Sys Rev Pharm 2019; 10(2):90-99
A multifaceted review journal in the field of pharmacy

Nephroprotective Potential Effect of Azilsartan in Renal Ischemia Reperfusion Injury /role VEGF Pathway

Aseel Noaman Ali[1], Murooj L. Altimimi[1], Haider Mahdi Al-Ardi[2],Najah R Hadi[1]
Kufa University, Faculty of medicine, Department of Pharmacology and Therapeutics, Najaf, Iraq
Kufa University, Faculty of medicine, Department of Cryosurgery, Najaf, Iraq

Article History: *Submitted: 07.08.2019* *Revised: 11.10.2019* *Accepted: 15.11.2019*

ABSTRACT
The impairment of blood flow to the kidney causes renal injury occurs by ischemia/reperfusion in surgical procedures, in which the kidneys remain without blood supply for some time. This is observed during kidney transplantation, vascular surgery of aorta and renal arteries, and in partial nephrectomy. Inflammation is major contributor to the pathogenesis of renal ischemia/reperfusion in acute kidney injury with an endothelium loss, causing the vascular dysfunction and the angiogenesis deregulation. Azilsartan is an angiotensin II receptor antagonist used in the treatment of hypertension. It demonstrates organ protective effects in hypertension. In this study, we aim to assess the ability of azilsartanto exert potential protective effects on the kidney I/R and subsequent kidney dysfunctions in the rat through the modulation of pro-inflammatory cytokinesand angiogenesis parameters (vascular endothelial growth factor receptor2 /vascular endothelial growth factor pathway). A total of 24 rats were randomly distributed into 4 groups (6 rats in each one) then they used in this experiment and the ischemia-reperfusion injury was induced in a rat model by bilateral renal artery clamping for 30 min and reperfusion for 2 h, and azilsartan (0.4 mg/kg) and vehicle was injected through intraperitoneally route before 30 min. prior of ischemia-reperfusion injury induction. Azilsartan administration could exert a protective role in the kidney against injury by significantly reducing the tissue levels of pro-inflammatory cytokines (interleukin 1 beta, Monocyte chemoattractant protein-1and high mobility group box protein 1), lessen the serum levels of renal function parameters (blood urea nitrogen and serum creatinine), reduction of the histopathological severity score of renal damage and up regulation the angiogenesis markers (Vascular endothelial growth factor receptor 2, caveolin-1 and cluster of differentiation 34). All changes in the study parameters were significant (p value≤ 0.05).

key words: nephron protection, histopathological effects, azilsartan

Correspondance:
Prof. Najah R. Hadi
A head of pharmacology and Therapeutic Department
Faculty of medicine, Kufa University
E-mail: drnajahhadi@yahoo.com

DOI: 10.5530/srp.2019.2.15

INTRODUCTION

Acute kidney injury (AKI) appeared as a clinically critical alarm which is linked to tremendous death proportions. Also, it acts as a substantial risk factor that intended for the progression to chronic kidney disorders (CKD) (Basile, 2018). It is a sudden reduction in kidney function leading to accumulation of waste products in the circulatory system like urea and creatinine (Deneen, 2010). AKI is one of the most common complications in patients who had been admitted in the hospitals, which is believed to be as consequence of ischemia-reperfusion injury (IRI) (Devarajan 2006; Chertow, Burdick et al. 2005; Park 2017). In IRI, it is important to distinguish between the two phases of the syndrome, which are differentiated but inseparable. The first phase “Ischemia”: it is the process by which the blood flow is restricted or interrupted for a certain period and the second “reperfusion”: it is the subsequent process by which the blood flow is restored and oxygen enters the organ (Panisello-Roselló and Roselló-Catafau 2018). The basic mechanisms of I/R-induced AKI aren't studied yet to be fully elucidated. The last reports have been demonstrated that I/R-induced AKI lead to tubular epithelial cells damage, thus triggering the inflammatory signaling pathway and a cascade of inflammatory responses. So ischemic AKI activated IL-1β, MCP-1 and the infiltration of the kidney by macrophages, many studies have reported that macrophages are involved in I/R-induced AKI damage and inflammation (Zheng, 2018), besides, High-mobility group box-1 protein (HMGB1) which is another proinflammatory cytokine that has been reported to be released by the kidney following IRI (Chen, 2011). An important characteristic of the rapid release for HMGB1 after the cellular injury suggesting that it may have an essential role in the early stages of damage signal propagation following the injury, also, it represents a striking example of the parsimony in biological systems (Bianchi 2007). As an innate response to renal I/R injury and AKI, stimulation of various cell survival signaling pathways had been activated, including an expression of VEGF (Jayle, Favreau et al. 2007; Ke and Costa 2006). Recently, it had been found that VEGFR2 is increased in renal I/R injury. So, as IRI in kidney induced an inflammation reaction inside the tissues, this would lead to the producing of the renal pro-inflammatory chemokines like Interleukins which is promoting the synthesis of VEGF (Burne 2003; Chen 2010; Tanaka, 2000; Lin, 2017). VEGFR2 are family of type IV receptor tyrosine kinase (RTKs), they act as a cell-surface receptor for VEGF, that had been existed on the external layer of the plasma membrane of the endothelial cells (Shibuya 2006). In like manner, leukocytes were performed using the cell surface markers as CD34 and VEGFR2 for confirmation of multi-lineage identification of putative endothelial progenitors (Bertolini, Shaked et al. 2006).Also, the regulation of VEGFR2 activity is achieved by caveolin-1 (the plasma membrane cholesterol "Cav-1") (Labrecque, Royal et al. 2003).These Signals essential for renal protection and repair immediately after I/R injury (Higgins, Kimura et al. 2008; Kelsen, He et al. 2012). Azilsartan is an angiotensin-receptor blocking (ARB) agent (Michel, Brunner et al. 2016). Azilsartan had known as TAK-491 or as TAK-536 for its prodrug, azilsartanmedoxomil. It used for antihypertensive treatment in patients 18 years of age or older (Kurtz, 2012). Azilsartan reverses the cerebrovascular remodeling and dysfunction in the diabetic rats, and decrease arterial stiffness (Abdel 2014).Also, it is hypothesized that azilsartan would lower mortality rates and the onset of cardiovascular disease (Hjermitslev, 2017). Additionally, azilsartan had a beneficial effect inthemyocardial I/R injury. The injury abolished by azilsartan through a specific modulation of endothelial nitric oxide synthase (eNOS) (Hjermitslev, 2017).

MATERIALS AND METHODS

Preparation of animals

In this study, 24 adult Swiss albino male rats (weigh up 250 - 350 g, with 18-24 weeks in age) were purchased from animal resource center, Science College/University of Duhok (UOD). The rats were healthy, and they had been kept in the animal house at Science faculty in University of Kufa (UOK) in wire-bottomed cages at temperature (24°C ± 2°C) until the experiments had been started with ambient humidity and alternative 12 hrs light-12hrs dark cycles. The rats had been received a standard diet of food with water. They had been acclimatized in a quarantine room for two weeks. All efforts were made to minimize the number of animals used and their suffering degree.

Ethical Statement

This study has been through agreeing with the guide and usage of laboratory animals' association for Laboratory animal science. All animals' considerations and conventions have been approved by animal care committee. All rats have been sacrificed under overdose anesthesia of xylazine and ketamine mixture. Also, after death a cardiac puncture had been performed (De Araujo, 2015). Rats had been subjected to bilateral renal ischemia for 30 min followed by 2hrs. reperfusion period (De Vries, 2004; Mizutani, 2000; Gulmen, 2009; Huang, 2015) azilsartan administrated in a dose of 0.4mg/kg (Matsumoto, 2014; Li, 2017) i.p 30 min. before IRI induction. The rats were randomized into equal 4 groups, 6 rats in each group, as following:

1. Sham group: animals have been underwent median laparotomy under anesthesia without renal pedicles clamping.
2. IRI group: animals have been underwent 30-min bilateral renal ischemia and 2-hr reperfusion.
3. Vehicle (DMSO) group for azilsartan (Kajiya, Ho et al. 2011): animals received DMSO by intraperitoneally injection at 30 min. before ischemia plus IRI.
4. Azilsartantreated group: rats have been pretreated with azilsartan (0.4 mg per kg) via intraperitoneal injection at 30 min. before ischemia reperfusion injury induction plus IRI.

Experimental procedure

The rats were weighed, anesthetized with ketamine 100mg/kg ip and xylazine hydrochloride (10mg/kg) ip (Feitoza et al., 2008; Can, Catak et al. 2015). After anesthesia and under sedation (5-10min), they had been placed on its back, fixed their limbs, and tail with stickers to ensure their stability during surgery. Hair in the abdomen had been shaved and the area disinfected. The reflexes had been checked through pinching the tail and the hind feet to ensure that the rats were adequately anesthetized. By making midline laparotomy incision to expose the abdomen and in order to expose the right and the left renal pedicles, the intestines had been retracted. Bilateral model of ischemia, the non-traumatic micro vascular clamps had been positioned around the renal pedicles. The abdomen had been covered using warm and moist gauze. The blood flow to the kidney had been returned after the clamps had removed and this had been confirmed visually. Then, the abdominal cavity incision had been closed in two layers (1ml normal saline "pre-warmed at 37°C had been administrated into the abdomen to counteract dehydration). The animals had been returned to their cages and allowed to get better with food and water. In post-surgery after 2hr.,the animals will be euthanized and both blood as well as tissue samples had been collected for analysis. Blood samples were collected directly from the heart and harvesting the kidneys for determination the experiment parameters(Hadi et al.,2018; Wei and Dong 2012;Wan et al., 2015).

Collection and preparation of samples

About 2-3 ml bloods have been gathered from the heart, then, the obtained serum had been used for the determination of urea, and creatinine by enzyme linked immunosorbent assay (ELISA). The kit of ELISA was commercially available according to the manufacturer's instructions. Homogenate kidney's tissue sampling had been used for measurements of IL-1β, MCP-1, HMGBox1, VEGFR2, CAV-1 and CD34. The homogenate had been centrifuged at 3000 xg at 4°C for 20 min. The supernatant had been assembled for determination of the above analysts by ELISA technique as per kits' instructions.

Renal function and histopathological analysis

Kidney function was examined by both of blood urea nitrogen and serum creatinine as it was described previously (Wei et al., 2007).The tissue sample had been placed without delay in10% formalin and it have been processed in paraffin tissue blocks (Yousif 2014). The tissue slides sections had been cut in about 5µm thick horizontal and stained with hematoxylin and eosin. Then, it had been sent to histopathology's for scoring estimation. After that, ablinded investigator to the experimental treatment groups evaluated an estimation of scores. Tissue sections were examined by light microscopy and graded for degeneration/necrosis. Using quantitative measurements had been accomplished for the assessing scoring system of tissue damage (Bancroft and Gamble 2002). The damage of tubular identified as swelling of tubular epithelial, brush border losing, vacuolar degeneration, and formation of the cast. The degree of damage in kidney was described through the subsequent criteria of tubules damage: 0, represents normal; 1, <25%;2, 25%–50%;3, 50%–75%; and 4,75%–100%. The degree of kidney injury was estimated at 40 to 100x magnification; the scores of histological changes of kidney had been evaluated as previously described (Zhang, et al., 2008).

Statistical analysis

Statistical analyses had been achieved by SPSS 24.0 for window Inc. Data had been stated as mean +_ SEM. Analysis of variance (ANOVA) had been applied to the multiple comparisons among all groups monitored by post hoc tests using LSD method. For histopathological renal changes, the Mann –Whitney U had been applied to calculate the statistical significance of difference between two groups with the kruskal-wallis test were had been achieved that headed for evaluation the statistical significance of difference across multiple groups in total severity score. In all tests P≤0.05 had considered statistically significant.

Effect of azilsartan on inflammatory parameters and angiogenesis parameters following renal IRI

It had been studied the effect of azilsartan on the inflammatory parameters (interleukin-1 beta, monocyte chemoattractant protein-1, high mobility group box-1) and the angiogenesis parameters (vascular endothelial growth factor receptor 2, caveolin-1, cluster of differentiation 34) following renal IRI.

Effect on interleukin-1 beta

Renal IRI causes significant increase in mean of tissue level of IL1β (1814.8±20.2 pg/ml, p value 0.001) when it compared with sham group (187.8±16.5 pg/ml, p value 0.001), while azilsartan cause significant decrease in mean of tissue level of IL1β (538.8±12.1 pg/ml, p value 0.001) when it compared with IRI group. Other comparisons were not significant, as shown in figure (1).

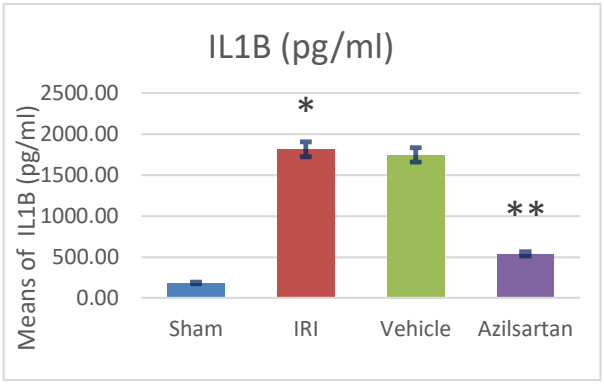

Figure1. Error bar chart show the effect of azilsartanon mean tissue IL1β level following renal IRI, expressed as mean ± SEM pg / ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

Effect on monocyte chemoattractant protein-1

Renal IRI causes significant increase in mean of tissue level of MCP-1(8.4±0.2 ng/ml, p value 0.001) when it compared with sham group (1.4±0.04 ng/ml, p value 0.001). Azilsartan cause significant decrease in mean of tissue level of MCP-1 (5.5±0.2 ng/ml, p value 0.001) when it compared with IRI group.Other comparisons were not significant, as shown in figure (2).

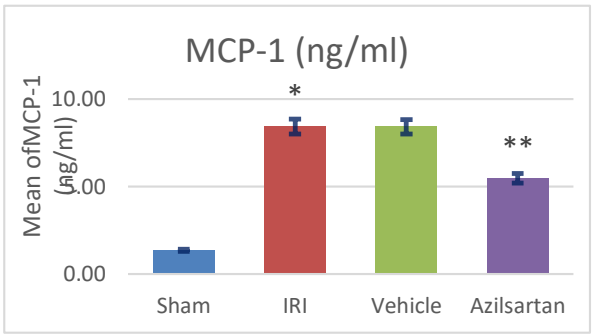

Figure 2. Error bar chart show the effect of azilsartan on mean tissue MCP-1 level following renal IRI, expressed as mean ± SEM ng / ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

Effect on high mobility group box-1

Renal IRI causes significant increase in mean of tissue level of HMGB1 (1904.2±27.4 pg/ml, p value 0.001) when it compared with sham group (158.7±11.0, p value 0.001) while azilsartan cause significant decrease in mean of tissue level of HMGB1 (779.1±39.7 pg/ml, p value 0.001) when it compared with IRI group. Other comparisons were not significant, as shown in figure (3).

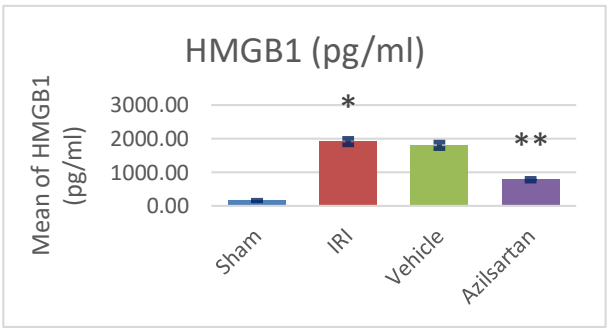

Figure 3. Error bar chart show the effect of azilsartan on mean tissue HMGB1 levelfollowing renal IRI, expressed as mean ± SEM pg / ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

Effect on vascular endothelial growth factor receptor 2

Renal IRI causes significant increase in mean of tissue level of VEGFR2 (6.6±0.5 ng/ml, p value 0.001) when it compared with sham group (2.9±0.1ng/ml, p value 0.001). In addition to that, azilsartan cause further significant increase mean of tissue level of VEGFR2 (9.2±0.1ng/ml, p value 0.001) when it compared with IRI group. Other comparisons were not significant, as shown in figure (4).

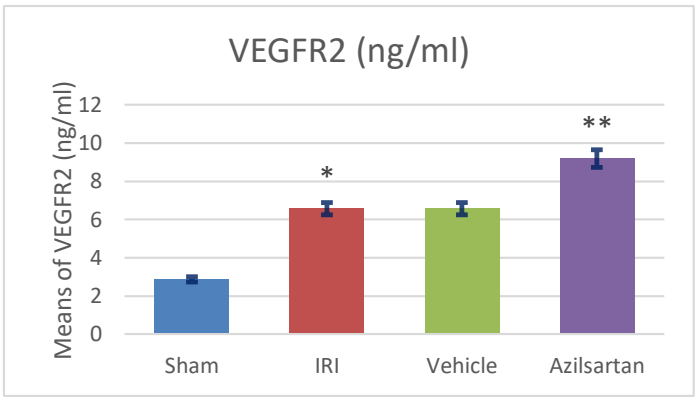

Figure4. Error bar chart show the effect of azilsartanon mean tissue VEGFR2 level following renal IRI, expressed as mean ± SEM ng /ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

Effect on caveolin-1

Renal IRI causes significant increase in mean of tissue level of CAV-1(4.5 ±0.2 ng/ml, p value 0.001) when it compared with sham group (1.9±0.2 ng/ml, p value 0.001). In addition to that, azilsartan cause further significant increase in mean of tissue level of CAV-1 (7.3±0.2ng/ml, p value 0.001) when it compared with IRI group. Other comparisons were not significant, as shown in figure (5).

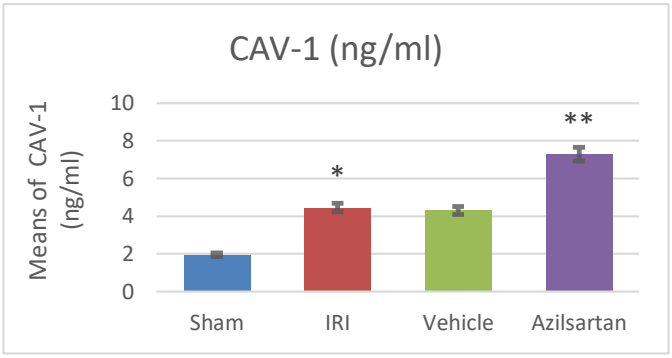

Figure 5. Error bar chart show the effect of azilsartnon mean tissue CAV-1 level following renal IRI, expressed as mean ± SEMpg/ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

Effect on cluster of differentiation 34

Renal IRI causes significant increase in mean of tissue level of CD34 (2.6±0.013 ng/ml, p value 0.001) when it compared with sham group (0.2±0.01ng/ml, p value 0.001). In addition to that, azilsartan cause further significant increase in mean of tissue level of CD34 (4.6±0.015 ng/ml, p value 0.001) when it compared with IRI group. Other comparisons were not significant, as shown in figure (6).

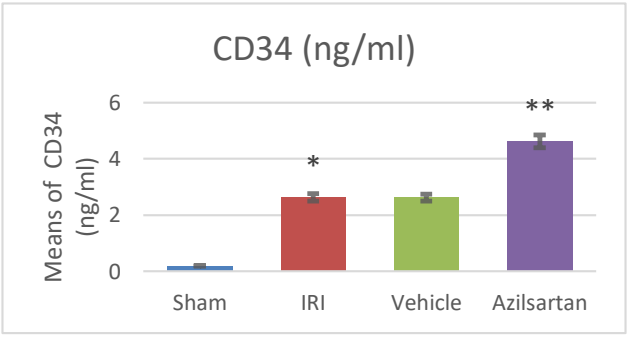

Figure 6. Error bar chart show the effect of azilsartan on mean tissue CD34 level following renal IRI, expressed as mean ± SEM ng / ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

Effect of azilsartanon blood urea nitrogen and serum creatinine following renal IRI

Renal IRI causes significant increase in mean of both blood ureanitrogen and serum creatinine (95.5±3.5mg/dl, 0.8±0.03 mg/dl, p value 0.001, 0.001 respectively) when it compared with sham group (20.0±1.1mg/dl, 0.2±0.04 mg/dl, p value 0.001, 0.001 respectively). Azilsartan causes significant decrease in mean of both blood ureanitrogen and serum creatinine (32.0±0.96mg/dl, 0.4±0.003 mg/dl, p value 0.001, 0.001 respectively) when it compared with IRI group. Other comparisons were not significant, as shown in figure (8) and figure (9).

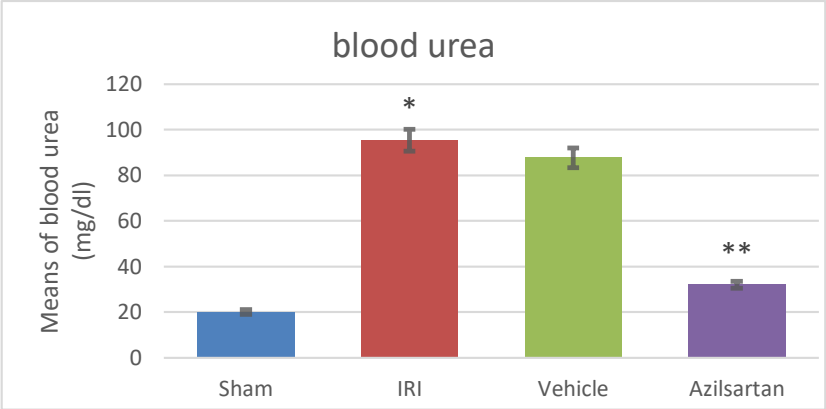

Figure 7. Error bar chart show the effect ofazilsartanon mean blood urea nitrogen following renal IRI, expressed as mean±SEM ng/ml, n=6 in each group, *p value ≤ 0.05 when it compared with the sham group, **p value ≤ 0.05 when it compared with IRI group.

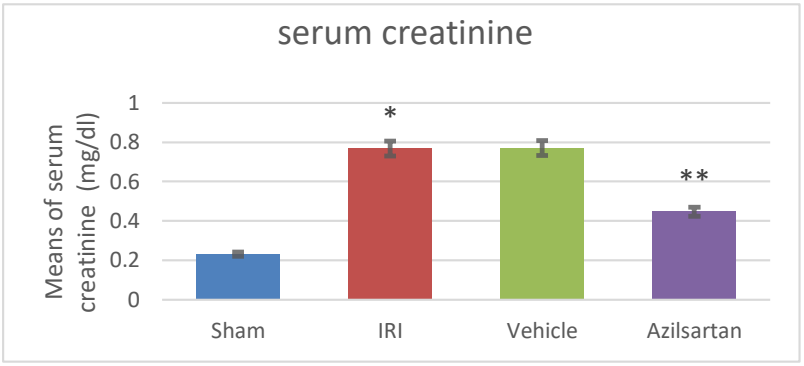

Figure 8.Error bar chart show the effect of on mean serum creatinine following renal IRI, expressed as mean ±SEM ng/ml, n=6 in each group, *p value ≤ 0.05 when compared with the sham group, **p value ≤ 0.05 when compared with IRI group.

HISTOPATHOLOGICAL FINDING

The Histopathological score in the four experimental groups had been shown in Figure (10) , IRI cause significant tissue damage (p value 0.001), pretreatment with azilsartancause amelioration of tissue damage (p value (0.002). In the figure (11), it had been shown the normal renal tubules without inducing IRI. While it had been shown the significant tissue damage that had caused by IRI in the figures (12) and (13) as mainly apoptotic/necrotic renal tubules, it had been shown in the figures (14) and (15) how the pretreatment with azilsartan caused significant reduction in necrosis of renal tubules that had caused by IRI.

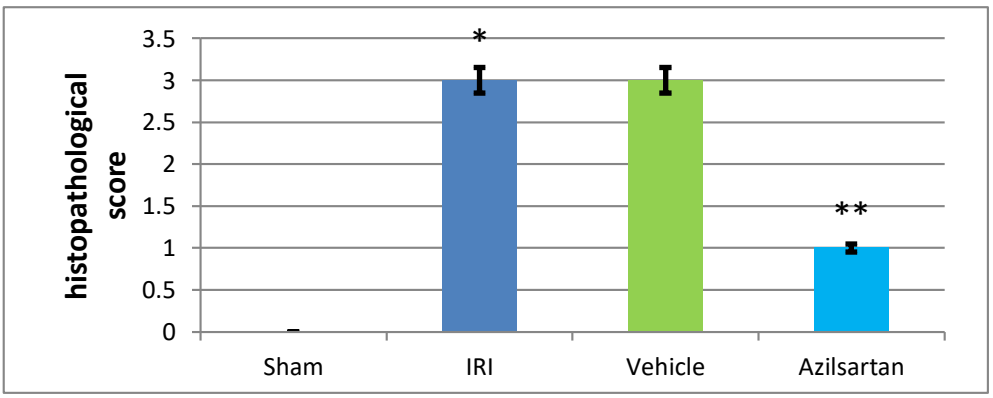

Figure 9. Error bar chart show the effect of azilsartanon mean histopathological scoring of kidney injury following renal IRI, expressed as mean ±SEM, n=6 in each group, *p value ≤0.05 when it compared with sham group, **p value ≤0.05 when it compared with IRI group.

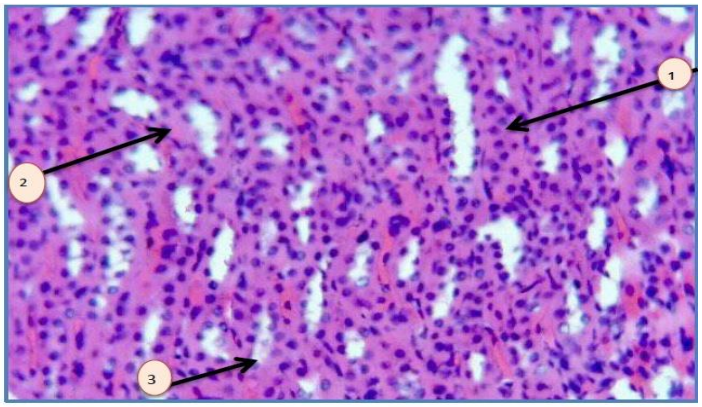

Figure 10. Photomicrograph of adult male rat kidney with H and E staining at x100 magnification for the sham-operated group without induced IRI(illustrating severity score zero as it shows normal renal tubules) as marked the number (1), the number (2) and the number (3) for the normal renal tubules with intact glomeruli (red raw) and normal Baumann capsule (blue raw).

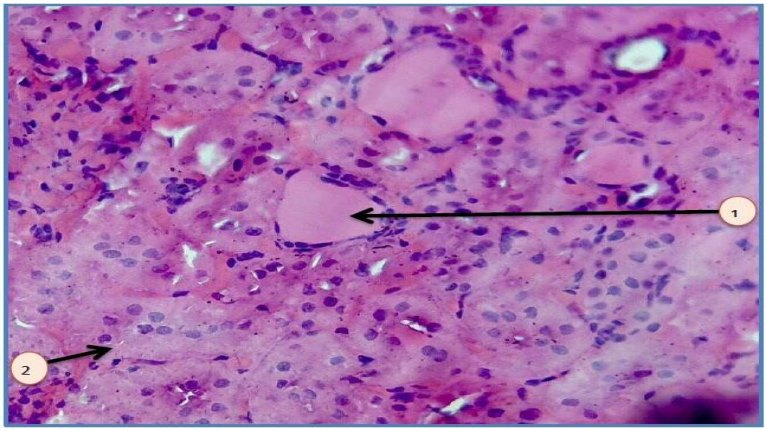

Figure 11. Photomicrograghof untreated ischemic adult male rat kidney with H and E staining at x40 magnification for the control group(with induced IRI) in which it showed morphological changes as cellular vacuolization, "nuclear shading "karyolysis, and moderate to severe necrosis (illustrating severity score three) as marked the number (1) for the eosinophilic cast and the number (2) for tubular cellular swelling.

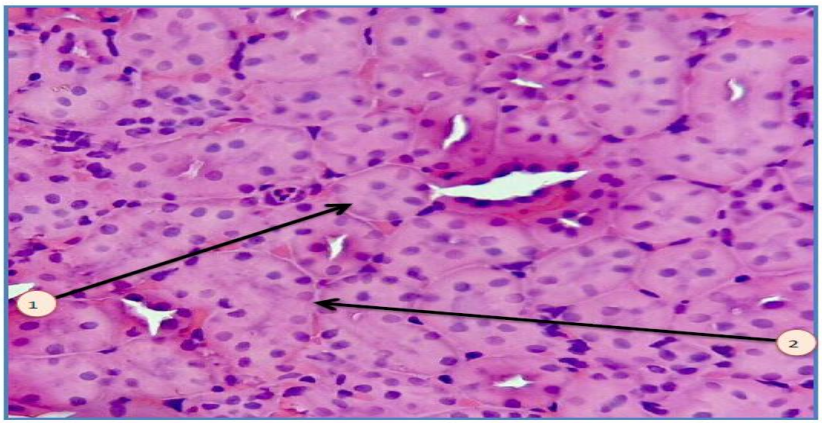

Figure 12: Photomicrogragh of adult malerat kidney with H and E staining at x40 magnification for the vehicle group (with induced IRI that were pretreated with DMSO) in which it showed morphological changes as cellular vacuolization, nuclear degeneration, and moderate to severe necrosis (illustrating severity score three) as marked the number (1) for hyper-eosinophilia and the number (2) for tubular cellular swelling. The entire field showed mostly necrotic renal tubules.

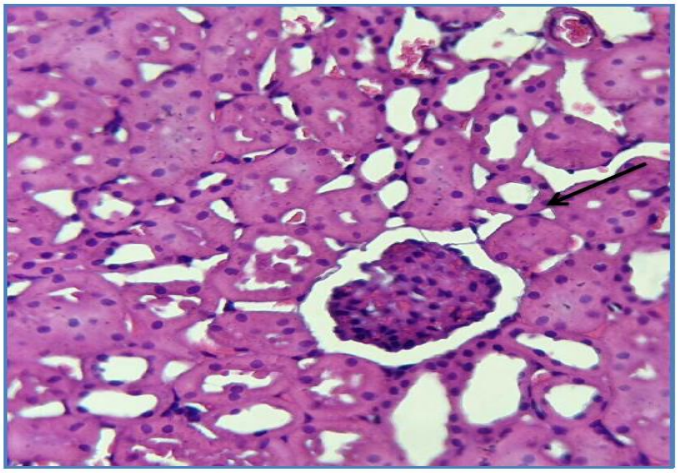

Figure 13. Photomicrograph of adult male rat kidney with H and E staining at x100 magnification for the azilsartan group with induced IRIand the pretreatment withazilsartan (illustrating severity score one) as marked with an arrow for the normal renal tubules.

DISCUSSION

Influence of renal IRI on the study's parameters

Acute renal failure had been generated due to ischemia with redistribution of blood flow which is a clinical and experimental condition branded with considerable declines in GFR, all-encompassing tubular destruction, tubular cell necrosis, glomerular injury, renal vasoconstriction and manifestation of tubular hindrance by cellular wastes (Moslemi, 2019; Yang, 2013; Bao, 2018). The sum of aspects that might devote to the grievance concomitant with ARF has been revealed in animal studies as inflammatory cascade (Gao, 2018). Renal tubular epithelial cells and endothelial cells' task have been debilitated and renal inhabitant leukocyte have been triggered that it had been caused additional vascular leak with fluid retention in interstitial space (Kumar 2018). An obvious notification had been included that the inflammation cascade represents a dynamic part in the predisposing of ARF. The Suppression for inflammation response had been explained by numerous researches that it had be responsible for renal IRI reduction and well-preserved of kidney tasks (Salvadori, 2015; Liu, 2019; Jung, Choi2019). Also, Dysregulation of angiogenetic process, deficiency of micro vascular bedding with O_2 deprivation had been complicatedly related with enrollment of kidney disorders. In spite of angiogenesis had been enhanced via hypoxia inducible factor stimulation, the micro vascular bedding deficiency upon O_2 deprivation had been persisted and progressed leading to anoxia and AKI. Anti-antigenic mechanisms had been highly stimulated, ineffectiveness of the endothelial progenitor cells (EPC_s) and then ecrotic/apoptotic renal tubules would lead to the inability for the expression of an appropriate extent from angiogenic factors which were restored capillary networks around renal tubules. All these events together had been explained the dysregulation of the angiogenesis process. So, Inducing the coordinated up regulation of the angiogenesis process could be a promising treatment strategy against renal disease (Tanaka, 2015; Tanaka, 2013; Yamakawa, 2003).

Dysregulation of Vascular endothelial growth factor receptor 2 after renal IRI

In this study, the renal tissue level of VEGFR2 was significantly increased in the IRI animals when it had been in a comparison to the sham-operated rats.VEGFR2 elevation have been reported in previous studies in which it was significantly increased after IRI (Kanellis, 2002; Ferrara 1999). In IRI, manifestations of VEGFR2 had been acted as the only mediator to mitogenic response neither VEGF nor VEGFR1 and the anti-apoptotic effects of it on ECs had be leading to preservation of endothelial unity with a probability of maintaining blood flow into ischemic areas (Gerber, 1997).While the hypoxia-responsiveness of VEGFR2 stays argumentative in vitro, this isn't the situation in vivo where some investigators had reported that it is profound. VEGFR2 seemed to assume a noteworthy job in VEGF-prompted mitogenesis and chemoattraction. Authorization of ligand to the receptor could happen in any intracellular part where it would approach extracellular spaces of VEGFR2, for example, the endoplasmic reticulum or the Golgi mechanical assembly. Enactment of the kinase action of receptor tyrosine kinases is known to prompt quick debasement, by means of either through lysosomal corruption or ubiquitination and proteasome-interceded corruption (Arbiser, Larsson et al. 2000). One could propose that VEGFR2 may have a main job in the shielding of the kidney from damage by saving micro vascular networks number that surrounded the renal tubules due to its angiogenic impacts. In spite of the way that inflammatory responses after VEGF-incitement are believed to be ascribed to VEGFR1, not VEGFR2, it particularly thought that VEGFR2 incitement raised the macrophage penetration and the disregarding of the eNOS articulation. On the other hand, some of studies showed that the renal macrophage penetration had been probably not going to be directed by the VEGFR2 incitement but it most likely was because of a roundabout impact from different cytokines that might be communicated in the impaired kidneys (Sato, Tanabe et al. 2011).

Influence of renal IRI on pro-inflammatory cytokine interleukin-1 beta

In this study the renal tissue level of IL-1β was significantly increased (p value ≤ 0.05) within control rats when it had been in a comparison to the sham-operated animals after IRI. This resulting data had been consistent with a previous study on renal IRI (Sakai, Nozaki et al. 2019; Yano, Nozaki et al. 2015). The leukocyte had been recruited to the area of renal

inflammation by the chemo attraction effect of IL-1β where it eventually leaded to the kidney damage (Torres-González, Cienfuegos-Pecina et al. 2018). During early phase of IRI, large amounts of ROS and pro-inflammatory cytokine (e.g.IL-1β and TNFα) had been produced by M1 macrophages, and a polarized Th1 immune response had been driven by them also which contribute to the injury (Kezić, 2017).

Up regulation monocyte chemoattractant protein-1 after renal IRI

In this study, the renal tissue level of MCP-1 was significantly increased (p value < 0.05) within the control animals when it had been in a comparison to the sham's rats. During IRI, MCP-1 is one of adhesion molecule that would enroll monocyte, memory T cells and dendrite cells to the site of damage resulting in a tubular injury (Chen, Lai et al. 2014). Renal proximal tubular cells can express MCP-1 as response to a range of pro-inflammatory provocations (Yoon and Kim 2015). A growing evidence indicate that the inflammatory reaction related with pro-inflammatory cytokine IL-1β, TNFα and chemo tactic cytokine (MCP-1) plays a major role in the impairment of the kidney functions following IRI (Fang, Liu et al. 2013).

Influence of renal IRI on high mobility group box-1

The other forceful pro‑inflammatory cytokine that participated in the pathogenesis of the various provocative inflammatory and infectious disorders including the promoting of the kidney damage after IRI, namely, HMGB1had significant increase in IRI rats (P < 0.05) as it had been in a comparison with sham's rats (Wu, Ma et al. 2010). It is consistent with that reported the components by which HMGB1 advanced the kidney harm. In this setting, it had been included an enactment of the body's defense mechanisms, it was bringing about the stimulation of the innate immune system, was causing in the production of inflammatory mediators as TNFα with MCP-1 (Zhang, Zhang et al. 2013). HMGB1 stimulate proinflammatory responses through TLR4 as proved by a study (Schaefer, 2005).

Influence of renal IRI on the cluster of differentiation 34

Bone marrow contains different types of stem cells, hematopoietic (HSCs), which express surface markers as CD34 (Orlic, 2003; Lagasse, 2000). In this study, CD34 had significant increase in IRI animals (P < 0.05) more than it had in the sham group (Peng, Yin et al. 2019).CD34+ stem/ancestor cells are enlisted to the harmed kidney for advanced survival, vascular recovery and recouped its services (Li, Cohen et al. 2010).

Influence of renal IRI on caveolin-1

Caveolin-1 as a notable basic protein of caveolae, influences an ischemia by controlling the cell signal transduction and as molecular vehicle.(Navarro, Anand-Apte et al. 2004). Abundant CAV-1 had been found in vascular endothelial cells and furthermore some in superficial layer of distal tubular epithelium (Mahmoudi, 2003). In this study, itwas significantly increased (p value < 0.05) within the control rats as it had been in a comparison to the sham-operated animals. It had prompted the vascular endothelial cell propagation by stimulation of the VEGFR2/CD34 and the eNOS expression that they had been increased in the IRI tissues of the rats where it had been eventually leading to the attenuating the damage as that reported in a previous study (Pang, Zhao et al. 2018).

Changes of blood urea nitrogen and serum creatinine after IRI

In this study, the renal tissue level of blood urea nitrogen and serum creatinine were significantly increased (p value < 0.05) within the control animals when they had been in a comparison to the sham-operated rats. These findings also had been demonstrated by other studies (Cao, 2018; Malek, 2018). Renal damage reduces glomerular capacity of the kidney and lead to increased serum level of metabolic byproducts. Among the metabolic waste products, blood urea nitrogen and serum creatinine are the most two important indexes for renal function alterations (Bagalad, 2017).

Histopathological Changes of renal tissue after renal IRI

Mean total of severity score of the sections of IRI group was significantly increased than the sham group (p value < 0.05). Also, this was explained in the previous studies (Nishikawa, Taniguchi et al. 2018; van Smaalen and Ellis 2019). The sham group exhibited normal kidney parenchyma. No signs of glomerular congestion neither renal tubular cells swelling or brush borders loss had been appeared in the sham-operated rats. On another hand, examination of sections from IRI group exhibited a distraction of the kidney structures leading to marked glomerular damage, glomerular congestion, inflammatory cell infiltration, epithelial atrophy, cells desquamation in the tubules, multiple hemorrhages in the intertubular space, vacuolization, edema of the tubules and vascular congestion among tubules.

Effect of azilsartan on renal IRI

Azilsartan is a quite new AT1receptor block represented for the management of any phase of hypertension. Previously, it was anonymous whether azilsartan would be joined to the angiotensin-converting enzyme inhibitors plus others angiotensin receptor blockers by the means of the chosen hypertensive agents for end-organ protection (Lam 2011). lately, the study had been reported that it improved glycemic status and reduces kidney damage associated with diabetes mellitus (Khan, 2014).More than that, renoprotective effects had been appealed due to azilsartan administration (de Araujo, Varela et al. 2015).

Influence of azilsartan on pro-inflammatory parameters (interleukin-1 beta, monocyte chemoattractant protein-1 and high mobility group box-1) after renal IRI

With that background and within this study, the clear anti-inflammatory effects of azilsartan had been demonstrated in IRI induced AKI which is supported the finding that pretreatment with azilsartan would reduce the proinflammatory cytokine (as: IL-1β, MCP-1) as well asHMGB1had been reduced by down regulation of TLR4 with reducing of TNF-α when it had been compared with the control group (Wu, Chen et al. 2007; Jin, O'Boyle et al. 2014; de Araujo, Varela et al. 2015). As mention in previous study, azilsartan had a property of multi functions that was famous in heading for hold back a definite kind of inflammatory

progressions via preventing Neutrophils movement, kidney macrophage infiltration and offsetting the overstated immunity response (Mahmood, Hussain et al. 2018). Pretreatment with azilsartan had reduced renal tissue level of IL-1β, MCP-1and HMGB1. These findings advocate that enfeeblement of kidney damage, caused via inflammation-replay for exogenous or endogenous insults, had been highly significant in azilsartan treated-groups compared to IRI group($p<0.05$)

Influence of azilsartan on angiogenesis parameters (vascular endothelial growth factor receptor 2, caveolin-1 and cluster of differentiation 34) after renal IRI

This first is study (to our knowledge) that describes the influence of azilsartan on VEGFR2/CD34 and CAV-1 levels. A significant further increase byazilsartan had been found in tissue levels of the VEGFR2/ CD34 and CAV-1 in IRI induced AKI when it compared with the control group (p value < 0.05). Therefore, it had been reported the diminishing of the vascular irritation, azilsartan applied helpful impacts in regarding of the endothelial reclamation, so, in that way, it avoiding its deterioration (Matsumoto, Shimabukuro et al. 2014). As mention earlier, Angiogenesis is the development of a fresh recruits of the vessels from the existed ones, it is an intricate procedure including extracellular framework debasement, endothelial relocation, multiplication, separation, and in the long run tube arrangement (Sapieha 2012; Priya, 2015). Growing of a fresh recruit of the vessels is a directed procedure where the leader is the endothelial tip cells that would start the vascular growing which trailed by stalk endothelial cells. This will intercede the vessel extension and lumen arrangement via activation of the VEGFR2/KDR receptor, is the real controller of the vascular development and their utilities. Also, tip cells have been appeared to express VEGFR2 (Gerhardt 2008; Bouchard and Mehta 2019). Thus, further up regulation of VEGFR2 that had been done by azilsartan plus its anti-inflammatory effect would be beneficial to compensate the endothelium loss inIRI induced AKI. So, understanding the pathway by which azilsartan excite the reparative angiogenesis may formulate a new remedial focus for the assurance of the vascular protection further than its realized control upon B.P. These results predisposed that azilsartan could own antigenic activity.

Effect of azilsartan on renal parenchyma, blood urea nitrogen and serum creatinine after renal IRI

Biochemical renal function and histopathological damage had been markedly improved by azilsartan (proteinuria, Albuminuria, nephrinuria, degeneration of tubule epithelium, tubular cast formation, glomerular injury, inflammatory cell infiltration, hemorrhage, vacuolization, edema and vascular congestion) that had reported when it compared with the control group (Hye, 2014). In addition to that, the results showed that pretreatment with azilsartan alone had significantly reduce the histopathological insults, the increments in serum creatinine level and the elevation of blood urea nitrogen level which had been formed after IRI induction. This is predisposing the idea of calling on the azilsartan as a kidney-guard due to its anti-inflammatory capability and antigenic ability. All these events were leading to preserving kidney's parenchyma from the damage and the upgrading its detoxification proficiency.

CONCLUSIONS

1. Pretreatment with azilsartan in rat model cause significant improvement of renal function (blood urea nitrogen and serum creatinine).
2. Reduction of pro inflammatory parameters (IL-1β, MCP-1and HMGB1).
3. Up regulation of angiogenesis parameters (VEGFR2, CD34 and CAV-1).
5. Amelioration of histopathological damage that caused by renal IRI.
8. These outcomes indicated a lowering of hyper-filtration, proteinuria, unsettle vascular structures and enfeeblement of the renal injury.

RECOMMENDATIONS

From the overall results, we can recommend the following:

1. Further documentation for the results using Immunohistochemistry analysis, western blot technique and flow cytometry analysis.
2. Studying the effect of on other biomarkers such as interleukin-11 (IL-11) for the prediction of progressive AKI.
3. Assessing the efficacy of azilsartanon other animal models.
4. Conducting a clinical trial on the effect of azilsartan in patient with renal disorders.

REFERENCES

1. Abdelsaid M, Coucha M, Ergul A. Cerebrovasculoprotective effects of azilsartan medoxomil in diabetes. Translational Research [Internet]. Elsevier BV; 2014 Nov;164(5):424–32. Available from: http://dx.doi.org/10.1016/j.trsl.2014.06.003
2. Bagalad B, Mohankumar K, Madhushankari G, Donoghue M, Kuberappa P. Diagnostic accuracy of salivary creatinine, urea, and potassium levels to assess dialysis need in renal failure patients. Dental Research Journal [Internet]. Medknow; 2017;14(1):13. Available from: http://dx.doi.org/10.4103/1735-3327.201138
3. Bancroft, J. D., & Gamble, M. (2002). Theory and Practice of Histological Techniques: Churchill Livingstone.
4. Basile D, Yoder M. Renal Endothelial Dysfunction in Acute Kidney Ischemia Reperfusion Injury. Cardiovascular & Hematological Disorders-Drug Targets [Internet]. Bentham Science Publishers Ltd.; 2014 Jul 23;14(1):3–14. Available from: http://dx.doi.org/10.2174/1871529x1401140724093505
5. Burne-Taney MJ, Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. American Journal of Physiology-Renal Physiology [Internet]. American Physiological Society; 2003 Jul;285(1):F87–F94. Available from: http://dx.doi.org/10.1152/ajprenal.00026.2003
6. Catak O, Can N, Turgut B, Demir T, Ilhan N, Kuloglu T, et al. Neuroprotective and antioxidant effects of ghrelin in an experimental glaucoma model. Drug Design, Development and Therapy [Internet]. Dove Medical Press Ltd.; 2015 Jun;2819. Available from: http://dx.doi.org/10.2147/dddt.s83067
7. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nature Reviews Immunology [Internet]. Springer Science and Business Media LLC; 2010 Nov

19;10(12):826–37. Available from: http://dx.doi.org/10.1038/nri2873

8. Chen J, John R, Richardson JA, Shelton JM, Zhou XJ, Wang Y, et al. Toll-like receptor 4 regulates early endothelial activation during ischemic acute kidney injury. Kidney International [Internet]. Elsevier BV; 2011 Feb;79(3):288–99. Available from: http://dx.doi.org/10.1038/ki.2010.381
9. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute Kidney Injury, Mortality, Length of Stay, and Costs in Hospitalized Patients. Journal of the American Society of Nephrology [Internet]. American Society of Nephrology (ASN); 2005 Sep 21;16(11):3365–70. Available from: http://dx.doi.org/10.1681/asn.2004090740
10. De Araújo AA, Varela H, de Medeiros CACX, de Castro Brito GA, de Lima KC, de Moura LM, et al. Azilsartan Reduced TNF-α and IL-1β Levels, Increased IL-10 Levels and Upregulated VEGF, FGF, KGF, and TGF-α in an Oral Mucositis Model. Bai C, editor. PLOS ONE [Internet]. Public Library of Science (PLoS); 2015 Feb 17;10(2):e0116799. Available from: http://dx.doi.org/10.1371/journal.pone.0116799
11. Devarajan P. Update on Mechanisms of Ischemic Acute Kidney Injury. Journal of the American Society of Nephrology [Internet]. American Society of Nephrology (ASN); 2006 May 17;17(6):1503–20. Available from: http://dx.doi.org/10.1681/asn.2006010017
12. Gerber H-P, Condorelli F, Park J, Ferrara N. Differential Transcriptional Regulation of the Two Vascular Endothelial Growth Factor Receptor Genes. Journal of Biological Chemistry [Internet]. American Society for Biochemistry & Molecular Biology (ASBMB); 1997 Sep 19;272(38):23659–67. Available from: http://dx.doi.org/10.1074/jbc.272.38.23659
13. Khan MAH, Necka J, Haines J, Imig JD. Azilsartan Improves Glycemic Status and Reduces Kidney Damage in Zucker Diabetic Fatty Rats. American Journal of Hypertension [Internet]. Oxford University Press (OUP); 2014 Mar 5;27(8):1087–95. Available from: http://dx.doi.org/10.1093/ajh/hpu016
14. Jung H, Choi EK, Baek SI, Cho C, Jin Y, Kwak KH, et al. The Effect of Nitric Oxide on Remote Ischemic Preconditioning in Renal Ischemia Reperfusion Injury in Rats. Dose-Response [Internet]. SAGE Publications; 2019 Apr;17(2):155932581985365. Available from: http://dx.doi.org/10.1177/1559325819853651
15. Ke Q, Costa M. Hypoxia-Inducible Factor-1 (HIF-1). Molecular Pharmacology [Internet]. American Society for Pharmacology & Experimental Therapeutics (ASPET); 2006 Aug 3;70(5):1469–80. Available from: http://dx.doi.org/10.1124/mol.106.027029
16. Lam S. Azilsartan. Cardiology in Review [Internet]. Ovid Technologies (Wolters Kluwer Health); 2011 Nov;19(6):300–4. Available from: http://dx.doi.org/10.1097/crd.0b013e31822e9ba3
17. Malek M, Maleki M. Protective effect of gastric distension preconditioning on renal ischemia/reperfusion injury in rats. Indian Journal of Nephrology [Internet]. Medknow; 2018;0(0):0. Available from: http://dx.doi.org/10.4103/ijn.ijn_342_16
18. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Bone marrow stem cells regenerate infarcted myocardium. Pediatric Transplantation [Internet]. Wiley; 2003 Apr;7:86–8. Available from: http://dx.doi.org/10.1034/j.1399-3046.7.s3.13.x.