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Insight into the role of Nrf-2/HO-1 hub in the protective effect of colchicine on renal ischemia-reperfusion induced distance organs dysfunction

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Abstract: Background: Injury of the kidney due to ischemia-reperfusion (IR) provokes damage in remote organs. The anti-inflammatory and antioxidant activities of colchicine have previously been proven. Objective: This study planned to assess the renal and cardiac dysfunctions during different reperfusion intervals after renal ischemia and explore the protective effect of colchicine focusing on the role of the Nrf-2/HO-1 axis. Materials and Methods: Thirty-two rats divided into four groups (8 rat/group) were used to investigate the effect of ischemia and different periods of reperfusion. Then, another thirty-two rats were divided into four groups (8 rat/group); sham, 45 minutes I/24 hours R, 45 minutes I/24 hours R / colchicine, colchicine, and examine the antioxidant and inflammatory status. Results: Renal IR worsens the kidney and cardiac functions, exaggerates lipid peroxide formation, and histopathological alterations markedly after (45 minutes I/24 hours R). Colchicine pretreatment recovers the kidney and cardiac functions markers and lessening lipid peroxidation. Moreover, renal IR significantly decreased the nuclear factor erythroid 2- related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1) expressions which alter the oxidant status and induce inflammation by increase the inflammatory mediator tumor necrosis factor-alpha (TNF-α). Colchicine pretreatment before renal IR significantly altered this insult by increase the levels of Nrf-2 and HO-1 which subsequent increment in TNF-α level. Conclusion: the vulnerability of the heart to injury after renal IR varies with the reperfusion period. In addition, colchicine conferred renal and cardioprotective effect against renal IR through its anti-inflammatory and antioxidant activities by modulation of Nrf-2/HO-1 hub.

Keywords: Ischemia-reperfusion, Cardiac injury, Renal injury, Oxidative stress, Rats, Colchicine, Nrf-2/HO-1

1. INTRODUCTION

Kidneys receive 20% of the cardiac output and are highly vulnerable to ischemia. Renal ischemia-reperfusion (IR) occurs due to a temporary decrease of the blood supply of the kidney accompanied by perfusion and re-oxygenation. Organ transplantation, cardiac arrest, and shock are frequently associated with renal IR injury [(1)]. One of the complications of renal IR is acute kidney injury (AKI) which is associated with decreased kidney functions and increase morbidity and mortality [(2, 3)].

IR injury is widely known to occur in two stages. The first stage is ischemia, which causes tissue damage via depletion of adenosine triphosphate (ATP), increase anaerobic metabolite, and excessive development of reactive oxygen species (ROS), with subsequent cellular dysfunction and death [(4)]. Since reperfusion is the second stage in which the hypoxic insult is reduced, it results in a particular form of injury response, typically referred to as "reperfusion injury" [(5)] .The kidney plays a decisive role in the preservation of homeostasis, that kidney disease can affect almost everybody systems [(6)]. However, heart failure has been found to be the main issue of life loss in this clinical condition, rather than renal failure itself [(7)]. AKI associated with cardiac dysfunction has been accompanying by decrease myocardial activity, hypoxia, oxidative stress, and renal IR-induced inflammation [(8)]. Reperfusion-induced changes in the thickness of the cardiac capillaries, loss of vasoregulatory function, initiation of neutrophils, the liberation of proteolytic enzymes, and ROS generation contribute to the interactions between blood components and vascular endothelium [(9)],viz., renal IR made damage to the heart as a remote organ. In addition, it was stated that

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the injures vary widely according to the reperfusion time [10, 11]).

Different mechanisms are mentioned to be implicated in remote organ incompetence such as oxidative stress and inflammation, though their exact pathophysiological character is not totally comprehended [12]. Nuclear factor erythroid 2-related factor 2 (Nrf-2) is referred to as the ‘‘main organizer’’ of the antioxidant response through the antioxidant response elements (AREs) and acts as a defensive transcription factor in IR injury [13]). When cells are attacked by ROS, Nrf-2 is quickly transported to the nucleus, phosphorylated, then phosphorylated Nrf-2 merges with AREs, which stimulate the expression of heme oxygenase 1 (HO-1) (14). HO-1 is a significant endogenous antioxidant and represents a vital protection system. HO-1, and its metabolites, can stop extreme oxidation of lipids and proteins by tracking ROS (15).

When Nrf-2 is stimulated in the nucleus, it moves toward the production of antioxidant enzymes which catch a lot of free radicals per second (16). Nrf-2 has been proposed to be the center of defense against oxidative stress in the pathophysiology of renal IR injury (17). In injured kidney, Nrf-2 and its associated target genes were upregulated, so stimulation of Nrf-2 hub would protect against IR injury (18). So, it was hypothesized that pharmacological activation of Nrf-2 might protect against IR injury.

Colchicine is a microtubule-disrupting agent that has anti-inflammatory and antioxidant features (19, 20) formerly used in the management of several inflammatory diseases including acute gout, Behcet’s disease, Mediterranean fever, and recurrent pericarditis (21). A few studies illustrated the effect of colchicine regarding different types of IR injury, skeletal muscle (20), testicular (22), and ovarian (19) IR injuries. The role of colchicine in cardiac injury following renal IR injury has not been addressed so far. Hence, according to the previous data, the present study was designed to assess the proper time interval of renal IR required to induce remote organ dysfunction (cardiac injury) by histological and biochemical examinations. Also, it is interesting to determine whether colchicine has a protective effect against these renal and cardiac dysfunctions induced by IR injury and unveil the possible role of the Nrf-2/HO-l axis.

2. Material and METHODS

2.1 Animals

Sixty-four male Sprague Dawley rats (200–250 g) were used in study, thirty-two rats used in each part. All rats were housed under controlled temperature conditions and a 12-hour lighting cycle. Rats were given free access to standard rat chow and water. Treatment of animals complies with the main guides of usage of laboratory animals, and the guides of the Ethics Committee affiliated to the College of Pharmacy, Al-Azhar University, Egypt (No. 142).

2.2 Drugs and chemicals

Colchicine used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA). It was dissolved in saline. All chemicals and solvents used in this study are of the commercially available excellent grade.

2.3 Inducement of renal ischemia-reperfusion

Following the method described by Chok et al, inducement of bilateral renal IR was carried out [23]. Using a warming pad, animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally). A midline abdominal cut was done, and the two renal pedicles were showing with minimal dissection and occluded for 45 minutes with the help of surgical clamps. After 45 minutes of ischemia, the surgical clamps were detached to permit blood flow (reperfusion period) for 2, 4, and 24 hours of reperfusion. In order to overcome the extreme loss of fluids during the induction of renal ischemia, the abdomen was momentarily closed. Within 5 minutes of the removal of the arterial clip, the color of the kidney was turned from dark purple to reddish-brown which is an indication of effective restoration of blood perfusion. All rats were carefully noticed till complete recovery from anesthesia.

2.4 Experimental design

This work is divided into two parts; the first part to assess whether the reperfusion time is a major factor in the cardiac injury following renal IR injury and to determine the most suitable reperfusion time for the second part of the study to illustrate the protective effect of colchicine. In the first part, thirty-two rats were divided into four groups (8 rats/each); group I (Sham): rats underwent all surgical procedures without any surgical inference, group II (45 minutes + 2 hours): rats exposed to renal ischemia for 45 minutes then 2 hours of reperfusion; group III (45 minutes + 4 hours): rats exposed to renal ischemia for 45 minutes then 4 hours of reperfusion; group IV (45 minutes + 24 hours): rats exposed to renal ischemia for 45minutes then 24 hours of reperfusion. Blood samples, kidney and heart tissues are used in this part to determine the renal and cardiac functions, lipid peroxide formation as well as histological examination as discussed later. In the second part, another thirty-two rats were divided into four groups (8 rats/each); group I (Sham): rats underwent all surgical procedures without any surgical inference, group II (IR): rats exposed to renal ischemia for 45 minutes then reperfusion for 24 hours, group III (IR + Colchicine): rats received colchicine (60 µg/kg) intraperitoneally for 7 successive days then rats were exposed to renal IR (as in group II), group IV (Colchicine): rats

received colchicine (60µg/kg) intraperitoneally for 7 successive days [24].

2.5 Collection and preparation of samples

After completion of the reperfusion period, blood samples from all animals were centrifuged at 4000 rpm for 15 minutes at 4 °C. After collection, the serum was used for the determination of kidney function tests and cardiac enzymes. After blood collection, all animals were euthanized, and the kidney and heart of each rat were removed. One part of renal and cardiac tissues was taken and fixed in 10% phosphate-buffered formalin for histopathological examination, and another part was homogenized using ice-cold saline to obtain the tissue homogenate. The third portion kept frozen at -80°C for Western blot analysis.

2.6 Renal functions assessment

According to the methods illustrated by Tabacce et al and Yaung and Fridman [25, 26] and using the colorimetric assay kits (Biomed-diagnostics, Cairo, Egypt), blood urea nitrogen (BUN) and serum creatinine (SCr) were detected respectively.

2.7 Estimation of cardiac functions

Serum creatine kinase MB (CK-MB) was determined by colorimetric assay kit (Biomed-diagnostics, Cairo, Egypt) following the method described by Gerhardt and waldenstorm [27] , and cardiac troponin I (cTn-I) was determined by an enzyme-linked immunosorbent assay kit (Mybiosource, USA) and the evaluations followed the manufacturer's advice Catalogue number: MBS727624.

2.8 Determination of lipid peroxide formation

Lipid peroxidation in cardiac and renal homogenates measured as malondialdehyde (MDA) contents was detected spectrophotometrically following the manufacturer's instructions mentioned by Ohkawa et al [28] by using the thiobarbituric acid reactive substances method.

2.9 Determination of inflammatory marker (TNF-α)

Renal and cardiac contents of tumor necrosis factor-alpha (TNF-α) were determined by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, USA) and followed the manufacturer's advices Catalogue number: RTA00

2.10 Western blot determination of Nrf-2 and HO-1

Renal and cardiac contents of nuclear factor erythroid 2- related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1) were detected by western blotting technique according to the method described by Harlow and Lane [29]. The Bradford method was used to determine protein concentrations. SDS-PAGE gel electrophoresis was used to separate proteins, which were then transferred to nitrocellulose membranes. The primary antibodies used are for Nrf-2 (Catalogue # PA5-27882**,** Thermo Fisher Scientific, USA), HO-1 (Cat #MA5-32042, Thermo Fisher Scientific, USA). A secondary horseradish peroxidase conjugated antibody (Thermo Fisher Scientific, USA; Catalog # G-21234) was used to visualize the protein-antibody complex. X-ray films were used to capture the chemiluminescence produced. A luminescent image analyzer (FUJIFILM LAS-4000, Japan) was used to scan the films, and bands were quantified using Image J software. For Nrf-2 and HO-1, the results were expressed as arbitrary units after normalization for β-actin protein.

2.11 Histopathological assessment

In 10% buffered formalin, renal and cardiac tissues were fixed for 24 hours. Then, dehydrated in different grades of alcohol, cleared in xylene, and embedded in paraffin wax. Using hematoxylin and eosin (H&E) stain, the paraffin sections (4 µm) were stained. To avoid bias, the cells were examined by a blinded pathologist using a light microscope.

3. RESULTS

3.1 Effect of different intervals of reperfusion on renal functions following renal ischemia

Induction of renal ischemia in experimental animals for 45 minutes followed by different intervals of reperfusion (2, 4, 24 hours) resulted in significant deterioration of renal functions manifested as elevated levels of SCr (Fig 1A) and BUN (Fig 1B) in comparison with the sham group, at $p < 0.05$. The highest growth in SCr and BUN levels was observed at (24 hours R/ 45 minutes I) as compared to the sham group.

3.2 Effect of different intervals of reperfusion on cardiac functions following renal ischemia

Induction of renal ischemia in experimental animals for 45 minutes then different intervals of reperfusion (2, 4, 24 hours) resulted in a significant raise in serum levels of CK-MB (Fig 1 C) and cTn-I (Fig 1 D) at 24 hours of reperfusion as compared to the sham group, at $p < 0.05$. There is no significant difference detected in cardiac functions at (2 and 4 hours) of reperfusion compared to the sham group.

3.3 Effect of different intervals of reperfusion on renal and cardiac contents of MDA following renal ischemia

Animals exposed to renal ischemia (45 minutes) and various periods of reperfusion (2, 4, 24 hours) showed significantly elevated levels of renal MDA, a marker of oxidative stress and lipid peroxidation as compared to the sham group (Fig 2 A). In contrast, cardiac MDA level was significantly increased at 24 hours reperfusion in comparison with the sham

group. There is no significant difference observed in cardiac contents of MDA at (2 and 4 hours) of reperfusion as compared to the sham group (Fig 2 B), all are carried out at $p < 0.05$.

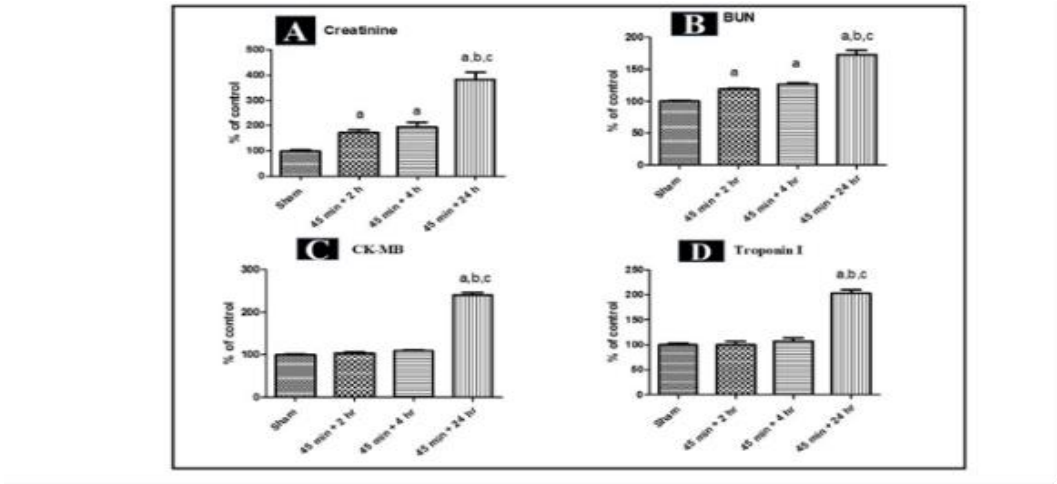


Figure 1: Influence of different intervals of reperfusion on serum creatinine (A), blood urea nitrogen (B), creatin kinase-MB (C) and cardiac troponin I (D) following renal ischemia

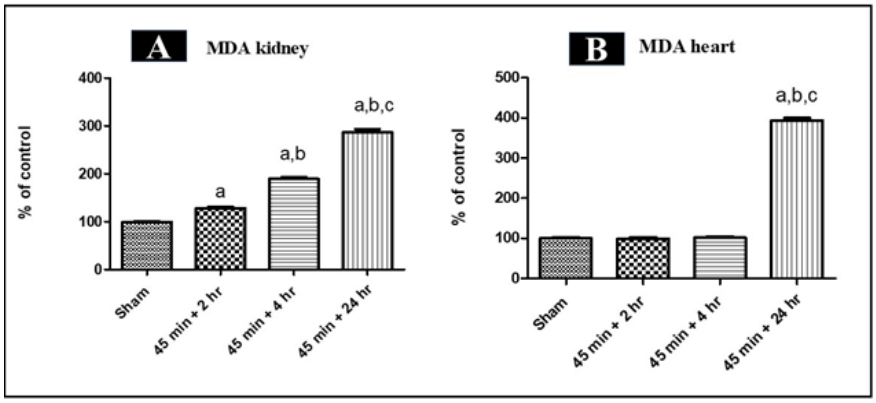


Figure 2: Effect of different intervals of reperfusion on renal (A) and cardiac (B) contents of MDA following renal ischemia

3.4 Histopathological alterations of the renal and cardiac tissues

Sections of kidney and heart tissue from the four studied groups were examined. As shown in Fig (3 A), the kidney of the sham group showed the normal histological structure of the glomeruli and tubules of the cortex. Renal ischemia for 45 minutes followed by 2 h of reperfusion showed periglomerular as well as perivascular hemorrhages associated with dilatation of the blood vessels and degeneration in the tubular lining epithelium at the cortical portion also multiple focal hemorrhages in between the degenerated tubules at the corticomedullary junction. After four h of reperfusion, the cortex showed congestion in the tufts of the glomeruli with perivascular hemorrhages. Focal hemorrhages were detected in between the degenerated tubules at the corticomedullary junction. The greatest damage was observed in kidney sections of animals subjected to reperfusion for 24 hours, which showed swelling and degeneration of the tubular lining epithelium and cystic dilatation in the tubules at the corticomedullary portion. On the other hand, Fig (3 B) showed extensive changes occur in heart tissues after 24 hours of reperfusion, where degeneration and swelling were noticed in the cells of the myocardium, in comparison with the sham group. However, after 2 hours and 4 hours of reperfusion, no structural alteration in heart tissue was observed.

3.5 Colchicine maintains the renal and cardiac functions following renal IR

Rats exposed to renal artery occlusion for 45 minutes followed by 24 hours reperfusion exhibited significant increase in serum levels of BUN and CK-MB compared to sham group, while colchicine administration before renal ischemia significantly declined the serum levels of BUN and CK-MB compared to IR group at $p < 0.05$, as shown in Fig (4 A, 4 B) respectively.

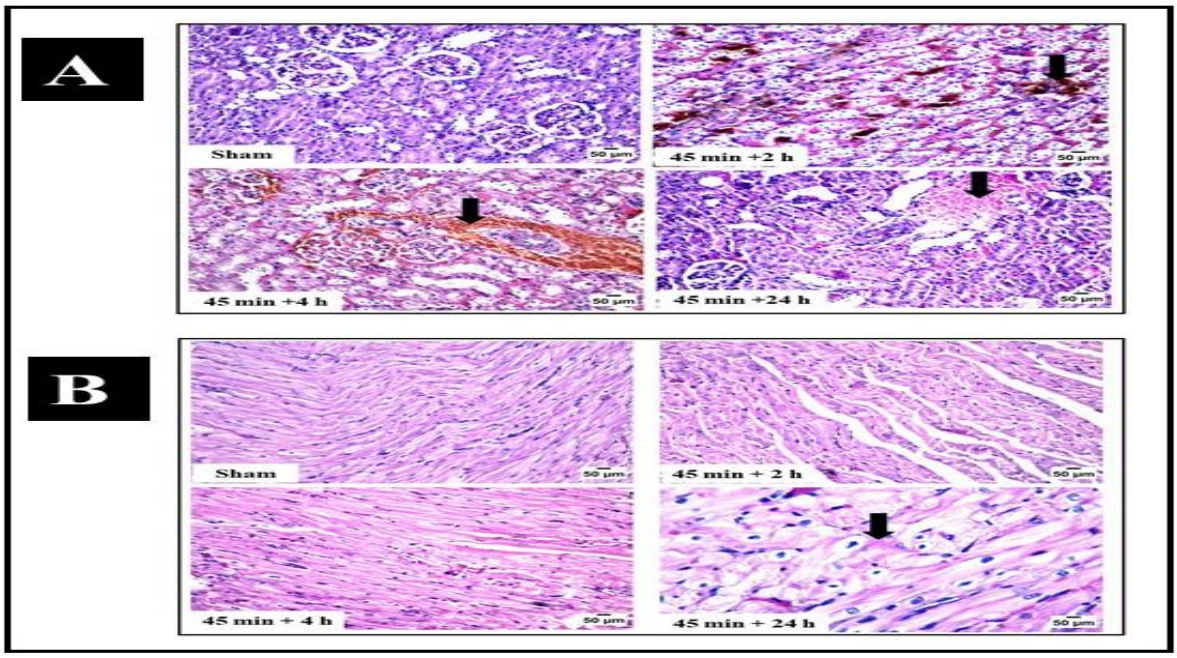


Figure 3: Transverse sections of different rat kidney (A) and heart (B) sections stained by H&E (X40)

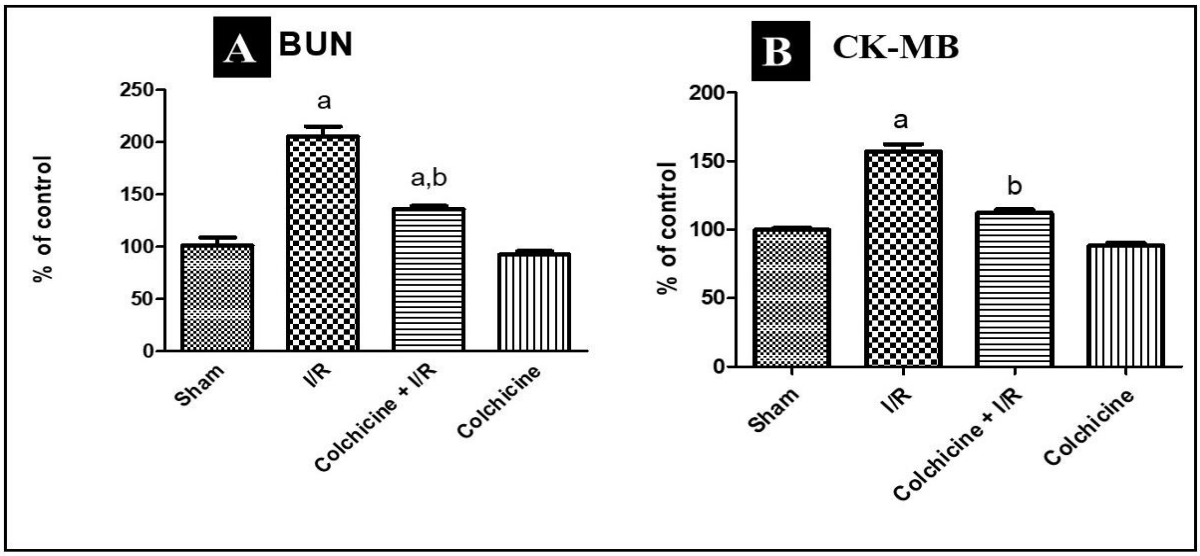


Figure 4: Effect of colchicine on serum level of BUN (A) and CK-MB (B) following renal ischemia-reperfusion

3.6 Colchicine decreased lipid peroxidation and inflammation in renal and cardiac homogenates following renal IR

Renal ischemia for 45 minutes and reperfusion for 24 hours significantly increased renal and cardiac contents of MDA compared to sham group, while pretreatment with colchicine before renal ischemia significantly decreased MDA contents in both renal and cardiac homogenates compared to IR group at $p < 0.05$, as shown in Fig (5 A). In addition, Fig (5 B) displays the inflammatory effect induced by IR where the inflammatory mediators TNF-α significantly increased in both homogenates after renal IR (45 minutes I/24 hours R). As a consequence of decreased lipid peroxidation, colchicine pretreatment decreased inflammation which manifested by decreased TNF-α contents in renal and cardiac muscles compared to IR group at $p < 0.05$.

3.7 Colchicine attenuates renal and cardiac dysfunctions after renal IR through Nrf-2/HO-1 hub

Renal artery occlusion in rats for 45 minutes followed by 24 hours reperfusion induced redox

imbalance in renal and cardiac muscle. Nrf-2 as a regulator of the antioxidant system in conjugation with its downstream regulator HO-1 were affected by renal IR. IR (45 minutes I/24 hours R) significantly decreased renal and cardiac contents of Nrf-2, HO-1 compared to sham group, while administration of colchicine before renal IR significantly increased renal and cardiac contents of Nrf-2, HO-1 compared to IR group at $p < 0.05$, as shown in Fig (6, 7) respectively.

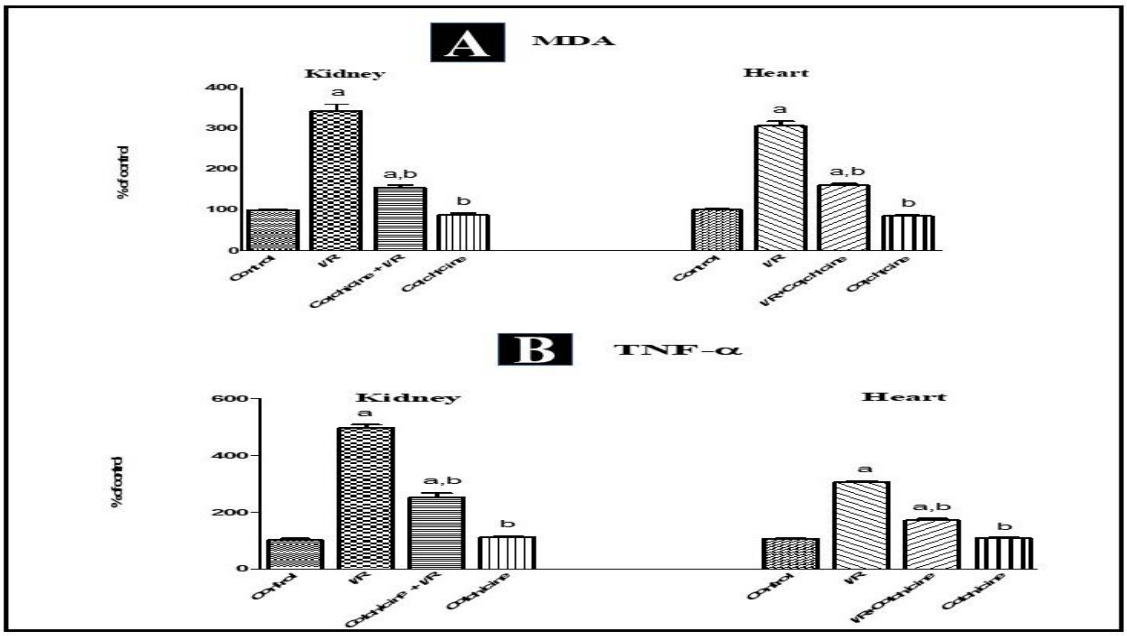


Figure 5: Effect of colchicine on renal and cardiac contents of MDA (A) and TNF-α (B) following renal ischemia-reperfusion

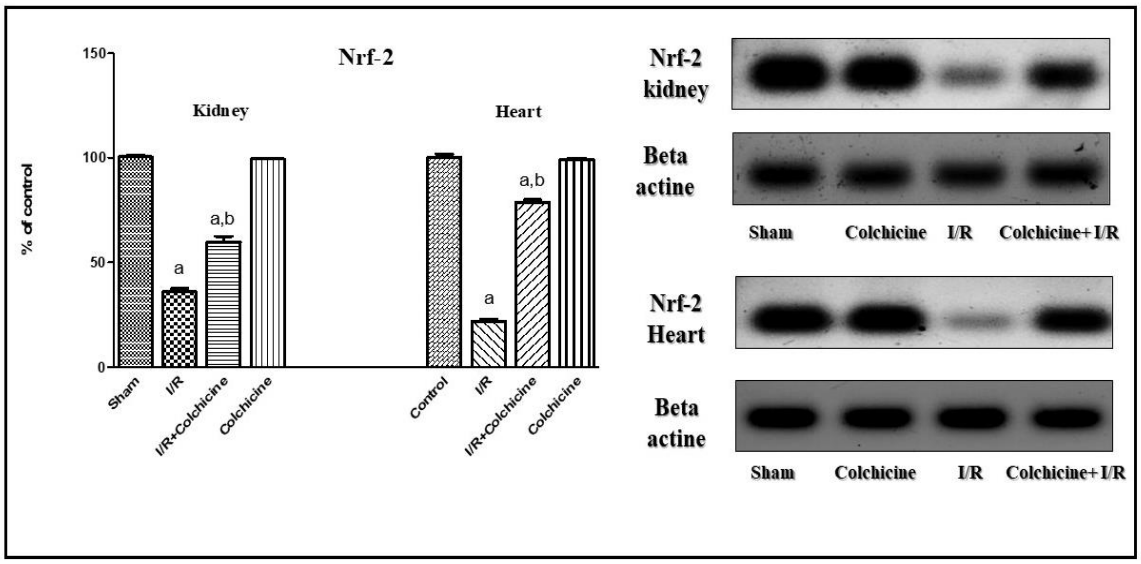


Figure 6: Effect of colchicine on renal and cardiac contents of nuclear factor erythroid 2- related factor 2 following renal ischemia-reperfusion

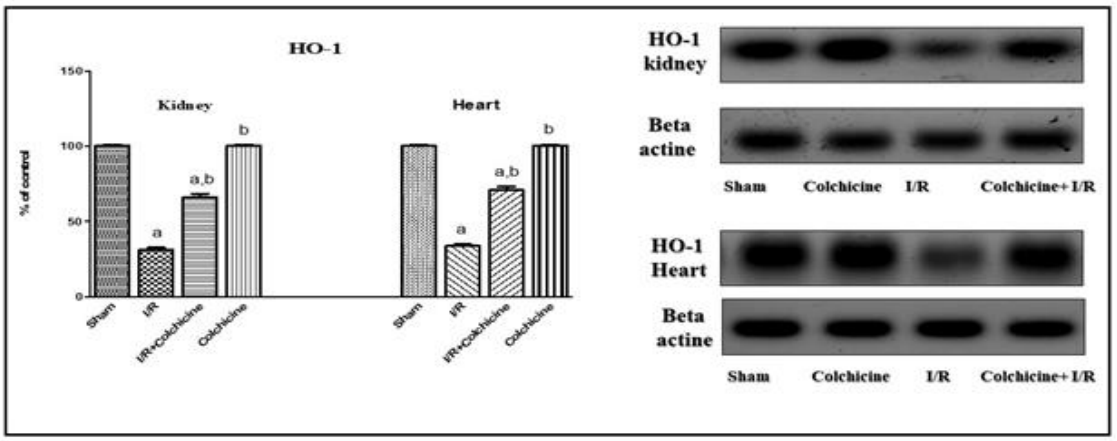


Figure 7: Effect of colchicine on renal and cardiac contents of heme oxygenase-1 following renal ischemia-reperfusion

4. Discussion

In IR injury, the blood source to an organ is limited then restored and the organ is re-oxygenated by the blood again. Renal IR damage occurs in a clinically relevant setting, such as hypotension, cardiac bypass surgery, transplantation of kidney with subsequent AKI [30]. It reviewed in the literature that renal IR injury is a pathological event that starts with ischemia, continues with oxidative stress, and progresses with inflammation [31].

In clinical practice, renal IR damage is a general organ disorder leading to changes in kidney functions in addition to induction of some organ dysfunctions. It may cause damage to the liver, pancreas, lungs, and heart. The cardiac tissue is considered the most sensitive tissue to renal IR with subsequent changes either pathologically or physiologically [32].

It has been reported that the sudden reperfusion of blood can lead to more and more exaggeration of injury which is not due to the first ischemia and can be modified by different treatments in the reperfusion period [10]. Seifi et al [11] also reported that the injures vary depending on the different time intervals of reperfusion. Several causes, mediators, and effectors are involved in the upregulation of endothelium adhesion molecules, transendothelial inflammatory cell migration, tissue edema, infarction, and apoptosis [11].

Ischemic AKI is a clinical and experimental syndrome with substantial decreases in the rate of glomerular filtration, necrosis of the tubular cells, severe tubular damage, glomerular injury and obstruction of tubules with cell debris [33]. In the present study regarding the effect of different reperfusion periods, there is a marked increase in BUN and SCr at different time intervals of reperfusion (2, 4 and 24 hours), which is consistent with other studies that showed significant increases in kidney function tests as evidence of renal damage after renal IR injury for different time intervals [34-36].

Concerning the cardiac injury following the renal IR, in the present study significant increase in the levels of serum troponin I and CK-MB following renal IR were observed only in reperfusion for 24 hours, which is an indicator of remote heart injury. Cardiac troponin I and CK-MB are vital markers in patients with renal failure [37]. These results confirmed by a previous study showed cardiac damage after renal IR in hyperlipidemic rats [38]. There is no significant difference was observed in the cardiac markers test after 2 hours and 4 hours of reperfusion.

During renal ischemia, generation of free radicals occur, however, the amount of free radical production is much higher in the reperfusion period, which subsequently leads to lipid peroxidation and increases the damage [39]. MDA is a good indicator of lipid peroxidation [40]. In the present study, all three periods of reperfusion caused an increase in renal MDA levels, but the increase after 24 hours was significantly very high and demonstrated more significant differences from the sham and other reperfusion groups; this result confirmed by previous studies [41, 42]. On the other hand, cardiac MDA showed a significant increase after 24 hours of reperfusion, while reperfusion for 2 hours and 4 hours showed no significant difference compared to the sham group. This result showed that there was cardiac damage caused by renal IR injury. The present results were following previous study which revealed a significant elevation in oxidative stress parameters due to myocardium injury after renal IR injury [7].

The histopathological examination of kidney and cardiac tissues confirms the present biochemical findings, which reveal that renal damage associated with renal IR leads to swelling and degeneration of the tubular lining epithelium and cystic dilatation the tubules at the corticomedullary portion, which is more severe after 24 hours reperfusion. Histopathological examination of the heart reveals that, 24 hours reperfusion-induced extensive changes

in heart tissues with degeneration and swelling were noticed in the cells of the myocardium. These histopathological changes confirmed by Nezamoleslami et al [42] who found that kidney tissues after renal IR injury showed tubular injury, interstitial hemorrhage, edema, and infiltration of lymphatic cells. Also, Alihemmati et al [32] found that renal IR injury-induced cardiac damage revealed abnormalities in cardiac muscles and growing connective tissue in the myofibril.

Depending on the documented literatures [10, 11] and all the previous mentioned results, we proved that the reperfusion period is an important factor to induce remote organ dysfunction (heart injury) and detect the proper time of reperfusion (24 hours) to assess the reno-cardioprotective effect of colchicine after renal IR.

Considering the favorable anti-inflammatory and antioxidant properties of colchicine [19, 20], the present study was aimed to investigate the protective effect of colchicine on cardio-renal dysfunction after renal IR injury. Colchicine has demonstrated a protective effects in several kidney models of crescentic nephritis [43], chronic cyclosporine nephrotoxicity [44], diabetic nephropathy [45], and hypertensive chronic kidney disease [46]. Pretreatment of colchicine significantly decreased the elevation observed in BUN after 45 minutes ischemia /24 hours reperfusion, which is in accordance with the results obtained from Sabry et al [47] which suggest that colchicine diminish kidney function markers in cyclosporine treated rats which induced nephrotoxicity. Colchicine pretreatment attenuated cardiac damage induced by renal IR injury, and significantly decreased CK-MB that elevated after 45 minutes ischemia/ 24 hours reperfusion. Deftereos, Giannopoulos [48] reported the decrease in serum creatine kinase as well as, decreasing the infarcted size in a patient cohort treated with colchicine in the acute myocardial infarction.

Nrf-2 is an vital factor in the organizing of several antioxidants, which keep the equilibrium of oxidation and reduction, and hinder apoptosis and inflammation [49]. When activated by ROS, Nrf-2 translocate into the nucleus and binds to antioxidant response components, causing phase II cytoprotective enzymes like HO-1 to be upregulated [50, 51]. Additionally, Nrf-2 activation improve the defense system against oxidative stress in tubular cells during the early and late stages of injury [52]. In cardiac ischemia-related injury, Nrf-2 and its target genes have been shown to protect the heart via the Nrf-2 pathway, certain antioxidants protect the heart from ischemia-induced cardiac injury. [53]. In consistent with these studies, our study revealed that renal and cardiac contents of Nrf-2 and HO-1were significantly decreased after renal IR injury.

Colchicine pretreatment increased renal and cardiac Nrf-2 and HO-1 contents compared with the IR group, suggesting that antioxidant effect of colchicine may protect kidney and heart from damage induced by renal IR injury. These data are in accordance with Dinesh and Rasool [54], who revealed that colchicine pretreatment significantly upregulated mRNA levels of transcription factor Nrf-2 and its phase II cytoprotective enzymes HO-1 in monosodium urate crystal stimulated RAW 264.7 macrophages. Recently, Awad et al supported the role of Nrf-2/HO-1 hub in the protective effect of colchicine IR where they reported that colchicine decreased renal IR injury induced liver damage in rats by over activation of Nrf-2 and HO-1 [55].

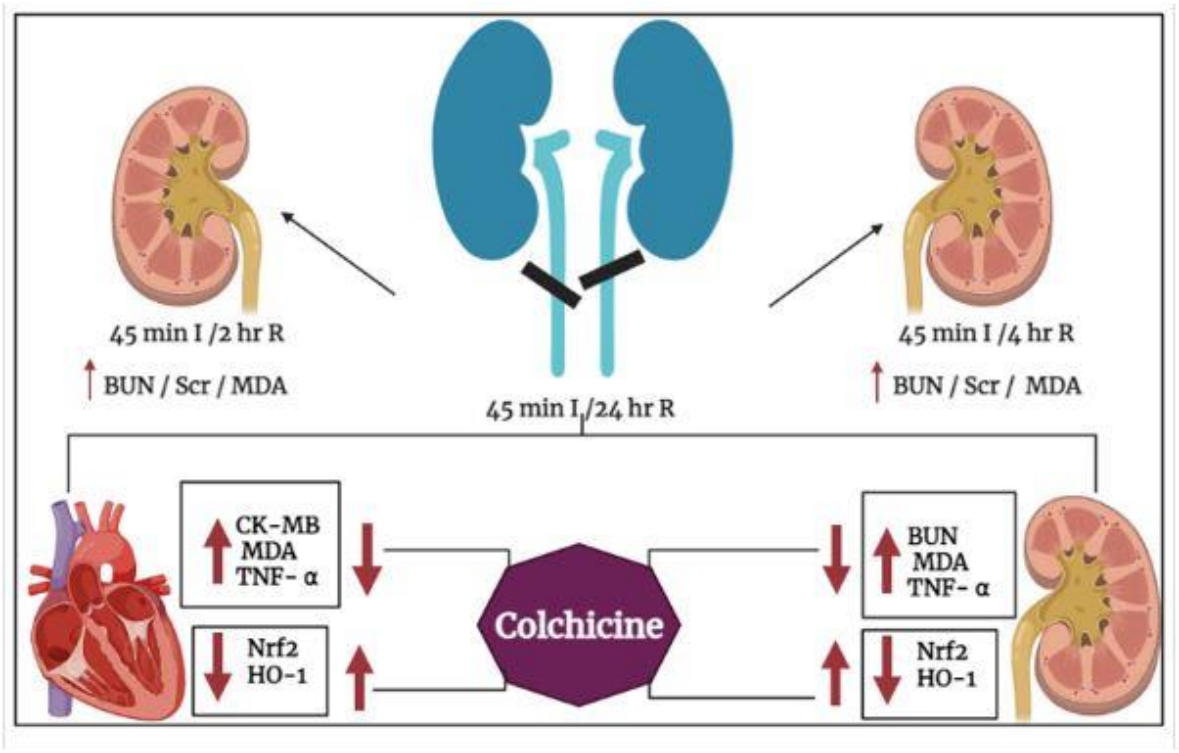
Moreover, due to the modulatory effect colchicine on Nrf-2/HO-1 and restore the balanced redox state, colchicine significantly reduced the elevation observed in renal and cardiac contents of MDA. The potential of colchicine to control oxidative stress has been widely explored [56]. Colchicine has been shown in previous studies to protect against lipid peroxidation by scavenging free radicals and reducing the risk of oxidative complications in albino rats with diclofenac sodium mediated hepatorenal toxicity [57]. Colchicine has protective role on preventing IR injury in testicle torsion animal model by suppressing MDA the important index of oxidative stress [58].

Inflammation is an essential pathophysiological progression in IR induced renal injury [30]. ROS production, neutrophil infiltration, and tubular cell stimulation all can activate the inflammatory cascade. In turn, proinflammatory cytokines, can encourage the localized tissue injury to remote injury through the neutrophil activation and infiltration [59]. Ischemic renal injury causes the release of inflammatory cytokines that primarily target the heart and cause circulatory changes as a result of the immune system's participation. [60]. Colchicine pretreatment protect heart and kidney from damage induced by renal IR, by decreased renal and cardiac contents of TNF-α, This is in line with a study that found colchicine can protect the lungs from LPS-induced lung damage in rats by suppressing TNF- α production [61], also Ozdemir et al [56] reported that colchicine protect against hyperoxia-induced lung injury in neonatal rats by suppressing TNF- α production.

Collectively. this data showed that Nrf-2 deficiency with its downstream regulator HO-1 exacerbated oxidative stress, inflammatory response, renal and cardiac damage, while pretreatment with colchicine augments Nrf-2/HO-1 cue which significantly inhibited oxidative stress, TNF-α induction, increment of MDA level, as well as the impaired kidney and heart functions caused by IR.

5. CONCLUSIONS

The fundamental outcomes of the present study illustrated that different time intervals of renal IR caused renal damage. Additionally, renal IR injury was associated with significant cardiac damage at 24 hours of reperfusion. Overall, the susceptibility of the heart to injury after renal IR vary with reperfusion time. In addition, the study unveils the role of Nrf-2/HO-1 trajectory in the antioxidant and anti-inflammatory effects of colchicine against renal and cardiac damage following renal ischemia and reperfusion.



Schematic diagram illustrates the different time intervals of renal IR caused renal damage, and the role of Nrf-2/HO-1 hub in the antioxidant and anti-inflammatory effects of colchicine against renal and cardiac damage following renal IR.

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Compliance with ethical standards: The research was performed in line with the ethical rules and policies set out by the Ethics Committee of Faculty of Pharmacy, (Girls), Al-Azhar University (No. 142) that is compatible with the NIH guidelines for laboratory animals.

Conflict of interest: The authors declare that they have no conflict of interest.

Authors' contributions: NT performed the experiment, collected the data, performed the graphical and statistical analysis, and wrote the manuscript. EM supervised the data analysis, writing, and revised the manuscript. NR shared in designing the research idea and the execution of the experiment and revised the manuscript. AA came up with the thesis's idea, supported and helped to overcome most of the work obstacles, including teaching the practical work techniques then revised the manuscript.

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