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Alogliptin Alleviates Ischemia/ Reperfusion Induced Kidney Injury by Attenuating Inflammation and Apoptosis in Rats Model

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Abstract: Ischemia/reperfusion injury is a complicated phenomenon leading to cellular damage via the biphasic process. Ischemia causes injury by reducing or entirely eliminating the energy supply required for homeostasis. Reperfusion exacerbates cellular damage through several proposed mechanisms, including inflammatory responses and the production of free radicals and oxygen. A total of 24 adult male rats were randomly divided into four groups(n=6). **Sham group**: Rats were anesthetized and had a surgery without ischemia-reperfusion induction. **I/R control group**: Rats underwent a same anesthetic procedure, followed by 40 min bilateral renal ischemia, then 2 hr. of reperfusion. **DMSO group**: Rats were received DMSO (as a solvent for alogliptin) ip injection at 24hr and 30 min. before surgery (an identical surgical technique as the control group). **Alogliptin treated group:** Rats were received alogliptin ip injection 10 mg/kg at 24h and 30 min. before surgery (an identical surgical technique as the control group). All rats had their blood samples and renal tissues collected the conclusion of the reperfusion phase. In blood samples, serum urea and creatinine were measured by the standard laboratory protocols, and by using ELISA method, KIM-1 measurement was taken in kidney tissues. Furthermore, kidney histopathological investigation was conducted by assessing the extent of degeneration, tubular dilation, interstitial lymphocyte infiltration, and loss of brush borders. It was concluded that kidney damage resulted from elevated urea, creatinine, and KIM-1 levels noted in blood samples and kidney tissues of rats in both control and DMSO groups in contrast with sham group. In comparison to the control and DMSO groups, rats in the treatment group showed decreasing levels of urea, creatinine, and KIM-1, Furthermore, the treated group with alogliptin also had decreased histopathological damage scores. these results suggest that alogliptin can prevent kidney damage as mainly evidenced by the decreased histopathological damage region, enhanced renal functions. Alogliptin's characteristics may facilitate its application in the management of acute kidney injury (AKI).

Keywords: Alogliptin, ischemia/reperfusion, urea, creatinine, and Kim-1

1.Introduction:

Ischemia and reperfusion constitute a morbid state that initially restricts the blood flow to an organ, then restores perfusion and reoxygenation simultaneously. A prolonged duration of ischemia causes lactate accumulation and anaerobic metabolism, which lower intracellular pH and ATP levels. Consequently, the strategy of ATPase-dependent ion transport will not work, leading to an excess of calcium within cells and mitochondria, swelling, rupture of cells, and death of cells by processes like necrosis, necroptosis, apoptosis, and autophagia **(Kalogeris et al., 2012)**. Raising oxygen levels during reperfusion stimulates oxidative stress, inflammatory cytokines, neutrophil recruitment, excitation, attachment, and immigration, all of which lead to ischemic injury (**Oliveira *et al*., 2018)**. Renal ischemia/reperfusion damage is the predominant etiology of acute kidney injury. Increased morbidity, mortality, and length of hospital stay are associated with it **(Chiang et al., 2020).** In acute renal disease causes urea levels to rise early, although serum creatinine is a more reliable indicator of renal function **(Gowda et al., 2010).** Following ischemia injury, the kidney cortex expresses more Kidney injury molecule-1(KIM-1) mRNA and protein, with a peak expression after reperfusion **(Ismail et al., 2015).** The underlying mechanism of renal I/R is extremely complicated. After reperfusion, several physiological and pathological alterations such as oxidative damage, inflammation, and metabolic dysfunction, follow the initial energy deficit **(Wang *et al*., 2020).** Alogliptin is a novel family of a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4), this is an oral anti-hyperglycemic agent. Most short-term studies indicate that DPP-4 inhibitors are safe and exhibit pleiotropic favorable effects. This DPP-4 inhibitor (also known as the "gliptin" class) can help persons with (T2DM) more effectively control their level of blood sugar **(Covington *et al*., 2008)**

Aim: Investigate the reno-protective effects of alogliptin on ameliorating the damage induced by renal ischemia reperfusion injury.

2.Methodology

2.1. Animal Groups the study effectively adhered to all considerations and conventions involving animals that were approved by the Animals Care Committee. The ethical clearance, with the number (12771) was approved in (19/5/2024), by the University of Kufa / Faculty of Pharmacy. A male white albino rats *(rat Rattus*) weighing 150-250g and aged 15-20 weeks were acquired from the Faculty of Science, UOK. The animal house was used as the home for the rats at Faculty of Science /UOK. The animals were stored in an isolated room in a group caging system, with temperature and humidity set at 25±2 C° and 60-65%, respectively, with a 12-hour light and 12-hour dark cycle. The experiment involved giving the rats tap water and a typical. A total of 24 adult male rats were randomly assigned to four groups (n=6). Sham **group**: Rats were anesthetized and had a surgery without ischemia-reperfusion induction. **I/R control group**: Rats underwent a same anesthetic procedure, followed by 40 min bilateral renal ischemia, then 2 hr. of reperfusion (Herrera-Luna et al., 2022). **DMSO group**: Rats were received DMSO (as a solvent for alogliptin) ip injection at 24hr and 30 min. before surgery (an identical surgical technique as the control group). **Alogliptin treated group**: Rats were received alogliptin ip injection 10 mg/kg at 24h and 30 min. before surgery (an identical surgical technique as the control group). The rats were sacrificed while being given general anesthesia.

2.2. Renal ischemia/reperfusion induction

Animals were given a ketamine (100mg/kg) and xylazine hydrochloride (10mg/kg) ip. to induce anesthesia **(Torres-González *et al*., 2018).** After the animals had got consciousness, the limbs were fastening, shaving the region around the incision, and cleaning with iodine spray. Then making retroperitoneal flank incision. Non-traumatic micro vascular clamps were used to perform bilateral renal occlusions for 40 minutes. After that, sterile gauze was placed over the rat. Closing the ischemic period and removing the clamp, the kidney will quickly turn from a dark maroon to a dark pink, signifying that reperfusion was accomplished. After reperfusion was verified, the wound was sutured and covered with sterilized gauze damped with 0.9% saline to prohibit the dehydration. After 2hr of reperfusion, extracting the kidneys for determination the parameters of the experiment then the rats were killed by a cardiac puncture. The kidney was cut lengthwise divided into two components, one of which was installed in 10% formaldehyde for

histopathological analysis and the other section was frozen at -80ċ with PBS solution for an ELISA investigation **(Herrera-Luna et al., 2022).**

2.3. Preparation of samples

2.3.1. Blood sample preparation for serum biomarkers assessment:

Approximately 4 milliliters of blood were extracted straight from the hearts of the rats after two hours of reperfusion while they were still under anesthesia. The serum was obtained by centrifuging the blood sample in a gel tube with no anticoagulant for ten minutes at 6000 rpm. The serum was then utilized to quantify creatinine and urea by standard laboratory protocols.

2.3.2. kidney tissue preparation for tissue proteins assessment: Division of the frozen kidney tissue into tiny pieces and used ice-cold PBS to wash it to remove blood and clots which may affect the measurements and then weight the tissue and homogenized by pestle and mortar in 1:10 (W/V) 0.1M cooled PBS (pH 7.4) contain both 1% Triton 100X and protease inhibitor cocktail. For further degradation the homogenate was sonicated by an ultrasonic cell disrupter. Finally, centrifugation the suspension according to manufacture procedure. Then, in order to ascertain the concentration of KIM-1, using available ELISA Kits **(Najah *et. al.*, 2020).**

2.3.3. Tissue preparation for histopathology: Fixed in 10% formaldehyde, dehydrated in a series of alcohols, cleansed with xylene and then placed in paraffin was a piece of kidney tissue. After paraffin embedding, Kidney tissues were divided into pieces that were 5 µm thick. Hematoxylin/eosin staining was then applied to the sections **(Li *et al.*, 2018).** A measure of histologic alterations was the proportion of damaged or injured renal tubules. This was the score for tissue injury: 0, no pathological changes, 1=<25%, 2= 25-50%, 3= 51-75%, 4= 76-100% **(Wei et al., 2010).**

2.3.4. Analysis of statistics: The experimental data were statistically analyzed using Tukey´s multiple comparisons in Graph Pad Prism version 8. The study employed one-way (ANOVA) to examine the significance of groups differences. P values <0.05 proved to be statistically significant, and the results were presented as mean ± standard deviation of mean (SDM). Changes in histopathology were measured by non-parametric (Kruskal -walis) test. Tukey´s multiple comparisons was performed to investigate significance differences $P < 0.05$ between groups.

3.Results

3.1. Effect of alogliptin on urea: As showed in (**figure 3.1**) there are a significant rise in urea concentration in control, and DMSO groups compared to sham group but there is no significant change among former groups. Treated rats with alogliptin was significantly reduced when in contrast to that of the control group.

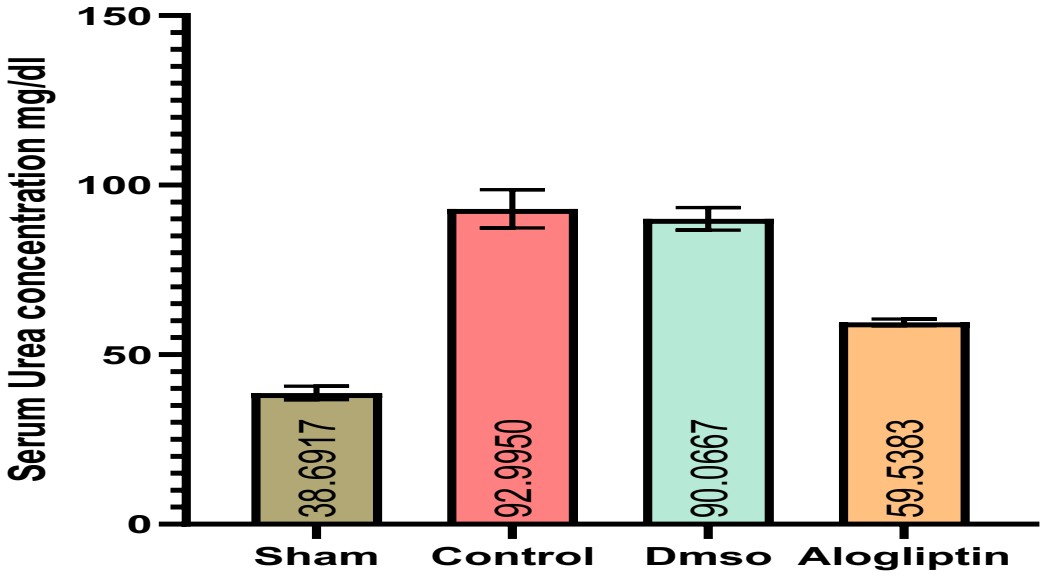

Figure 3.1: Mean of urea levels among of the study groups.

2.Effect of alogliptin on creatinine

Creatinine levels exhibit a substantial variance between sham group with control, and DMSO groups. From another perspective, control, and DMSO groups were not significantly different in the mean values of creatinine. The alogliptin-treated rats showed a considerable reduction when compared to the control group (**figure 3.2**)

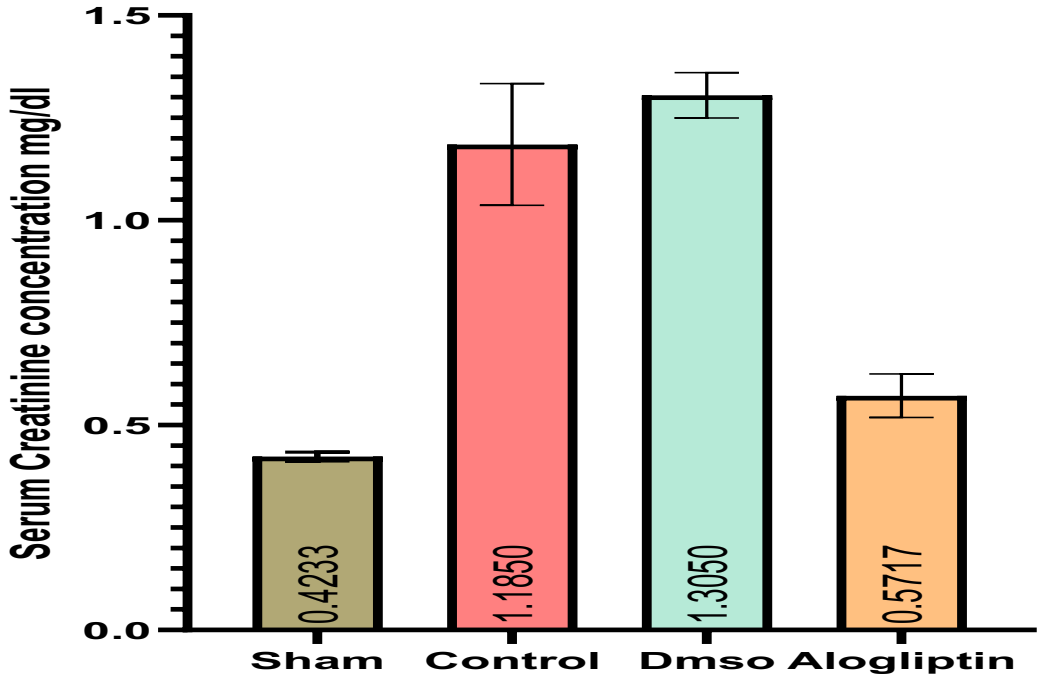

Figure 3.2: Means of creatinine levels among of the study groups.

3.Effect of alogliptin on kidney injury molecule-1(KIM-1)

KIM-1 appeared significantly increase in control, and DMSO groups in contrast to sham group. From other point of view, control, and DMSO groups were not significantly different in the mean values of kim-1. Pretreated rats with alogliptin result in a significant reduced in kim-1 when compared to that of the control group (**figure 3.3**).

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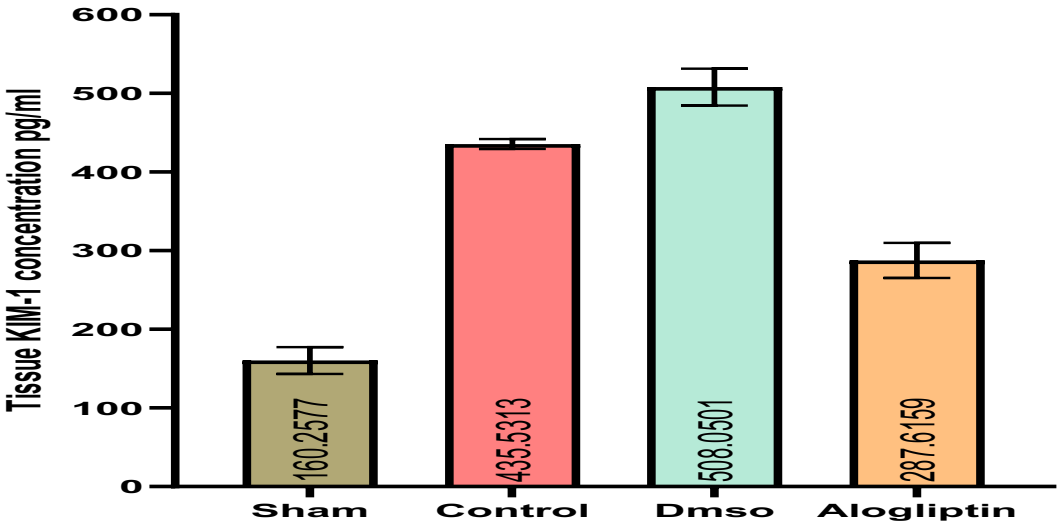

Figure 3.3: Means of kim-1 levels among of the study groups.

3.7. Histopathological findings

3.7.1. Percentage of renal tubular damage

The control, and DMSO groups showed a significant histological change (percentage of renal tubular damage) < 75% compared with normal tissue of sham group. Further comparison that according to the histopathology and scoring system, it was found that the mean score of renal tubular damage, was significantly lower in alogliptin group compared to control and DMSO groups (**figure 3.7**).

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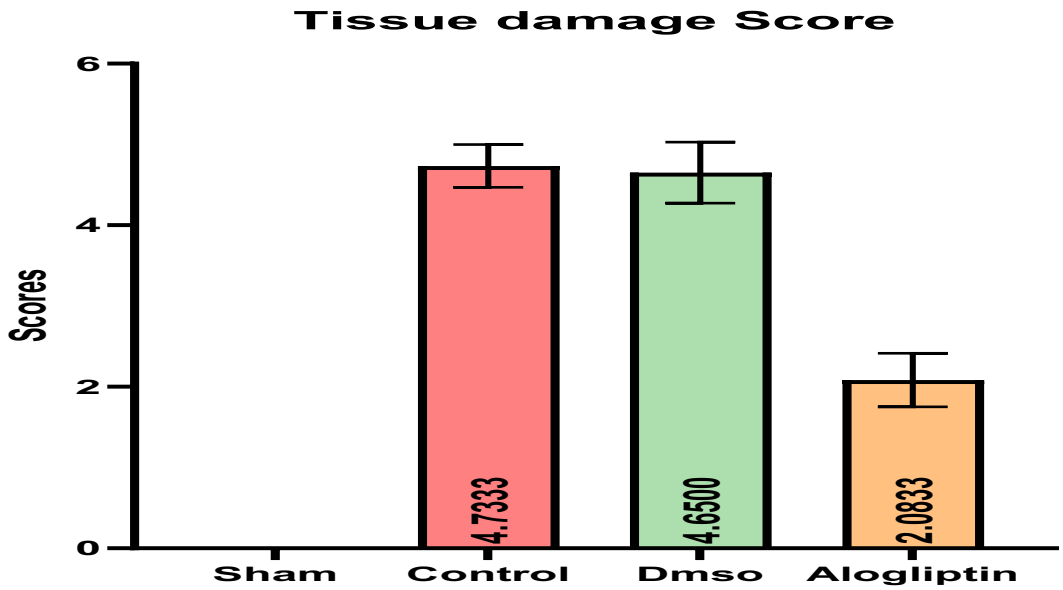

Figure 3.7: Mean score of histopathological renal tubular damage.

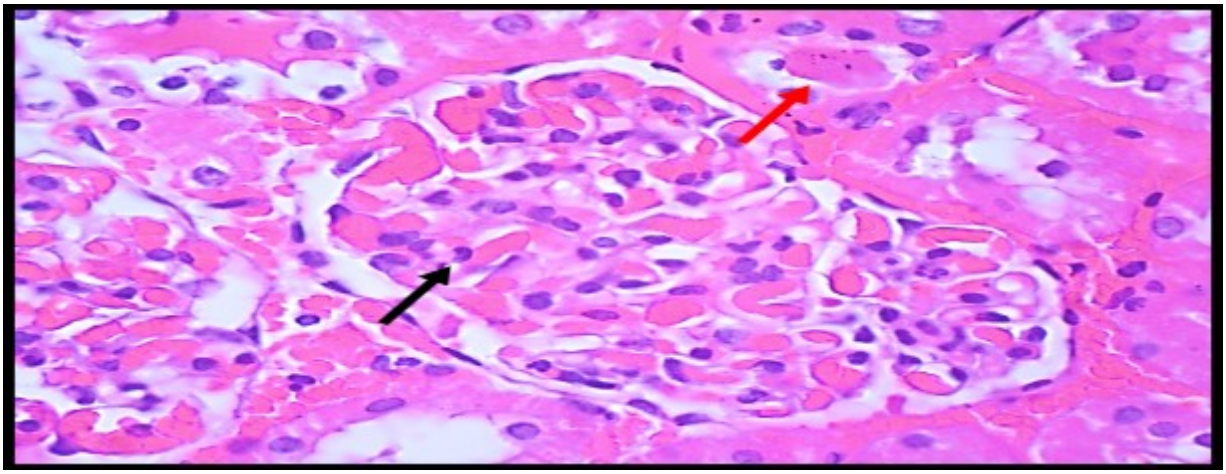

Figure 3.8: The Histopathological features in different study

Sham 40X Figure . The histological section in the renal tissue for rat in sham group. The section shows normal glomeruli (glomerular tuft and capsule , **black arrow**) and normal proximal renal tubules (**red arrow)** There are no noticeable lesions in the tissue. H&E dye is

applied to the tissue, and a digital camera is used to take a picture and light microscope with magnifications of **40X.**

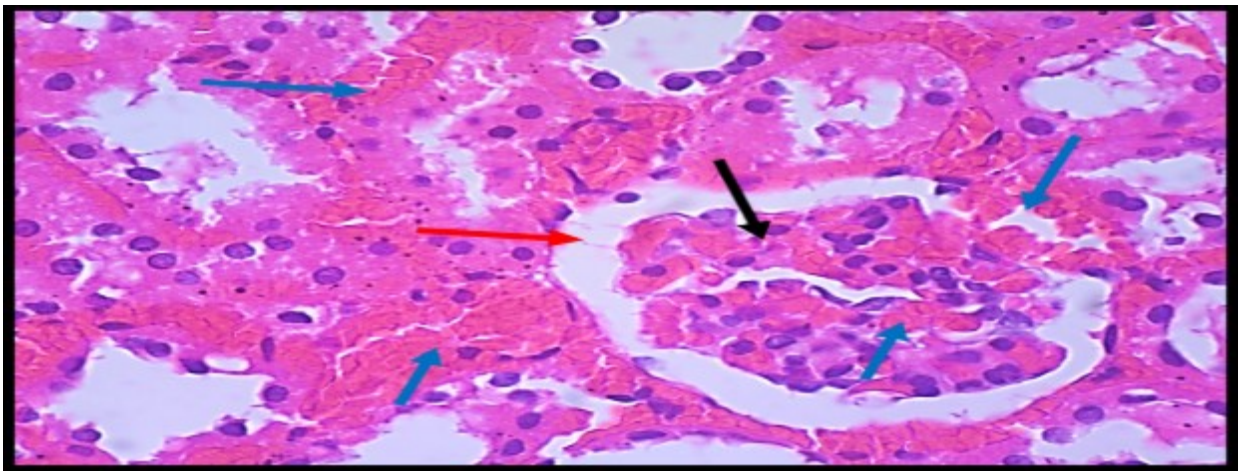

Figure 3.9

Control Ischemia 40X. Figure. The histopathological section of a rat kidney in control group. A section displays clear atrophied lesion in the glomeruli (glomerular tuft, **black arrow**) with increasing glomerular space (**red arrow**), and the tissue shows RBCs accumulation inside glomeruli and proximal renal tubules (**blue arrows**). The glomeruli show atrophied lesion with increasing of glomerular space and complete necrosis in the glomerular tuft for some glomeruli (**red arrows**). H&E dye is applied to the tissue, and a digital camera is used to take a picture and light microscope with magnifications of **40X**.

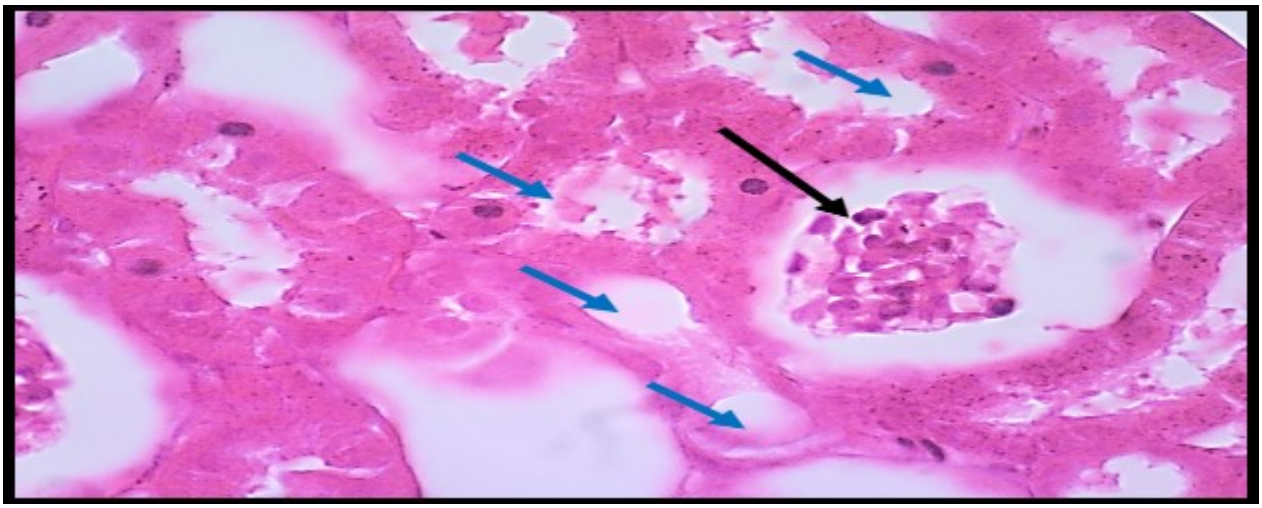

Figure 3.10

DMSO.40X. Figure. The histopathological section in the kidney of rats in DMSO group. A section displays severe glomerular damage (glomerular tuft necrosis and damage of glomerular capsule, **black arrow**). This section displays sever damage in the epithelial layers that lining both proximal and distal renal tubules (**blue arrows**). H&E dye is applied to the tissue and a digital camera is used to take picture and light microscope with magnifications of **40X**.

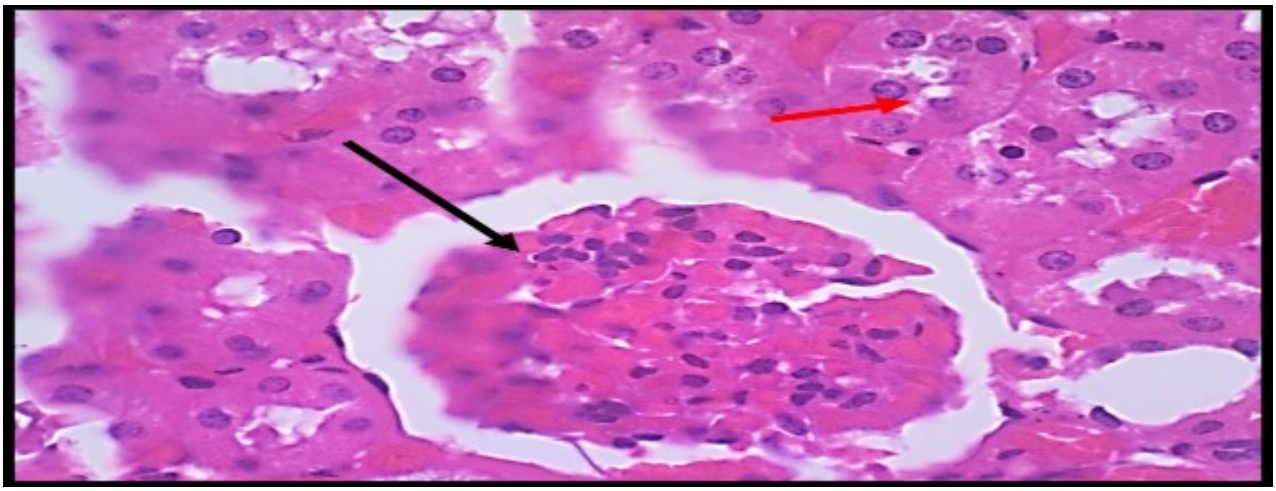

Figure 3.11

Alogliptin 40X Figure. The histological section in the renal tissue for rat in alogliptin group. The section shows normal glomeruli (glomerular tuft and capsule, **black arrow**) and normal proximal renal tubules (**red arrow**) There are no significant lesions in the tissue. H&E dye is applied to the tissue and a digital camera is used to take picture and light microscope with magnifications of **40X**.

4.Discussion: IRI has a major effect on graft life and function, making it a crucial factor determining the results of renal transplantation. Intermittent reduction and restoration of the kidney's blood flow causes IRI, which results in inflammation and cellular damage. Given that IRI is an important contributor to Delayed Graft Function (DGF), which can lengthen hospital stays and raise the chance of rejection **(Lasorsa *et al*., 2024).**

4.1.Effect of renal I/R on urea, creatinine and KIM-1 levels: In this study, serum urea, serum creatinine and tissue KIM-1 concentrations were markedly raised in both control and vehicle groups relative to sham group. This findings are compatible with the research by Han et al.,in 2021 that showed significantly higher serum urea and creatinine concentrations were seen in (I/R) group in mice (subjected to both kidneys being ischemic and then reperfusion) in contrast to the sham group. The control group exhibited significantly higher expressions of KIM-1 in immunohistochemistry compared to the sham group **(Han *et al*., 2021).**

4.1.2. Effect of alogliptin on urea, creatinine and KIM-1 levels: In this study, we have observed that the pretreatment of the rats with alogliptin 24 hours and 30 min. has led to a significantly lower levels of serum urea, creatinine and tissue KIM-1 when compared to the control and vehicle groups. These findings are similar to what Sakr and Kamel (2023) found in their study where they investigated the possible reno-protective benefits of alogliptin as an example of gentamicin-induced nephrotoxicity in rats, The urea and creatinine concentrations in the serum both dramatically decreased in comparison to the control group **(Sakr and Kamel, 2023).**

4.4. Renal parenchyma

4.4.1. Effect of alogliptin on renal parenchyma: In this study, the histopathological examination in the alogliptin group displayed normal glomeruli (glomerular tuft and capsule), normal proximal renal tubules and there are no significant lesions in the tissue indicating that alogliptin has considerably decreased the severity of the renal injury when compared to both control and vehicle groups. Also, these results agree with research by Botros et al., in (2024) who studied to assess the effect of alogliptin on doxorubicin (DOX)-induced renal tissue toxicity. Compared to the DOX-treated group, which showed variable degrees of tubular epithelial cell destruction and the necrosis of their epithelial lining, the histological alterations in renal tubules, glomeruli, and interstitial tissues improved **(Botros *et al*., 2024)**.

Conclusion: The study's findings on alogliptin (DPP4 inhibition) provide a foundation for considering these agents in clinical practice for the prevention and treatment of kidney injuries, potentially leading to improved patient outcomes

Recommendation: We recommended to use alogliptin to protect kidney tissue against renal ischemia and reperfusion injury.

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