusion increased the histological damage (B), contrasted with the sham group (A). Serelaxin decreased the histological damage induced by ischemia reperfusion (C). Quantification of kidney damage scores (D). (*,**,*** mean $p < 0.05$, 0.01, and 0.001 respectively)

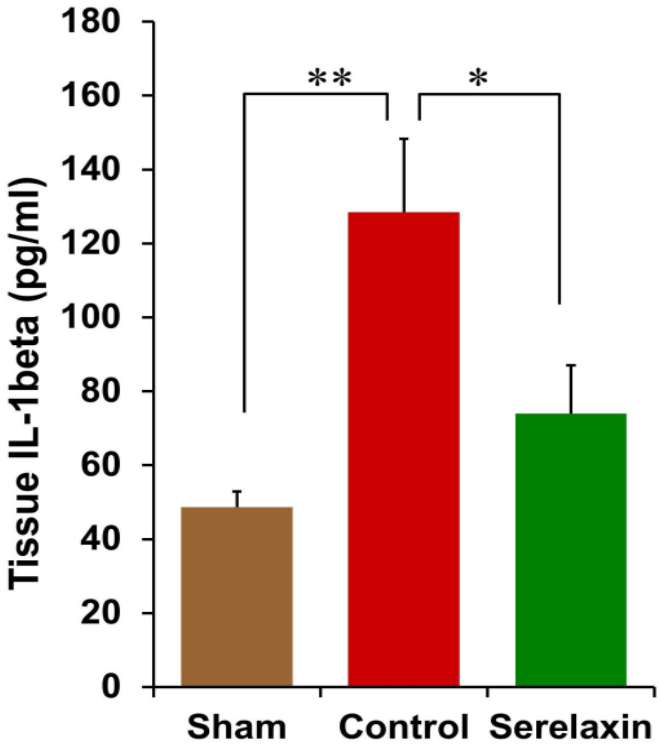
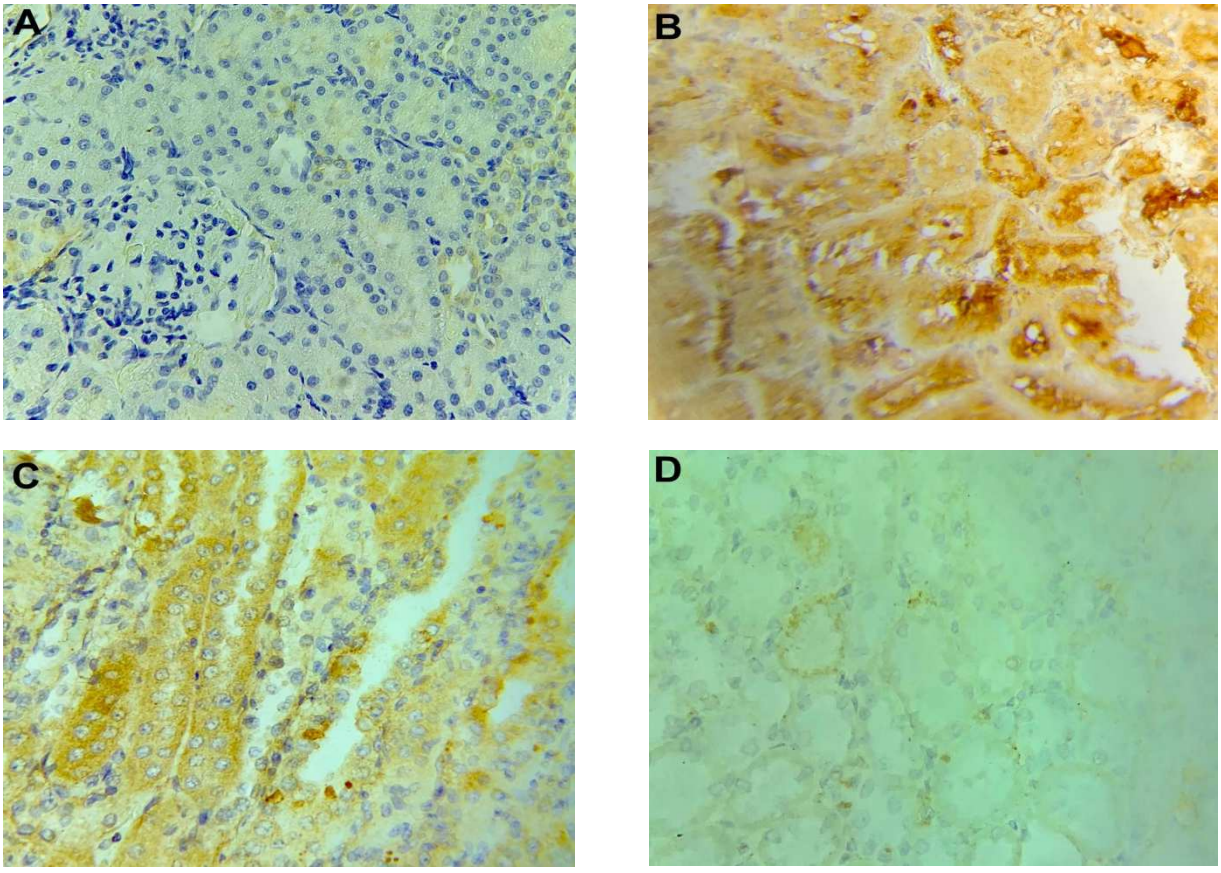


Fig. 3: Tissue IL-1β levels of rats of different groups. (*,**,*** mean $p < 0.05$, 0.01, and 0.001 respectively)



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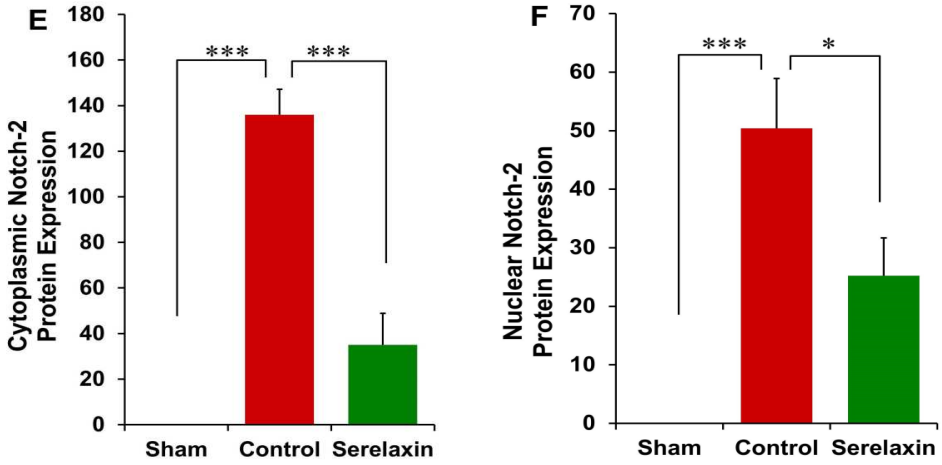

Fig. 4: Immunohistochemistry staining images and expression levels of kidney tissues for Notch-2 protein in different groups (original magnification ×400). (A) Sham group, negative cytoplasmic and nuclear stain Notch-2. (B and C) Control group, the expression of Notch-2 in kidney tissue after IR via IHC staining. Kidney IR induced the raising of Notch-2 expression within the cytoplasm and nucleus of tubular cells. (D) Serelaxin group, negative cytoplasmic and nuclear stain Notch-2. (E and F) H-scores of protein expressions of cytoplasmic and nuclear Notch-2 were appeared significantly reduced in serelaxin group compared with control group. (*,**,*** mean $p < 0.05$, 0.01, and 0.001 respectively)

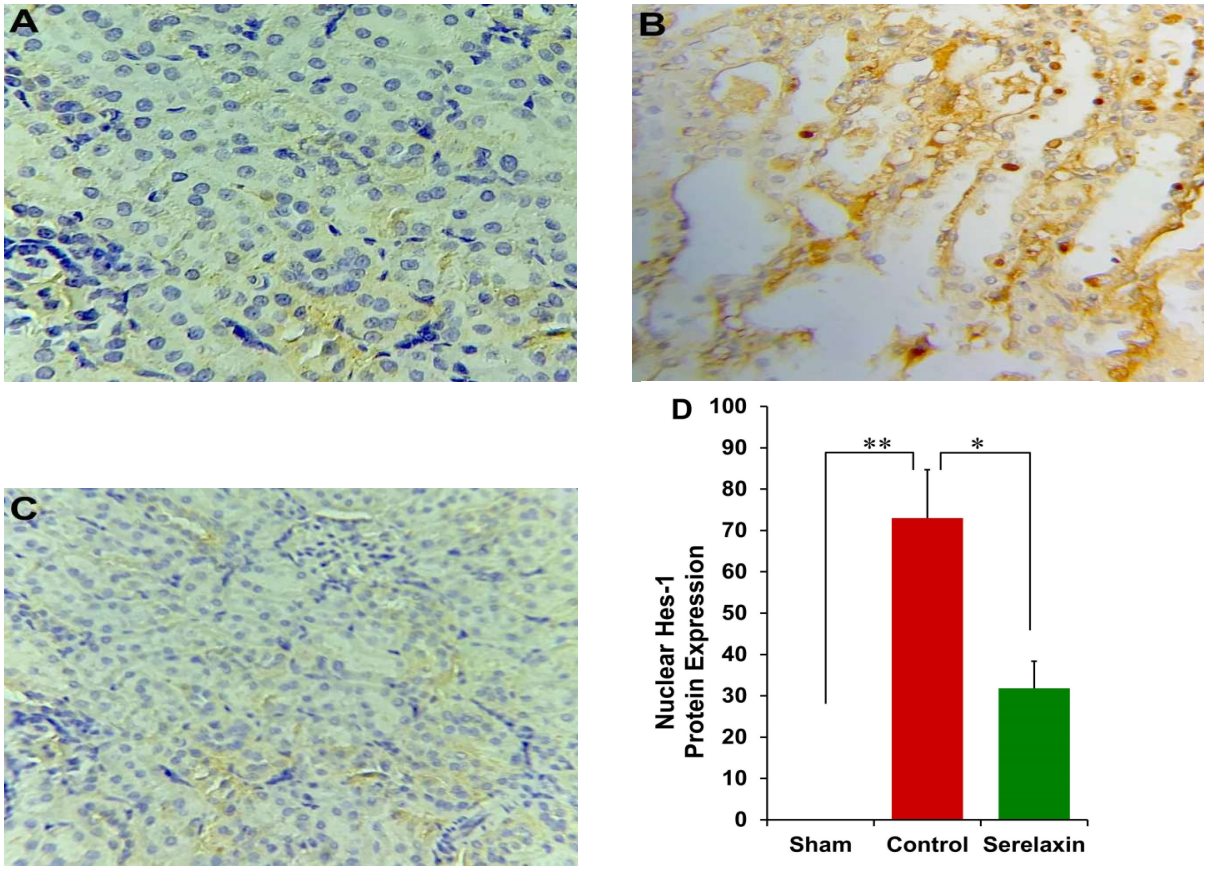

Fig. 5: Immunohistochemistry staining images and expression levels of kidney tissues for Hes-1 protein in different groups (original magnification ×400). (A) Sham group, negative nuclear stain Hes-1. (B) Control group, expression of the Hes-1 in kidney tissue after IR via IHC staining. Kidney IR induced the raising of Hes-1 expression within the nucleus of renal cells. (C) Serelaxin group, the number of Hes-1 positive cells was reduced in serelaxin group. (D) H-scores of protein expressions of nuclear Hes-1 were appeared significantly reduced in serelaxin group compared with control group. (*,**,*** mean $p < 0.05$, 0.01, and 0.001 respectively)

In this study, the rats underwent renal ischemia reperfusion revealed increasing in renal function tests (S.u and S.cr) in addition to occurrence of significant pathological changes in kidney tissue, which indicates occurrence of renal dysfunction. This is in agreement with Najah *et al*., (2020) study, that demonstrated 180 min ischemia and reperfusion resulting in increased S.u, S.cr, and injury score in control group compared with sham group (Najah *et al.,* 2020).

Our findings displayed that serelaxin has protective effects against renal IRI via decreasing the level of Su, Scr, and diminishing of histological damage of renal tubules. This outcome is in agreement with a preceding study that serelaxin is able to decrease not only plasma urea and creatinine concentrations but also prevent histological injury (Li *et al.,* 2021). Another study by Collino *et al*. (2013) reported that administration of recombinant human relaxin during reperfusion prevented the rise in the serum concentrations of both urea and creatinine, in addition to significantly reducing the renal histological abnormalities, resulting in a mitigation of the renal dysfunction and damage (Collino *et al.,* 2013).

This nephroprotection may produce primarily from the repression of inflammation that associated with IR, as appeared by our results and/or by inhibition of Notch-2 signaling pathway. This is compatible with a previous study that indicates the inhibition of Notch-2/Hes-1 signaling mitigates the severeness of the renal tubular injury, ameliorates kidney function, and reduces the level of pre-inflammation factors (Duan and Qin, 2020). Wang *et al*. (2022) study showed that the overexpression of miR-149-5p guards the rat brain against ischemia and reperfusion injury by regulating Notch2-mediated inflammatory and apoptotic pathways (Wang *et al.,* 2022).

The present study finds that the IHC examination of protein expression of activated Notch-2 and Hes-1 is not detected in the kidney tissue of sham group of rats. Nevertheless, the kidneys underwent IR showed significant surge in protein expression level of cleaved Notch-2 and Hes-1. These findings disclose that Notch signaling pathway is triggered after ischemia and reperfusion. These results are consistent with Kobayashi *et al*. (2008) study, which appeared that the cleaved Notch-2 and Hes-1 elevated at both levels of mRNA and protein expression after 60 min of unilateral ischemia via ligation of the left kidney artery (Kobayashi *et al.,* 2008). Albéri *et al*., work exhibited that neonatal ischemia prompted by unilateral carotid clamping in P12 mice associated with a strong increase in Notch-2 protein expression. The work indicated that this aberrant induction of the Notch-2 signaling may be neurotoxic (Albéri *et al*., 2010).

The current study demonstrated that serelaxin significantly reduced the cytoplasmic and nuclear cleaved Notch-2 and nuclear Hes-1 protein expression in pretreated group when compared with control group.

To the best of the researcher's knowledge, the current paper is the first one that studied the impact of serelaxin on Notch-2/Hes-1 pathway in IR induces renal injury model. The serelaxin may have a direct or indirect effect on these proteins by different pathways. One of these pathways is through inhibition of NF-kB. Several of studies indicate that there is an interaction between both Notch and NF-kB signaling pathways. These papers display that Notch controls the transcription of NF-kB signaling pathway components. Otherwise, enhancing the expression of the p52/RelB rises the Notch activation plus Hes levels (Marquez-Exposito *et al.,* 2018). This is compatible with Xueping *et al*., (2023) study has shown that serelaxin strongly blocks the activation of the NF-kB signaling pathway and decreases the expression of IkBα and P65/p50 (Xueping *et al.,* 2023).

Other underlying molecular mechanism that may explain the effect of serelaxin on Notch-2/Hes-1 is by reducing IL-1β, as shown in this study, which is one of the factors or mediators that regulate of Notch signaling. This is in line with Hua *et al*., (2013) study that showed IL-1β and TNF-α have a regulatory effect on the Notch signaling pathway in rat nucleus pulpous (NP) cells. Interestingly, IL-1β or TNF-α treatment induces expression of Notch-1 and Notch-2 mRNA. Notch-1 expression exhibits a delayed rise compared with Notch-2, which is induced early after treatment. Furthermore, the expression of the target genes of Notch pathway, like Hey-1, Hey-2, and Hes-1, is also raised after treatment with both cytokines. In addition to that, the NP cells pretreated with IL-1β or TNF-α showed increase in the level of cleaved Notch-1 and Notch-2. Other previous studies indicated that Notch signaling is enhanced in stem cells and other kinds of the cells in response to cytokines (Hua *et al*., 2013).

Taken together, decreasing IL-1β and inhibitory effect on NF-kB of serelaxin can explain the reduction of expression of cleaved Notch-2 and Hes-1 in serelaxin pretreated group compared with control. The study at hand gives further proof that inactivation of the Notch signaling pathway can rescue the kidney defects in ischemia reperfusion induced injury rats, pointing that this may possibly be utilized as a treatment approach.

The inflammatory reaction is a prime component in the pathophysiology of the renal IRI, producing kidney tissue injury via liberating numerous of mediators. Several studies showed that the renal damage during IR period results in the generation of proinflammatory cytokines. IL-6 and IL-1β are both inflammatory downstream and can hurt kidney cells directly. The complex interaction among the endothelial and epithelial cells, the cytokines, and inflammatory biomediators leads to sustained damage through acute tubular necrosis. Therefore, early and effec-

tive suppression of inflammatory reaction is significant strategy for both prevention and treatment of the kidney injury (Qiu *et al*., 2019).

In this study, our results demonstrated that ischemia reperfusion-induced inflammatory cytokine, IL-1β, increased significantly in control group compared with sham. Consistent results were observed in several of papers that refer to IR leading to raise the concentration of IL-1β in kidney tissue (Tan *et al.,* 2018; Tang *et al.,* 2022; Chen *et al.,* 2023).

Besides the effect of serelaxin on Notch-2/Hes-1 signaling pathway, the current study exhibited that it exerts beneficial influences against renal ischemic injury by decreasing inflammation through reducing the production of IL-1β in the kidney tissue of animals exposed to IR injury. Similar to this study, previous studies displayed the role of serelaxin as a potent suppressor for inflammation via inhibitory impacts on the expression of the cytokines (TNFα, IL-1β, IL-6, and others) (Wang *et al.,* 2017; Xueping *et al.,* 2023; Alana *et al.,* 2024).

CONCLUSION

The study posits evidence that Notch-2/Hes-1 signaling plays a significant role in induction and progression of the inflammation and development of renal dysfunction and kidney damage following IRI, that may create a new goal for treating of acute kidney injury and prevention of kidney rejection. We further revealed the impacts of inhibiting inflammation and Notch signaling on ischemia reperfusion injury. These findings suggest that serelaxin can effectively inhibit the inflammation and Notch-2 pathway and ameliorate renal function. The study provides new visions to support the possible benefit of the serelaxin as a novel therapeutic goal for prevention of renal I/R injury and its complications.

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Not applicable.

Authors' contributions
Thu-Alfeqar Tweij: conceived and designed the project, implemented the experiments on rats, acquired and analyzed the data, and wrote the manuscript draft. Esraa Alyasiry: participated in gathering and assessing the data, and revised the manuscript. Atheer Al-Zurfi and Shahad Rabeea: were contributed in data interpretation, making figures, and formatting and revising the original manuscript.

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Data availability statement
All data arose and/or analyzed in the current research are included in this paper.

Ethical approval
The procedures that involving animal were executed in conformity with ethical criterion that were approved by the Animals Care Committee, and according to the directives of the University of Kufa/Ethics Committee. (No. 22675).

Conflict of interest
There is no conflict of interest.

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