Time Course of Renal Ischemia/Reperfusion and Distance Organ; Lung Dysfunction in Male and Female Rats

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Research

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Abstract

Background

Renal ischemia/reperfusion injury (IRI) may influence distance organ such as lung. The severity of IRI induced - lung injury can be affected by gender. The aim of this study was to determine the role of gender in IRI induced- lung injury in different of renal reperfusion time.

Methods

80 male and female Wistar rats were assigned into 8 groups; 4 groups in each gender including sham, renal ischemia (ISC) for 45 min by clamping renal vessels followed by 3 hr, 24 hr or 48 hr reperfusion.blood samples were obtained for measuring the serum level of blood urea nitrogen (BUN), creatinine (Cr), nitrite, and malondialdehyde (MDA). The kidneys and lung tissues were removed and used for MDA and nitrite measurements and the histological changes evaluation.the percentage of lung water content was calculated.

Results

In both genders, the rise in Cr and BUN reached the peak at 24 h reperfusion. In 3 h reperfusion female rats, lead to significant increase in serum level of nitrite compared with males. In male rats subjected to 3 h reperfusion, the renal MDA level increased but not seen in females. The enhanced lung tissues damages were depended to reperfusion time in both genders. The water content of lung was reduced in 3 h of reperfusion groups.

Conclusions

IRI caused kidney and lung dysfunction depends on reperfusion time. Considering gender difference, female gender may be more sensitive to alteration of nitrite level compared with males. It seems that the effect of IRI is more rapid in males than females.

Background

Renal ischemia/reperfusion injury (IRI) is known as a frequent devastating complication in clinic which results acute kidney injury (AKI) [1]. In the post-ischemic kidney, the release of vasoconstrictive agents increase and vasodilatory responses decrease. For example, endothelial nitric oxide synthase (eNOS) function is lost following renal ischemic injury [2]. In addition, the reoxygenation process during the reperfusion period contributes to more pathological conditions by release of ROS and inflammatory factors in circulation which results kidney tissue damage and also leads to distant organ failure [1, 3]. So the impact of renal IRI on remote organs such as lung, brain, liver, heart, gut and other organs is well

recognized in patients with AKI that increase the rate of morbidity and mortality [3, 4]. The lung capillary network is a complex system, so increasing of pulmonary vascular permeability, interstitial edema, alveolar hemorrhage and existence of leukocytes in lung occure after renal IRI, and the mortality rate has been reported to be about 80% when lung injury combined with AKI [5, 6].

The incidence, prevalence, and progression of renal IRI induced ARI is altered by gender [7, 8] . Some studies have reported that female is more resistant to renal IRI compared with male [9, 10], and estrogen as a most effective factor in progressing ischemic insult may cause these differences. Because it acts as an antioxidant and chiefly increases the activity of antioxidant enzymes like superoxide dismutase [11, 12]. This sex hormone also enhances synthesis of nitric oxide (NO), and consequently the renal blood flow (RBF) will increase [10]. Muller et al. has reported that RBF recovers slowly after IRI in male due to more increasing of renal vascular resistance (RVR) compared to female [13] .

There are a number of evidences that the severity of kidney and lung injury after renal IRI can be affected by reperfusion time alteration. The deterioration of renal function was less apparent initially for several hours, while rapid change occurred in 24 hours [14, 15]. However, other study indicated that the most severely injured period was associated with 48 hour reperfusion time [16]. Others studies have also examined the side effects of renal IRI on lung tissue during different time periods of reperfusion in male rat, and they found the majority of damage after 24 and 48 hours of reperfusion [14, 17]

With regards to gender differences in renal IRI and about the possible association between lung dysturbances, the mechanism of IRI induced- lung injury in male and female needs to be further investigated, and this study was assigned to investigate gender differences in IRI induced- lung injury in different reperfusion time

Together, our results demonstrate that that sex effects and the time of reperfusion may be important factors to consider clinical therapeutic of renal IRI and its impact on remote organs.

Methods

80 male and female Wistar rats (weighting 200 ± 20g) were kept at a constant temperature of 23-25 ºC and 12h light/12h dark cycle. The rats were fed with rat chow and water ad libitum. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985). The rats were purchased from Animal House of Hormozgan University of Medical Sciences, Bandar Abbas, Iran. The protocol of experiment was approved in advance by the Hormozgan University of Medical Sciences Ethics Committee (HUMS.REC.1396.138).

Experimental protocol

The animals were randomly assigned into 8 experimental groups, n=10 in each groups, (4 groups of male and 4 groups of female: Group1 (sham): male rats were exposed to surgery without ischemia

process. Group 3, 5 and 7 (named ISC3hr, ISC24hr, and ISC48hr): male rats were subjected to renal ischemia with 3 hr, 24 hr or 48 reperfusions respectively. Groups 2, 4, 6 and 8 were subjected to same procedure of the groups 1, 3, 5 and 7 respectively except female rats used instead of male. To obtain renal ischemia, the animals body weights (BW) were measured and they were anesthetized with chloral hydrate injection (450 mg/kg; ip), the kidneys were carefully excised. Special care was done to avoid damage to the organs. The both kidney arteries and veins were occluded for 45 min by clamping.

The occurrence of ischemia was visually confirmed by the observation of renal blanching. After 45 min, the clamps were removed with care to initiate kidney reperfusion. The kidneys were observed for about 10 min to ensure reperfusion. The animals that didn't have adequate restoration of blood flow into kidneys were excluded from the study. Then, the skin and tissue were sutured and the animals were kept in the animal room under direct observation for 3 hours, 24 hours and 48 hours as assigned.

Collection of blood sample by cardiac puncture

At the end of reperfusion times, the animals were anesthetized again, with chloral hydrate injection (450 mg/kg; ip, and the blood samples were obtained via heart puncture with 22G needle and 5 ml syringe. The animals were sacrificed humanly with over dose administration of anesthetic drug. An incision was made in the chest and abdomen with a surgical blade to obtain kidney and lung tissues.

Blood samples were centrifuged at 2500 g for 10 minutes to obtain serum samples for measuring the serum level of blood urea nitrogen (BUN), creatinine (Cr), nitrite, and malondialdehyde (MDA). Serum samples were sorted at -80 ºC until measurement.

Preparation of kidney and lung tissue

The kidneys and lung tissues were also removed and weighted immediately. The kidney weight (KW) was normalized to the BW, and reported as tissue weight (kW)/100 g of BW. The right kidney and lung tissues were fixed in 10%formalin for histopathological investigation. The removed left kidney was transferred into liquid nitrogen very quickly and then was stored at -80 ºC refrigerator until measurement. In addition, a small part of kidney and lung tissues were weighted and homogenized and centrifuged at 15,000 g for 2 min, and the supernatant was used for MDA and nitrite measurements.

To evaluate the effects of IRI on lung histology, the right bronchi was tied with 3 -0 silk and one needle was placed in the right bronchi. Then, 1 ml of 10% formalin was instilled into the right lung to achieve inflation ex-vivo. Finally, it was removed and again fixed in 10% formalin for pathological examinations. To determine lung water content (index of edema), a sample of left lung tissue was taken and was immediately weighed as lung wet weight.

Measurement of pulmonary water content

Pulmonary edema was measured by determination of lung water content. The left lung was dried in the oven under 100°C for at least 48 hours until constant weight was obtained. The percentage of lung water

content was calculated by the following formula:

Water content% = [(Lung wet weight – Lung dry weight)/ Lung wet weight] × 100

Biochemical assay

The serum levels Cr and BUN were determined using quantitative diagnostic kits (Pars Azmoon, Iran). Serum and kidney nitrite levels (stable metabolite of NO) were measured by using an assay kit (Promega Corporation, USA) The nitrite concentration of samples was determined by comparison with the nitrite standard reference curve. The serum and kidney levels of MDA were quantified according to the thiobarbituric acid (TBA) method [18].

Histopathological procedures

The kidney and lung tissue were fixed in 10% formalin solution, and embedded in paraffin for histopathological staining. The Hematoxylin and Eosin stain was applied, and to determine the kidney damage, presence of tubular atrophy, ischemic necrosis, inflammation, vessels congestion, hyaline casts, vacuolization, and debris were evaluated. Based on the damage intensity and damage percentage, the samples were scored as 1-4 while score zero was assigned to normal tissue.

To determine the lung tissue damage, presence of congestion, inflammation, and fibrosis were evaluated and graded.

Statistical Analysis

The data are presented as Mean ± SEM. Differences among groups in serum levels of BUN, Cr, NO, and MDA; and kidney levels of MDA and NO, kidney weight, lung water content were compared with each other by tow-way ANOVA followed by the Tukey post hoc test. Due to the qualitative nature of scoring, Kruskal-Wallis tests with Mann-Whitney were used to compare the pathological damage score of the groups. Values of $P< 0.05$ were considered statistically significant.

Results

Effect of reperfusion time on renal function

By compare with sham groups, the renal ischemia animals showed significant enhancement in the serum level of BUN and Cr at 24 hr reperfusion (Pgroup < 0.0001) in both male and female rats (Fig. 1). KW also have an enhancement trend in 3 and 24 hr after IR, and after 48 hr of reperfusion return near basal level in male and female rats.

Effect of reperfusion time on nitrite and MDA level

The significant increase was seen in plasma nitrite level of female rats at 3 hr reperfusion point (Pgroup = 0.025), and a reduction trend was observed following 24 and 48 hr of reperfusion period compare with

sham groups, while this observation was not occurred in male animals (Pgender = 0.007). The nitrite concentration in kidney of both genders was not change in different time of reperfusion.

To consider of plasma MDA concentration, there isn't any significant difference in 3, 24, 48 hr reperfusion groups compare with sham groups in female and male rats, but in male rats underwent IRI, we observed a significant difference in renal MDA trend compare with female (Pgender = 0.033). So that in male rats, the factor increased in 3 h reperfusion group then decreased in 24 and 48 hr reperfusion group. (Fig. 2)

Effect of reperfusion time on renal and lung histological changes and water content of lung in male and female rats during renal IR

Histology score of kidney obtained from summation of tubular atrophy, ischemic necrosis, inflammation, vessels congestion, hyaline casts,vacuolization, and debris histologic score of kidney was significant increased during 24 and 48 reperfusion time compare with 3 h reperfusion and sham-operated groups. This trend was similar in male and female groups. (Fig. 3, 4)

In histology score of lung was measured (base on intensity of damage) by summation of congestion, inflammation, and fibrosis. It was enhanced reperfusion time dependently in both gender especially 24 and 48 reperfusion time. (Fig. 3, 5)

The water content of lung was reduced in 3h of reperfusion then significant increases were observed during 24 and 48 h reperfusion in both gender (Pgroup = 0.06). (Fig. 2)

Discussion

The purpose of the present study was to determine the severity of different times of reperfusion following renal ischemia and its impact on kidney and lung histological changes in male and female rats. The peak elevation of serum BUN and Cr were occurred at 24 h reperfusion then return to the normal at 48 h reperfusion in both male and female groups. The renal of MDA level increase in 3 h reperfusion with a reduction trend at 24 and 48 h time point in male rats but not in female, but the peak elevation of serum nitrite level take place in 3 h reperfusion in female rats and this trend was not observed in males. The severity of renal, lung histological damage was increase in reperfusion phase time dependently in both genders.

As known, the rise in plasma Cr is used typically as diagnosis of clinical renal IR. The peak elevation of creatinine occurred 24 h after the ischemic injury due to existence of lag period between the onset of renal damage with the following elevation in Cr [15] in accordance to our results that the rise in Cr and BUN reached the peak at 24 h reperfusion in both gender. So that change in renal function markers was depend on kidney reperfusion time and the mechanism by which proved AKI.

NO plays an important role in renal vascular tone and hemodynamics [19]. Nitrite is a stable metabolite of NO that involves the nearly 70% of oxidative product of NO [2]. It is well documented that there are sex differences in renal NO expression that way females have higher levels of eNOS compared to males [19].

In this study, female rats show the peak elevation of serum nitrite level in 3 h reperfusion and then reduced at 24 and 48 h time point. Therefore, nitrite production depends on reperfusion time in female rats but not in males. Park et al. reported that fundamental NOS activity is higher in females than in males in rat model of renal IR injury, and estrogen administration increases NOS activity while testosterone decreases it [20]. The research that had done by Ping Lu et al. on liver revealed that serum NO level of basal and post-ischemic was higher in female than male rat; also in accordance with our study in first hours of reperfusion time nitrite level was increased [21]. In addition, other researches in line with our study demonstrated that the maximum epithelial NOS (iNOS) and eNOS activity occur 2 and 4 hours after reperfusion, respectively and then the level of these enzymes reduce [2, 22]. In our previous studies similar to present research, serum and kidney level of nitrite was decreased after 72 hours of reperfusion following IRI in rat model [23, 24]. Therefore, the imbalance between the activity of iNOS and eNOS is an important contributor to the pathology of AKI and female gender exhibit a sensitive to nitric oxide system during renal IR.

In addition, the important mechanism of renal insult after IR is determined an imbalance between oxidant and anti-oxidant pathway called, oxidative stress. In this situation, overproduction of oxidant agent such as ROS lead to MDA release as a marker of lipid production [25]. It is important to consider the gender mechanism of AKI by which contribute male is particularly susceptible to glomerular and tubular dysfunction compared to females [26]. In line with this point, in the current study, we identified that IRI increased the production of kidney MDA of male rats in 3 h after reperfusion bot not in females. Kiris et al was observed enhanced MDA 30 and 60 min following reperfusion [27] also another study showed the peak of MDA enhancement take place 6 h reperfusion time [28]. These data was similar of our study suggesting that MDA increase the first hours of reperfusion and then reduced. This reduction may be due to improvement antioxidant capacity. The lungs with large and complex capillary net can be affected by unknown uremic toxins as renal reperfusion products. Studies demonstrated increasing of pulmonary vascular permeability, interstitial edema, alveolar hemorrhage and existence of leukocytes in lung as a remote organ after renal ischemia reperfusion. Mechanisms of AKI-induced lung injury can be dysregulation of water clearance, inflammatory reaction, immune response, oxidative stress, and apoptosis. Moreover The epithelial transporters of salt and water of rat lungs down regulated that can result to decreasing alveolar fluid clearance during renal ischemia [4–6, 29]. The result of the present study showed that damage score of lung and kidney significant increased during 24 and 48 reperfusion time compare with 3 h reperfusion and sham-operated groups with the same trend between male and female groups. The result is in agreement with reported study by Kramer in male rat [17]. Previous studies have examined side effects of ischemia reperfusion injury on lung tissue during different time periods of reperfusion in male rat. They found the majority of damage in male rat lungs 24 and 48 hours following of ischemia reperfusion injury [17]. During the ischemia, vascular endothelium, interstitial compartments and epithelium cells are involved. So, the disturbed structural integrity of tissue have been observed. The evidence from lung tissue of disturbed alveolar epithelium barrier and capillary endothelium with accumulation of inflammatory cells in the air spaces and parenchyma suggesting that pulmonary cellular apoptosis as a consequence of IRI [30].

Overacting and migration of neutrophils to other organs is the important immune process in response to multiple organs failure of IRI. Capillaries reperfusion of tissue occludes with the neutrophils which results in tissue necrosis. recently studies indicates that migration of these cells into the interstitial matrix drives not only vascular permeability and renal damage but also broadening distance organ insult such as lung histological change. Also systemic secretion of proinflammatory cytokines, chemokines, RAS and produce highly toxic peroxynitrite production can worsen the pathological situation [6, 30, 31].

Taken together, some evidence indicates that complexity of kidney–lung crosstalk exists by regulating acid-base balance, oxygen carrying capacity, and control of blood pressure through the renin-angiotensin system so that these mechanisms can provide the pathophysiology of lung injury associated with IRI. In this regarding, abnormal protein homeostasis. ,reduction of lung capillary oncotic pressure, disrupt capillary integrity, decreased expressions of the epithelial sodium channel (ENaC), the sodium-potassium-ATPase pump and volume overload in respiratory system result from renal dysfunction [30, 32].

We showed that water content of lung in 3 hours after IR decreased in both of gender. In our previous study 96 hours after IR lung edema didn't happen but we found pathological damages such as the presence of necrosis, congestion, inflammation and hemorrhage in lung like what we observed in the present study [23, 24]. It means that the changes of water content in the first time of reperfusion may be related to alternation of hydrostatic pressure at the time of reperfusion.

Conclusion

Together, our results demonstrate that renal IRI caused kidney and lung dysfunction depends on reperfusion time. Considering gender difference, female gender may be more sensitive to NO system compared with males. Male exhibit more rapid IRI injury by lipid peroxidation mechanism than females. So that sex effects and the time of reperfusion may be important factors to consider clinical therapeutic of renal IRI and its impact on remote organs.

Abbreviations

IRI: Renal ischemia/reperfusion injury; BUN:Blood urea nitrogen; Cr:Creatinine; MDA:Malondialdehyde; ISC:Renal ischemia; AKI:Acute kidney injury; ROS:Oxygen species; eNOS:Endothelial nitric oxide synthase; NO:Nitric oxide; RVR:Renal vascular resistance; KW:The kidney weight

Declarations

Ethics approval and consent to participate

This article is taken from a research project which was approved by the Ethics Committee of Hormozgan University of Medical Sciences (Ethics Code: HUMS.REC.1396.138).

Consent for publication

Not applicable.

Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request(aghdas. dehghani@yahoo.com)

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

AD and FA performed the majority of This work such as experimental design ideas, implementation of the research, preparation of the manuscript, the interpretation of the data and data analyses. AAAP worked in histopathological investigation. MN performed the interpretation of the data, preparation of the manuscript. All authors discussed the results, then they read and approved the final manuscript.

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Figures

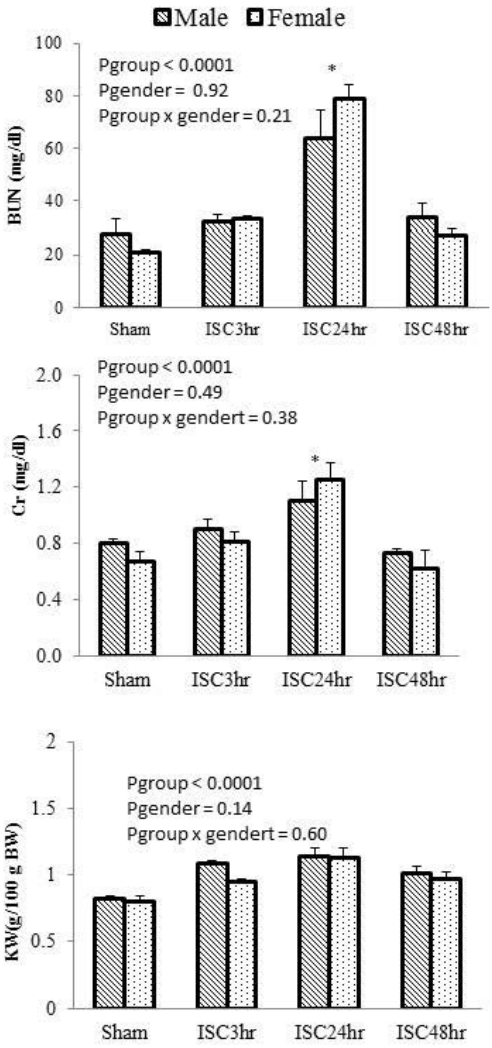

Figure 1

The serum levels of BUN and Cr , and normalized KW. TheP-values were derived from tow way ANOVA. Data are presented as mean ± SEM. BUN,blood ureanitrogen; Cr,creatinine; KW,kidney weight *p<0.05 ISC24hr group vs other groups in both gender #p<0.05 ISC48hr group vs sham and ISC3hr groups in both gender

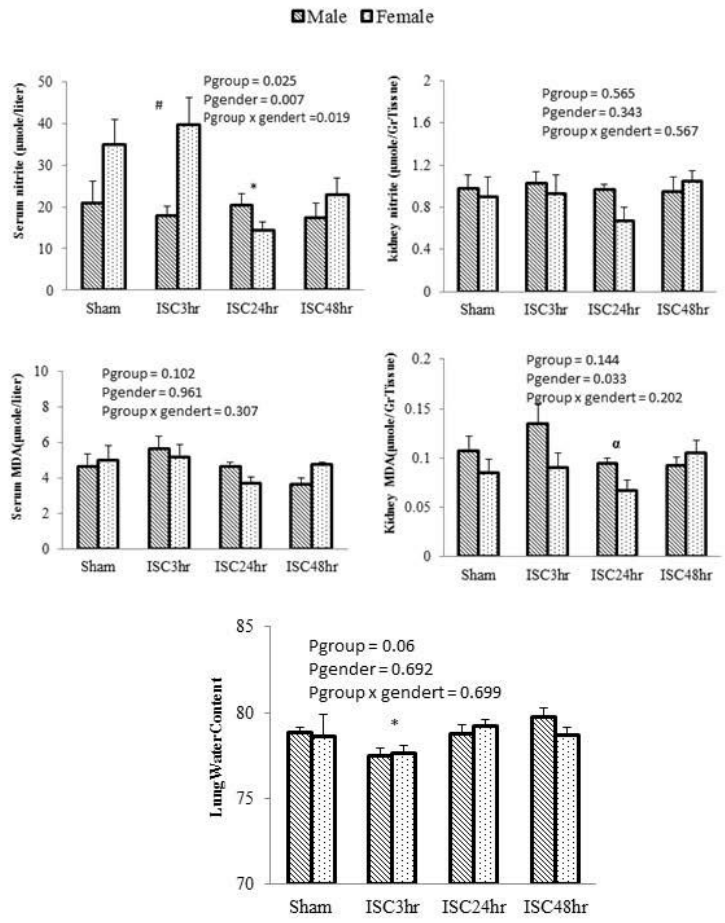

Figure 2

The serum and renal levels of nitrite and malondialdehyde(MDA), and The lung water content TheP-values were derived from tow way ANOVA.and t test Data are presented as mean ± SEM *p<0.05 ISC24hr group vs sham and ISC3hr groups in both gender analized by tow way ANOVA and tukey posthoc #p<0.05 maler group vs female groups after 3 h reperfusion(ISCH3hr) analized by t test αp<0.05 maler group vs female groups after 24 h reperfusion(ISCH3hr) analized by t test

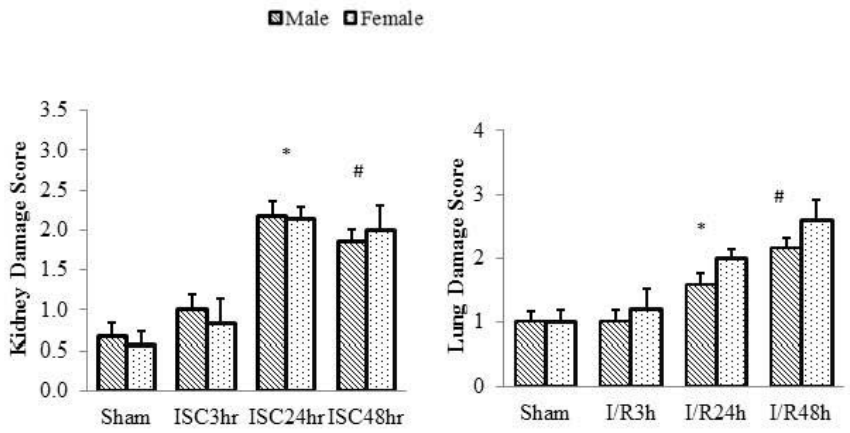

Figure 3

Lung and kidney score damage. Data are presented as mean ± SEM. TheP- values were derived from Kruskal-Wallis tests *p<0.05 ISC24hr group vs sham and ISC3hr groups in both gender #p<0.05 ISC48hr group vs sham and ISC3hr groups in both gender

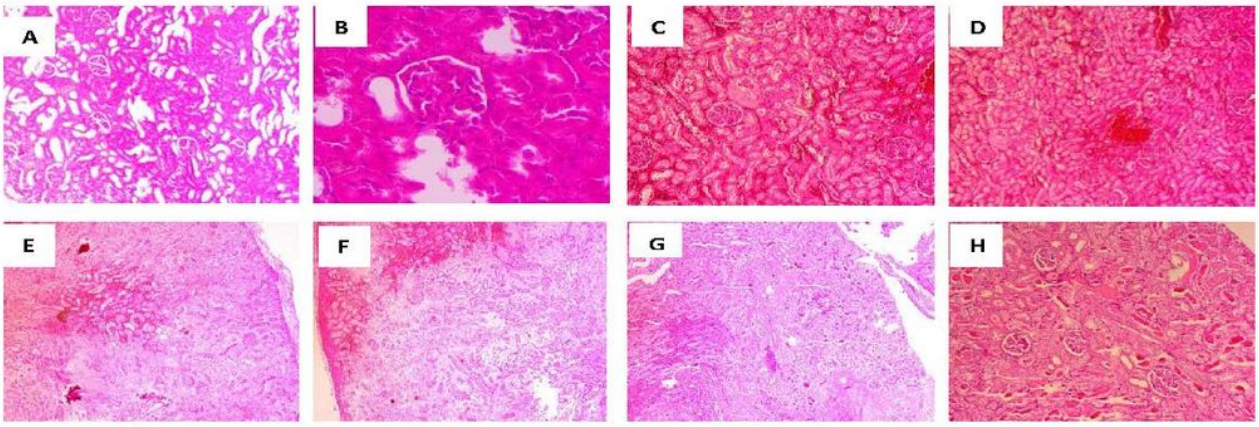

Figure 4

Renal histology shows tubular atrophy, ischemic necrosis, inflammation, vessels congestion, hyaline casts,vacuolization, and debris was significant increased during 24 and 48 reperfusion time compare with 3 h reperfusion and sham-operated groups. A& B(male and female sham),C&D(male and female ISC3hr), E&F(male and female ISC24hr), G&H(male and female ISC48hr)

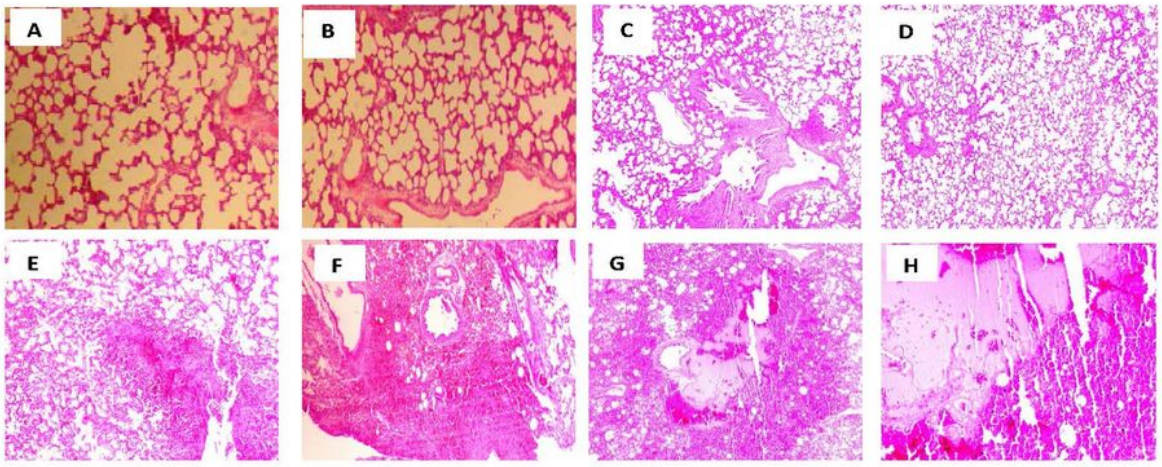

Figure 5

Lung histology shows congestion, inflammation, and fibrosis. These were enhanced during 24 and 48 reperfusion time compare with 3 h reperfusion and sham-operated groups. A& B(male and female sham),C&D(male and female ISC3hr), E&F(male and female ISC24hr), G&H(male and female ISC48hr).