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Diacerein Mitigates Renal Ischemia/Reperfusion Injury via Inhibition of Renal Inflammation, Dendritic Cells Maturation and Apoptosis: The Role of TLR4/NF-κB/NLRP3/IL-1β Signaling Pathway

La Diacereína Mitiga la Lesión por Isquemia/Reperfusión Renal a través de la Inhibición de la Inflamación Renal, la Maduración de las Células Dendríticas y la Apoptosis: El Papel de la vía de Señalización TLR4/NF-κB/NLRP3/IL-1β

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SUMMARY: One of the reasons for acute kidney damage is renal ischemia. Nevertheless, there are limited protective and therapeutic approaches for this problem. Diacerein is an anti-inflammatory drug characterized by numerous biological activities. We aimed to determine the ameliorative impact of diacerein on renal ischemia/reperfusion injury (I/R) condition, exploring the underlying mechanisms. Twenty-four male rats were allotted into four groups (n= 6): sham group; Diacerein (DIA) group; I/R group, in which a non-crushing clamp occluded the left renal pedicle for 45 min, and the right kidney was nephrectomized for 5 min before the reperfusion process; I/R + diacerein group, injected intraperitoneally with 50 mg diacerein/kg i.m 30 minutes prior to I/R operation. Ischemia/reperfusion was found to affect renal function and induce histopathological alterations. The flow cytometry analysis demonstrated an elevated expression of innate and mature dendritic cells in I/R renal tissues. Moreover, upregulation in the expression of the inflammatory genes (TLR4, Myd88, and NLRP3), and overexpression of the pro-inflammatory cytokines (IL-1β), apoptotic (caspase-3) and pyroptotic (caspase-1) markers were observed in I/R-experienced animals. The aforementioned deteriorations were mitigated by pre-I/R diacerein treatment. Diacerein alleviated I/R-induced inflammation and apoptosis. Thus, it could be a promising protective agent against I/R.

KEY WORDS: Diacerein; Renal ischemia; Dendritic cells; Oxidative stress; Inflammation; Apoptosis.

INTRODUCTION

Renal ischemia is one of the sever clinical problems that face patients admitted to hospitals (Palant *et al*., 2017), for renal transplantation, cardiovascular surgery (Ahmadiasl *et al*., 2014), partial nephrectomy, and renal tumors operation (Martin *et al*., 2012). It is a principal reason for acute renal failure, that progress to chronic renal failure (Malek & Nematbakhsh, 2015), affecting all the histological structure of the kidney, including the glomerulus, interstitium, tubules, and blood vessels (Zuk & Bonventre, 2016). Several studies report that reperfusion-induced renal tubular damage may be due to a large influx of calcium, reactive oxygen species (ROS), apoptosis, inflammatory cytokines (Gu *et al*., 2018).

Toll-like receptors (TLR) are a big family of transmembrane recognition proteins which incorporate ten members in humans. TLR4 is one of the important pattern-

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recognition receptors in renal ischemia. It was demonstrated to trigger the inflammatory response observed in experimental-induced ischemia-reperfusion injury effectively. It is activated by its attachment to the damage-associated molecular patterns (DAMPs) with subsequent activation of nuclear factor- kB (NF-κB), which upregulates the tumor necrosis factor-alpha (TNFα), pro-inflammatory cytokines, interleukin 6 (IL6), and interleukin-1 beta (IL1β) production (Yang *et al*., 2019). In addition, it is reported that renal tubular cell apoptosis is one of the most pathological processes during ischemia/reperfusion-induced renal damage (Kroemer *et al*., 2010). Thus, recent therapeutic approaches for renal I/R may have originated from research targeting inflammatory process, and apoptosis.

Diacerein, one of the anthraquinone derivatives, is a nonsteroidal anti-inflammatory drug with multiple pharmacological effects, such as anti-apoptotic and antioxidant activities (Bharti *et al*., 2017). It exerts its action by suppressing the IL-1 converting enzyme, a crucial limiting enzyme for releasing IL-1β cytokine, with blocking IL-1β receptor binding. Furthermore, it inhibits the expression of the pro-inflammatory mediator inducible nitric oxide synthase (iNOS) and subsequently, the synthesis of nitric oxide (NO) (Abdel-Gaber *et al*., 2018). Diacerein has been recognized to have a protective effect against doxorubicin and cisplatin-induced acute kidney injury via its anti-inflammatory and antioxidant properties (Refaie *et al*., 2016). Additionally, a former report demonstrated the ameliorative impact of diacerein and its active metabolite rhein on acetaminophen-induced nephrotoxicity and hepatotoxicity (Zhao *et al*., 1997). Ji *et al*. (2005) announced that treatment with 150 mg/kg/day of rhein protected the kidney in the early phase of glomerulosclerosis by attenuating the activities of caspase-3 and NF-κB. To our knowledge, no study verified the underlying anti-inflammatory and antiapoptotic mechanisms for the renoprotective impact of diacerein against renal ischemia. Hence, we carried out this research to evaluate the possible protective impact of diacerein on renal ischemia and its underlying mechanisms.

MATERIAL AND METHOD

Animals: Twenty-four male albino rats (200-220 g) aged (16-20 weeks) were brought from the National Research Center (Dokki, Giza, Egypt). Then, they were housed in three separate steel cages, one week before the experiment as an acclimatization span in the Faculty of Veterinary Medicine, Mansoura University, Egypt. They were kept in standard laboratory conditions at 24 ± 2 ºC with a 12-h light/dark cycle and food with free access to water throughout the experiment. Our experimental procedures were accepted by the Ethics Committee of Faculty of Veterinary medicine, Mansoura University (Approval No R/109). And all animal experiments were complied with the ARRIVE guidelines and carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Chemicals: Diacerein was obtained from (Eva Pharma, Egypt) and dissolved in 1 % carboxymethylcellulose (1 % CMC).

Renal I/R injury model: Renal ischemia-reperfusion was performed according to Zahran *et al*. (2015). In brief, the rats were anesthetized by being injected intraperitoneally with 4 % phenobarbitone with a dose of 10 ml/g body weight. The rats were placed in heating pads during the operation to maintain their temperature at 37 ºC. The operation started with a midline abdominal incision; then, the left renal pedicle was clamped by microaneurysm forceps for 45 min to occlude the renal pedicle to achieve maximum renal damage, without affecting the life of the rats. Five minutes before the end of the operation, the right kidney was removed; after that, the renal clamps were removed to ensure reperfusion, which was confirmed in the next 5 min of the operation by changing the color of the kidney to bluish color. Then 1 ml of 37 °C saline was injected into the abdominal cavity; after that, the abdominal incision was sutured in both layer; and 24 h after the perfusion, the rectal temperatures were maintained at 37 ºC. Finally, the left kidney was harvested.

Experimental groups: Rats were assigned into four groups (6 rats /group): Group I Sham group, we subjected rats to the same procedure of the surgery with right kidney discectomy without occluding the left renal pedicle; Group II sham group treated with diacerein with a dose (50 mg/kg i.m); Group II I/R group, in which the left renal pedicle occluded by a non-crushing clamp for 45 min and its right kidney was exposed to nephrectomy 5 min prior to the reperfusion process (Zahran *et al*., 2015); Group IV I/R+DIA: rats of this group were injected intraperitoneally with 50 mg diacerein /kg i.m 30 minutes before surgical operation in accordance with Abdel-Gaber *et al*. (2018).

Collection of blood and urine samples and harvested kidney specimens: After the operation, the rats were kept in 4 separate metabolic cages for 24 h to obtain urine samples to measure creatinine clearance and KIM-1. At the end of 24 hours for reperfusion, the animals were anesthetized intraperitoneally with 150 mg/kg body weight of sodium pentobarbitone, the blood samples were obtained from retro-orbital venous plexus, and the serum was separated by centrifugation and kept at -20 °C for further measuring

creatinine and blood urea nitrogen (BUN). Additionally, the left kidneys of different groups were collected and divided into three sections. We homogenized the first section in cold phosphate buffer saline (PBS) (Ph 7.4) and then centrifuged at 3000×g. We used the supernatant to measure oxidative stress biomarkers, and IL12 by ELISA, making flow cytometry to detect mature dendritic cells. We stored the second section at -80 °C for real-time PCR tests for gene expression measurement. Their last section part was kept in 10 % formalin for histopathological investigation: (Hematoxylin and Eosin), immunohistochemical analysis.

Renal function markers: KIM-1 protein expression in 24 hours of urine was measured by commercial ELIZA kits (Catalog Number: RKM29-K01) following the manufacturer's instructions. The levels of creatinine and BUN in serum were measured by an auto-analyzer (CX 7; Beckman, Foster City, CA, USA). Additionally, creatinine clearance (Cr Cl) was estimated as follows:

$$\text{Cr Cl (ml/min)} = \frac{\text{Urine volume } \left(\frac{\text{ml}}{24\text{h}}\right) x \; Urine\ creatinine\ concnetration\ (\frac{mg}{dl})}{plasma\ creatinine\ (\frac{mg}{dl})}$$

RT-PCR Assessment: Extraction of homogenized renal tissue total RNA was evaluated using direct-zol RNA (Cat# R2072., USA). The total RNA was reversely transcribed into complementary DNA (cDNA) by RT-PCR kit (Cat# 12594100, Thermo Fisher Scientific, Waltham, MA, USA), followed by PCR. 96-well plate StepOne instrument (Applied Biosystem, USA) was utilized in a thermal profile. After the RT-PCR run, data were presented in Cycle threshold (Ct) for the target genes and housekeeping gene. The normalization for variation in the expression of target genes: NFκB, TLR4, NLRP3, and MYD88 was carried out as per the mean critical threshold (CT) expression values of GAPDH housekeeping gene via the ΔΔCt method. The relative quantitation (RQ) of every target gene was calculated using the 2-ΔΔCt method.

Histopathological examination: The renal tissues were fixed in 10 % buffered formalin. After that, they were dehydrated by ascending grades of ethanol and then immersed in a paraffin block. Later, a 5 µm thickness section of renal tissues was cut and stained with H&E and examined by light microscopy (400x). The quantitative evaluation of tubular injury in I/R lesions according to the study done by Patel *et al*. (2004).

Immunohistochemical assay of inflammatory cytokines (IL-1β), apoptotic marker (caspase-3), pyroptotic marker (caspase-1): Immunohistochemical measurement for inflammatory cytokines (IL-1β), apoptotic marker (caspase-3) and pyroptosis marker (caspase-1) was performed according to Ramos-Vara *et al*. (2008). Positive area staining was carried out in renal tissue (mean area) (estimated by taking the average values from ten fields at ten magnifications) for every renal tissue region by ImageJ software).

Flow cytometry of the dendritic cells and detection of their inflammatory cytokine IL-12 by ELISA: We used single-cell suspension from renal tissue to assess dendritic cell maturation and abundance by staining it with fluorochrome-labeled antibodies: anti-CD11c (PE) and MHC-2 (FITC). Then, we conducted flow cytometry analysis by (Accuri c6 Becton Dickenson, Germany). We measured the levels of IL-12 in the supernatants of renal homogenates by ELISA kits (Catalog No. LS-F23156) following the manufacturer's guidelines.

Statistical analysis: Data were exhibited as mean ± SD. Shapiro-Wilk test was used to examine the data normality. one-way ANOVA was utilized, followed by Tukey's *post hoc* test for comparison between experimental groups. The threshold for significance was adjusted at $p< 0.05$. GraphPad Prism 9 Software was utilized for data analysis.

RESULTS

Impact of Diacerein on renal function markers: As shown in Table I, renal ischemia significantly affected renal function by increasing serum creatinine, urea, and 24 hours urine level of kidney injury molecule -1 (KIM-1) and decreased creatinine clearance relative to sham rats. In contrast, diacerein-treated rats significantly improved renal function by decreasing the serum level of creatinine, urea, and 24 hours urine KIM-1 and had a remarkable increase in creatinine clearance counterweight to ischemic rats ($p< 0.001$).

Table I. The impact of diacerein on renal functions (serum creatinine, blood urea nitrogen (BUN), creatinine clearance (CrCl), KIM-1 in different experimental groups.

Experimental groups	Sham	DIA	I/R	I/R+DIA
Serum creatinine (mg/dl)	0.63 ± 0.04	0.72 ± 0.05	$2.97 \pm 0.18^{\&\&}$	$1.43 \pm 0.32^{\&\$\$\$}$
Serum BUN (mg/dl)	31.2 ± 2.77	35.7 ± 2.41	$246 \pm 12.56^{\&\&\&}$	$106.6 \pm 36.86^{\&\&\$\$\$}$
Creatinine Clearance (ml/min)	0.272 ± 0.01	0.288 ± 0.01	$0.016 \pm 0.00^{\&\&}$	$0.088 \pm 0.03^{\&\&\$\$\$}$
KIM-1 (ng/ml)	0.278 ± 0.31	0.293 ± 0.31	$1.2 \pm 0.15^{\&\&}$	$0.546 \pm 0.10^{\&\&\$\$\$}$

Data are expressed as mean ± SD. $^{\&\&\&}p < 0.001$, $\&\&p < 0.01$ and $^{\&}p < 0.05$ significant vs sham group, $^{\$\$\$}p < 0.001$, and $^{\$\$}p < 0.01$ significant vs I/R group.

Influence of diacerein on renal morphology. By examining the renal tissue using H and E staining, we found that the sham and diacerein groups rats (Figs. 1A, B) showed normal glomeruli (G) and tubules (T). In contrast, in the I/R group, (Figs. 1C, D and E) there was great affection in the histology of the renal tissues in the form of severe congestion of BV around the glomeruli, swelling of renal tubular cells, loss of brush-border membranes, necrosis, and interstitial edema. This affection was significantly improved by pretreatment with diacerein which showed mild epithelial cells degeneration in a few tubules (Fig. 1F). By scoring the tubular injury, we found that pretreatment with 50mg diacerein significantly alleviated ($p < 0.001$) the tubular necrosis concerning I/R group (Fig. 1G).

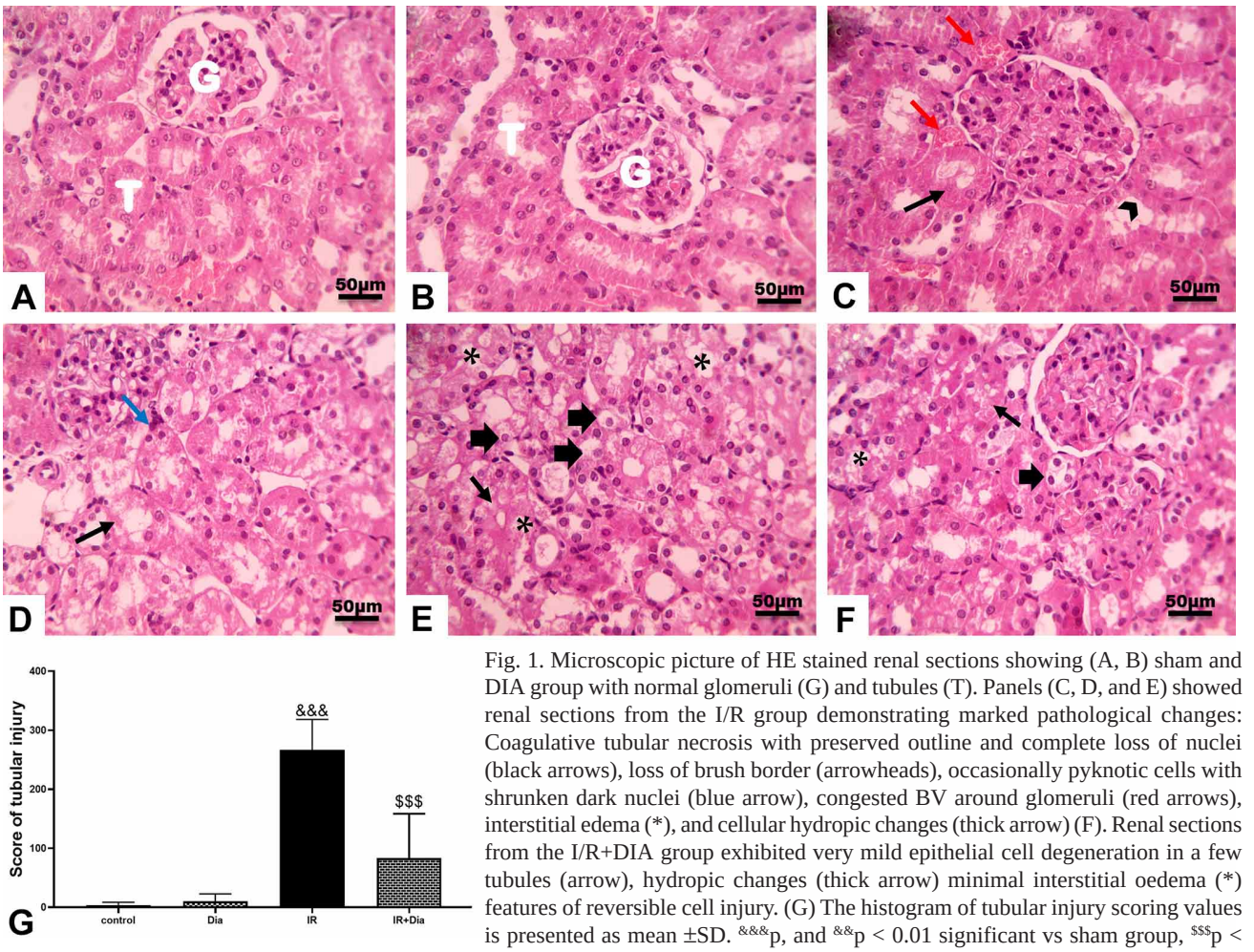

Fig. 1. Microscopic picture of HE stained renal sections showing (A, B) sham and DIA group with normal glomeruli (G) and tubules (T). Panels (C, D, and E) showed renal sections from the I/R group demonstrating marked pathological changes: Coagulative tubular necrosis with preserved outline and complete loss of nuclei (black arrows), loss of brush border (arrowheads), occasionally pyknotic cells with shrunken dark nuclei (blue arrow), congested BV around glomeruli (red arrows), interstitial edema (*), and cellular hydropic changes (thick arrow) (F). Renal sections from the I/R+DIA group exhibited very mild epithelial cell degeneration in a few tubules (arrow), hydropic changes (thick arrow) minimal interstitial oedema (*) features of reversible cell injury. (G) The histogram of tubular injury scoring values is presented as mean ±SD. $^{\&\&\&}p$, and $^{\&\&}p < 0.01$ significant vs sham group, $^{\$\$\$}p < 0.001$ significant vs I/R group (H&E, X: 400, Bar 50 µm).

Effect of diacerein on the inflammatory pathway (TLR4/NF-κB/NLRP-3) gene expression and pro-inflammatory cytokines (IL-1β) immunoexpression. The expression of TLR4, MYD88, NF-κB, and NLRP3 genes in renal tissues at the mRNA level was significantly elevated in the I/R groups (Figs. 2A, B, C and D), indicating exaggerated inflammatory response, which subsequently resulted in upregulating nuclear transcription factor NF-κB, increasing the protein level of pro-inflammatory cytokine immunoexpression of IL-1β (Fig. 3C) in relation to the sham group. Presurgical administration of diacerein markedly downregulated ($p< 0.001$) the genes and the immunoexpression of inflammatory pathway compared to I/R group, indicating the powerful anti-inflammatory character of diacerein against renal ischemia.

Effect of diacerein on the inflammatory response mediated by renal dendritic cells (DCs) population and IL-12 immune expression. By examining DCs in renal tissue by flow cytometry, either innate DCs through its receptor CD11c expression or mature DCs through its receptor MCH-II expression, we found that renal ischemia/reperfusion markedly upregulated the expression of innate DCs and mature DCs (Figs. 4C and G respectively) in

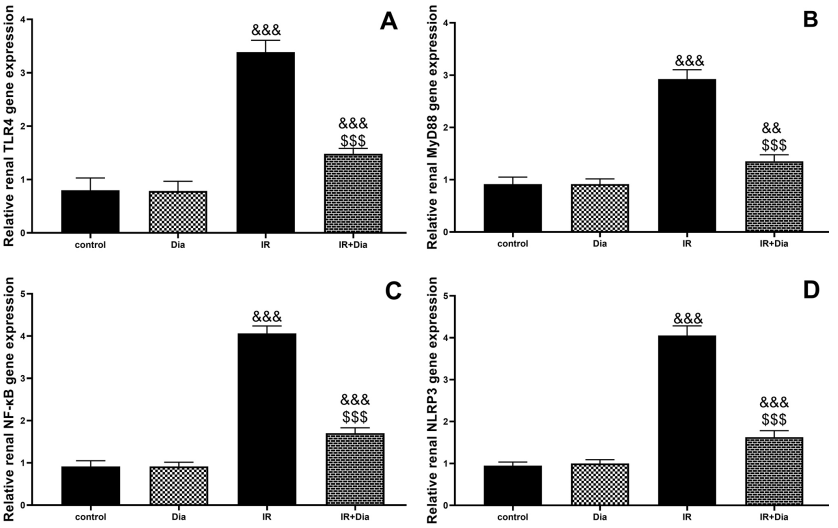

Fig. 2. Effect of diacerein treatment on the expression of antioxidant genes A) TLR4 B) MYD88 C) NFκB D) NLRP3 in different groups of rats. $^{\&\&\&}p < 0.001$ significant vs sham group, $^{\$\$\$}p < 0.001$ significant vs I/R group.

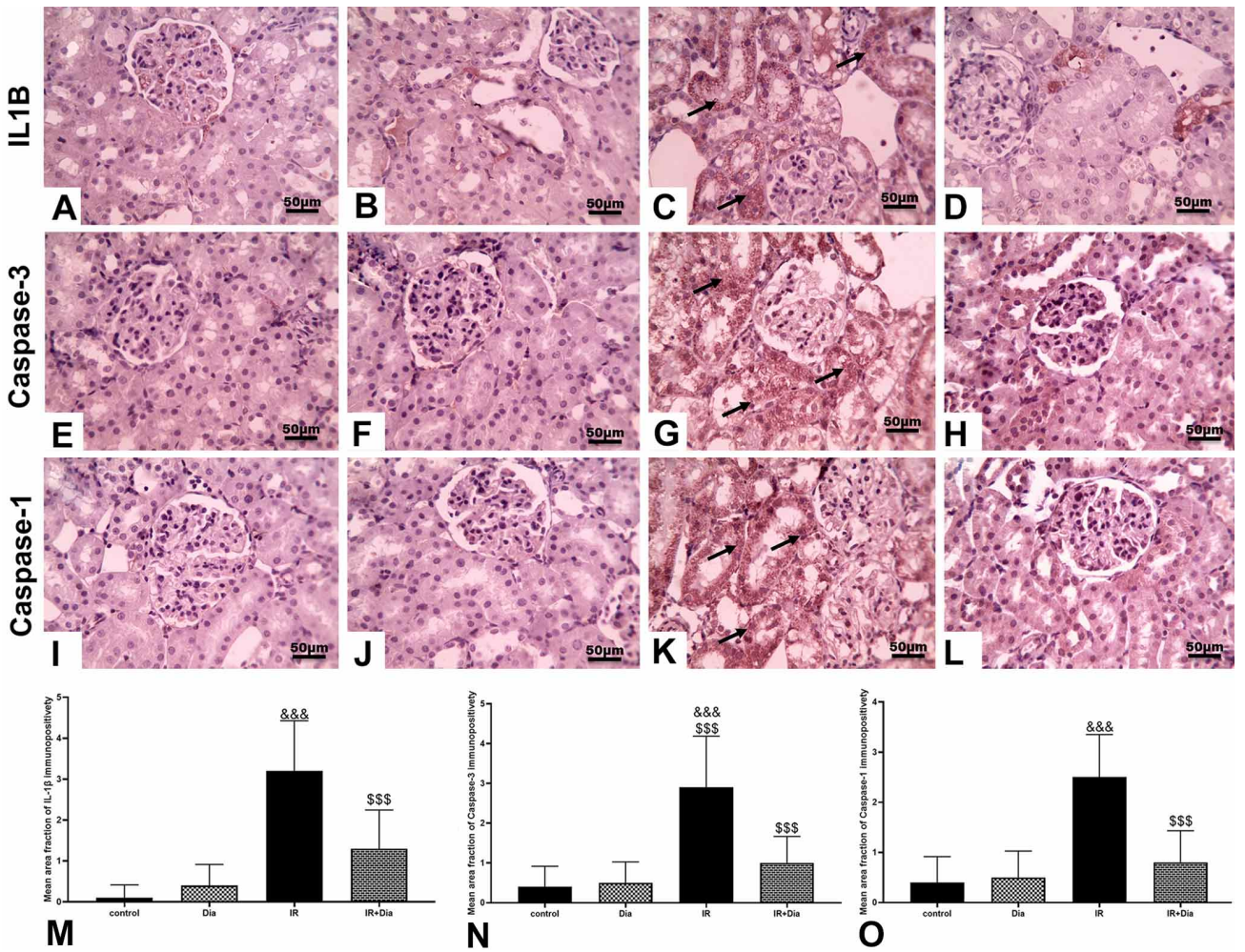

Fig. 3. Photomicrographs of immunostained renal sections against IL-1β, Caspase-3, Caspase-1 demonstrate a negative tubular reaction in sham (A, E, I) and DIA groups (B, F, J) groups, a strong positive brown cytoplasmic reaction in the tubular epithelial cells (Black arrows) in the I/R group (C, G, K), and very minimal reaction (Black arrows) in I/R+DIA group (D, H, L). (M, N, O) panel reveals the histogram of the area fraction of immunopositivity. $^{\&\&\&}p < 0.001$ significant vs sham group, $\$^{\$\$}p < 0.001$ significant vs I/R group. (IHC counterstained with Mayer's hematoxylin. X:400, Bar 50 µm).

comparison with the sham group. Diacerein treatment significantly downregulated ($p < 0.001$) the expression of innate and mature DCs (Figs. 4D and H respectively) in relation to the I/R group. As a result of this downregulating effect of diacerein on DCs, the secretion of the inflammatory marker (IL-12) was downregulated (Fig. 4K) compared to the ischemic group, resulting in more inhibition of the inflammatory process.

Effect of diacerein on apoptosis and pyroptotic markers (Caspase-3, caspase-1 immunoexpression). Renal tissue apoptotic and pyroptotic cell marker caspase-3 and caspase-1 at the protein level was significantly elevated in the I/R group (Figs. 3G and K) in comparison to the sham group. Diacerein treatment showed a strong anti-apoptotic property by decreasing ($p < 0.001$) the caspase-3 positive cells compared to the renal ischemic group.

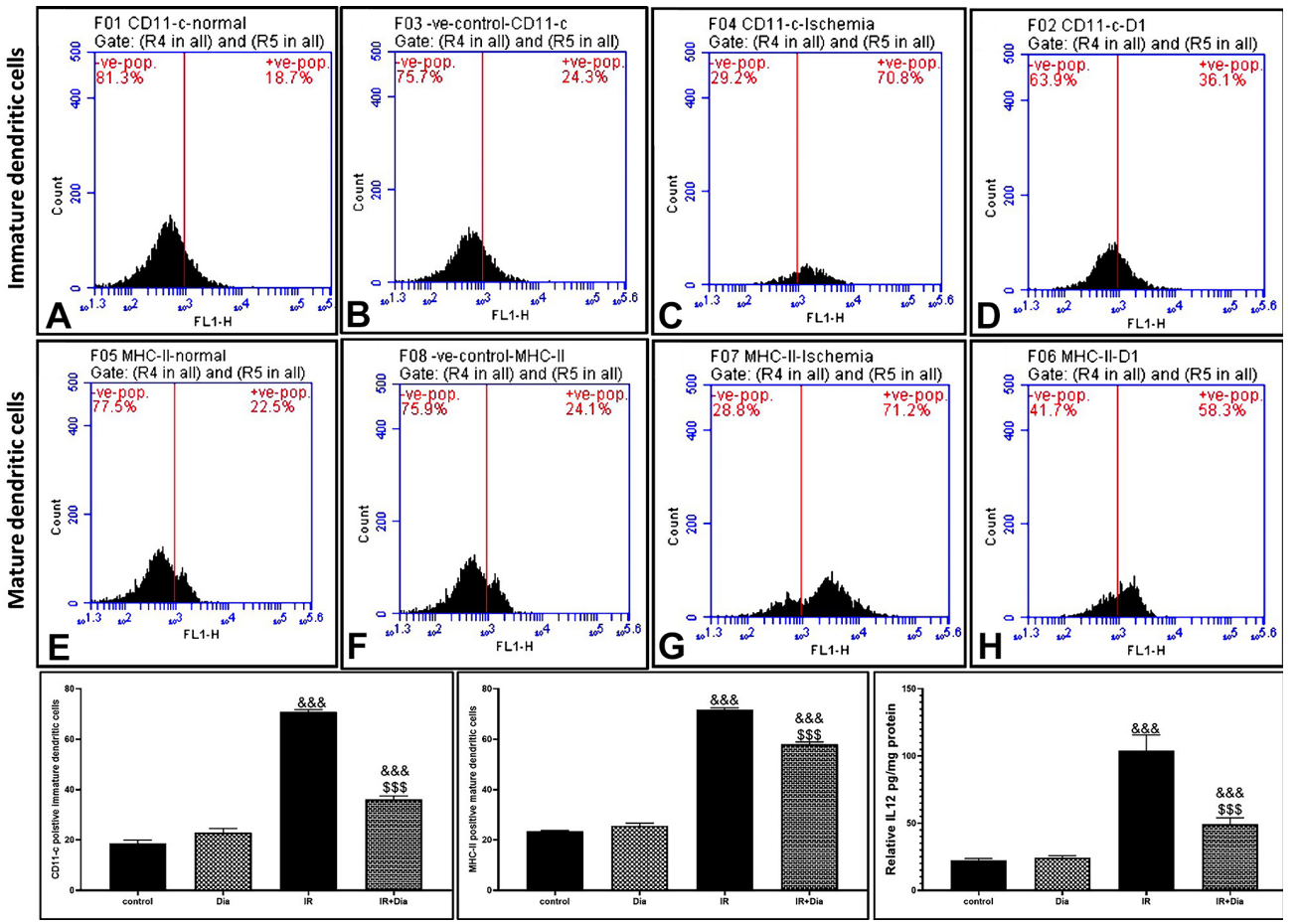

Fig. 4. The flow cytometric analysis of single-cell suspensions of the kidney for CD11-C positive immature and MHC-II positive mature dendritic cell in different experimental groups shows normal expression for sham (A,E) and DIA group (B,F), marked expression in I/R group (C,G) and moderate expression in I/R+DIA group (D,H); (I) Histogram of CD11-c positive immature cells; (J) Histogram of MHC-II positive mature cells; (K) The ELISA results showing the level of IL-12 in different groups. $^{\&\&\&}p < 0.001$significant vs sham group, $^{\&\&\&}p < 0.001$, significant vs I/R group.

DISCUSSION

Renal ischemia/reperfusion injury is one of the major complications of renal transplantation, renal vascular surgeries, aortic clamping, and shock. Many reports discussed the underlying pathophysiological pathways and their impact on renal function (Sharfuddin & Molitoris, 2011). Nevertheless, the full pathophysiology of renal ischemia has not been fully understood.

The first goal of our study was to examine the renoprotective of diacerein against renal ischemia. We found

that renal ischemia/reperfusion deteriorated renal function by increasing serum creatinine, urea, and 24 hours urine level of KIM-1, as the first indicator of acute kidney injury, with decreasing creatinine clearance; similar results were reported by Awadalla *et al*. (2021). According to Goldfarb *et al*. (2001), the elevation of serum creatinine is associated with renal medullary injury, which explained by the fact that reperfusion produces anoxia, which leads to lipid peroxidation and may be the causative agent of apoptosis and cellular necrosis (Shimizu *et al*., 2016). However, pretreatment with diacerein showed statistical improvement in renal function markers serum creatinine, urea, creatinine clearance, and KIM-1. These findings agree with those of Barakat *et al*. (2021). To the best of our knowledge, this is the first work declaring the downregulatory effect of diacerein on KIM-1 secretion. This study showed considerable improvement in the histopathological picture in ischemic acute kidney injury. Similar results reported by Refaie *et al*. (2016). DIA renoprotective regarded to its anti-inflammatory and anti-apoptotic effects, preserving renal tubular epithelial cell damage.

The second goal of our work is to explore the underlying renoprotective mechanisms of diacerein against renal ischemia. In our study, DIA pretreatment decreased IL-1β immunoexpression, inhibiting renal inflammation (Athanasopoulos *et al*., 2016).

TLR4 receptors are essential pattern-recognition transmembrane proteins in monocytes, macrophages, and renal tubular epithelial cells activated by recognition and binding to DAMPs released from damaged cells after exposure to ischemia (Du *et al*., 2019), leading to activation of Myd88 and NF-κB. This nuclear transcription factor activates the production of pro-inflammatory cytokines. In our study, we found that renal ischemia increased mRNA expression of TLR4, MyD88, and NF-κB with subsequent overproduction of protein level of proinflammatory cytokine (IL-1b). This finding agrees with Wu *et al*. (2007). In contrast, treatment with diacerein downregulated the expression of mRNA of TLR4, MyD88, and NF-κB, decreasing the protein level of pro-inflammatory cytokine (IL-1β). These results are consistent with Ibrahim *et al*. (2021). These outcomes demonstrated that diacerein has a powerful renal anti-inflammatory character through two dependent ways first by its inhibitory effect on TLR4/NF-κB- pathway with its subsequent secretion of pro-inflammatory mediators, and second, via its well-known direct blockage action against IL-1β receptors which starts the renal ischemic inflammatory flu.

Dendritic cells are the most abundant leukocyte in renal tissue and their activation with inflammatory cytokines production leads to renal damage. We found that 45 minutes of renal ischemia upregulated innate and mature DCs and their inflammatory marker IL12. This finding agrees with Song *et al*. (2018). Diacerein pretreatment significantly downregulated the expression of innate and mature DCs and their inflammatory cytokine IL12 because diacerein has an inhibitory impact on the NF-κB transcription factor, which has a positive regulator of the maturation of DCs (Lopez *et al*., 2015). To our knowledge, we are the first to discuss the regulatory effect of diacerein on renal dendritic cells.

Regarding the renoprotective effect of diacerein on renal tubular epithelial cells survival, we found increased immunoexpression of caspase 3 in the I/R group in comparison to the sham. This finding is consistent with Yang *et al*. (2010). Diacerein treatment significantly downregulated caspase-3 immunoexpression in the ischemic group. This finding agrees with those of Refaie *et al*. (2016). Pyroptosis is a highly inflammatory form of lytic programmed cell death. We found that renal ischemia significantly increased the mRNA gene expression of NLRP3 in renal tissue and the immunoexpression of caspase 1, which promoted pyroptosis. This finding is consistent with Sun *et al*. (2020). DIA treatment markedly downregulated the expression of NLRP3 mRNA and caspase-1 immunoexpression, in keeping with Refaie *et al*. (2022), indicating that diacerein might have an anti-apoptotic effect on ischemic renal tubular cells. Inhibition of inflammasome activation by diacerein with its subsequent activation of caspase-1, changes proactive IL-1β into active IL-1β supporting the well-known theory of the specific targeted inhibitory effect of diacerein on IL-1 β by direct locking of its receptor. The inhibition of NFκB phosphorylation, NLRP3 inflammasome inhibition, and autophagic stimulation has an inhibitory effect against the maturation and secretion of pro-inflammatory cytokines (Saitoh *et al*., 2008). The anti-apoptotic effect of diacerein in renal ischemia may be due to its upregulating and downregulating effects on renal autophagic and inflammatory processes, respectively.

CONCLUSIONS

Administration of diacerein markedly attenuated renal I/R injury in rats. This ameliorative effect of diacerein could be attributed to inhibition of TLR4/NF-κB/NLRP3 inflammatory pathway with subsequent depression of IL-1b proinflammatory cytokine secretions and dendritic cells maturation. Moreover, diacerein improved renal tubular epithelial cell survival by reducing apoptotic caspase-3, and pyroptotic caspase-1 cellular death. Hence, diacerein could be a promising protective agent against renal I/R.

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RESUMEN: La isquemia renal es una de los motivos del daño renal agudo. Sin embargo, los enfoques protectores y terapéuticos para este problema son limitados. La diacereína es un fármaco antiinflamatorio caracterizado por numerosas actividades biológicas. Nuestro objetivo fue determinar el impacto de mejora de la diacereína en la condición de lesión por isquemia/reperfusión renal (I/R), explorando los mecanismos subyacentes. Veinticuatro ratas macho se distribuyeron en cuatro grupos (n= 6): grupo simulado; grupo de diacereína (DIA); grupo I/R, en el que una pinza no aplastante ocluyó el pedículo renal izquierdo durante 45 min, y el riñón derecho fue nefrectomizado durante 5 min antes del proceso de reperfusión; Grupo I/R + diacereína, inyectado por vía intraperitoneal con 50 mg de diacereína/kg i.m. 30 min antes de la operación I/R. Se encontró que la isquemia/reperfusión afecta la función renal e induce alteraciones histopatológicas. El análisis de citometría de flujo demostró una expresión elevada de células dendríticas innatas y maduras en tejidos renales I/R. Además, se observó una regulación positiva en la expresión de los genes inflamatorios (TLR4, Myd88 y NLRP3) y una sobreexpresión de las citoquinas proinflamatorias (IL-1β), marcadores apoptóticos (caspasa-3) y piroptóticos (caspasa-1) en animales con experiencia en I/R. Los deterioros antes mencionados fueron mitigados por el tratamiento previo a la diacereína I/R. La diacereína alivió la inflamación y la apoptosis inducidas por I/R. Por lo tanto, podría ser un agente protector prometedor contra I/R.

PALABRAS CLAVE: Diacereína; Isquemia renal; Células dendríticas; Estrés oxidativo; Inflamación; Apoptosis.

REFERENCES

Abdel-Gaber, S. A.; Mohammed, R. K. & Refaie, M. M. M. Mechanism mediating the protective effect of diacerein in ischemia-reperfusion-induced testicular injury in rats. *Life Sci., 209*:57-62, 2018.

Ahmadiasl, N.; Banaei, S.; Alihemmati, A.; Baradaran, B. & Azimian, E. The anti-inflammatory effect of erythropoietin and melatonin on renal ischemia-reperfusion injury in male rats. *Adv. Pharm. Bull., 4(1)*:49-54, 2014.

Athanasopoulos, P.; Mastoraki, A.; Papalois, A.; Nastos, C. & Kondi-Pafiti, A.; Kostopanagiotou, G.; Smyrniotis, V. & Arkadopoulos, N. Expression of inflammatory and regenerative genes in a model of liver ischemia/reperfusion and partial hepatectomy. *J. Invest. Surg., 29(2)*:67-73, 2016.

Awadalla, A.; Hussein, A. M.; El-Far, Y. M.; Barakat, N.; Hamam, E. T.; El-Sherbiny, M.; El-Shafey, M. & Shokeir, A. Effect of zinc oxide nanoparticles and ferulic acid on renal ischemia/reperfusion injury: possible underlying mechanisms. *Biomed. Pharmacother., 140*:111686, 2021.

Barakat, N.; Barakat, L.; Zakaria, M. M. & Khirallah, S. M. Diacerein ameliorates kidney injury induced by cisplatin in rats by activation of Nrf2/Ho-1 pathway and Bax down-regulation. *Saudi J. Biol. Sci., 28(12)*:7219-26, 2021.

Bharti, R.; Dey, G.; Banerjee. I.; Dey, K. K.; Parida, S.; Kumar, B. N.; Das, C. K.; Pal, I.; Mukherjee, M.; Misra, M.; *et al*. Somatostatin receptor targeted liposomes with Diacerein inhibit IL-6 for breast cancer therapy. *Cancer Lett., 388*:292-302, 2017.

Du, Y.; Qian, B.; Gao, L.; Tan, P.; Chen, H.; Wang, A.; Zheng, T.; Pu, S.; Xia, X. & Fu, W. Aloin preconditioning attenuates hepatic ischemia/reperfusion injury via inhibiting TLR4/MyD88/NF- k B signal pathway in vivo and *in vitro. Oxid. Med. Cell. Longev., 2019*:3765898, 2019.

Goldfarb, M.; Abassi, Z.; Rosen, S.; Shina, A.; Brezis, M. & Heyman, S. N. Compensated heart failure predisposes to outer medullary tubular injury: studies in rats. *Kidney Int., 60(2)*:607-13, 2001.

Gu, L.; Tao, Y.; Chen, C.; Ye, Y.; Xiong, X. & Sun, Y. Initiation of the inflammatory response after renal ischemia/reperfusion injury during renal transplantation. *Int. Urol. Nephrol., 50(11)*:2027-35, 2018.

Ibrahim, M. A.; Abdelzaher, W. Y.; Ibrahim, Y. F.; Ahmed, A. F.; Welson, N. N.; Al-Rashed, S.; Batiha, G. E. & Abdel-Aziz, A. M. Diacerein protects rats with liver ischemia/reperfusion damage: Down-regulation of TLR4/NFk-B signaling pathway. *Biomed Pharmacother., 134*:111063, 2021.

Ji, Z. Q.; Huang, C. W.; Liang, C. J.; Sun, W. W.; Chen, B. & Tang, P. R. Effects of rhein on the activity of caspase-3 in kidney and cell apoptosis on the progression of renal injury in glomerulosclerosis. *Zhonghua Yi Xue Za Zhi, 85(26)*:1836-41, 2005.

Kroemer, G.; Marino, G. & Levine, B. Autophagy, and the integrated stress response. *Mol. Cell, 40(2)*:280-93, 2010.

Lopez, S.; Gomez, E.; Torres, M. J.; Pozo, D.; Fernandez, T. D.; Ariza, A.; Sanz, M. L.; Blanca, M. & Mayorga, C. Beta-lactam antibiotics affect human dendritic cells maturation through MAPK/NF-kB systems. Role in allergic reactions to drugs. *Toxicol. Appl. Pharmacol., 288(3)*:289-99, 2015.

Malek, M. & Nematbakhsh, M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. *J. Renal Inj. Prev., 4(2)*:20-7, 2015.

Martin, G. L.; Warner, J. N.; Nateras, R. N.; Andrews, P. E.; Humphreys, M. R. & Castle, E. P. Comparison of total, selective, and nonarterial clamping techniques during laparoscopic and robot-assisted partial nephrectomy. *J. Endourol., 26(2)*:152-6, 2012.

Palant, C. E.; Amdur, R. L. & Chawla, L. S. Long-term consequences of acute kidney injury in the perioperative setting. *Curr. Opin. Anaesthesiol., 30(1)*:100-4, 2017.

Patel, N. S. A.; Cuzzocrea, S.; Chatterjee, P. K.; Di Paola, R.; Sautebin, L.; Britti, D. & Thiemermann, C. Reduction of renal ischemia-reperfusion injury in 5-lipoxygenase knockout mice and by the 5-lipoxygenase inhibitor zileuton. *Mol. Pharmacol., 66(2)*:220-7, 2004.

Ramos-Vara, J. A.; Kiupel, M.; Baszler, T.; Bliven, L.; Brodersen, B.; Chelack, B.; Czub, S.; Del Piero, F.; Dial, S.; Ehrhart, E. J.; *et al*. Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. *J. Vet. Diagn. Invest., 20(4)*:393-413, 2008.

Refaie, M. M. M.; Amin, E. F.; El-Tahawy, N. F. & Abdelrahman, A. M. Possible protective effect of diacerein on doxorubicin-induced nephrotoxicity in rats. *J. Toxicol., 2016*:9507563, 2016.

Refaie, M. M. M.; El-Hussieny, M. & Abdelraheem, W. M. Diacerein ameliorates induced polycystic ovary in female rats via modulation of inflammasome/caspase1/IL1b and Bax/Bcl2 pathways. *Naunyn Schmiedebergs Arch. Pharmacol., 395(3)*:295-304, 2022.

Saitoh, T.; Fujita, N.; Jang, M. H.; Uematsu, S.; Yang, B. G.; Satoh, T.; Omori, H.; Noda, T.; Yamamoto, N.; Komatsu, M.; *et al*. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature, 456(7219)*:264-8, 2008.

Sharfuddin, A. A. & Molitoris, B. A. Pathophysiology of ischemic acute kidney injury. *Nat. Rev. Nephrol., 784)*:189-200, 2011.

Shimizu, S.; Tsounapi, P.; Dimitriadis, F.; Higashi, Y.; Shimizu, T. & Saito, M. Testicular torsion-detorsion and potential therapeutic treatments: A possible role for ischemic postconditioning. *Int. J. Urol., 23(6)*:454-63, 2016.

Song, N.; Zhang, T.; Xu, X.; Lu, Z.; Yu, X.; Fang, Y.; Hu, J.; Jia, P.; Teng, J. & Ding, X. miR-21 protects against ischemia/reperfusion-induced acute kidney injury by preventing epithelial cell apoptosis and inhibiting dendritic cell maturation. *Front. Physiol., 9*:790, 2018.

Sun, W.; Li, A.; Wang, Z.; Sun, X.; Dong, M.; Qi, F.; Wang, L.; Zhang, Y. & Du, P. Tetramethylpyrazine alleviates acute kidney injury by inhibiting NLRP3/HIF 1a and apoptosis. *Mol. Med. Rep., 22(4)*:2655-64, 2020.

Wu, H.; Chen, G.; Wyburn, K. R.; Yin, J.; Bertolino, P.; Eris, J. M.; Alexander, S. I.; Sharland, A. F. & Chadban, S. J. TLR4 activation mediates kidney ischemia/reperfusion injury. *J. Clin. Invest., 117(10)*:2847-59, 2007.

Yang, B.; Hosgood, S. A.; Harper, S. J. & Nicholson, M. L. Leucocyte depletion improves renal function in porcine kidney hemoreperfusion through reduction of myeloperoxidase+ cells, caspase-3, IL-1b, and tubular apoptosis. *J. Surg. Res., 164(2)*:e315-24, 2010.

Yang, L.; Jiang, L.; Jiang, D.; Liu, B. & Jin, S. The protective effects of salvianolic acid A against hepatic ischemia-reperfusion injury via inhibiting expression of toll-like receptor 4 in rats. *Arch. Med. Sci., 15(6)*:1599-607, 2019.

Zahran, M. H.; Hussein, A. M.; Barakat, N.; Awadalla, A.; Khater, S.; Harraz, A. & Shokeir, A. A. Sildenafil activates antioxidant and antiapoptotic genes and inhibits proinflammatory cytokine genes in a rat model of renal ischemia/reperfusion injury. *Int. Urol. Nephrol., 47(11)*:1907-15, 2015.

Zhao, J. J.; Rogers, J. D.; Holland, S. D.; Larson, P.; Amin, R. D.; Haesen, R.; Freeman, A.; Seiberling, M.; Merz, M. & Cheng, H. Pharmacokinetics and bioavailability of montelukast sodium (MK-0476) in healthy young and elderly volunteers. B*iopharm. Drug Dispos., 18(9)*:769-77, 1997.

Zuk, A. & Bonventre, J. V. Acute kidney injury. *Ann. Rev. Med., 67*:293-307, 2016.

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