Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-249
Volume 106 Issue 6

Curcumin Modulates Renal Ischemia Reperfusion Injury via Down Regulation of AKT mTOR Pathway

Najah R Hadi[1]*, Muroog Luay[1], Munther Abusauda[2], Rusul Falah[1] and Hayder Alaridi[1]

[1]Faculty of Medicine, University of Kufa, Iraq
[2]College of Pharmacy, Islamic University, Iraq

Abstract

The study aimed to investigate the potential protective effect of curcumin on renal IRI in rats model by regulation of Akt/mTOR pathway.

Materials and Methods: In this study, adult male rats with 15-28 weeks in age and 300-350 g in weight divided randomly into 4 equal groups (6 rats in each group):sham group, control group, vehicle group and curcumin treated group. Rats were exposed to bilateral renal ischemia for 30 min by renal pedicles clamping and 2 hr reperfusion. Curcumin was administrated intraperitoneally at dose of 60 mg/kg 45 min before ischemia. At the end of reperfusion, collection of blood and renal tissue samples was performed for measurement of the following parameters:

Interleukin-1β (IL-1β), caspase-3, F2-isoprostane, mTOR, p-mTOR, Akt and p-Akt estimated in homogenization of frozen renal tissues by using ElISA.

Serum urea and creatinine by using colorimetric method.

Histopathological analysis.

Results: At the end of experiment, the serum levels of urea and creatinine and renal levels regarding p-mTOR, p-Akt, IL-1β, caspase-3, and F2-isoprostane are recognized to be significantly ($p<0.05$) elevated in control group when compared with sham group. Histopathological analysis of the renal tissues recognizes a sever renal injury in control group and there is a significant role of curcumin in ameliorating this injury by minimizing the serum level of urea and creatinine, tissue level of IL-1β, caspase-3, F2-isoprostane except for p-mTOR and p-AKt levels which were significantly ($p<0.05$) higher in comparison with that of control group.

Conclusion: From the overall results, curcumin significantly diminishes renal ischemia reperfusion injury in the rat model through Akt/mTOR pathwayby its effect as anti-oxidant, anti-inflammatory and anti-apoptotic.

Correspondence to: Najah R Hadi, Professor, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Iraq; E-mail: drnajahiraq@gmail.com

Citation: Najah R Hadi, Muroog Luay, Munther Abusauda, et al. (2020) Curcumin Modulates Renal Ischemia Reperfusion Injury via Down Regulation of AKT mTOR Pathway. Prensa Med Argent, Volume 106:6. 249. DOI: https://doi.org/10.47275/0032-745X-249.

Received: March 24, 2020; **Accepted:** April 13, 2020; **Published:** April 18, 2020

Introduction

Renal ischaemia reperfusion injury is caused by a generalized or regional impairment of oxygen supply to, and removal of waste products from, kidney cells [1]. Ischemia reperfusion injury considers as a principle source of AKI [2], which is still associated with high morbidity and mortality [3]. Kidney is a high perfusion organ and is sensitive to ischemic insults and ischemia/reperfusion. Renal ischemia reperfusion injury frequently occurs in clinical conditions, for example, hypovolemic shock, kidney transplantation, partial nephrectomy, trauma and vascular surgery [4,5].

Although the exact pathophysiology of renal IRI is unclear, multiple pathways, which include reactive oxygen species (ROS) generation, inflammation, apoptosis or necrosis are involved [6]. Following ischemia, blood restoration to ischemic tissue brings about additional damages by generation of reactive oxygen species and activation of immune response which prompt inflammation, that is characterized by mobilization of inflammatory cells, upregulation of cell-adhesion molecules and production of altered chemokine and cytokine [7,8]. In this way, it is critical to research how to prevent and treat IRI. Therapeutic strategies planned to repress ischemia/reperfusion injury may significantly limit AKI and the advancement of chronic kidney disease.

Interleukin-1β is an inflammatory cytokine that modulates immunity and mediates inflammation. IL-1β has numerous impacts in host defenses and in a wide variety of diseases pathogenesis [9].

Cytokines synthesis as large precursor proteins is done by numerous cell types of both the peripheral and central immune system, including monocyte and lymphocytes [10]. The production of interleukin-1β needs, in addition to the synthesis of new NF-κB dependent pro IL-1β, a second signal which provokes activation of caspase-1, a pro-inflammatory caspase [11]. Caspase-1 is reliable for proteolysis activation of pro IL-1β into mature and bioactive form.

Rise of pro-inflammatory cytokines, IL-1β, has been shown in acute renal failure induced by renal IRI [12]. The renal tubular epithelial cells generate pro-inflammatory cytokines, for example, IL-1β that aggravate inflammation subsequent to renal IRI [13] by attraction and activation of leukocytes to sites of injury [14]. Recent studies recommend that cytokines not only prompt generation of ROS yet in addition themselves are induced by ROS. Nevertheless, later information connecting the NF-κB induction to the intermediate and nonlethal levels of ROS, as the latter are available in an enormous quantities during reperfusion [15]. Akt/mTOR pathway is a serine threonine kinase that integrates the intracellular and extracellular signals; mTOR assumes a potential role in regulation of proliferation, growth, and survival. mTOR pathway is activated in different cellular processes, for example, insulin resistance and tumor formation. This is featured through growing use of its inhibitor rapamycin in the pathological settings, such as organ transplantation and solid tumor [16], mTOR comprises two obvious multiprotein complexes including mTOR complex-1 (mTORC-1) as well as mTOR complex-2 (mTORC-2). Growth factor/ PI3K/ Akt pathway is a well determined mTOR regulator. Growth factors, for example, insulin and insulin growth factor (IGF) activate their related receptor tyrosine kinase (RTK) and eventually activate PI3K/Akt signaling, which is negatively regulated by a tumor suppressor protein named phosphates and tensing homolog (PTEN) [17]. Activated Akt directly causes phosphorylation and thereby inhibition of tuberous sclerosis complex (TSC) heterodimers which consists of TSC1 and TSC2. TSC serve as mTORC1 inhibitor, therefore inhibition of TSC via Akt dependent phosphorylation prompts the activation of mTORC1. The most significant role of mTOR complex-2 is mainly phosphorylation and thereby activation of Akt to enhance cell survival, growth, and proliferation. Inflammatory biomarkers for example, IL-1β, signal to mTOR through TSC complex. This biomarker inactivates TSC, leading to mTOR activation (Lee et al. 2007). mTOR activation by loss of TSC or by inflammatory stimuli leads to diminished NF-kB, a transcription factor involved in regulation of inflammatory biomarkers, providing a feedback loop to confine excessive inflammation [18]. So mTOR activation limits inflammatory response in renal ischemia reperfusion injury by NF-κB blocking [19]. Since mTOR activates by growth factor and inhibits by ATP exhaustion, its activity is presumably highly repressed throughout the ischemic phase of IRI, when there is a marked reduction in the availability of growth factors and cell ATP, and activated during reperfusion phase of IRI by abrupt availability of growth factors and ATP following the period of profound insufficiency. Curcuminis aprimary natural compound presented in rhizome of Curcuma longa [20]. Curcuma longa is utilized due to its antioxidant [21], anti-inflammatory [22], antimicrobial, antimutagenic, and anticancer properties [23]. In the recent years it has been demonstrated that most of the impacts of Curcuma longa are largely attributed to curcumin, with a significant impacts against allergies, diabetes, arthritis, Alzheimer's disease [24], and cardiovascular disaeses (CVDs) [25]. Curcumin can scavenge several of free radicals forms, for example, reactive nitrogen and oxygen species. In addition, it can inhibit the ROS-creating enzymes, for example, cyclooxygenase and lipoxygenase [26]. Curcumin downregulates the expression of TNF-α induced IL-1ß, as well as TNF-α induced NF-κB inhibition [27]. In addition curcumin has been appeared to inhibit IL-1ß production [28].

Materials and Methods

Preparation of Animals

In this study, a type of Wistar albino rats were used with 15-28 weeks in age and 300-350g in weight purchased from the animal resources center/college of veterinary medicine/Duhok University. Animals were kept in animal house at Kufa University, temperature controlled at 24 ± 2°C with the fit 12h light: 12h cycle dark. The rats got a standard diet of food with water. The experiments were started following 2 weeks of acclimatization in quarantine room. Retaining the rats in the study after approval set up by the Animal Care and Research Committee/ University of Kufa upon presenting the compelled applications.

Place and Period of Experiment study

The experiment study was performed in the research's laboratory in department of Pharmacology and Therapeutics and centre of cancer researchers in College of Medicine/Kufa University. The experiment study was persisted for 5 months.

Ethical consideration

This study was achieved according to the Guide for Care and Use of Laboratory Animals Association for Laboratory Animals Science. Approval by Animals Care Committee was done for all animals' consideration and conventions. The pain and discomfort must be decreased. Utilization of anesthesia was only as required with avoiding repeated surgeries on same animal. The rats were sacrificed under ketamine and xylazine mixture anesthesia with efforts to decrease the suffering of animals.

Design of study

Adult male of Wistar albino rats were divided randomly into 4 groups (6 rats /group) as following:

1. I/R (control) group: Rats exposed to bilateral renal ischemia for 30 min [29] followed by 2 hr reperfusion [30].

2. Vehicle group: Rats received DMSO a vehicle for curcumin through intraperitoneal (I.P.) injection and subjected to 30 minute ischemia and reperfusion for 2 hr.

3. Sham group: Rats exposed to the same surgical procedure with exception of ischemia induction.

4. Curcumin pretreated group: Rats pretreated with 60 mg/kg of curcumin [31]. I.P. injection 45 min before ischemia and exposed to 30 minute of bilateral renal ischemia and 2 hr reperfusion.

Selected morphological and biochemical parameters will be followed in the sham operated rats and those exposed to IRI and pretreated with DMSO and curcumin.

Preparation of Drugs

Curcumin: Pure Curcumin powder was purchased from sigma-Aldrich Company

Formula name: (E, E) 1,7-bis (4-Hydroxy 3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Diferulylmethane.

Empirical Formula: [(HOC6H3 (OCH3) CH=CHCO] 2CH2

Molecular Weight: 368.38

This product is soluble in ethanol 10mg/ml, DMSO 11mg/ml according to sigma /Aldrich package insert.

Experimental Model of Renal I/R Injury

Rats were received anesthetic of ketamine l.P. injection (100 mg/kg) and l.P. injection of xylazine (10 mg/kg) [31,32]. All procedures were accomplished by sterilized instruments. Under sedation within

5-10 min, rats were set on their back, the limbs and tail were fixed using stickers to assure stability during surgery. The skin disinfected and the hair of the abdomen region was shaved. To ensure that the rats were adequately anesthetized, the hind feet and the tail were pinched to check the reflexes. Abdominal cavity exposed through a median laparotomy incision and both renal pedicles cautiously isolated. Non-traumatic vascular clamps were used to perform a bilateral renal occlusion for 30 minutes, the prompt color change of the kidneys denoting the stoppage of blood flow and successful occlusion. Also, through this procedure, to keep the animal well hydrated 1ml of normal saline was used to be administered into the abdomen, then abdomen covered by moist and warm gauze [33,34]. During reperfusion period, clamps were withdrawn and the blood flow to the kidney was restored with visual confirmation of blood return [35]. At that point, the incision of abdominal cavity was sutured in two layers [8]. In post-surgery after recovery from anesthesia, rats were transported to their cage with some food accessible on floor with checking and monitoring general appearance. Then, after 2 hr the animals ethically sacrificed by using deep anesthesia and samples of blood and tissues collected for analysis [36].

Collection and preparation of sample

Preparation of blood sample for measurement of renal function: At the end of experiment, rats were anesthetized and the blood samples were collected from heart and placed in plane tube at 37°C without using anticoagulant and left for 30 minute, then it was centrifuged at 3000 rpm for 10 minute [36], and finally the serum utilized for the determination of serum creatinine and urea.

Preparation of tissue for measurement of inflammatory, apoptotic and oxidative stress markers: Ice-cold normal saline was used to wash the kidney in order to evacuate any blood and the kidney was stored in deep freeze (-80°C), after that homogenization was executed by weighing renal tissues (previously stored in deep freeze) with high intensity-ultrasonic liquid processor in phosphate-buffered saline (1:10 w/v) which comprised protease inhibitor cocktail and Triton X-100 (1%) [37]. The supernatant was collected after centrifugation of homogenate at 3000g for 20 minute at 4°C for determination of caspase-3, IL-1β, f2-isoprostane, mTOR, p-mTOR, Akt and p-Akt levels by means of ELISA technique.

Tissue sampling for histology analysis and damage scores: The renal tissue was fixed in 10% formalin, dehydrated in alcohol, then cleared in xylene and embedded in paraffin block. The slide sections of tissue were cut horizontally about 5-µm of thickness and stained by using hematoxylin and eosin (H and E), then sent to histopathology's for histological examination [38]. An investigator was blinded to experimental groups to perform an evaluation of scores. Grading of degeneration/necrosis was done by examining tissue sections using light microscopy.

Quantitative measurements was used to assess the scoring system of tissue damage [39]. The damage of tubules recognized as swelling of tubular epithelial, losing of brush border, formation of cast and vacuolar degeneration. The degree of kidney damages were identified through the subsequent criteria: 0 denotes normal, 1 denotes<25% damage of tubules, 2 denotes 25-50%, 3 denotes 50-75%, 4 denotes>75% damage of tubules [40].

Statistical Analysis

Statistical analysis was done using SPSS for windows (version 24). The data were expressed as mean ± SEM. Analysis of Variance (ANOVA) and LSD post-hoc test were used for multiple comparisons. Analysis of histopathological data were done using Mann Whitney U and Kruskal-Wallis test. In all tests, P-value was considered significant at <0.05.

Results

Effect of Curcumin on Inflammatory Marker (IL-1β)

Control group significantly increased the mean± SEM of renal level of IL-1β (955.21±2.59, p value 0.001) in comparison to sham group (158.77±1.90), while control and vehicle groups exhibited insignificant difference between them (p value 0.166). In comparison to control group, curcumin significantly decreased the mean± SEM of renal level of IL-1β (448.71±1.55, p value 0.001) (Figure 1).

Effect of Curcumin on Apoptotic Marker (caspase-3)

Control group significantly increased the mean± SEM of renal level of caspase-3 (12.69±0.039, p value 0.001) in comparison to sham group (2.55±0.057), while control and vehicle groups exhibited insignificant difference between them (p value 0.186). In comparison to control group, curcumin significantly decreased the mean± SEM of renal level of caspase-3 (6.42±0.042, p value 0.001) (Figure 2).

Effect of Curcumin on Akt / mTOR Pathway

Effect on renal Akt level: The renal level of Akt showed insignificant (p value >0.05) differences between the four experimental groups (Figure 3).

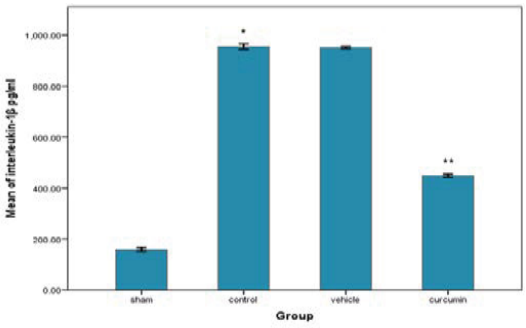

Figure 1: Error bar chart showing the effect of curcumin on renal IL-1β level (pg/ml) expressed as mean ± SEM (6 animals in each group).

*P value <0.05 when control compared with sham;

**P value <0.05 when curcumin compared with control).

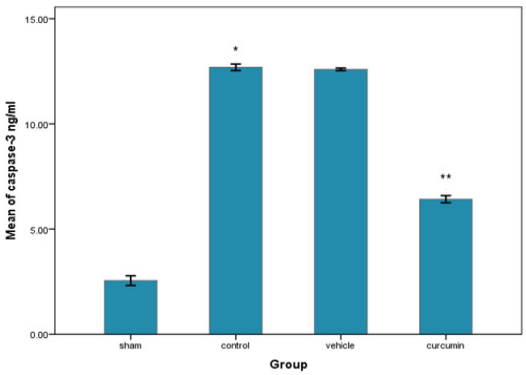

Figure 2: Error bar chart showing the effect of curcumin on renal caspase-3 level (ng/ml) expressed as mean ± SEM (6 animals in each group

*P value <0.05 when control compared with sham;

**P value <0.05 when curcumin compared with control).

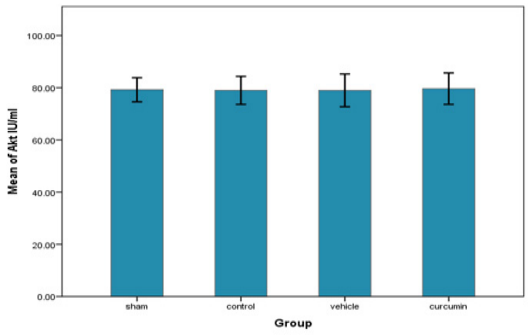

Figure 3: Error bar chart showing the effect of curcumin on renal Akt level (IU/ml) expressed as mean ± SEM (6 animals in each group)
* P value <0.05 when control compared with sham;
** P value <0.05 when curcumin compared with control).

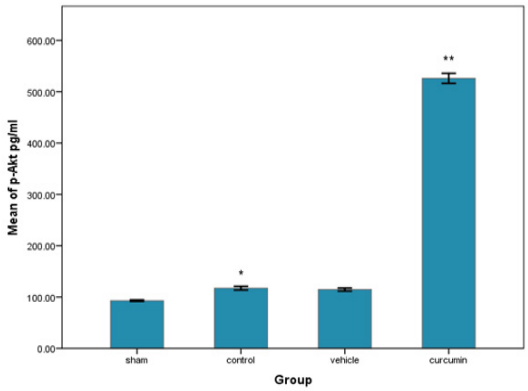

Figure 4: Error bar chart showing the effect of curcumin on renal p-Akt level (pg/ml) expressed as mean ± SEM (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

Effect on renal p-Akt level: Control group significantly increased the mean± SEM of renal level of p-Akt (117.11±0.92, p value 0.001) in comparison to sham group (92.87±0.39), while control and vehicle groups exhibited insignificant difference between them (p value 0.219), in comparison to control group, curcumin significantly increasedthe mean± SEM of renal level of p-Akt (526.14±2.44, p value 0.001) (Figure 4).

Effect on renal p-Akt/Akt level: Control group significantly increased the mean± SEM of renal level of p-Akt/Akt (1.48±0.026, p value 0.006) in comparison to sham group (1.17±0.016), while control and vehicle groups exhibited insignificant difference between them (p value 0.761).

In comparison to control group, curcumin significantly increased the mean± SEM of renal level of p-Akt/Akt (6.61±0.116, p value 0.001) (Figure 5).

Effect on renal mTOR level: The renal level of mTOR showed insignificant (p value >0.05) differences between the four experimental groups (Figure 6).

Effect on renal p-mTOR level: Control group significantly increased the mean±SEM of renal level of p-mTOR (3076847.60±118609.57, p value 0.001) in comparison to sham group (1045944.57±14418.86), while control and vehicle groups exhibited insignificant difference between them (p value 0.064).

In comparison to control group, curcumin significantly increased the mean± SEM of renal level of p-mTOR (6130540.36±9218.20, p value 0.001) (Figure 7).

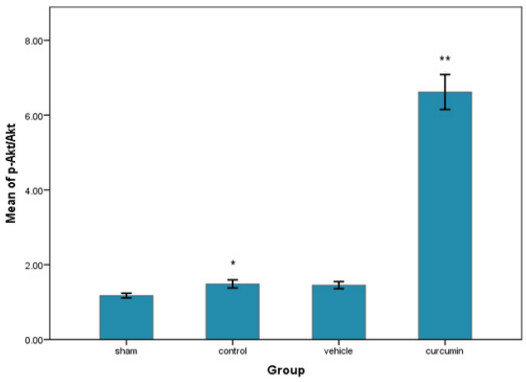

Figure 5: Error bar chart showing the effect of curcumin on renal p-Akt/Akt level expressed as mean ± SEM (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

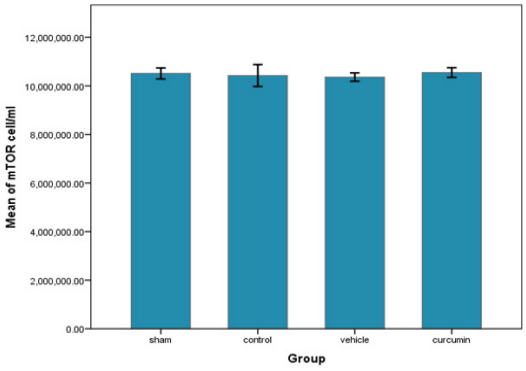

Figure 6: Error bar chart showing the effect of curcumin on renal mTOR level (cell/ml) expressed as mean ± SEM (6 animals in each group).
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

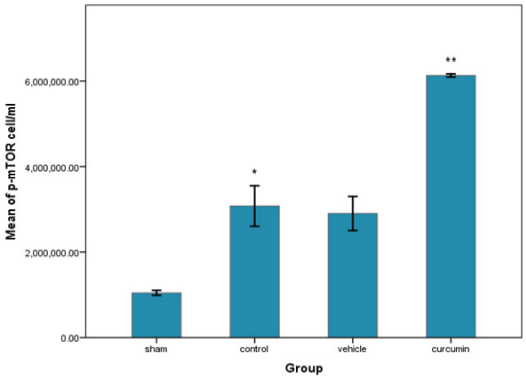

Figure 7: Error bar chart showing the effect of curcumin on renal p-mTOR level (cell/ml) expressed as mean ± SEM (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

Effect on renal p-mTOR / mTOR level: Control group significantly increased the mean± SEM of renal level of p-mTOR/ mTOR (0.295±0.011, p value 0.001) in comparison to sham group (0.099±0.001), while control and vehicle groups exhibited insignificant difference between them (p value 0.105). In comparison to control group, curcumin significantly increased the mean± SEM of renal level of p-mTOR / mTOR (0.581±0.002, p value 0.001) (Figure 8).

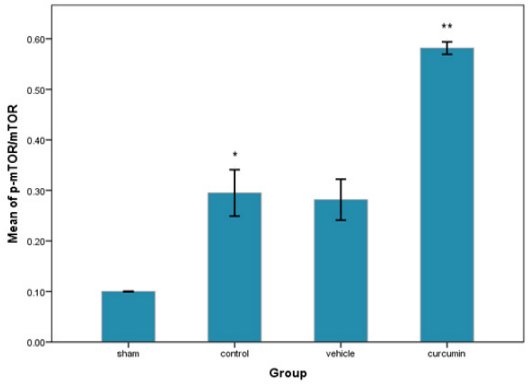

Figure 8: Error bar chart showing the effect of curcumin on renal p-mTOR / mTOR level expressed as mean ± SEM (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

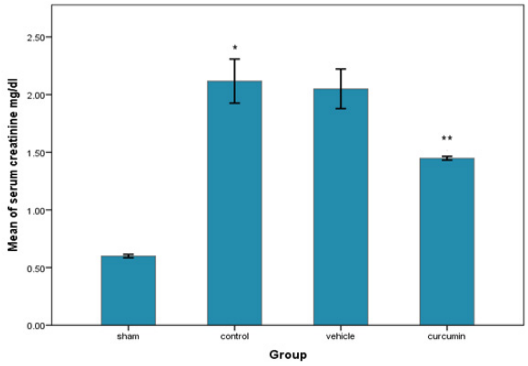

Figure 9: Error bar chart showing the effect of curcumin on serum creatinine level (mg/dl) expressed as mean ± SEM of six experimental groups (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumincompared with control).

Effect of Curcumin on Renal Function

Effect on serum creatinine level: Control group significantly increased the mean± SEM of serum level of creatinine (2.1167±0.0477, p value 0.001) in comparison to sham group (0.60±0.0036), while control and vehicle groups exhibited insignificant difference between them (p value 0.085). In comparison to control group, curcumin significantly decreased the mean± SEM of serum creatinine level (1.4483±0.0040, p value 0.001) (Figure 9).

Effect on blood urea level: Control group significantly increased the mean± SEM of blood urea level (91.17±1.5793, p value 0.001) in comparison to sham group (22.33±0.7149), while control and vehicle groups exhibited insignificant difference between them (p value 0.460). In comparison to control group, curcumin significantly decreased the mean± SEM of blood urea level (57.50±0.4281, p value 0.001) (Figure 10).

Effect of curcumin on oxidative stress marker (F2-isoprostane)

Control group significantly increased the mean± SEM of renal level of F2-isoprostane (232.62±1.417, p value 0.001) in comparison to sham group (56.38±2.960), while control and vehicle groups exhibited insignificant difference between them (p value 0.078). In comparison to control group, curcumin significantly decreased the mean± SEM of renal level of F2-isoprostane (125.87±1.006, p value 0.001) (Figure 11).

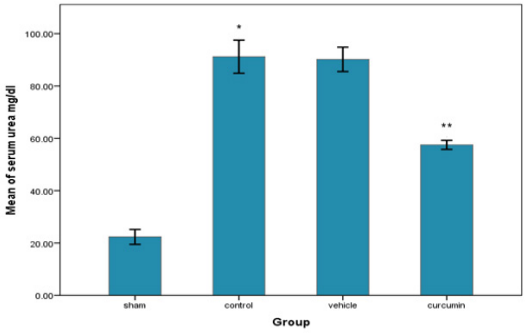

Figure 10: Error bar chart showing the effect of curcumin on urea level (mg/dl) expressed as mean ± SEM (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

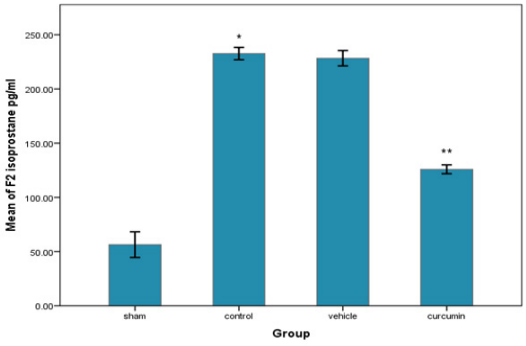

Figure 11: Error bar chart showing the effect of curcumin on renal F2-isoprostane level (pg/ml) expressed as mean ± SEM (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

Histopathological finding

There was no significant changes appeared in the renal tissue of sham group. Control and vehicle groups showed a statistically insignificant differences between them, renal IRI resulted in significant (p<0.05) and sever tissue damages when compared to sham group. Pretreatment with curcumin resulted in significant (p<0.05) amelioration of renal tissue damages when compared with control group. Figure 12 showing histopathological scores of six experimental groups. Figure 13 showed normal renal structure in the sham group. Figure 14 and 15 showed significant tissue damages caused by renal IRI. Figure 16 showed the significant amelioration of tissue damage with pretreatment of curcumin (Figures 12-16).

Discussion

Acute kidney injury is a rapid decline in renal function which causes retention of urea and nitrogen waste product with inability to preseve renal function [41]. Acute kidney injury develops from toxic, obstructive or ischemic insults, for example, renal IRI which is still connected with high morbidity and mortality [3,8]. The pathophysiology of renal IRI includes multiple pathways such as oxidative stress, inflammation and apoptotic cell death [6]. In the pathogenesis of ischemia-induced AKI, infiltration of inflammatory and immune cells as well as production of inflammatory cytokine lead to the necrosis and apoptosis of renal tubular epithelial cells [42]. It is likely to be a potential therapeutical strategy to execute anti-inflammatory and antioxidant agents to treat renal injury after I/R. We estimated the protective effects of curcumin, quercetin and their combination against experimental renal IR.

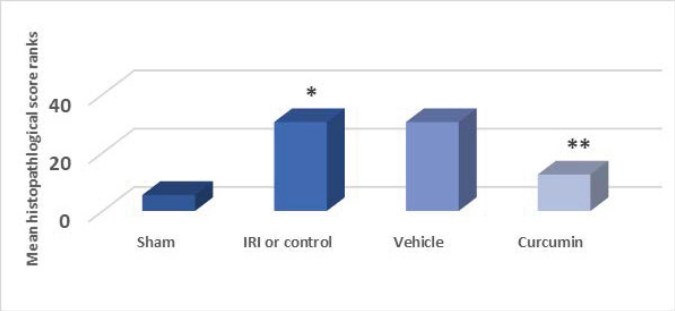

Figure 12: Bar chart showing the effect of curcumin on mean histopathological rank score (6 animals in each group)
*P value <0.05 when control compared with sham;
**P value <0.05 when curcumin compared with control).

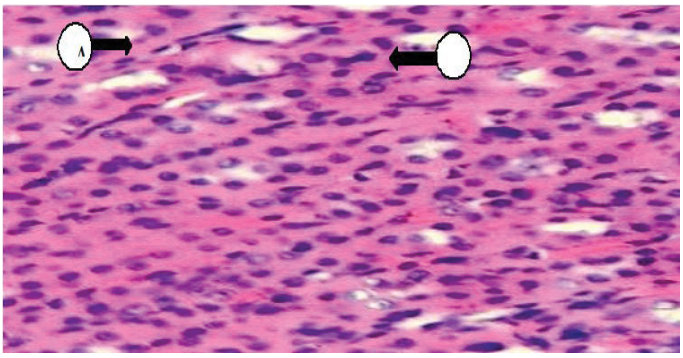

Figure 13: Photomicrograph of renal tissue section of sham group showing normal renal tubules (1) and (2), the renal section was stained with H and E with X40 magnification, the severity score 0.

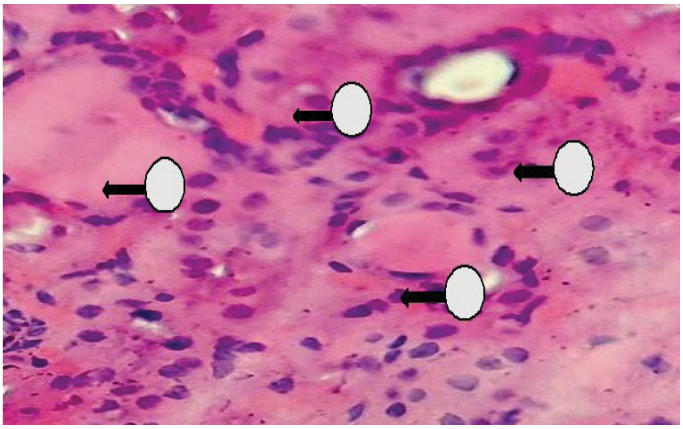

Figure 14: Photomicrograph of renal tissue section of control group showing the tubular cellular swelling (1) and (2), eosinophilic casts (3) and (4), the renal section was stained with H and E with X40 magnification, the severity score 3.

Effect of Renal IR on Inflammatory Marker (IL-1β)

In this study the renal level of IL-1β was elevated in IR group in comparison to that of the sham group. Furthermore, the present study showed that the inflammatory process assumes key role in pathophysiology of renal IRI. This resulting data is consistent with previous study which showed that IL-1β level was elevated in control group compared with sham group when rats exposed to renal ischemia followed by reperfusion [27]. The renal level of IL-1β in mice of IR group was significantly elevated in comparison with sham group [34]. Neutrophil cell joins to the activated endothelial cell and aggregates in the kidney during IRI, as right on time as 30 minutes of the reperfusion

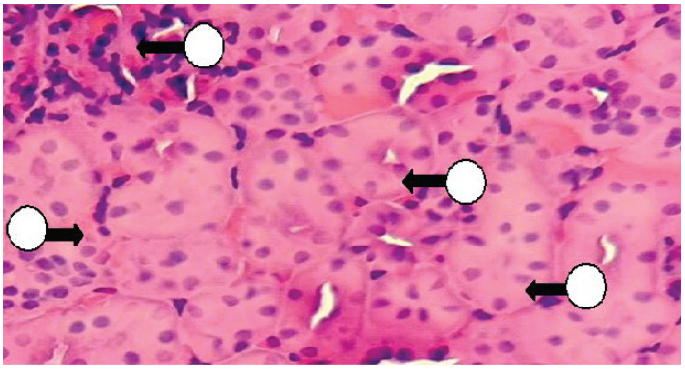

Figure 15: Photomicrograph of renal tissue section of vehicle group showing the tubular cellular swelling (1) and (2), damaged glomerulus (3) and karyolysis (4), the renal section was stained with H and E with X40 magnification, the severity score 3.

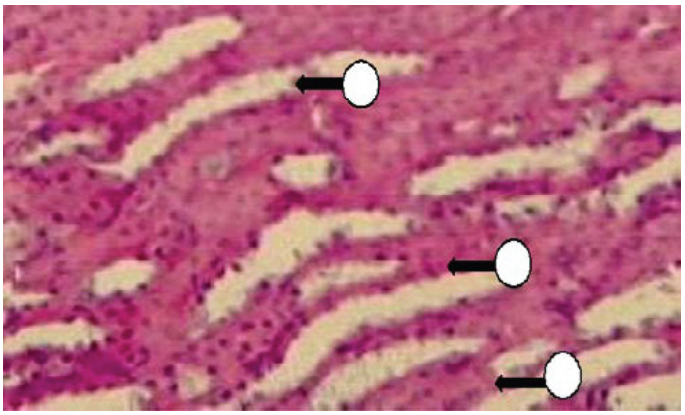

Figure 16: Photomicrograph of renal tissue section of curcumin treated group showing the normal renal tubules (1), (2) and (3), the renal section was stained with H and E with X40 magnification, the severity score 0.

phase. They generate ROS and inflammatory cytokines, which prompts vascular permeability and diminished the integrity of TEC triggering renal damage [43,44].

Effect of Curcumin on Inflammatory Cytokines (IL-1β)

The present study demosetrated that curcumin has clear anti-inflammatory effect in IR-induced AKI, which was supported by finding that curcumin pretreatment significantly reduced pro-inflammatory IL-1β when compared with control group. These results are in settlement with previously confirmed following renal ischemia/reperfusion there was a significant elevation in both serum and renal tissue production of IL-1β in the control group. While curcumin pretreatment significantly diminished the production of IL-1β systemically and in the damaged renal tissue in comparison with I/R group. An increment in renal concentration of pro-inflammatory cytokine, IL-1β, was observed after exposure to cisplatin-induced nephrotoxicity, however curcumin treatment significantly decreased the level of cytokine.

Effect of Renal IR on Apoptotic Marker (caspase-3)

This study proved that a significant elevation of caspase -3 renal level was founded in I/R group when compared with sham group. Furthermore, the present study proved that apoptosis assumes an essential role in pathophysiology of renal IRI. These outcomes are in settlement with existing study that confirmed the level of caspase 3 was markedly improved in control group when compared with sham group after renal IRI in rats model.Renal IR caused significantly better caspase-3 level in control as compared with sham group when rats

subjected to bilateral renal pedicles clamping followed by reperfusion. Apoptosis of TEC is an initiated aspect of renal IRI [45] and although the ischemic insult alone may prompts renal apoptosis, blood reinstitution during reperfusion also causes a regeneration of ROS and elevation of celluar apoptosis [46].

Effect of Curcumin on Apoptotic Marker (caspase -3)

The study showed that there was a marked reduction in the caspase-3 renal level of curcumin treatment group in comparison to control group. This result indicated that curcumin could reduce apoptosis in renal tissue after IRI. This result is consistent with the prevailingstudywhichshowed that the renal level of caspase -3 higher in the IR control group, while curcumin pretreatment appreciably decreased the expression of caspase-3. Fan and followers showed that IR caused upregulation of caspase-3 expression in the kidneys, while the expression of caspase-3 was markedly diminished in mice receiving I.P. curcumin compared to control.

Effect of Renal IRI on Oxidative Stress Marker (F2-isoprostane)

This study demonstrated that renal tissue level of F2- isoprostane was significantly elevated in IR group in comparison with sham group. In addition, the present study revealed that oxidative stress assumes an essential role in pathophysiology of renal IRI. This resulting outcome is in settlement with previous study which indicated that the level of F2-isoprostane significantly elevated in the renal transplantation indicating that oxidative stress is a critical response during this procedure. Furthermore, Carlström and followers exhibited that F2-isoprostane significantly elevated after unilateral nephrectomyof rats model. Previous studies reported that the underlying mechanisms of renal IRI are mainly by ROS production and oxidative stress [47]. Clinical and experimental studies suggested that F2-isoprostane was the potent reliable parameter indicating oxidative stress [48].

Effect of Curcumin on Oxidative Stress Marker (F2-isoprostane)

In this study, renal level of F2-isoprostane significantly decreased subsequent to curcumin treatment in comparison to I/R group, this clearly demonstrating the potential antioxidant effect of curcumin against renal IRI. This result is in agreement with study which confirmed that treatment with curcumin significantly lowered the increment in ROS cellular level and the level of oxidative stress marker, F2-isoprostane, when the adult male rats subjected to cardiac ischemia / reperfusion. Curcumin treatment found to be beneficial in preventing cisplatin-induced nephrotoxicity by downregulating ROS production and significantly reducing F2-isoprostane level. Up until now, there may not be a prior study analyzing curcumin effect on F2-isoprostane in renal IRI of animal model.

Effect of Renal IR on Akt Activation

In this study, the renal level of p-Akt and p-Akt/Akt were significantly elevated in the control in comparison to the sham group. Whereas Akt level was insignificantly different in comparison to sham group.This resulting data are consistent with the previous studywhich exhibited that in comparison with sham group, p-Akt level increased and the level of Akt not significantly changed after subjecting the male mice to bilateral renal pedicles clamping for 30 minute, followed by removing clamps to allow blood reperfusion [36]. Reoxygenation following hypoxia of renal tissue causes a transient activation of PI3K/Akt signaling pathway and thereby improving the proliferation of tubular cells. Reperfusion of blood to an organ subsequent to diminished blood supply puts the metabolically active tissue in danger for injury. Akt pathway, a potential mediator of IRI, is activated after IR occasions [49]. Activating Akt pathway in renal I/R could repress the downstream inflammatory elements expression, for example, interleukin-1 and associated with tissue protection by inhibiting apoptosis [33]. Therefore, Akt considers as a potential pathway and if controlled by targeted therapy could be a potent alleviator of IRI.

Effect of Curcumin on Akt Activation

This study showed a significant increase in the p-Akt level and p-Akt/Akt in curcumin treated group when compared with control group. Whereas the renal tissue level of Akt was not significantly different as compared with control group. This outcome is in agreement with monitoring study which suggested that curcumin treatment significantly diminished apoptosis in experimental acute kidney injury through activation of Akt signaling pathway which is expressed as an increment of p-Akt level and equal Akt level when curcumin treated group compared with control group. This results demonstrated, for the first time, that curcumin exerts nephroprotection against renal IRI by activation of Akt.

Effect of Renal IR on mTOR Activation

In this study the renal tissue level of p-mTOR and p-mTOR /mTOR significantly elevated in the control compared to sham group. Whereas no significant difference found in the renal level of mTOR compared with sham. This resulting data are consistent with previous study that indicateda reperfusion subsequent to hypoxia of myocardial tissue caused higher expression of p-mTOR levels and p-mTOR/mTOR in IR group compared with the sham group.Lieberthal and followers proved that mTOR activity is absent or low in the normal renal tissue but elevates markedly after ischemia-reperfusion injury. Previous studies affirmed that systemic activation of Akt/mTOR promotes the nephroprotection after IRI, which leads to improve recovery in the kidney through attenuation of lipid peroxidation and inflammation as well as inhibition of pro-apoptotic mediators and activation of anti-apoptotic mediators [50,51].

Effect of Curcumin on mTOR Activation

In this study, curcumin treated group showed a higher increase in renal level of p-mTOR and p-mTOR/mTOR in comparison with control group. Whereas the renal tissue level of mTOR was insignificantly different in comparison with control group. These end results are in settlement withprevious study that indicated the treatment with curcumin was effective in improving the neurological function and brain tissue infarction after cerebral IRI via activation of Akt/mTOR signaling in rats model. Although existing studies had indicated that curcumin has a potential protective effect in renal IRI, the molecular mechanism of nephroprotection was not clear [31,52]. For these reasons, we thought that curcumin may exert nephroprotection by activation of Akt / mTOR pathway in renal IRI.

Effect of Renal IR on Renal Function

The present study demonstrated that level of serum urea and creatinine in control group was elevated in comparison to sham group. These results are consistent with previous studywhich revealed that renal I/R increased serum creatinine and BUN when compared to sham group in rats model. Tan and followers found that the levels of serum creatinine and BUN were both significantly elevated in the IR rats compared to sham group.

Effect of Curcumin on Renal Function

This study found that curcumin significantly decreased urea and creatinine levels when compared with control group, demonstrating that curcumin improved the renal function after IRI. This results are in consistence with previous results indicated that curcumin treatment showed a potential improvement of renal function by decreasing urea and creatinine level in rats with IRI [31]. Rogers and followers indicated that mice receiving curcumin were protected against IR induced acute kidney injury as reflected by a significant lowering in serum creatinine and urea levels.

Effect of Renal IR on Renal Parenchyma

In this study, there were no noticeable changes in the renal tissue of sham group. By contrast, histological examination of IR group displayed a marked damage of renal tissue, for example, dilatation of the Bowman's capsule, lack of brush borders, tubular cellular swelling, necrotic areas, kareolysis, eiosinophilic cast and glomerular modifications. These changes are in agreement with previous study outcomes which showed a normal kidney structure in sham group, while in IR group the renal sections appeared with marked changes and injuries such as glomerular degeneration, tubular lumen dilation, hemorrhage, inflammatory cell infiltration, epithelial atrophy and tubular casts [53].

Effect of Curcumin on Renal Parenchyma

The present study demonstrated that there was improvement of renal injury in curcumin treatment group when compared with control group. These results verified the protective role of curcumin against renal IRI through histopathological findings. These consequences are consistent with existing study [52] revealed thatI/R caused prominent tissue damages including tubular cellular swelling, tubular atrophy and inflammatory cells infiltration. While curcumin treatment previous to renal ischemia alleviate the renal damage.

Conclusion

From the overall results, curcumin significantly diminishes renal ischemia reperfusion injury in the rat model through Akt/mTOR pathway by its effect as anti-oxidant, anti-inflammatory and anti-apoptotic.

References

1. Le Dorze M, Legrand M, Payen D, Ince (2009) The Role of the Microcirculation in Acute Kidney Injury. Curr Opin Crit Care 15: 503-508.
2. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW (2008) Acute Kidney Injury, Mortality, Length of Stay, and Costs in Hospitalized Patients. J Am Soc Nephrol 16: 3365-3370.
3. Kaddourah A, Basu RK, Bagshaw SM, Goldstein SL (2017) Epidemiology of acute kidney injury in critically ill children and young adults. N Eng J Med 376: 11-20.
4. Abreu LD, Kawano PR, Yamamoto H, Damião R, Fugita OE (2011) Comparative Study between Trimetazidine and Ice Slush Hypothermia in Protection against Renal Ischemia/Reperfusion Injury in a Porcine Model. Int Braz j Urol 37: 649-656.
5. Wang Y, Seto SW, Golledge J, Therapeutic Effects of Renal Denervation on Renal Failure, Curr Neurovas Res 10: 172-184.
6. Sethi K, Rao K, Bolton D, Patel O, Ischia J (2018) Targeting HIF-1α to prevent renal ischemia-reperfusion injury: does it work? Int J Cell Biol.
7. Eltzschig HK, Eckle T (2011) Ischemia and Reperfusion-from Mechanism to Translation. Nat Med 17: 1391.
8. Danobeitia JS, Ziemelis M, Ma X, Zitur LJ, Zens T (2017) Complement Inhibition Attenuates Acute Kidney Injury after Ischemia-Reperfusion and Limits Progression to Renal Fibrosis in Mice. PLoS ONE 12: 1-20.
9. Libby P (2007) Interleukin-1 Beta as a Target for Atherosclerosis Therapy: Biological Basis of CANTOS and Beyond, J Am Coll Cardiol 70: 2278-2289.
10. Allan SM, Tyrrell PJ, Rothwell NJ (2005) Interleukin-1 and neuronal injury. Nature Reviews Immunology 5: 629-640.
11. Sun Q, Scott MJ (2016) Caspase-1 as a multifunctional inflammatory mediator: noncytokine maturation roles. J Leukoc Biol 100: 961-967.
12. Simmons EM, Himmelfarb J, Sezer MT, Chertow GM, Mehta RL, et al. (2004) Plasma cytokine levels predict mortality in patients with acute renal failure. Kidney Int 65: 1357-1365.
13. Daha MR, Van Kooten C (2000) Is the Proximal Tubular Cell a Proinflammatory Cell?, Nephrol Dial Transpl 15: 41-43.
14. Ramesh G, Reeves WB (2004) Inflammatory cytokines in acute renal failure. Kidney Int 66: S56-S61.
15. von Knethen A, Callsen D, Brüne B (1999) Superoxide Attenuates Macrophage Apoptosis by NF-KB and AP-1 Activation That Promotes Cyclooxygenase-2 Expression. J Immunol 163: 2858-2866.
16. Laplante M, Sabatini DM (1999) mTOR Signaling at a Glance J Cell Sci 122: 3589-3594.
17. Kim YC, Guan KL (2015) mTOR: A Pharmacologic Target for Autophagy Regulation, J Clin Invest 125: 25-32.
18. Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, et al. (2007) IKKβ Suppression of TSC1 Links Inflammation and Tumor Angiogenesis via the MTOR Pathway. Cell 130: 440-455.
19. Kezic A, Becker JU, Thaiss F (2013) The Effect of MTOR-Inhibition on NF-KB Activity in Kidney Ischemia-Reperfusion Injury in Mice, Transplant Proc 45: 1708-1714.
20. Aggarwal BB, Kumar A, Bharti AC (2003) Anticancer Potential of Curcumin: Preclinical and Clinical Studies, Anticancer Res 23: 363-398.
21. Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, et al. (2011) Antioxidant and Vascular Protective Effects of Curcumin and Tetrahydrocurcumin in Rats with L-NAME-Induced Hypertension, N-S Arch Pharmacol 383: 519.
22. Lestari ML, Indrayanto G (2014) Curcumin. Profiles Drug Subst Excip Relat Methodol 39: 113-204.
23. E Wright L, B Frye J, Gorti B, N Timmermann B, L Funk J (2013) Bioactivity of Turmeric-Derived Curcuminoids and Related Metabolites in Breast Cancer, Curr Pharm Des 19: 6218-6225.
24. Chin D, Huebbe P, Pallauf K, Rimbach G (2013) Neuroprotective Properties of Curcumin in Alzheimer's Disease-Merits and Limitations, Curr Med Chem 20: 3955-3985.
25. Jiang S, Han J, Li T, Xin Z, Ma Z, et al. (2017) Curcumin as a Potential Protective Compound against Cardiac Diseases, Pharmacolog Res 119: 373-383.
26. Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, et al. (2007) Lutgendorf, Curcumin Inhibits Tumor Growth and Angiogenesis in Ovarian Carcinoma by Targeting the Nuclear Factor-KB Pathway. Clin Cancer Res 13: 3423-3430.
27. Cho JW, Lee KS, Kim CW (2007) Curcumin Attenuates the Expression of IL-1β, IL-6, and TNF-α as Well as Cyclin E in TNF-α-Treated HaCaT Cells; NF-KB and MAPKs as Potential Upstream Targets. Int J Mol Med 19: 469-474.
28. Das L, Vinayak M (2014) Curcumin Attenuates Carcinogenesis by down Regulating Proinflammatory Cytokine Interleukin-1 (IL-1α and IL-1β) via Modulation of AP-1 and NF-IL6 in Lymphoma Bearing Mice. Int Immunopharmacol 20: 141-147.
29. Bayrak O, Bavbek N, Karatas OF, Bayrak R, Catal F, et al. (2008) Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys. Nephrol Dial Transpl 23: 2206-2212.
30. Matthijsen RA, Huugen D, Hoebers NT, De Vries B, Peutz-Kootstra CJ, et al. (2017) Myeloperoxidase Is Critically Involved in the Induction of Organ Damage after Renal Ischemia Reperfusion. Am J Pathol 171: 1743-1752.
31. Zhang J, Tang L, Li GS, Wang J (2018) The Anti-Inflammatory Effects of Curcumin on Renal Ischemia-Reperfusion Injury in Rats. Ren Fail 40: 680-686.
32. Aktoz T, Aydogdu N, Alagol B, Yalcin O, Huseyinova G, et al. (2009) The protective effects of melatonin and vitamin E against renal ischemia-reperfusion injury in rats. Renal failure 29: 535-542.

33. Yang C, Zhao T, Lin M, Zhao Z, Hu L (2013) Helix B surface peptide administered after insult of ischemia reperfusion improved renal function, structure and apoptosis through beta common receptor/erythropoietin receptor and PI3K/Akt pathway in a murine model. Exp Biol Med 238: 111-119.

34. Hu C, Li L, Ding P, Li L, Ge X, et al. (2018) Complement Inhibitor CRIg/FH Ameliorates Renal Ischemia Reperfusion Injury via Activation of PI3K/AKT Signaling. J Immunol 201: 3717-3730.

35. Wang F, Yu G, Liu SY, Li JB, Wang JF, et al. (2011) Hydrogen-rich saline protects against renal ischemia/reperfusion injury in rats. J Surg Res 167: e339-e344.

36. Liu HB, Meng QH, Huang C, Wang JB, Liu XW (2015) Nephroprotective Effects of Polydatin against Ischemia/Reperfusion Injury: A Role for the PI3K/Akt Signal Pathway. Oxid Med Cell Longev 2015.

37. Matsuyama M, Funao K, Kawahito Y, Sano H, Chargui J, et al. (2008) Study of Cysteinyl Leukotriene-1 Receptor in Rat Renal Ischemia-Reperfusion Injury, Transplant Proc 40: 2149-2151.

38. Yousif NG (2014) Fibronectin Promotes Migration and Invasion of Ovarian Cancer Cells through Up□regulation of FAK–PI 3 K/A Kt Pathway. Cell Biol Int 38: 85-91.

39. Waseem T, Duxbury M, Ito H, Ashley SW, Robinson MK (2008) Exogenous Ghrelin Modulates Release of Pro-Inflammatory and Anti-Inflammatory Cytokines in LPS-Stimulated Macrophages through Distinct Signaling Pathways. Surgery 143: 334-342.

40. Jiang M, Liu K, Luo J, Dong Z (2010) Autophagy Is a Renoprotective Mechanism during in Vitro Hypoxia and in Vivo Ischemia-Reperfusion Injury, Am J Pathol 176: 1181-1192.

41. Thomas ME, Blaine C, Dawnay A, Devonald MA, Ftouh S, et al. (2015) The Definition of Acute Kidney Injury and Its Use in Practice, Kidney Int 87: 62-73.

42. Ornellas FM, Ornellas DS, Martini SV, Castiglione RC, Ventura GM, et al. (2017) Bone Marrow-Derived Mononuclear Cell Therapy Accelerates Renal Ischemia-Reperfusion Injury Recovery by Modulating Inflammatory, Antioxidant and Apoptotic Related Molecules. Cell Physiol Biochem 41: 1736-1752.

43. Bonventre JV, Yang L (2011) Cellular pathophysiology of ischemic acute kidney injury. The Journal of clinical investigation 121: 4210-4221.

44. Jang HR, Rabb H (2009) The Innate Immune Response in Ischemic Acute Kidney Injury, Clin Immunol 130: 41-50.

45. Daemen MA, de Vries B, Buurman WA (2002) Apoptosis and Inflammation in Renal Reperfusion Injury. Transplantation 73: 1693-1700.

46. Qiao X, Chen X, Wu D, Ding R, Wang J, et al. (2005) Mitochondrial pathway is responsible for aging-related increase of tubular cell apoptosis in renal ischemia/reperfusion injury. J Gerontol A Biol 60: 830-839.

47. Di Nardo M, Ficarella A, Ricci Z, Luciano R, Stoppa F, et al. (2013) Impact of Severe Sepsis on Serum and Urinary Biomarkers of Acute Kidney Injury in Critically Ill Children: An Observational Study. Blood Purificat 35: 172-176.

48. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, et al. (2005) Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 38: 698-710.

49. Zhang J, Zou YR, Zhong X, Deng HD, Pu L, et al. (2015) Erythropoietin Pretreatment Ameliorates Renal Ischaemia-Reperfusion Injury by Activating PI3K/Akt Signalling, Nephrology 20: 266-272.

50. Wei Q, Zhao J, Zhou X, Yu L, Liu Z, et al. (2019) Propofol Can Suppress Renal Ischemia-Reperfusion Injury through the Activation of PI3K/AKT/MTOR Signal Pathway. Gene 708: 14-20.

51. Zhang G, Wang Q, Wang W, Yu M, Zhang S, et al. (2018) Tempol Protects Against Acute Renal Injury by Regulating PI3K/Akt/MTOR and GSK3β Signaling Cascades and Afferent Arteriolar Activity, Kidney Blood Press Res 43: 904-913.

52. Erturk N, Elbe H, Dogan Z, Aktas S, Demirbilek S, Ozturk F. Curcumin prevents renal oxidative stress and tissue damage induced by renal ischemia/reperfusion in rats. Int Surg J 25: 3192-3197.

53. Kenan Kinaci M, Erkasap N, Kucuk A, Koken T, Tosun M (2012) Effects of Quercetin on Apoptosis, NF-KB and NOS Gene Expression in Renal Ischemia/Reperfusion Injury, ExpTher Med 3: 249-254.