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Nephroprotective Potential Effect of Quercttin in Renal Ischemia Reperfusion Injury in Rat Model (Role of Akt/m TOR Pathway)

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ABSTRACT
Ischemia reperfusion is determined by a limitation of blood supply to the organs and subsequent blood restoration and reoxygenation. Renal ischemia reperfusion injury (IRI) is a potential element in the pathogenesis of acute kidney injury arising from oxidative stress, inflammation and apoptosis of renal tubular cells. The mammalian target of rapamycin (mTOR) pathway activates after IRI and contributes to renal repair and regeneration. Quercetin is a flavonoid compound that presents in many fruits and vegetables. Quercetin is widely used for its anti-inflammatory and anti-oxidant effects. The present study aimed to investigate the potential protective effect of quercetin on renal IRI in rat model by regulation of Akt/mTOR pathway. At the end of experiment, the serum levels of urea and creatinine and renal tissue levels regarding p-mTOR, p-Akt, IL-1β, caspase-3, and F2-isoprostane were recognized to be significantly (*p value*<0.05) elevated in control group when compared with sham group. Histopathologic alanalysis of the renal tissues recognized a severe renal injury in control group and there was a significant role of quercetin in ameliorating this injury by minimizing the serum levels of urea and creatinine, tissue levels of IL-1β, caspase-3, F2-isoprostane except for p-mTOR and p-AKt levels which were significantly (*p value*<0.05) higher in comparison with that of control group. In addition, treatment of quercetin significantly (*p<0.05*) reversed the histopathologic severity scores of renal tissue damage. From the overall results, we concluded that quercetin significantly diminished renal ischemia reperfusion injury in the rat model through Akt/mTOR pathway by its effect as anti-oxidant, anti-inflammatory and anti-apoptotic.

Keywords: IRI, manti-inflammatory effect, AKt /m TOR IRI
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INTRODUCTION
Renal ischemia 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, which is still associated with high morbidity and mortality (2-3). 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 (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, that is reliable for proteolysis activation of pro IL-1β into mature and bioactive form (11). 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 that aggravate inflammation subsequent to renal IRI by attraction and activation of leukocytes to sites of injury (13). Akt/m TOR pathway is a serine threonine kinase that assumes a potential role in regulation of proliferation, growth, and survival. M TOR pathway is activated in different cellular processes, for example, insulin resistance and tumor formation (14). M TOR comprises two obvious multiprotein complexes including m TOR complex-1 (mTORC-1) as well as m TOR complex-2 (mTORC-2) (15). Growth factor/ PI3K/ Akt pathway is a well determined m TOR regulator. Growth factors activate their related receptor and eventually activate PI3K/Akt signaling (16). Activated Akt directly causes phosphorylation and inhibition of tuberous sclerosis complex (TSC) heterodimeric which consists of TSC1 and TSC2. TSC serve as mTORC1 inhibitor, therefore inhibition of TSC via Akt dependent phosphorylation prompts the activation of mTORC1 (17). The most significant role of m TOR complex-2 is mainly phosphorylation and activation of Akt to enhance cell survival, growth, and proliferation (18-19). Inflammatory biomarkers for example, IL-1β, signal to m TOR through TSC complex. This biomarker inactivates TSC, leading to m TOR activation that diminishes NF-kB, providing a feedback loop to confine excessive

inflammation (20). So m TOR activation limits inflammatory response in renal ischemia reperfusion injury by NF-κB blocking (21). Since m TOR 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 (22). Quercetin is a flavonoid compound obtained in fruits and vegetables. Rich sources are black tea, onion, various berries and apples (23), and also some of commercial food supplements (24). Quercetin exerts specific biological effects such as improvement of physical/mental performance and reduction of infection risk (25). Quercetin has anti-inflammatory effect by blocking NF-kB activation, and therefore attenuates pro-inflammatory cytokines expression (26). Furthermore quercetin is proved to exert some potential benefits to disease resistance and overall health such as anti-carcinogenic, antioxidant, antiviral, and psychostimulant activity, in addition to its ability to inhibit lipid peroxidation and platelet aggregation (27).

MATERIALS AND METHOD

Preparation of animals

In the present study, a type of Wistar Albino rats had been used with 15-28 weeks in age and 300-350g in weight were 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 required applications.

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 rats were sacrificed under ketamine and xylazine mixture anesthesia with efforts to decrease the suffering of animals.

Design of study

Adult male rats were divided randomly into four groups (6 rats /group) as following:

1. I/R (control) group: Rats were exposed to bilateral renal ischemia for 30 minute (28) (29) followed by 2 hr reperfusion (30).
2. Vehicle group: Rats were received DMSO a vehicle for quercttin (31) through intraperitoneal (I.P.) injection and subjected to 30 minute ischemia and reperfusion for 2 hr.
3. Sham group: Rats were exposed to the same surgical procedure with exception of ischemia induction.
4. Quercetin pretreated group: Rats were pretreated with 50 mg/kg of quercttin (32) I.P. injection at 1 hr before ischemia and exposed to 30 minute of bilateral renal ischemia and 2 hr reperfusion.

PREPARATION OF DRUGS

Quercttin

Pure quercttin powder was purchased from Sigma-Aldrich Company

Empirical Formula: $C_{15}H_{10}O_7 \cdot xH_2O$

Molecular Weight: 302.24 (anhydrous basis)

This product is soluble in DMSO 200 mg/ml according to Sigma /Aldrich package insert.

Experimental model of renal I/R injury

Rats were received anesthetic of ketamine I.P. injection (100 mg/kg) and I.P. injection of xylazine (10 mg/kg) (33). All procedures were accomplished by sterilized instruments. Under sedation within 5–10 minute, 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 was 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 (29), 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 (34) (35). During reperfusion period, clamps were withdrawn and the blood flow to the kidney was restored with visual confirmation of blood return (36). At that point, the incision of abdominal cavity was sutured in two layers (37). 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 (38).

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 (38), and finally the serum utilized for the determination of serum level of creatinine and urea.

Preparation of tissue for measurement of inflammatory, apoptotic and oxidative stress markers

Homogenization of renal tissues was executed with high intensity-ultrasonic liquid processor in phosphate-buffered saline (1:10 w/v) which comprised protease inhibitor cocktail and Triton X-100 (1%). The supernatant was collected after centrifugation of homogenate at 3000g for 20 minute at 4°C (39) (40), and used for determination of caspase-3, IL-1β, F2-isoprostane, m TOR, p-m TOR, Akt and p-Akt levels by means of ELISA technique.

Tissue sampling for histology analysis and damage scores

The renal tissue of the left kidney 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 (41). 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. 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 was 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 (42).

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 quercttin on inflammatory marker (IL-1β)

Control group significantly increased the mean±SEMof 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, quercetin significantly decreased the mean±SEM of renal level of IL-1β (478.58±2.37, p value 0.001).

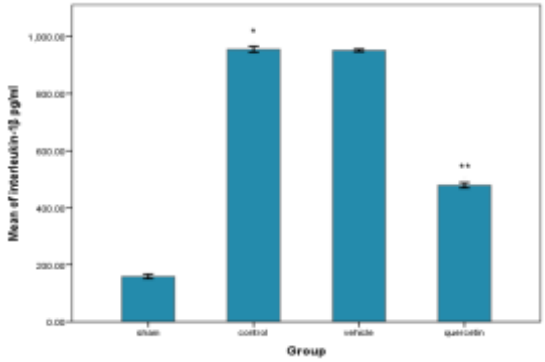

Figure 3.1 Error bar chart showing the effect of quercetin on renal IL-1β level (pg/ml) expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect of quercttin 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, quercetin significantly decreased the mean±SEM of renal level of caspase-3 (7.84±0.083, p value 0.001).

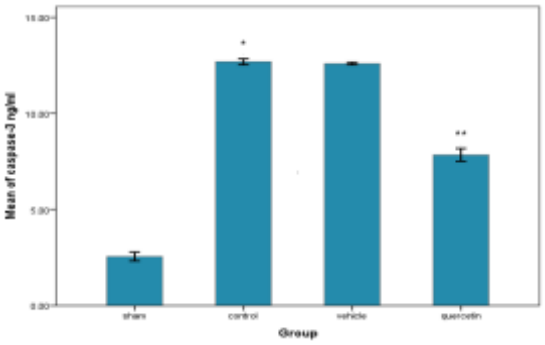

Figure 3.2 Error bar chart showing the effect of quercetin on renal caspase-3 level (ng/ml) expressed as mean ± SEM (6 animals in each group) (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect of quercttin 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, quercetin significantly decreased the mean±SEM of renal level of F2-isoprostane (139.80±0.696, p value 0.001).

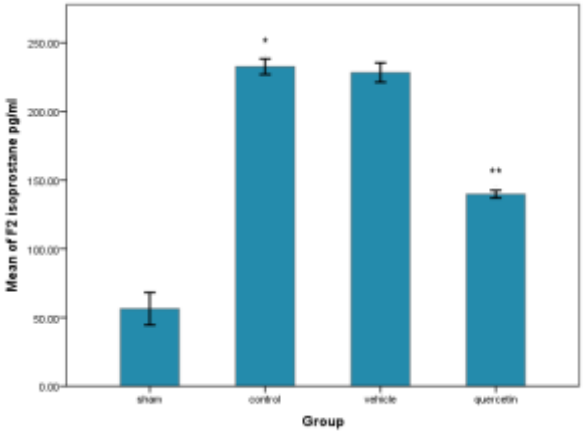

Figure 3.3 Error bar chart showing the effect of quercetin on renal F2-isoprostane level (pg/ml) expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect of quercttin on Akt /m TOR pathway

Effect on renal Akt level

The renal level of total Akt showed insignificant (p value >0.05) differences between the four experimental groups.

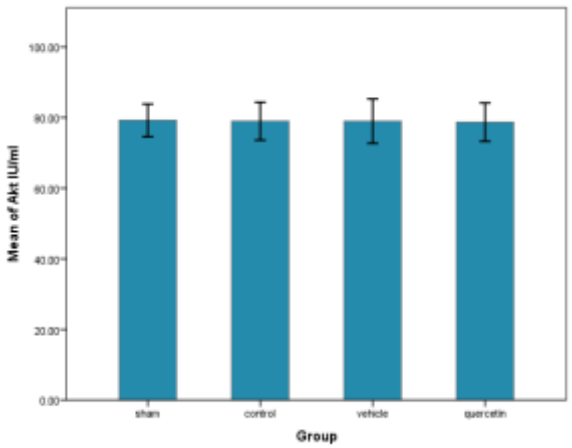

Figure 3.4 Error bar chart showing the effect of quercetin on renal Akt level (IU/ml) expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

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, quercetin significantly increased the mean±SEM of renal level of p-Akt (359.34±1.95, p value 0.001).

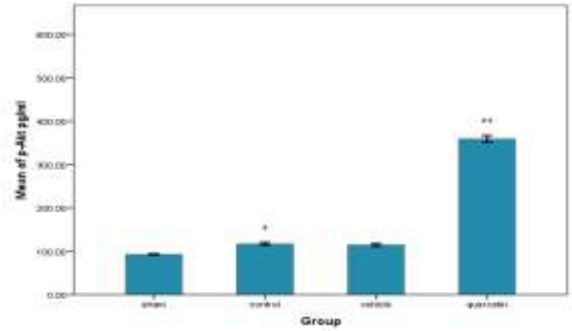

Figure 3.5 Error bar chart showing the effect of quercetin on renal p-Akt level (pg/ml) expressed as mean ± SEM (6 animals in each group).(* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

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, quercetin significantly increased the mean±SEM of renal level of p-Akt/Akt (4.57±0.073, p value 0.001).

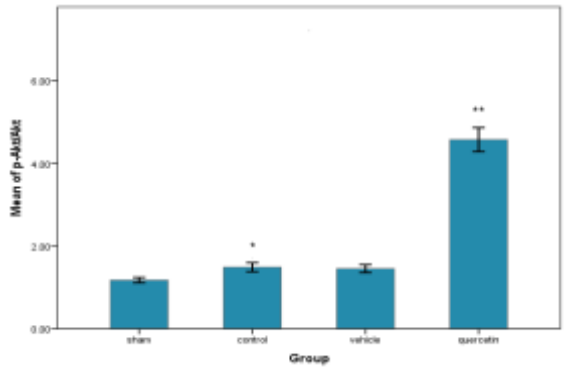

Figure 3.6 Error bar chart showing the effect of quercetin on renal p-Akt/Akt level expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect on renal m TOR level

The renal level of total mTOR showed insignificant (p value >0.05) differences between the four experimental groups.

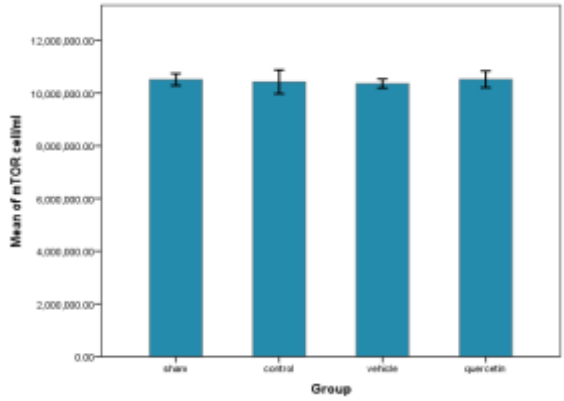

Figure 3.7 Error bar chart showing the effect of quercetin on renal mTOR level (cell/ml) expressed as mean ± SEM (6 animals in each group).(* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect on renal p-m TOR 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, quercetin significantly increased the mean±SEM of renal level of p-mTOR (3592833.15±13483.43, p value 0.001).

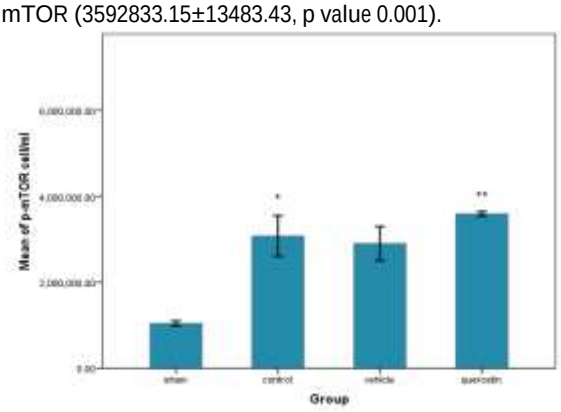

Figure 3.8 Error bar chart showing the effect of quercetin on renal p-mTOR level (cell/ml) expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect on renal p-m TOR / m TOR 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, quercetin significantly increased the mean± SEM of renal level of p-mTOR / mTOR (0.342±0.002, p value 0.001).

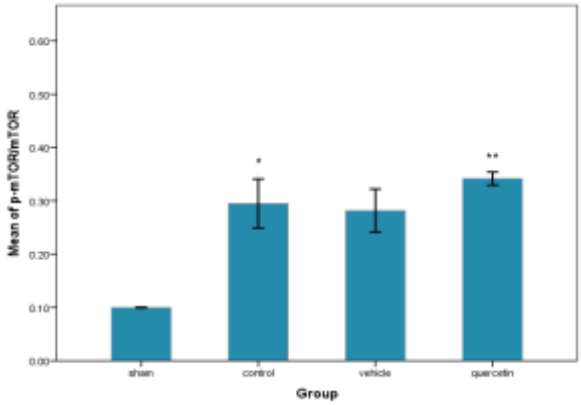

Figure 3.9 Error bar chart showing the effect of quercetin on renal p-mTOR / mTOR level expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect of quercttin 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, quercetin significantly decreased the mean±SEMof serum creatinine level (1.5300±0.0051, p value 0.001).

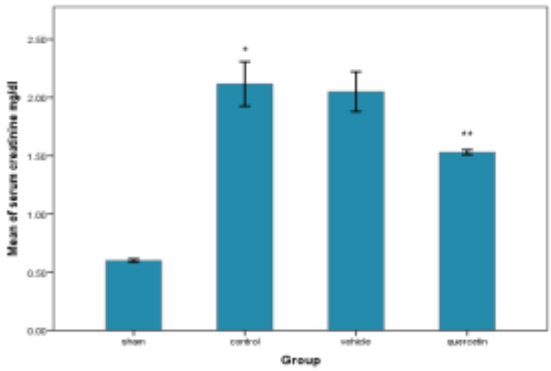

Figure 3.10 Error bar chart showing the effect of quercetin on serum creatinine level (mg/dl) expressed as mean ± SEM of six experimental groups (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

Effect on blood urea level

Control group significantly increased the mean±SEMof 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, quercetin significantly decreased the mean±SEM of blood urea level (62.83±0.4772, p value 0.001).

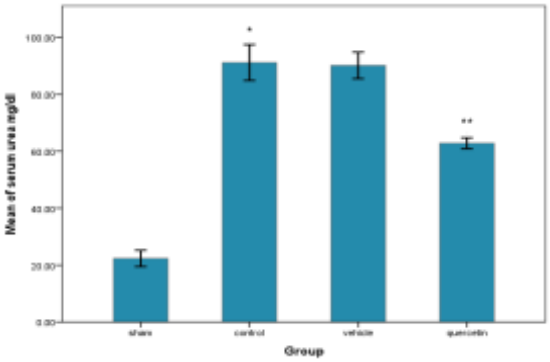

Figure 3.11 Error bar chart showing the effect of quercetin on urea level (mg/dl) expressed as mean ± SEM (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

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 severe tissue damages when compared to sham group. Pretreatment with quercetin resulted in significant (p<0.05) amelioration of renal tissue damages when compared with control group. Figure (3.12) showing histopathological scores of six experimental groups. Figure (3.13) showing normal renal structure in the sham group. Figure (3.14) and (3.15) showing significant tissue damages caused by renal IRI. Figure (3.16) showing the significant amelioration of tissue damage with pretreatment of quercetin.

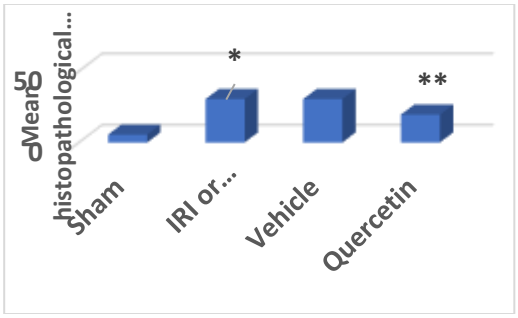

Figure 3.12 Bar chart showing the effect of quercetin on mean histopathological rank score (6 animals in each group). (* P value <0.05 when control compared with sham, ** P value <0.05 when quercetin compared with control).

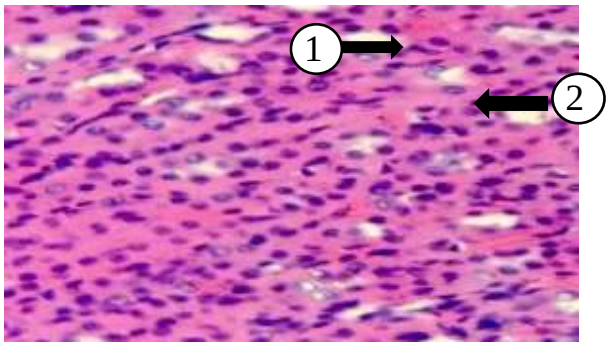

Figure 3.13 Photomicrograph of renal tissue section of sham group showing normal renal tubules (1) and (2), the renal section was stained with H and E with X40 magnification, the sevirity score 0.

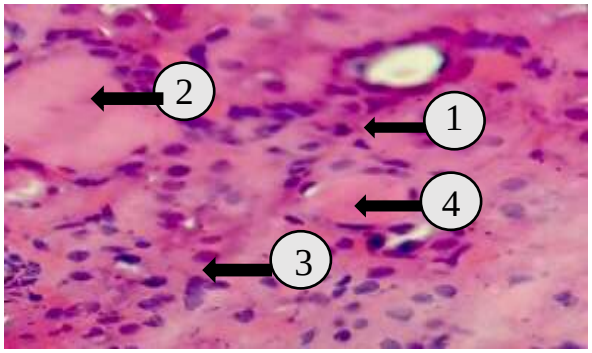

Figure 3.14 Photomicrograph of renal tissue section of control group showing the tubular cellular swelling (1) and (2), eosinophilic casts (3) and (4), the renal section was stained with H and E with X40 magnification, the sevirity score 3.

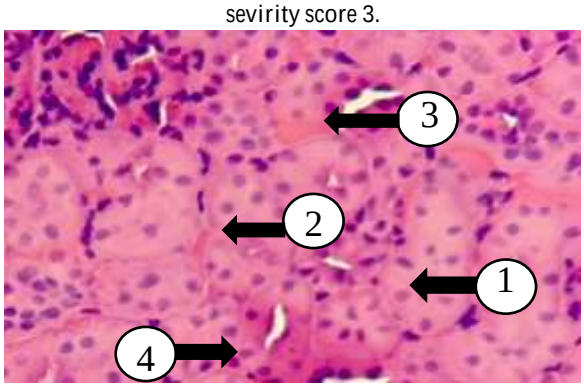

Figure 3.15 Photomicrograph of renal tissue section of vehicle group showing the tubular cellular swelling (1) and (2), damaged glomerulus (3) and karyolysis (4), the renal section was stained with H and E with X40 magnification, the sevirity score 3.

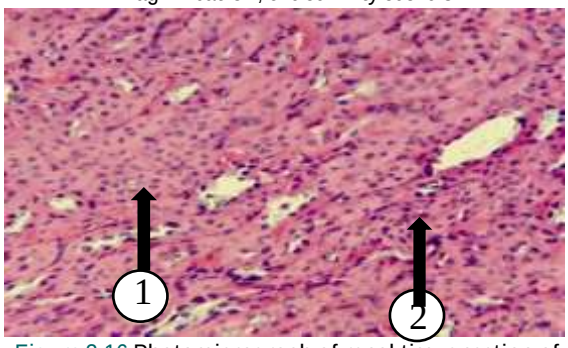

Figure 3.16 Photomicrograph of renal tissue section of quercetin treated group showing the normal renal tubules (1) and (2), the renal section was stained with H and E with X40 magnification, the sevirity score 1.

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 (43). Acute kidney injury develops from toxic, obstructive or ischemic insults, for example, renal IRI which is still connected with high morbidity and mortality (3) (37). 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 (44). 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 quercetin against experimental renal IR.

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 (45). The renal level of IL-1β in mice of IR group was significantly elevated in comparison with sham group (35). Neutrophils cell joins to the activated endothelial cell and aggregates in the kidney during IRI, as right on time as 30 minutes of the reperfusion phase. They generate ROS and inflammatory cytokines, which prompt vascular permeability and diminished the integrity of TEC triggering renal damage (46-47).

Effect of quercttin on inflammatory cytokine (IL-**1β**)

In this study, quercetin pretreatment significantly reduced pro-inflammatory cytokine (IL-1β) when compared with IR group. This result suggested that quercetin could alleviate renal IRI through anti-inflammatory effect. According to existing evidence quercetin could alleviate lead induced kidney injury through attenuation of IL-1β expressions which was upregulated in the rat kidney of lead treatment group (48). To the best of our knowledge, there is no information available about the influence of quercetin on inflammatory mediator (IL-1β) in renal IRI.

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. This outcome is in settlement with existing studythat confirmed the level of caspase 3 was markedly improved in control group when compared with sham group after renal IRI in rats model (45). 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 (49).

Apoptosis of TEC is an initiated aspect of renal IRI 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 (50)(51).

Effect of quercttin on apoptotic marker (caspase -3)

According to existing evidences, anti-apoptotic action of quercetin in term of decreasing caspase-3 had been reported in a retinal IR model by Arikan and followers who revealed that when quercetin administrated to rats previous to retinal ischemia, caspase-3 was significantly reduced compared to control group (52). Furthermore, when rats subjected to coronary artery clamping followed by reperfusion, caspase 3 was markedly elevated, while quercetin treatment diminished caspase 3 when compared to the IR group (31). Because quercetin treatment was founded to exert cardioprotection and neuroprotection in the IR model by anti-apoptotic effect, we thought that it may also be beneficial to protect kidney from I/R injury. Hence, we aimed to analyze this relation in the this study. Significant decrease in the level of caspase-3 noted in quercetin group compared with IR group in this study. Thus, it suggests for the first time that quercetin has potential nephroprotective effect in the IR induced renal damage by its anti-apoptotic effect.

Effect of renal IR 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 studywhich indicated that the level of F2-isoprostane significantly elevated in the renal transplantation indicating that oxidative stress is a critical response during this procedure (53). Furthermore, Carlström and followers exhibited that F2-isoprostane significantly elevated after unilateral nephrectomyof rats model (54). Previous studies reported that the underlying mechanisms of renal IRI are mainly by ROS production and oxidative stress (55-56).Clinical and experimental studies suggested that F2-isoprostane was the potent reliable parameter indicating oxidative stress (57).

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

In this study, it appeared that quercetin pretreatment significantly decrease F2-isoprostane compared with I/R group. This result clearly demonstrating the potential antioxidant effect of quercetin against renal IRI. Up until now, there may not be a prior study analyzing quercetin 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 study which 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 (38). Reoxygenation following hypoxia of renal tissue causes a transient activation of PI3K/Akt signaling pathway and thereby improving the proliferation of tubular cells (58).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 (59). 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 (34). Therefore, Akt considers as a potential pathway and if controlled by targeted therapy could be a potent alleviator of IRI.

Effect of quercttin on Akt activation

The outcomes of the previous study suggested that quercetin treatment attenuated myocardial I/R injury and modulated apoptosis in rats via activation of Akt signaling pathway and increased phosphorylated form of Akt (31). Treatment with quercetin could significantly protect against apoptosis and improve neurological function following cerebral ischemia by upregulation of Akt signaling pathway which is expressed as an increment of p-Akt level and equal Akt level when quercetin treated group compared with I/R group (60-61). However it is unclear whether the Akt pathway mediates the nephroprotection of quercetin. The present study confirmed for the first time that the renal level of p-Akt and p-Akt/Akt was increased significantly in quercetin treated group compared to control group. Whereas the renal tissue level of Akt was not significantly different when compared with control group.

Effect of renal IR on m TOR 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 is consistent with previous study that indicated a 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 (62). Lieberthal and followers proved that mTOR activity is absent or low in the normal renal tissue but elevates markedly after ischemia-reperfusion injury (63). 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 (64-65).

Effect of quercttin on m TOR activation

This study found a significant increase in p-mTOR renal level and p-mTOR/mTOR in the quercetin group in comparison to control group. Whereas the renal tissue level of mTOR was not significantly different in comparison to control group. This results demonstrated, for the first time, that quercetin exerts nephroprotection against renal IRI by activation of mTOR pathway.

Effect of renal IR on renal function

The present study demonstrated that serum level of urea and creatinine in control group was elevated in comparison to sham group. These results are consistent with previous study which revealed that renal I/R increased serum creatinine and BUN when compared to

sham group in rats model (66). Tan and followers found that the levels of serum creatinine and BUN were both significantly elevated in the IR rats compared to sham group (67).

Effect of quercttin on renal function

The present study showed that pretreatment with quercetin significantly decreased urea and creatinine serum level in comparison with control group, demonstrating that quercetin was maintaining renal function after IRI. These results are consistent with the prevailing study showing that treatment of rats with quercetin substantially reduced the levels of BUN and creatinine associated with IRI (68). Creatinine and BUN elevated in experimentally induced chronic kidney disease. However, treatment with quercetin resulted in a lowering of creatinine and blood urea nitrogen (69).

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 (70).

Effect of quercttin on renal parenchyma

In this study, quercetin treatment caused decline in renal tissue damage when compared with control group. This result established the protective role of quercetin against renal IRI through histopathological findings. These consequences are in agreement with prevailing study showed that renal I/R caused extensive changes in liver and kidney tissues. I/R group showed loss of brush borders, enlargement of Bowman's space and necrosis of the hepatic and renal epithelial cells. However, quercetin treatment improved hepatic and renal histopathological damages in comparison with I/R group (71).

CONCLUSION

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

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