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Evaluation the Kidney Protection Effect of Rosuvastatin against I\R injury in male rats. Assessment the Effect of Rosuvastatin on mTOR gene

Ahmed M. Abdul Hameed[1*], Murooj L. Altemimi[2]

[1]Department of Pharmacology and Toxicology**,** Faculty of Pharmacy, Jabir Ibn Hayyan Medical University, Al Najaf Al-Ashraf, Iraq.

ORCID ID: 0000-0002-8745-4616

Email ID: ahmed.m.abdulhamid@jmu.edu.iq

[2]Department of Pharmacology & Therapeutics, Faculty of Medicine, University of Kufa, Iraq.

ORCID ID: 0009-0006-6697-7529

Email ID: Murooj.altameemi@uokufa.edu.iq

[*]**Corresponding Author:**

Ahmed M. Abdul Hameed

Department of Pharmacology and Toxicology**,** Faculty of Pharmacy, Jabir Ibn Hayyan Medical University, Al Najaf Al-Ashraf, Iraq.

ORCID ID: 0000-0002-8745-4616

Email ID: ahmed.m.abdulhamid@jmu.edu.iq

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ABSTRACT

Background: In certain situations, such as sepsis, myocardial infarction, ischaemic stroke, and acute kidney damage (AKI), ischaemia reperfusion injury (IRI) appears to be the main cause of morbidity and mortality. A decrease in the blood supply to the ischaemic organ during ischaemia results in hypoxia and a slowdown of the outflow of metabolic waste products, which allows the buildup of carbon dioxide (CO2) and other debris. Chronic severe ischaemia and hypoxia cause structural and functional changes in the microvascular system. When the ischaemic tissue is rapidly restored with fresh blood that is high in oxygen and nutrients, this causes damage and oedema and also causes the endothelial layer of capillaries to create reactive oxygen species (ROS), which intensifies the inflammatory process and the NFκB signalling pathway. Complement protein is secreted along with inflammatory or immune cells, such as lymphocytes, neutrophils, and macrophages, as well as inflammatory components like tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin-1β (IL-1β), and interferon gamma.

Objective: This animal work is done to investigate the effectiveness of Rosuvastatin in attenuating renal injury during ischemia reperfusion through modulation of mTOR expression gene.

Method: 28 Wister Albino rats were randomly assigned to four equal groups, (N=7): **Sham:** Rats undergone laparotomy without ischemia. **Control:** Rats undergone laparotomy with bilateral RIRI for 30-minute following two hours of reperfusion. **Vehicle:** Rats given an intraperitoneal injection of DMSO three days before induction of RIRI. **Rosuvastatin:** Rats received an intraperitoneal injection of Rosuvastatin three days prior to RIRI.

Results: In comparison to the vehicle and control, the sham had significantly lower tissue levels of TNFα, IL-1β, F2 Isoprostane, BAX, and KIM-1; the results also showed that Rosuvastatin had significantly lower tissue levels of Bcl2 and mTOR, TNFα, IL-1β, BAX, and KIM-1; and the histopathology showed that Rosuvastatin could significantly reduce kidney damage.

Conclusion: The AR therapy group significantly reduced renal I/R damage in the adult male rats' bilateral renal I/R due to their pleiotropic effects, which include anti-inflammatory, anti-oxidant, and anti-apoptotic qualities, according to the study's overall findings.Additionally, by increasing the expression of the mTOR gene in ischaemic renal tissues, they prevented necrosis and apoptosis.

Keywords: *Rosuvastatin, RIRI, Bcl-2, BAX, mTOR.*

1. INTRODUCTION

When an organ encounters a momentary reduction or suspension of blood flow, followed by a restoration of perfusion, the net impact of an inflammatory process is ischaemia reperfusion damage (IRI) [1]. Numerous clinical circumstances, including organ transplantation, heart and vascular surgery, shock, drug-induced ischaemia, and sepsis, can result in IRI [2][3].

Since it is closely related to graft rejection, IRI is regarded as one of the key difficulties in organ transplantation. IRI is the cause of 10% of early transplant failures. High rates of acute and chronic graft rejection are also associated with IRI [4]. The first ischaemic insult during IR produces tissue damage and/or death, which is primarily dictated by the degree and length of the blood flow disruption. Following reoxygenation, reactive oxygen species (ROS) are generated, which triggers IRI events and results in a severe inflammatory response, apoptosis, and necrosis of irreversibly injured cells [5][6].

The immune system and inflammation have a major impact on the pathogenesis of renal IRI. Immune system involvement is thought to be the source of both acute kidney injury and long-term structural alterations like interstitial fibrosis or repair [7]. Inflammatory cells might worsen kidney injury by attracting leukocytes, increasing adhesion molecules, and generating mediators like cytokines, chemokines, ROS, and eicosanoid [8].

Two important examples of cytokines are TNFα and IL1β. Activated macrophages are the primary producers of TNFα, one of the main pro-inflammatory mediators or cytokines, but other innate and adaptive immune cells, including as mast cells, eosinophils, T and B lymphocytes, neutrophils, and natural killer cells, can also release it [9]. Additionally, cells other than immune cells—such as neurones, adipose tissues, cardiac myocytes, endothelial cells, fibroblasts, and mesangial cells in glomeruli—form and release it.[10][11][12][9].

Other cytokines, such as IL6, IL-1β, and IL-8, can be released when TNFα activates macrophages [13][14].Growth regulation, cell and tissue differentiation, apoptosis, and the cell cycle are all significantly impacted by TNFα [10][15][16].

During inflammatory responses, innate and adaptive immune cells release IL-1β, a pro-inflammatory mediator thought to belong to the IL-1 family. The pathophysiology and aetiology of AKI and other conditions like pancreatitis are significantly influenced by the endogenous polypeptide cytokine IL-1β, which is produced and secreted by a variety of cell and tissue types [17]. Although circulating monocytes are the main source of IL-1β synthesis and release, kidney parenchymal cells can also create it in small amounts under specific circumstances, and natural killer cells, neutrophils, macrophages, and dendritic cells within tissues can all produce considerable amounts of it [18][19]. Proteolytic activation, an enzyme process, is necessary to change the pro-IL-1β, which is inert by nature and has 266 amino acids, into the active version of IL-1β, which is composed of 153 amino acids [18].

A range of enzymatic activation processes, such as the release of reactive oxygen species (ROS) from mitochondria during cell ischaemia reperfusion injury, the leakage of proteases enzyme from lysosomes, and changes in the intracellular concentration of calcium and potassium ions (increased Ca ions influx and K efflux), can activate IL-1β depending on the type of body cell. Injured kidney cells produce and activate IL-1β, which binds to its receptor (IL1R1) to start the recruitment, activation, and infiltration of more innate and adaptive immune cells. Furthermore, it increases the release of cytokines and chemokines by renal epithelial cells. These findings have been documented in numerous research studies [20][21][22][23][24].

There are a number of functional indicators, or clinical laboratory tests, available to evaluate renal function and determine whether GFR is normal. Kidney Injury Molecule-1 is one of these particular functional tests (KIM1). A transmembrane glycoprotein called Kidney Injury Molecule-1 is currently an essential biomarker for detecting kidney damage, especially acute kidney injury (AKI). In healthy kidneys, KIM1 expression is minimal; however, after renal damage, it significantly rises in proximal tubular epithelial cells [25]. According to Sabbisetti et al. (2014) and Van Timmeren et al. (2007), this rise renders KIM1 a useful diagnostic for the early diagnosis and prognosis of AKI [26][27]. The potential of KIM1 to predict AKI before more well-known indications, including serum creatinine, exhibit noticeable alterations highlights the biomarker's importance. KIM1 is therefore helpful in clinical settings for managing renal damage and prompt intervention [28].

One important pathway that contributes to the pathophysiology of IRI is oxidative stress (OS), which increases the production of reactive oxygen species (ROS) [29]. ROS are tiny, potentially harmful molecules that react very quickly. By reacting with biological elements such lipids and proteins of the cell membrane, carbohydrates, thiols, and DNA, they produce lipid peroxidation, enzyme inactivation, glutathione oxidation, organic radicals, and cell death. However, ROS, especially H2O2, can help tissues mostly because of their normal function in cell signalling. Therefore, ROS levels in a cell must be tightly regulated [30][31].

To biosynthesise arachidonic acid, free radicals catalyse the creation of beneficial chemicals known as isoprostanes, rather than using cyclooxygenases. A recent study conducted by the National Institutes of Health (NIH) in the United States found that isoprostanes are reliable indicators of oxidative stress [32][33][34]. F2-isoprostane is currently regarded as one of the

most helpful markers in vivo for assessing oxidative stress and lipid peroxidation because of its great specificity and stability [35]. Therefore, using ROS scavengers and antioxidant medicines to inhibit or block this pathogenic pathway or limit the generation of free radicals is the primary and most important technique to prevent tissue damage during renal ischaemia reperfusion. Additionally, this will protect the tissues from damage and death [36].

One important mechanism that controls a variety of biological processes is the macromolecular protein phosphatidylinositol 3-kinase\protein kinase B\mammalian target of rapamycin (PI3K/Akt/mTOR) signalling pathway. Additionally, it is essential for certain physiological functions and pathological responses [37]. The PI3K/Akt/mTOR signalling pathway is crucial for enhancing cell survival, proliferation, and metabolism, which in turn protects kidney cells from harm. Several growth factors and cytokines activate this pathway by binding to their specific receptors and activating phosphoinositide 3-kinase, or PI3K. Akt (protein kinase B) is then drawn to the plasma membrane by PI3K-generated phosphatidylinositol (3,4,5)-trisphosphate (PIP3), where it completely activates [38]. The pro-apoptotic proteins caspase-9 and BAD are among the downstream substrates that Akt phosphorylates and deactivates when it is activated [39]. When acute kidney injury (AKI) occurs, this anti-apoptotic action is especially helpful because renal tubular cells are especially vulnerable to apoptotic cell death. Research has shown that after ischaemia or nephrotoxic shocks, PI3K/Akt pathway stimulation can dramatically lower apoptosis in renal tubular cells [40].

Moreover, the mTOR (mammalian target of rapamycin) component of this system controls autophagy, cell division, and proliferation. There are two complexes of mTOR: mTORC1 and mTORC2. While mTORC2 controls cytoskeletal structure and cell viability, mTORC1 stimulates protein synthesis and suppresses autophagy [41]. The development of renal fibrosis in chronic kidney disease (CKD) has been linked to autophagy dysregulation. Through preserving cellular homeostasis and lowering oxidative stress, activation of the PI3K/Akt/mTOR pathway can regulate autophagy and avoid renal fibrosis [42].

One of the final processes that occur in the ischaemic injured parenchymal tissues of the kidney is apoptosis. Apoptosis, which is considered a type of planned cell death, eventually occurs in both healthy and sick cells [43]. There are two types of apoptosis: pathologic and physiological. Physiological apoptosis can occur under normal conditions, such as when harmful or damaged cells are removed, or as cells age or lose their usefulness. Unwanted sick cells that undergo substantial DNA degradation, such as those exposed to cytotoxic medications, radiation, viruses, cancer cells, and severe damage from ischaemia and hypoxia, can be eliminated by pathologic apoptosis [44]. The activation of caspase enzymes is necessary for both of the primary apoptotic pathways. These mechanisms are extrinsic, or death receptor, and intrinsic, or mitochondrial [43].
The anti-apoptotic protein Bcl-2 enhances cell survival by blocking numerous apoptotic triggers through its interactions with the pro-apoptotic proteins BAX and BAK [45][46][47]. Apoptosis can be managed or regulated by maintaining the stability and balance of the mitochondrial membrane, controlling its permeability, and stopping the release or leakage of death chemicals such cytochrome c [48]. Bcl-2 has other biological roles in regulating the dynamics of mitochondria, the fusion of mitochondrial membranes, insulin release, and other metabolic processes in the beta cells of the pancreas [49].

An imbalance between cell survival, division, and death will ultimately lead to tumour growth, especially in tissues with high division activity, such as breast, lung, and prostate cancer, melanoma, and chronic lymphocytic leukaemia [50][51][52]. This imbalance can be caused by dysregulation of Bcl-2 levels or activity, misbalancing, or a defect or damage to the Bcl-2 gene.
Because Bcl-2 plays a critical role in preventing the apoptosis of parenchymal kidney cells during renal I/R, it is considered a good biomarker that should be measured during this model of studies (renal I/R model in rats) to assess the severity of injury and to estimate the protection role of the treatment [53].

BAX is considered a pro-apoptotic endogenous agent that causes apoptosis through the intrinsic pathway by promoting the caspase cascade to cause apoptosis and increasing the permeability of the mitochondrial membrane to release cytochrome c. It is related to proteins in the Bcl-2 family [54]. By causing necrosis and apoptosis, BAX contributes to the development of kidney fibrosis and cell death in a variety of renal diseases. Anti-apoptotic medications that block BAX activity can prevent these effects [55][56][57][58][59]. Normally found in the cytoplasm, BAX changes its shape and moves to the organelle membrane, especially the mitochondrial membrane, when apoptosis is initiated [60][61][62][63][64]. Consequently, cytochrome c and several pro-apoptotic proteins will be produced by the mitochondria. Then, cytochrome c will cause caspase-9, a part of the intrinsic cascade of apoptosis, to become active. The active form of caspase-9 activates caspase-3, which in turn intensifies the other caspase cascade to start the intrinsic apoptotic process [65]. Because BAX plays a crucial role in triggering apoptosis during renal I/R injury and aggravating other kidney diseases, it is considered a vital biomarker to assess the extent of damage to kidney parenchymal tissues as well as to estimate the protection and treatable effects of anti-apoptotic agents used in these conditions [66].

Rosuvastatin's potent inhibitor of HMG-CoA reductase makes it a common cholesterol-lowering medication. Its primary pharmacological action is inhibiting HMG-CoA reductase, an enzyme necessary for cholesterol synthesis. By inhibiting the enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol, rosuvastatin efficiently reduces the liver's production of cholesterol [67]. This inhibition improves the clearance of LDL cholesterol from the bloodstream by

upregulating low-density lipoprotein (LDL) receptors on hepatocyte surfaces [68]. Rosuvastatin is beneficial for several lipid markers in addition to lowering low-density lipoprotein (LDL). By increasing HDL cholesterol and decreasing triglycerides, it helps provide a more favourable total lipid profile [69]. Rosuvastatin has also been shown to reduce inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP), which is a risk factor, for cardiovascular events on its own [70].

2. MATERIAL AND METHOD

Site and Ethical Consideration of the Research

The study was done in the department of pharmacology and toxicology \ Faculty of Pharmacy \ University of Kufa and in Middle Euphrates Unit for Cancer Researches \ Faculty of Medicine \ University of Kufa. The study was accepted by Committee center of Bioethics in the University of Kufa and its representative in Faculty of Pharmacy. Whole procedures were done according to the recommendations of the Committee.

Animal Grouping

28 mature Wister Albino rats weighing between 220 and 350 grammes and 20 to 25 weeks of age were used in this investigation. They were obtained from the Ministry of Health's Centre of Control and Pharmaceutical Research. Before the operations began, the animals were kept in the Faculty of Science/University of Kufa's animal house for 14 days at a temperature of 20–25 degrees Celsius, 60–65% humidity, and a 12-hour light/dark cycle. The rats also had unrestricted access to food and water. Rats were randomly assigned to four equal groups for this investigation, with seven rats in each group. The groups were as follows:

1. **Sham group:** For the same amount of time, all seven rats received the same anaesthetic and surgical treatments for ischaemia and reperfusion without ischaemia reperfusion induction. Blood samples and renal tissues were gathered.
2. **Control group:** following a 30-minute bilateral renal ischaemia and a median laparotomy performed under anaesthesia on all seven rats, renal tissues and blood samples were taken two hours following reperfusion [71][72][73].
3. **Vehicle group:** Three days prior to the induction of RIRI [74], all seven-albino rats received an intraperitoneal injection of DMSO. They then experienced bilateral renal ischaemia for 30 minutes and reperfusion for two hours [75][76]. At last, both kidneys were removed.
4. **Rosuvastatin group:** Three days before to the induction of RIRI [74], all seven-albino rats received an intraperitoneal injection of Rosuvastatin 10 mg/kg [77]. They then experienced bilateral renal ischaemia for 30 minutes and reperfusion for two hours [75][76]. At last, both kidneys were removed.

Renal ischemia Reperfusion Injury Rat Model

Intraperitoneal injections of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride were utilised to anaesthetise every rat. To keep the rat body temperature at roughly 37 oC, the animals were put on a heat plate. After trimming and cleaning the abdomen region with an antiseptic to prevent infection, the midline incision was made, exposing the renal pedicles by first slicing the abdominal skin and then the abdominal muscle. Using non-traumatic vascular clamps to clamp the left and right renal pedicles for 30 minutes. To maintain adequate hydration, one millilitre of warm, sterile saline was injected into the peritoneal cavity. Following the conclusion of the ischaemic period, the clamps were taken off in order to reperfuse, stitch, and cover the wound with sterile gauze dampened with regular saline to prevent dehydration. Following two hours of reperfusion, the suture was opened, and roughly three millilitres of blood were extracted from the heart. This was followed by a bilateral nephrectomy, during which the kidney was cleaned of blood using precooled phosphate buffer saline (PBS). Ultimately, the rat was killed by puncturing its heart [78]. The left kidney was divided in half sagittally. For biomolecular evaluation, the first half was stored in a deep freezer. For histological and immunohistochemical evaluation, the second half was embedded in paraffin after being placed in 10% formalin.

Preparation of the Drug

The drug was prepared immediately before using by dissolved in DMSO **(Solubility: In DMSO: 20 mg/ml)** as descripted by manufacturer (Medchemexpress).

Assessment of Tissue TNFα, IL1β, F2 isoprostane and KIM1

The tissue was initially homogenised using a mortar and pestle with 1:10 (W/V) 0.1 M of precooled PBS (PH 7.4) with 1% of the protease inhibitor cocktail and 1% Triton 100X after the frozen kidney part was broken up into small pieces and cleaned with cold PBS [79][80]. The homogenate was put through a high-intensity ultrasonic liquid processor to further break down the cell membranes for optimal homogenisation. The homogenate was then centrifuged for 10 minutes at 4 oC and 10,000 rpm. The ELISA Sunlong kit was used to measure the levels of TNFα, IL1β, F2 isoprostane, and KIM1 in the supernatant.

Assessment of Tissue mTOR Gene Expression by RT-qPCR

1. Total RNA Extraction Using Easy-spin™ (DNA free) Total RNA Extraction Kit.
2. cDNA Synthesis (Using AddScript cDNA Synthesis Kit).
3. Preparation of Primers.
4. Primers Used in this Study [81][82].

Host	Gene		5'-3'	Product (bp)	Accession number	Reference
Rattus	mTOR	F	ACGCCTGCCATACTTGAGTC	113	XM_03289 4667.1	**Osqueei et al., 2023**
Rattus		R	TGGATCTCCAGCTCTCCGAA			
Rattus	GAPDH	F	ATGACTCTACCCACGGCAAG	89	NM_01700 8	**Kunst et al., 2012**
Rattus		R	CTGGAAGATGGTGATGGGTT			

5. Protocol of GoTaq® RT-qPCR System for Real-Time qPCR (Gene expression assay).

Histopathological Analysis

The left kidney was drained, cleaned, and then fixed in paraffin before being sliced into 5-micrometer-thick pieces using a rotary microtome. The tissue section was then fixed on slides, stained with haematoxylin and eosin dye, and covered to get ready for microscopic inspection. Two skilled pathologists assessed renal tissue damage in a blind manner while taking into account six randomly chosen fields. The sections were categorised using a scale design to evaluate the extent of renal injury, including vascular and tubular necrosis degeneration, eosinophilic cast formation, loss of brush boundary, swelling of renal epithelial cells, and desquamation of epithelial cells into the lumen. Five scores made up the scoring system that was employed: 0 for normal kidney tissue, 1 for less than 25% renal damage, 2 for 25%–50% kidney damage, 3 for 50%–75% kidney damage, and 4 for more than 75% kidney damage [83].

Immunohistochemistry assessment

In order to evaluate Bcl-2 and BAX in kidney tissue, immunohistochemistry was used. Sections embedded in 5 µm paraffin were stained using the immunostaining technique. In short, the sections underwent deparaffinization, rehydration, retrieval buffer exposure to restore the antigen, and 3% H2O2 inhibition of endogenous peroxidase activity. Overnight at 4 oC, the sections were treated with either the Bcl-2 or BAX polyclonal antibody (1:200, bioassay). Following washing, the slices were exposed to horseradish peroxidase for 30 minutes, followed by an hour of incubation with conjugated secondary antibody. The sections were then incubated for eight minutes with fresh 3, 3′-diaminobenzidine. Lastly, the counterstain was haematoxylin stain. Next, use a microscope to view the staining. By multiplying the stained area's intensity and percentage, the H-score method (which ranges from 0-300) was used to determine the protein expression of Bcl-2 or BAX. A score of 0–3 was assigned to the stain intensity: 0 denoted no staining, 1 weak staining, 2 moderate staining, and 3 severe staining. From 0% to 100%, the percentage of stained cells was rated [84].

Statistical Analysis

Microsoft Windows Inc.'s GraphPad Prism version 8.0.2 was used for statistical analysis. The mean±SD was used to display the data. One-way analysis of variance, or one-way ANOVA, was used to perform multiple comparisons across all groups. To determine if there were statistically significant differences between the different study groups in the mean H.score for IHC-P and the total severity score (mean score) for histological renal abnormalities, the Kruskal-Wallis test was utilised. All comparisons and tests were considered statistically significant if $P < 0.001$.

3. RESULTS

Rosuvastatin Improve Renal Function Parameter

Rats in control and vehicle groups exhibited a considerable elevation in tissues level of KIM1 in comparison with sham group. Rosuvastatin pretreatment group was significantly alleviate the kidney tissues content of KIM1 comparing with control and vehicle groups (Figure 1).

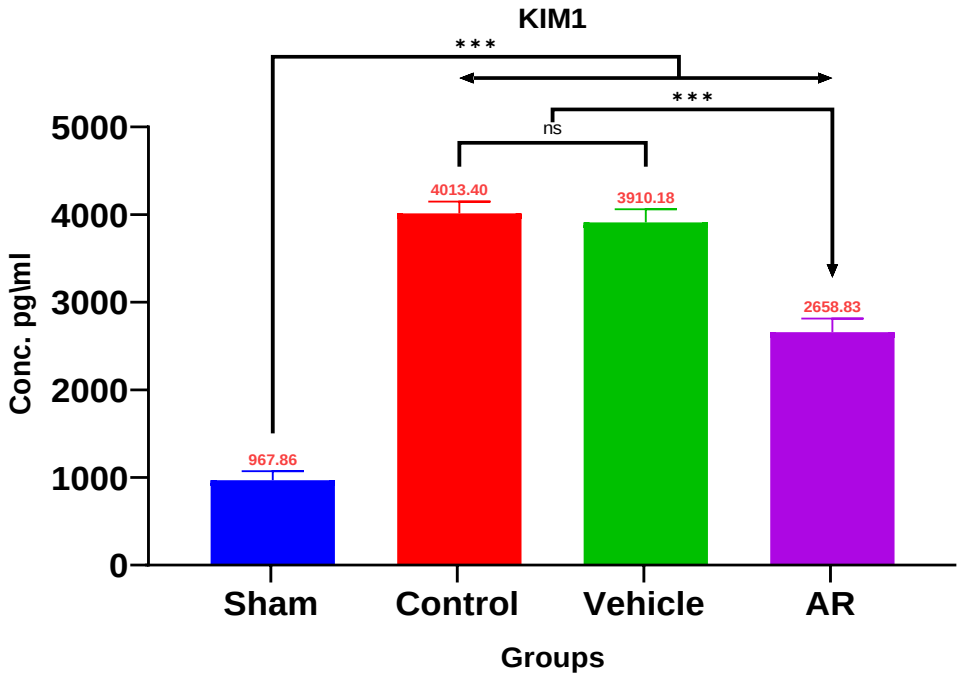

Figure (1): The statistical analysis of KIM1 concentrations mean (pg/ml) in renal tissues in the four experimental study groups at the finishing of the research (No of rats = 7 in each study group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

Rosuvastatin Attenuated Oxidative Stress and Alleviate F2 isoprostane in Renal Tissue

In our animal research, we stated that the renal tissue content of F2 isoprostane in sham group was significantly ($p < 0.001$) lower than that content in both control and vehicle groups. The renal tissue amount of F2 isoprostane of AR pretreated group was significantly ($p < 0.001$) lower than that level in both control and vehicle groups (Figure 2).

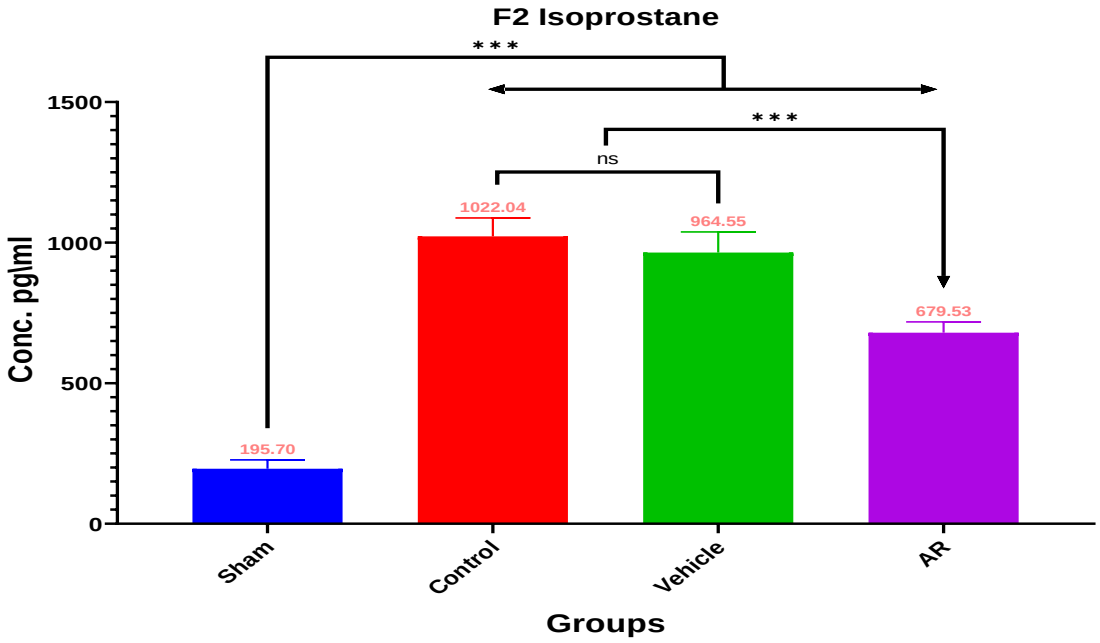

FIGURE (2): THE STATISTICAL ANALYSIS OF F2 Isoprostane concentrations mean (pg/ml) in renal tissues in the four experimental study groups at the finishing of the research (No of rats = 7 in each study group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

Rosuvastatin Decreased the Inflammatory Markers in Renal Tissue (TNFα and IL1β)

Protein level of the inflammatory mediators, TNFα and IL1β, were increased significantly in kidney homogenate of control and vehicle rats in comparison with sham rats. Three consecutive days of IP injection of 10 mg/kg of Rosuvastatin significantly diminished the level of TNFα and IL1β in comparison with control and vehicle rats (figure 3 and 4).

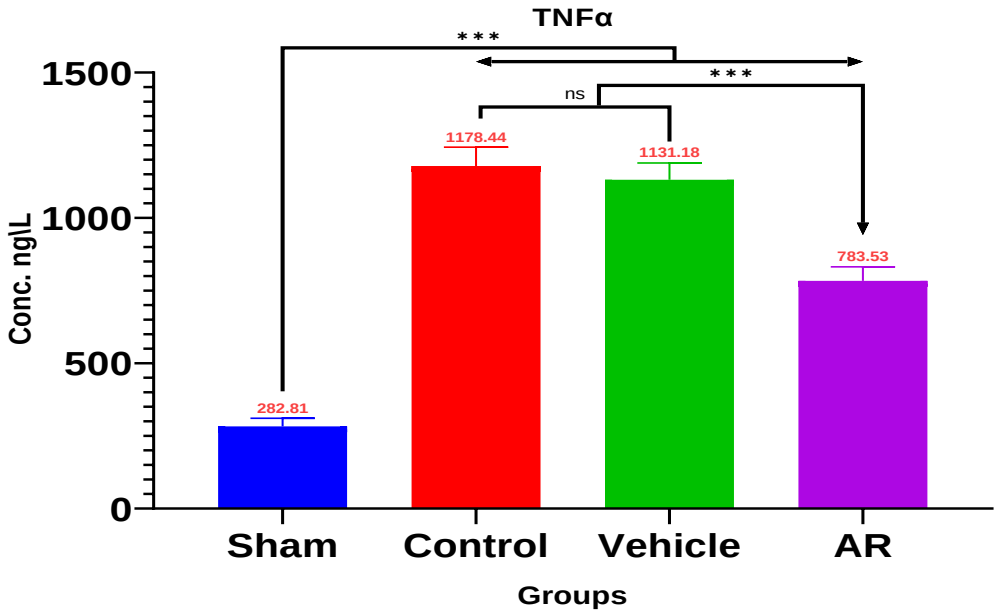

Figure (3): The statistical analysis of TNFα concentrations mean (ng/L) in renal tissues in the four animal study groups at the finishing of the research (No of rats = 7 in each study group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

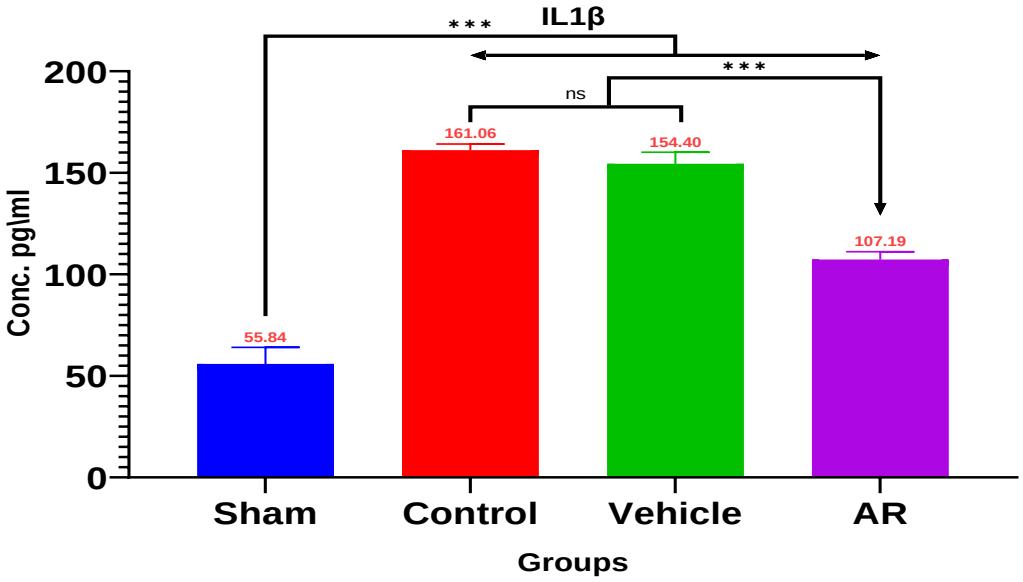

Figure (4): The statistical analysis of IL-1β concentrations mean (pg/ml) in renal tissues in the four animal study groups at the finishing of the research (No of rats = 7 in each study group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

Rosuvastatin Upregulated Bcl-2 Expression

In this investigation, we proved that the renal tissue Bcl-2 amount of the sham group was significantly ($p < 0.001$) greater than that of both control and vehicle groups. The AR pretreatment group's renal tissue Bcl-2 amount was significantly ($p < 0.001$) higher than the control and vehicle groups' levels (Figure 5 and 6).

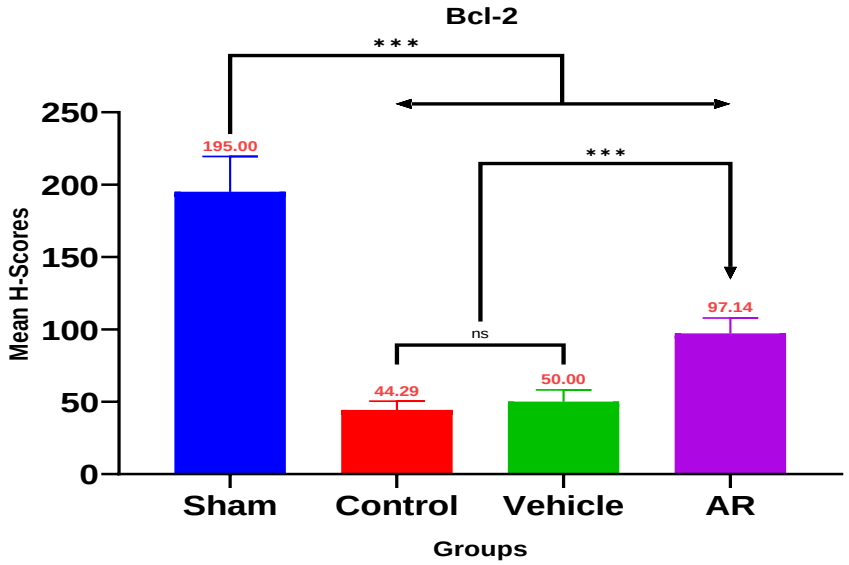

Figure (5): Mean H.scores of Bcl-2 in renal tissue of the four experimental groups at the end of the study (No of animals = 7 in each group).

Sham group vs. vehicle group, ***P.value < 0.001

Sham group vs. control group, ***P. value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

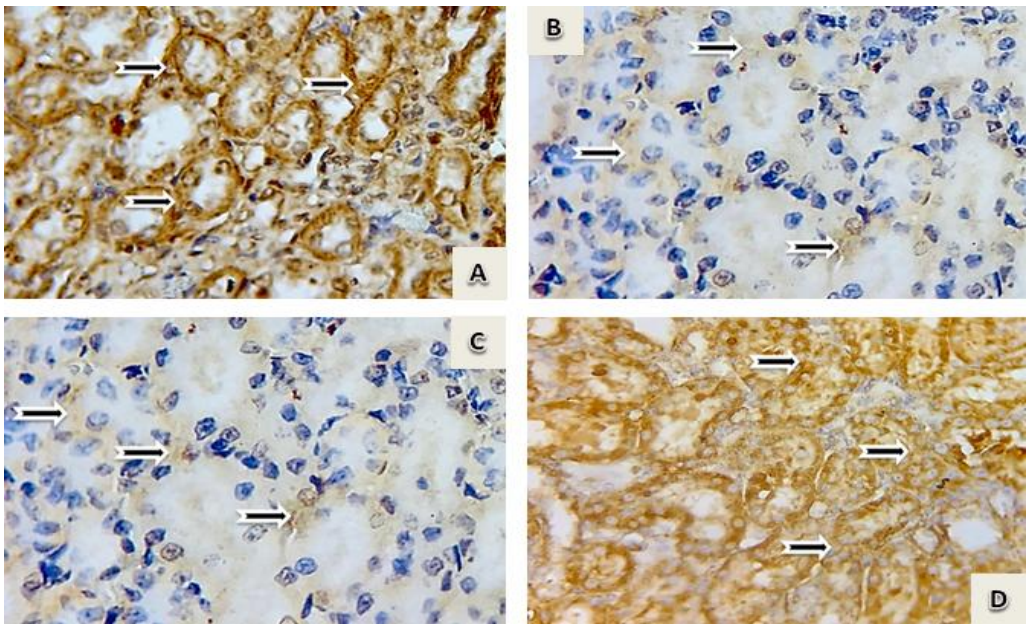

Figure (6): **A)** A cross section of left kidney represented a strong positive cytoplasmic brown stain of Bcl-2 protien (blue arrows) × 400. Sham group. **B)** A cross section of left kidney showed a slightly positive cytoplasmic brown stain of Bcl-2

protien (black arrows) × 400. Control group. **C)** A cross section of left kidney appeared a slightly positive cytoplasmic brown stain of Bcl-2 protien (black arrows) × 400. Vehicle group. **D)** A cross section of left kidney appeared a strong positive cytoplasmic brown stain of Bcl-2 protien (black arrows) × 400. AR treated group.

Rosuvastatin Downregulated BAX Expression

In the time of our work, we discovered that the pro-apoptotic biomarker (BAX) was substantially ($p < 0.001$) less expressed in the renal tissues of the sham group than it was in the vehicle and control groups. The renal tissue level of BAX of AR pretreated group was significantly ($p < 0.001$) lower than those levels in both control and vehicle groups (figure 7 and 8).

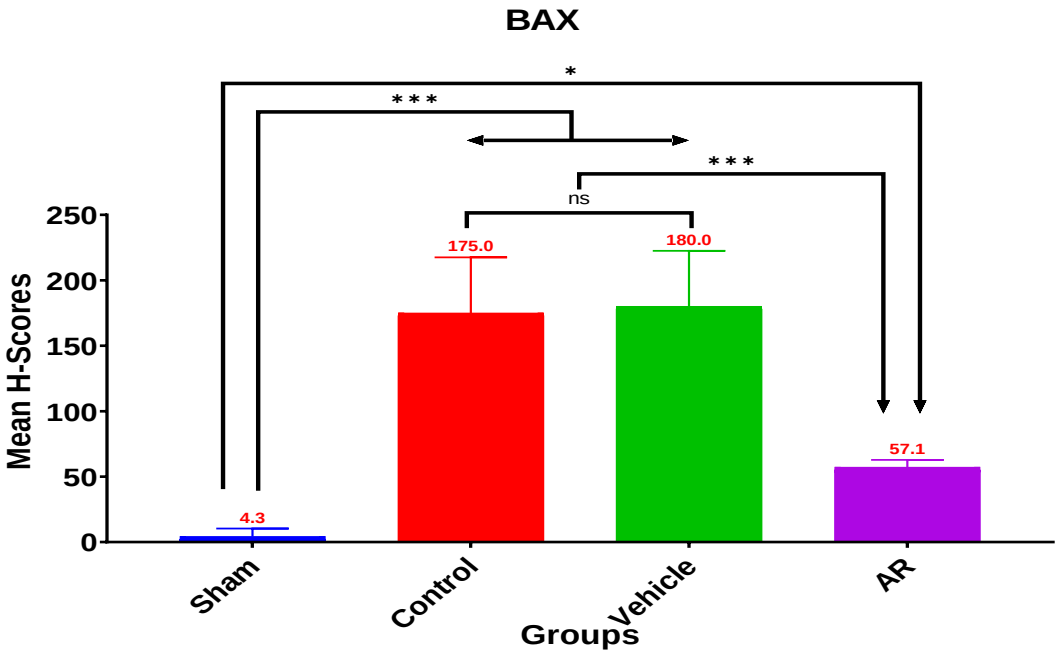

Figure (7): Mean H.scores of BAX in renal tissue of the four experimental groups at the end of the study (No of animals = 7 in each group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

AR vs. Sham group, *P.value = 0.016

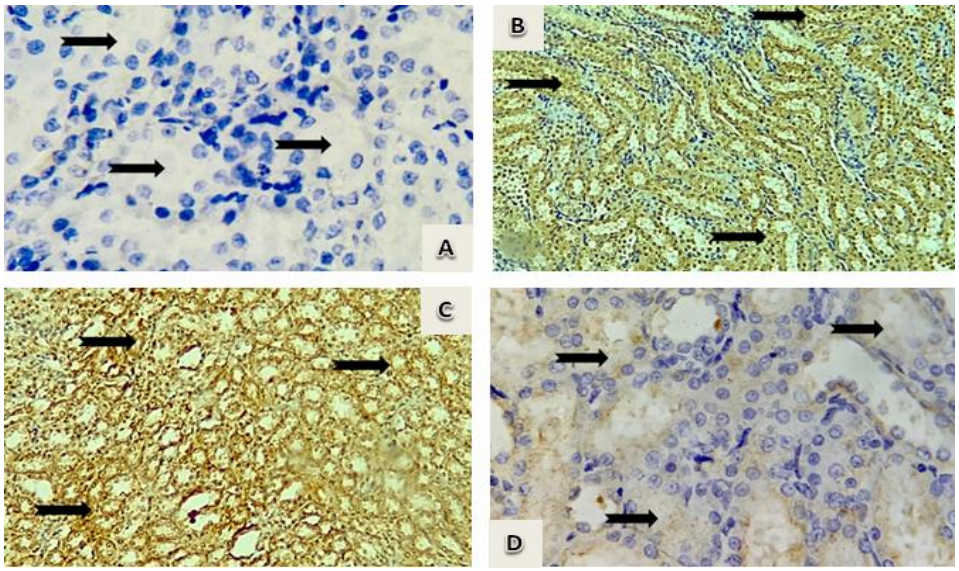

Figure (8): **A)** A cross section of left kidney showed BAX negative cytoplasmic stain (black arrows) × 400. Sham group

(Zero H.Score). **B)** A cross section of left kidney showed BAX highly intense positive cytoplasmic stain, brown stain (black arrows) × 100. Control group. **C)** A cross section of left kidney showed BAX highly strong positive cytoplasmic stain, brown stain (black arrows) × 100. Vehicle group. **D)** A cross section of left kidney showed BAX slightly brown stain (black arrows) × 400. AR treated group.

Rosuvastatin upregulatred the kidney tissues expression of mTOR gene

We stated in this animal work that there is no substantial variation in renal tissues mTOR gene expression between control and vehicle groups ($p > 0.001$). Furthermore, the renal tissue amount of mTOR protein of AR pretreated group was significantly ($p < 0.001$) more than those levels in both control and vehicle groups (Figure 9).

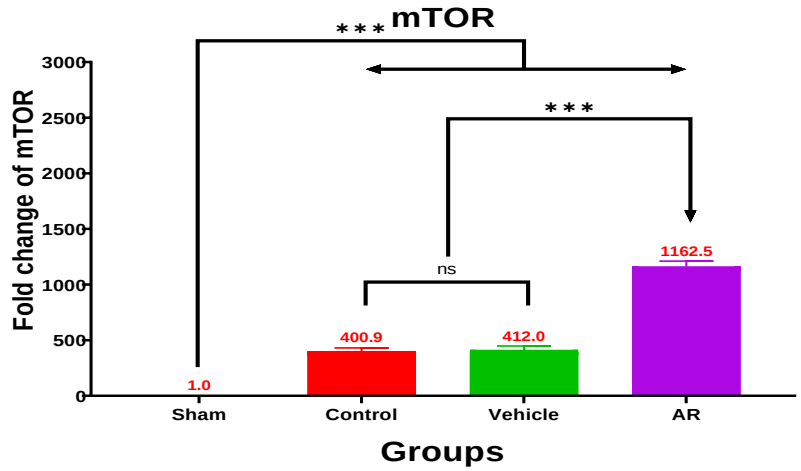

Figure (9): Mean of fold change of mTOR gene in renal tissue of the four experimental groups at the end of the study (No of animals = 7 in each group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

Rosuvastatin Minimized Kidney Injury

Histopathological examination presented no renal injury in the sham group. On the other hand, in control and vehicle groups, an increased number of damaged tubules and cell dilatation were noticed in comparison with the sham group ($P < 0.001$). AR pretreated group showed little histological change in contrast to the control and vehicle groups ($P < 0.001$) (figure 10 and 11).

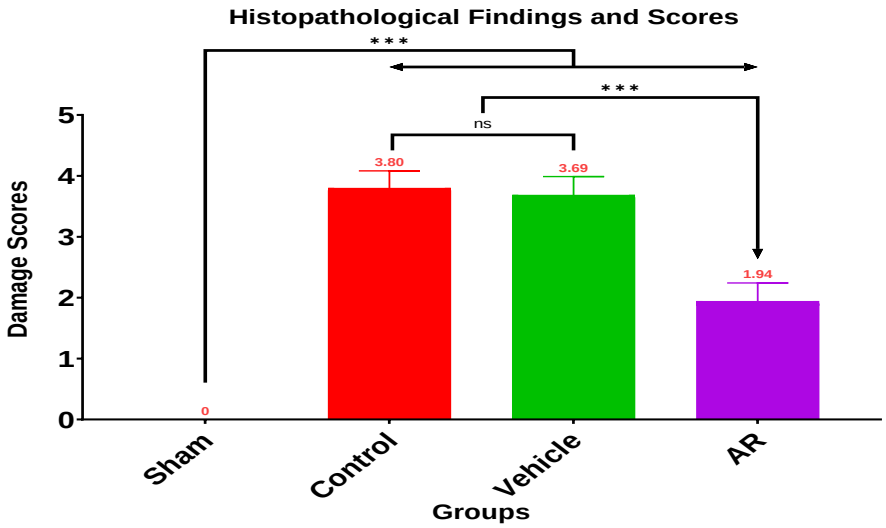

Figure (10): Score severity mean of renal tissue histopathology of the four experimental groups at the end of the study (No of animals = 7 in each group).

Sham group vs. vehicle & control groups, ***P.value < 0.001

AR vs. vehicle & control groups, ***P.value < 0.001

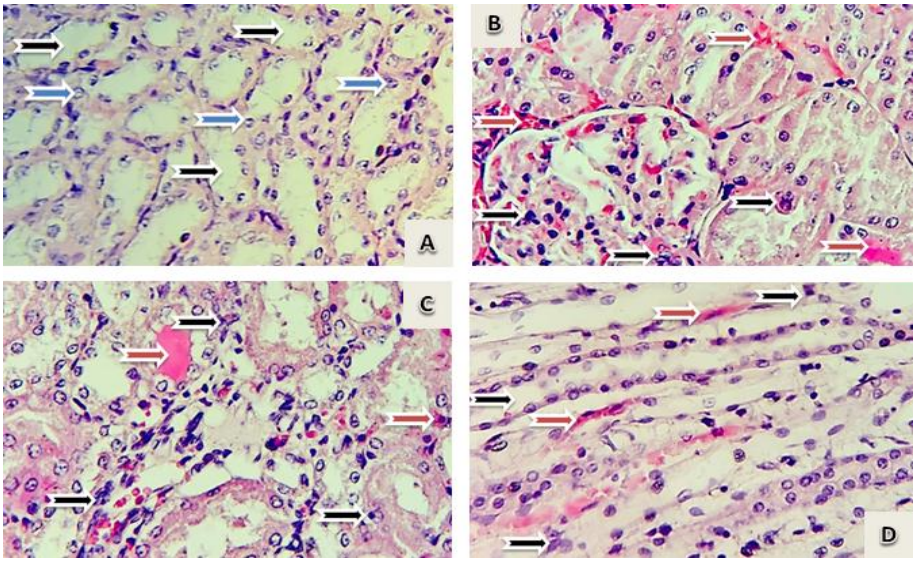

Figure (11): **A)** A microscopic cross section of left kidney represented normal tissues histology, normal renal tubules (black arrow), normal cell size (blue arrow) and there are no cast formation, cells odema or loss of brush boarder. Sham group. H & E stain × 400. **B)** A microscopic cross section of left kidney represented score 4 tissues modifecations including severe cellular odema, cytoplasmic eosinophilia (black arrows) and strongly eosinophilic cast (red arrows). Control group. H & E stain × 400. **C)** A microscopic cross section of left kidney represented score 4 tissues modifecations including severe cellular odema, cytoplasmic eosinophilia (black arrows) and strongly eosinophilic cast (red arrows). Vehicle group. H & E stain × 400. **D)** Microscopic cross section of left kidney represented score 2 tissues modefications including few eosinophilic cast (red arrows), moderate cellular odema and tubular dilatation (black arrows). AR treatment group. H & E stain × 400.

4. DISCUSSION

IRI is thought to be one of the most important factors that has a major impact on the morbidity and mortality of many diseases, such as sepsis, ischaemic stroke, acute kidney injury, and MI. In certain situations, such as organ transplantation and major surgery, when it may affect healing and clinical results, IRI is also seen as a serious issue. Because ischaemia decreases blood flow to vital organs, hypoxia (low oxygen concentration), a decrease in the availability of nutrients, and the buildup of CO2 and debris might result. Prolonged hypoxia and ischaemia will lead to structural changes and micro-blood vessel malfunction. After rapid reperfusion, a significant amount of blood will flow to the ischaemic organ, which may cause the organ to face several challenges. The inflammation process is one of the problems that might aggravate the tissues and lead to additional difficulties, ROS generation, and apoptosis [85][86].

So in our work, we tested the nephroprotective influence of Rosuvastatin, against control renal IRI experimentally.

Effect of Rosuvastatin on Kidney Injury Molecule-1 (KIM1)

According to this experimental work, rosuvastatin pretreatment before ischaemia induction significantly ($P < 0.001$) reduces the level of KIM1 in renal tissues in comparison to the levels in the vehicle and control groups. According to this study, after renal IRI development, rosuvastatin preserves renal tissues and function parameters in a rat model. This outcome is consistent with previous studies. Rosuvastatin treatment shielded the kidney from oxidised LDL damage in chronic kidney disease (CKD) and was shown to reduce the level of KIM1 in a recent experimental research on CKD rats [87].

Rosuvastatin's Impact on the Kidney Parenchyma

This study shown that, in comparison to the vehicle and control groups, the degree of kidney injury is significantly ($P < 0.001$) reduced when Rosuvastatin, an HMG-CoA reductase inhibitor, is administered prior to ischaemia induction. The vehicle and control groups' mean score intensity indicated severe kidney damage, while the Rosuvastatin-pretreated group's mean score intensity indicated mild to moderate impairment. Our findings are in line with those of other studies.

According to recent research by Shafik et al. (2023), when Rosuvastatin is administered concurrently to a group of rats that are taking Colistin for six days in a row, it can cause renal injury, prevent the severity of Colostin-induced nephrotoxicity, preserve the renal parenchyma, and lessen the severity of tubular injury, necrosis, cast formation, and tubular dilatation compared to the untreated control group, which exhibits all these histological changes in a high degree [88].

Effect of Rosuvastatin on the Inflammatory Mediators (TNFα and IL-1β)

This animal study found that premanagement with Rosuvastatin prior to the onset of ischaemia can significantly ($P < 0.001$) lower the concentrations of inflammatory molecules (TNFα and IL-1β) in ischaemic renal tissues, in contrast to the levels of cytokines linked to inflammation in the vehicle and control groups. Rosuvastatin reduces inflammation in renal tissues that have undergone ischaemia and reperfusion, which is consistent with this finding.

These results are consistent with other studies. Rosuvastatin can lower TNFα, IL-1β, and NF-κB levels in rats given Cisplatin to cause nephrotoxicity, according to an experimental study by Saad et al. (2024) [89]. An additional experimental study demonstrated that administering Rosuvastatin at two different doses to a group of rats receiving three intraperitoneal injections of thioacetamide per week for six weeks to induce liver fibrosis can lower the levels of TNFα, IL-1β, and NF-κB in the liver tissues compared to the group receiving thioacetamide alone [90].

Effect of Rosuvastatin on F2 Isoprostane, Oxidative Stress, and Lipid Peroxidation

Considering the outcomes of this lab experiment, pretreatment with Rosuvastatin before renal ischaemia reperfusion induction can significantly ($P < 0.001$) lower the content of F2 Isoprostane in ischaemic renal tissues in comparison to the concentrations of this oxidative stress biomarker in the vehicle and control groups. This finding suggests that rosuvastatin has an anti-oxidative effect and reduces lipid peroxidation and ROS production in injured renal tissues following ischaemia and reperfusion.

The results of this animal study are consistent with a number of earlier investigations. Thej et al.'s (2024) clinical study, which involved T2DM patients, found that taking Rosuvastatin for 12 weeks significantly reduced oxidant biomarkers, such as F2 isoprostane level, malondialdehyde (MDA), and protein carbonyl content (PCC). These biomarkers are linked to a reduction in oxidative stress and the production of ROS [91].

Impact of Rosuvastatin on Anti-apoptotic Marker (Bcl-2) and Pro-apoptotic Marker (BAX)

Owing to our discoveries, pretreatment with Rosuvastatin before ischaemia reperfusion induction can significantly ($P < 0.001$) change the ratio of BAX/Bcl-2 in injured renal tissues when compared to both the vehicle and control groups. It can reduce the amount of pro-apoptotic marker (BAX) and increase the concentration of anti-apoptotic marker (Bcl-2) in kidney tissues. This indicates that rosuvastatin has antiapoptotic properties and can prevent necrosis and apoptosis in damaged kidney tissues.

These results coincide with those of other works. Rosuvastatin can preserve the viability of cardiaomyocytes, increase the concentration of anti-apoptotic marker (Bcl-2) and decrease the amount of pro-apoptotic marker (BAX), and prevent the loss of cardiac muscles due to apoptosis and necrosis, according to a laboratory experiment conducted on male rats that had their cardiomyocytes injured by isoprenaline [92]. A study on cultured human coronary artery endothelial cells (HCAECs) was conducted by Wang et al. (2020). In this study, CoCl2 is used to trigger apoptosis in these colonized coronary artery endothelial cells. The results showed that by increasing the expression of the anti-apoptotic marker Bcl-2 and decreasing the expression of the pro-apoptotic marker BAX, rosuvastatin therapy reduced the apoptosis of HCAECs produced by CoCl2 [93].

Impact of Rosuvastatin on mTOR Protien

This study established that the pretreatment group with Rosuvastatin had considerably ($P < 0.001$) higher levels of PI3K\Akt and mTOR gene expression in renal tissues compared to the vehicle and control groups. According to the results of this lab animal study, rosuvastatin protects the kidneys by upregulating the expression of the protective molecular signalling pathway (PI3K\Akt\mTOR) in damaged renal tissues. Our findings are consistent with those of other study. According to a recent study, giving Rusovastatin to a group of rats that had cardiac ischaemia reperfusion injury increased cell viability, raised PI3K\Akt\mTOR and Bcl 2 expression levels, and lowered cleaved-caspase3 and BAX [94].

Ethical Approval

All procedures involving the handling and experimentation on rats, as well as the conducted tests, were carried out in compliance with the applicable guidelines and regulations for the ethical use of animals \ University of Kufa **(20547 in 29/8/2024).** The animals were housed in the animal facility at the College of Sciences, University of Kufa.

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