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# Effects of Pirfenidone on Renal Ischemia-Reperfusion Injury in a Mice Model: Reno-protective Effect

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***ABSTRACT***

*This study look to the nephroprotective properties of pirfenidone in adult male mouse model with renal ischemia-reperfusion injury (IRI). Adult mice (12-15 weeks age and weigh 25-35 g) were separated at random to four groups: sham, ischemia, vehicle (DMSO), and pirfenidone (300 mg/kg orally, 30 minutes preceding to ischemia). Renal IRI produced by 30 minutes of bilateral ischemia and 2 hours of reperfusion. In order to assess renal function and oxidative stress, serum urea, creatinine and F2-isoprostane was checked. TNF-α as well as NF-κB levels in renal tissue also was checked for reactions related to inflammation. Histopathological grades evaluated structural kidney injury. When compared to sham group, the ischemia and vehicle groups revealed significant higher levels of renal function, oxidative stress, and inflammatory markers. Pirfenidone lowered all biochemical indicators dramatically, while improving histological damage scores, resulting in near-normal architecture. The study found that pirfenidone provide significant nephroprotection in renal IRI by acting as anti-inflammatory and antioxidant.*

**Keywords: Pirfenidone, Renal Ischemia-Reperfusion Injury, Urea, Creatinine, NF-kB, TNF-α, and $F_2$-Isoprostane.**

**Article Information**

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## INTRODUCTION

Ischemia refers to a condition characterized by tissue oxygen deprivation and reduced clearance of metabolites. Reperfusion, the process of restoring blood flow to ischemic tissues, can paradoxically result in tissue injury [(1)]. This implies a decline in an organ's flow of blood, followed by re-oxygenation and blood flow restoration. Injury is expected after sepsis, infarction, or transplantation of organs, and this occurrence speeds up tissue damage by starting a sequence of inflammatory processes including reactive oxygen species, chemoknes, cytokines, and leukocyte activation [(2)]. Ischemia/reperfusion injury (IRI) frequently affects various organs, including renal system, heart, skeletal muscle, pulmonary, and cerebral [(3)]. Clinical manifestations of ischemia reperfusion (I/R) encompass systemic inflammatory response syndrome as well as resulting from the disruption of the dynamic balance among pro-inflammatory and anti-inflammatory responses [(4)]. Serum levels of urea, creatinine, interleukin-18 (IL-18), and interleukin-6 (IL-6) are significantly elevated in this condition [(5)]. 20% to 30% of renal graft failure in clinical settings is due to IRI [(6)].

Acute kidney injury (AKI) is a disorder characterized by fast renal dysfunction and increased mortality, both of which are exacerbated by IRI in the kidney [(7)], Acute kidney failure (AKF) occurs in the renal system and is exacerbated by a reduction in the flow of blood (renal ischemia), which leads to a declined oxygen supply to the organ (renal hypoxia), that caused reduction in renal output and glomerular filtration rate (GFR), induces

excessive production and deposits of extracellular matrix proteins (ECM) which can influence the long-term prognosis of renal fibrosis, creation chronic kidney disease (CKD), and led to end-stage kidney failure [8,9], It is increasing morbidity and mortality; as the kidney is one of the organs susceptible to IRI because of its particular tissue structure and function and high oxygen demand [10,11], consequently it becomes problematic since its highly mortality rate, lack of effective treatment, and sustained or progressive decline of renal function immediately following the acute phase of ischemia [12,13].

Pirfenidone is anti-inflammatory, antioxidant and antifibrotic effects [14]. Several in vitro investigations show pirfenidone reduces cellular damage in IPF by scavenging ROS and inhibiting lipid peroxidation [15], in addition, its antioxidant property of the substance is substantiated by the ongoing production of NO, which is an active component in the defense against free radicals [14]. PFD has positive effects on IRI in many organs such as testis, kidney, liver, lung and small intestine (16, 17), anti-inflammatory action in RIRI (18) through downregulation pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, IFN-γ, and platelet derived growth factor (PDGF), facilitates the synthesis of anti-inflammatory agents and inhibits the aggregation of diverse inflammatory cells, such as lymphocytes, macrophages, and neutrophils [15, 19]. NF-κB controls cellular activities and is linked to a condition called septic shock problems, chronic inflammatory illnesses, multiorgan dysfunction, and viral infections [20]. NF-κB increases the synthesis of proinflammatory cytokines, such as TNF-α, IL-12, and IL-1β [21]. RIRI increases the production of proinflammatory cytokines, which trigger NF-κB. TNF-α then binds to its receptor and activates NF-κB, developing positive feedback mechanism for NF-κB regulation (upregulation). This signaling loop plays an essential part in the pathophysiology of RIRI [22], NF-kB is also activated in glomerular core cells such as podocytes and mesangial, tubular, as well as endothelial cells in vivo and in vitro following renal ischemia (RI) or interaction with triggers of inflammation [23].

TNF-α is a proinflammatory cytokine secreted by immune cells, including T lymphocytes, as well as non-immune cells like fibroblasts [24]. Elevated levels of TNF-α in patients with renal failure (RF), sickle cell disease, and myeloproliferative neoplasms (MPNs) [25]. That is through TNF-α interacts with its receptor and activates NF-kB, playing a crucial role in the pathogenesis of RIRI. [26].

F2-isoprostanes are prostaglandin analogues generated through the peroxidation of polyunsaturated arachidonic acid in the presence of free radicals. [27]. The 8-Iso-Prostaglandin F2α (8-iso-PGF2α) is the majority frequent tested isomer for F2-isoprostanes quantitation [28]. F2-isoprostanes elevated in the rat bleomycin/induced renal ischemia-injury model, and stimulated myofibroblast differentiation of rat lung fibroblasts [29].

## METHODS

**2.1. Animal Preparation:** Twenty-eight adult male mice weighing (25–35) g, aged (12–15) weeks, acquired from the Iraqi Center for Cancer Research, these mice lived in the animal facility and were maintained in the Science Collage /Kufa University in the house of the animals, they were kept in cages of period cycle of a (12-hr.) light and (12-hr.) dark, maintaining temperatures between 22 and 24 °C and the humidity values between 60 and 65 %. Mice received a standard access of water and nutrient. The investigation was carried out within The Laboratory Research in Clinical Department at the Pharmacy College at Kufa University has been operational since October 15, 2024, and will continue till February 15, 2025.

**2.2. Ethical consideration:** All experimental methods in this study were granted permission by Kufa University's Institutional Animal Care and Use Committee (IACUC) following the

filing of the required documentation (3178 on 4/2/2025).

**2.3. Study Design:** Mice were acclimated for a week before being randomly separated into 4-groups: **sham, Ischemia, DMSO,** and **pirfenidone** (n = 7/group):

- **Sham group:** Mice were subjected to the same anesthesia, and the kidneys underwent the same laparotomy procedures same that in ischemia group except of ischemia induction; this group had been used as group for the negative laparotomy control.
- **Ischemia group:** Mice were anesthetized, and the kidneys undergo a flank laparotomy, followed by 30 minutes of bilateral renal ischemia and 2 hours of reperfusion.
- **Vehicle group**: Mice gave equivalent volume DMSO as vehicles for pirfenidone by oral gavage 30min. before ischemia/reperfusion.
- **Pirfenidone group:** drug pre-treated group, mice administered PFD (300mg per kg) by the oral gavage route of administrational 30 min. before ischemia/reperfusion.

**2.4. Ischemia protocol in mice:** 0.01 mg per g of xylazine plus 0.1 mg per g of ketamine, they were injected by the intraperitoneal route (I.P) to mice to induce anesthesia [30]. Mice were selected for the induction of ischemia by the application of a renal clamp, a (1.5 cm) vertical flank incision was made employing surgical scissors, dissecting layer by layer through the cutaneous, fascia, and muscle section, a (0.3 cm) diameter instrument was utilized to displace the kidney from the retroperitoneal fat, to expose the renal hilum; a cotton swab was used to incise the peri-nephric fat along the kidney's midline, to make room for the pedicle clamp; cotton swabs or tweezers were put into the renal hilar fat above and below the renal pedicle for 30 min., this induced ischemia on both sides. The wound was closed using the surgical suture with a diameter of 4-3, after that, 2 hours of reperfusion, and finally at end of reperfusion, the mice has been verified.

**2.5. Preparation of Pirfenidone**: The pirfenidone powder (raw materials) dosage is a 300 mg/kg/PO [30] It is soluble in 100 mg/ml of DMSO, which serves as the experiment's standard vehicle, then it is stored and subsequently diluted in normal saline 0.9% (use as a suitable medium in this study) directly prior to use, in accordance to the manufacturer's instructions, Target-Mol [31].

**2.6.Sample Collection:**

**2.6.1. Blood Sample:** The blood samples have been gathered by cardiac punctures before verifying mice. The blood sample was collected without anticoagulant, and it was put within a gel tube and stay to stand at around 25°C for 1-hr., to separate the serum; was centrifuged for ten minutes at 6000 rpm; it could be used to evaluate the values of serum urea as well as serum creatinine by employing a spectrophotometric method and $F_2$-Isoprostane level by using commercial ELISA kits.

**2.6.2. Tissue Sample:** Renal organ tissues were stored at -80°C before being homogenized using a high-intensity ultrasonic liquid processor in a 1:10 W:V solution of PBS (phosphate buffered saline) with 1% Triton X-100 and the protease inhibitor cocktail (PIC) included in it [32]. After centrifuging the homogenate at 5000 rpm for 10 minutes at 4°C, the supernatants were collected and utilized to evaluate renal tissue level of TNF-α and NF-kB via employing ELISA kits.

**2.6.3. Histological Examination:** Renal organ tissues sections had fixation within 10% of the formaldehyde, then exposed to dehydration through a series concentration of alcohol, after that undergone clearing with xylene also embedding within paraffin, subsequently they sectioned into slices of 5-*m*m in thickness, these sections of the tissue slices were stained with hematoxylin and eosin prior to examine under a light microscope [33] , and the histopathological examination was conducted at original magnifications ranging from (X100 to X400) [34]. Histologic changes were identified by the

percentage of injured kidney tubules/renal tissue damage was evaluated by utilizing the subsequent scoring system:

- **Score-0**: signifies the absent of damage (0% damage). There is no evidence of tubular damage, and the histology is normal.
- **Score-1:** indicates that the damage is (less than 25%). There is a localized necrosis that is accompanied by interstitial edema.
- **Score-2**: indicate that the damage ranging from (25% to 50%). Diffused swelling of the nephrogenic tissues.
- **Score-3:** indicates that the damage ranges from (50% to 75%). Leukocytes are infiltration and contraction bands.
- **Score-4:** indicates that the damage (exceeds 75%). Hemorrhage, leukocyte infiltration, cellular edema, cytoplasmic eosinophilia, and congestion of the vascular system [35].

**2.7. Statistical Analysis:** GraphPad Prism 8.1 software (GraphPad Software, La Jolla, CA, USA) was used to analyze the data in this study. Every outcome is shown as (mean ± Standard Error Mean (SEM)). unless stated specified otherwise. A One-Way Analysis of Variance (ANOVA) was performed, followed by the Bonferroni multiple comparison test for post hoc analysis of the data. Histopathological changes were contrasted among the groups using a non-parametric test, followed by Dunn's-post hoc test. Statistical significance was established at data ($P < 0.05$) for all analyses.

## RESULTS

Ischemia continued for 30 minutes, followed by 2 hours of reperfusion. A 30 min. before ischemia, the mice received prior treatment with DMSO (as a vehicle), pirfenidone (as drug), or no any treatments group as in the ischemia and sham. Various biochemical as well as histopathological biomarkers were measured to evaluate the degree and score of the injury as following:

**3.1. The Effect of PFD on TNF-α Level:** The ischemia group had significantly more renal tissue levels of TNF-α comparing to the sham group ($P < 0.001$). There were no statistically significant differences in renal tissue of TNF-α levels was noticed between the vehicle and ischemia groups (Figure-1). The PFD group showed significant lower in the renal tissue level of TNF-α contrasted with both vehicle and ischemic ($P<0.001$).

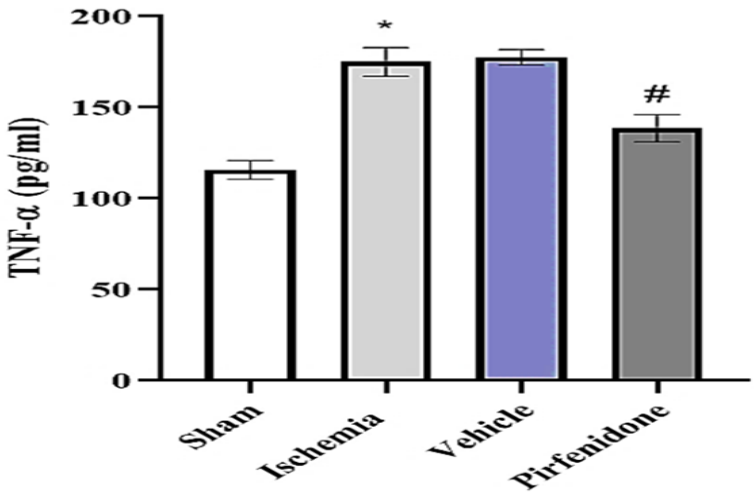


**Figure-1: Tissue level of TNF-α in this study groups (n=7).**
*** Significant versus sham group, (*P*<0.001).**
**# Significant versus ischemic or vehicle groups, (*P*<0.001).**

**3.2. The Effect of PFD on NF-kB Level:** Renal tissue levels of NF-kB in the ischemia group had a significantly higher than contrasted with sham group, (P< 0.001). Renal tissue NF-kB levels did not significantly change between the ischemia and vehicle groups (Figure-1). The PFD group showed significant lower in the renal tissue level of NF-kB contracted with both vehicle and an ischemic group (P<0.001).

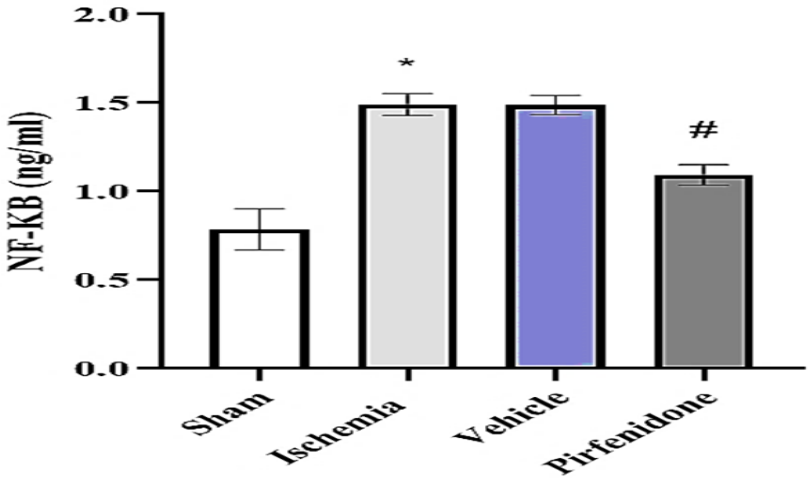


**Figure-2: Renal tissue level of NF-kB in this study groups (n=7).**
*** Significant versus sham group, (*P*<0.001).**
**# Significant versus ischemic or vehicle groups, (*P*<0.001).**

**3.3. The Effect of PFD on $F_2$-Isoprostane Level:** The serum $F_2$-isoprostane levels in the ischemia group had significantly higher compared to the sham group, (P < 0.001). Serum F2-Isoprostane levels were not different statistically significant between the ischemia and vehicle groups (Figure-3). The PFD-treated group had significant lower in serum levels of $F_2$-Isoprostane in comparison with the vehicle as well as an ischemic groups (P<0.001).

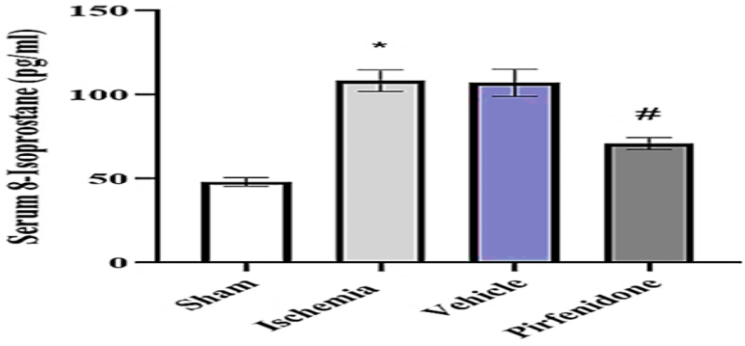


**Figure-3: Serum $F_2$-Isoprostane level in this study groups (n=7).**
*** Significant versus sham group, (*P*<0.001).**
**# Significant versus ischemic or vehicle groups, (*P*<0.001).**

### 3.4. The Effect of PFD on Urea Level:

The serum urea levels in the ischemia group had significantly higher compared to the sham group, ($P < 0.001$). Serum urea levels did not differ statistically significant between the ischemia and vehicle groups (Figure-4). The PFD-treated group had significant lower in serum levels of urea in comparison with both vehicle and an ischemic group, ($P<0.001$).

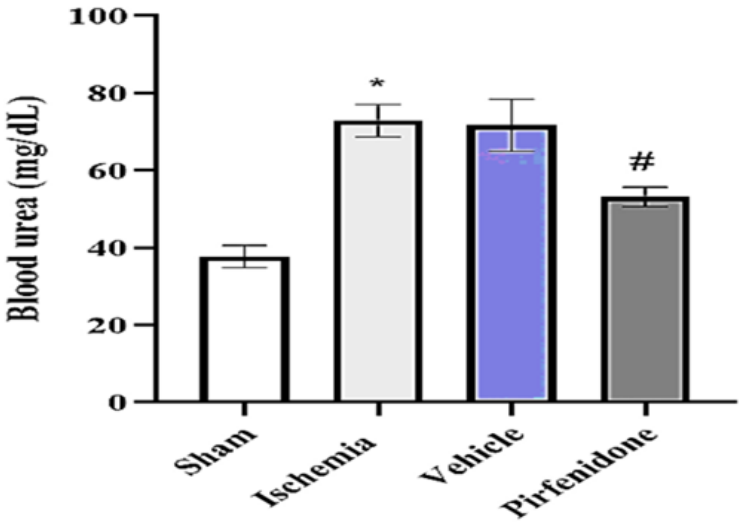


**Figure-4: Serum urea level in this study groups (n=7).**
*** Significant versus sham group (*P*<0.001).**
**# Significant versus ischemic or vehicle groups (*P*<0.001).**

**3.5. The Effect of PFD on Creatinine Level:** The serum urea levels in the ischemia group had significantly higher compared to the sham group ($P < 0.001$). Serum urea levels did not differ statistically significant between the ischemia and vehicle groups (Figure 4). Compared to the ischemia and vehicle groups, the PFD-treated group's blood urea levels were noticeably lower ($P<0.001$).

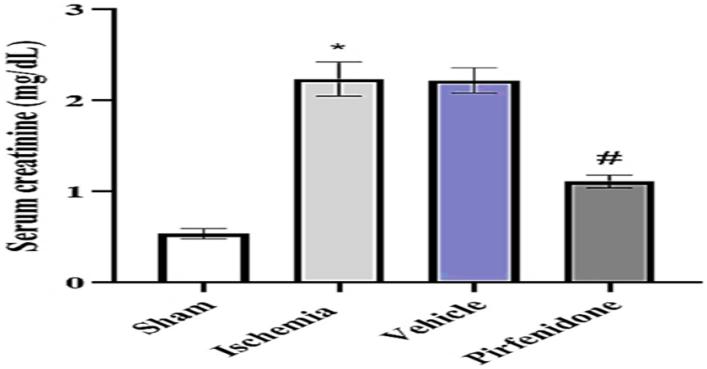


**Figure-5: Serum creatinine level of study groups 9n=7).**
*** Significant versus sham group, (*P*<0.001).**
**# Significant versus ischemic or vehicle groups, (*P*<0.001).**

**3.6. Histopathological Changes of Renal Tissue in Study Group:** The histopathologicalexamination of renal tissue was performed to substantiate the reno-protective effects of PFD. Each group comprised seven mice, with a minimum of four sections evaluated per mouse according to the Zingarelli protocol.Renal tissue of sham group showed normal architecture histology of renal tubules (Figure-6 A and B) with no damaged area (0% renal tubular damage), score-0 ($P$<0.001) (Figure-10).

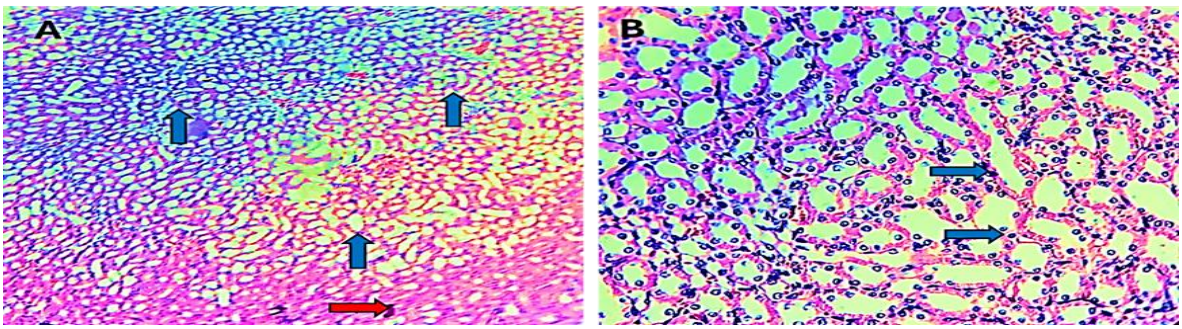


**Figure-6: Histopathological examination of the renal section for sham group. Mice kidney with score-0 (0% renal tubular damage). Damaged area (red arrow), normal histology of renal tubules (blue arrows). The section stained with Hematoxylin& Eosin. A. 100x. B. 400x**

While, in ischemic renal tissues had a significantly high severe renal injury (90% renal tubular damage), score-4 (Figure 7 A, B, and C) characterized by cellular swelling, cytoplasmic eosinophilia, vascular congestion, hemorrhage, leukocyte infiltration, and inflammation (P < 0.001) (Figure-10).

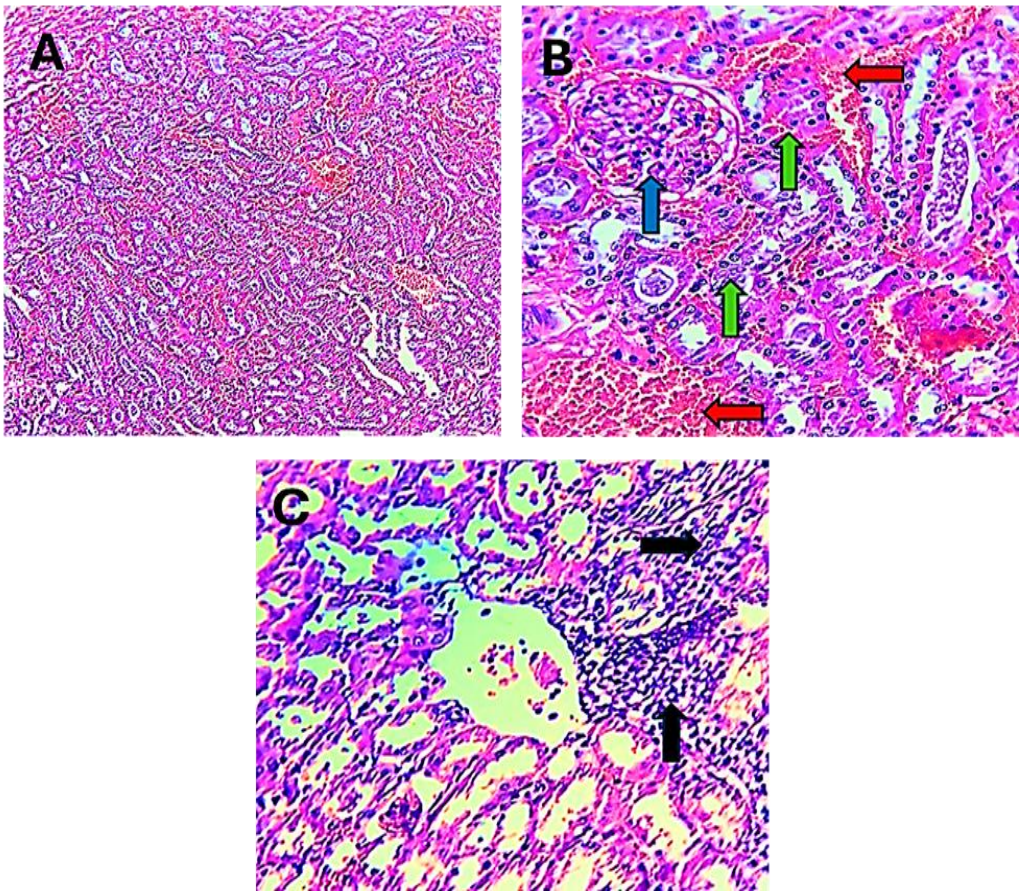


**Figure-7: Histopathological examination of the renal section for Ischemic group. Mice kidney with score-4 (90%) renal tubular damage. Cellular swelling and cytoplasmic eosinophilia (green arrows), leukocyte infiltration (blue arrow), vascular congestion and hemorrhage (red arrows), and inflammation (black arrow). The section stained with Hematoxylin& Eosin. A. (100x.); B. and C. (400x.)**

Moreover, the vehicle group had a significantly high severe renal injury (90% renal tubular damage) score of 4 (Figure 3.8—A, B, C, and D). characterized by the cellular swelling, cytoplasmic eosinophilia, vascular congestion, hemorrhage, leukocyte infiltration, and inflammation. (P<0.001) (Figure-10).

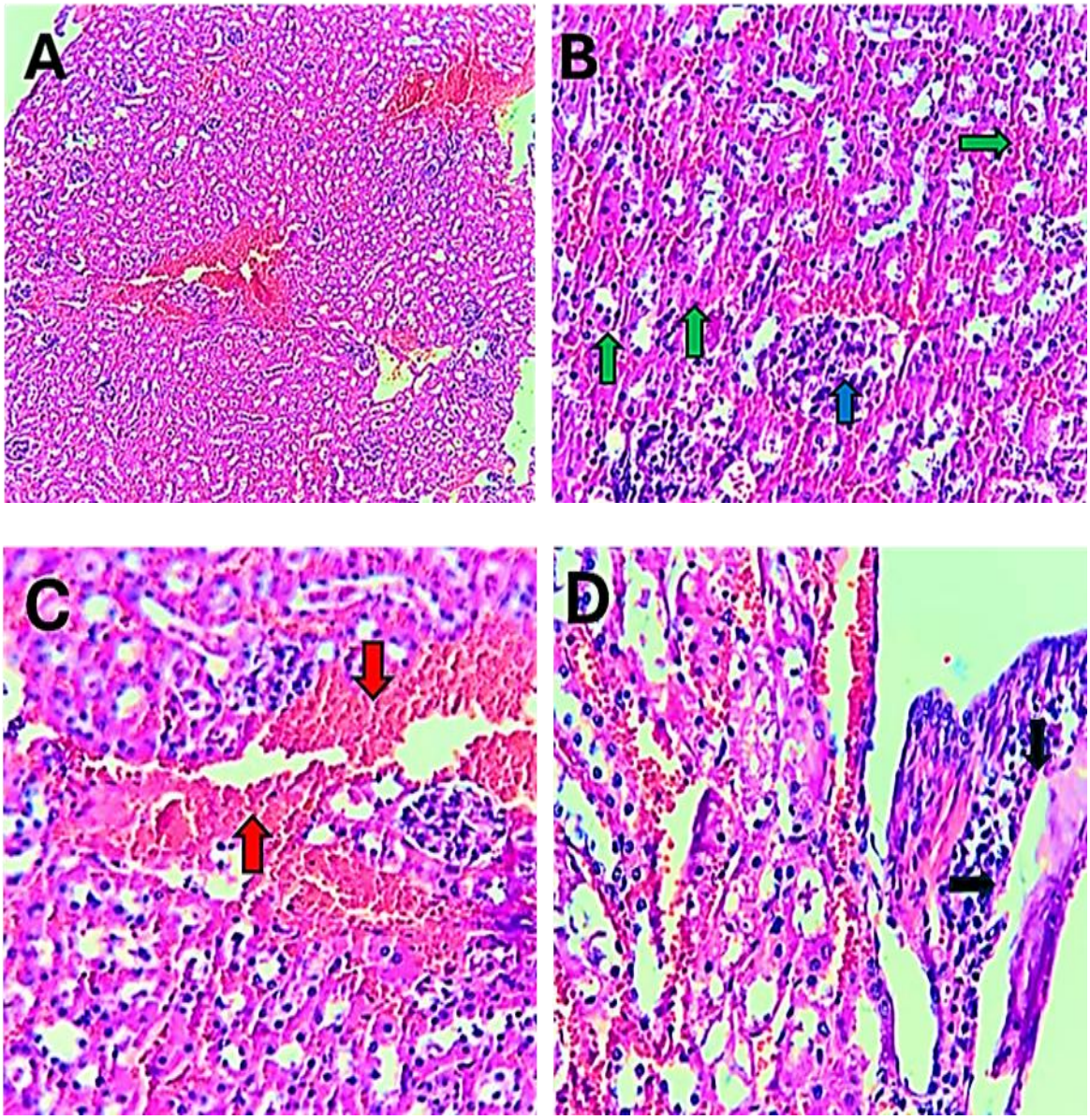


**Figure 8: The histopathological examination of the kidney section for vehicle (DMSO) group. Mice kidney with score-4 (90%) renal tubular damage. Cellular swelling, cytoplasmic eosinophilia (green arrows), inflammation (black arrows), leukocyte infiltration (blue arrow), and vascular congestion and hemorrhage (red arrows). The section stained with Hematoxylin& Eosin. A. (100x.); B., C, and D. (400x.).**

The PFD-treated renal tissues revealed a mild renal injury with score-2 (less than 30%) renal tubular damage (Figure-9 A, B, and c) and a significantly lower average histopathological score than the ischemic and vehicle groups (P<0.001) (Figure-10).

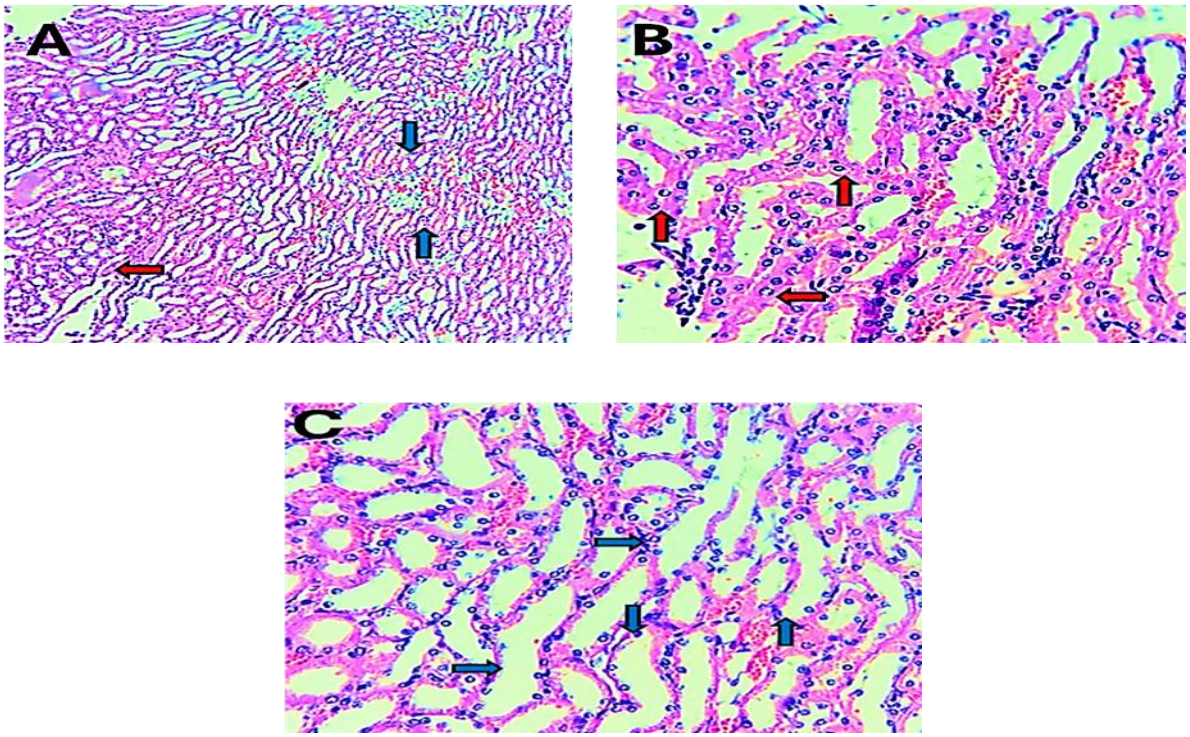


**Figure-9: Histopathological examination of the kidney section for Pirfenidone group. Mice kidney with score-1 (less than 25%) renal tubular damage. Damaged area (red arrows), normal renal tubules (blue arrows). The section stained with Hematoxylin& Eosin. A. (100x.); B. and c. (400x.).**

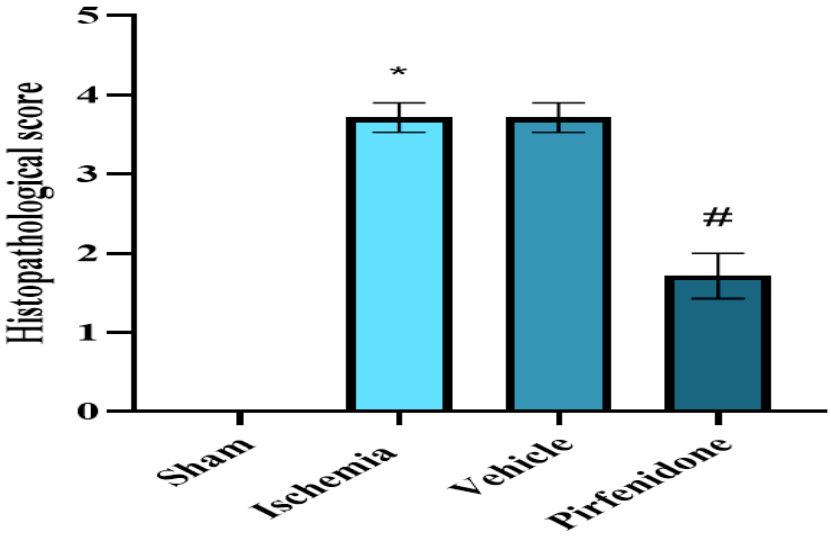


**Figure 3.10: Histopathological scores in this study groups.**
*** Significant versus sham group, (*P*<0.001).**
**# Significant versus ischemia or vehicle groups, (*P*<0.001).**

## DISCUSSION

Renal ischemia reperfusion injury (RIRI) is a multifaceted process that commences with the hypoperfusion phase, marked by reduced local oxygen and nutrient supply to the kidney, triggering a cascade of deleterious cellular and molecular reactions predominantly in tubule epithelial cells [35]. This subsequently results in hypoxic and inflammatory damage that affects renal parenchyma [37]. Along with elevated reactive oxygen species, free radical generation, cellular metabolic dysfunctions characterized by reduced ATP and glucose levels, apoptosis, and necrosis, ultimately contributed to the degradation of tubular cells. However, it is the reduction in renal blood flow that initiates this process [38,39]. Substantial evidence indicated that inflammation was a significant contributor to RIRI [40].

The study found elevated levels of TNF-α in kidney tissue considerably greater than the sham group in both the ischemia and vehicle groups (P<0.001). The PFD-treated group, on the other hand, had significant much lower levels of TNFα in their kidney tissue than the vehicle and ischemia groups.

Researchers Holderied et al., (2020) found that TNF-α levels significantly upregulated in mice that had RIRI after 35 and 45 min. of ischemia and then went down again after 24 h. of reperfusion [41]. Kocaturk et al., (2020) showed in a rat model, that TNF-α levels are significantly elevated in the I/R group in contrast to both the sham and normal control groups [42]. Schreurs et al., (2019) discovered that TNF-α often stimulates cell proliferation at modest levels. However, at elevated levels, it suppresses cell proliferation and induces apoptosis [43]. In contrast, Hazem et al., (2022) did a study that showed PFD reduces cytokines released by macrophages, such as TNF-α [44]. Liu & Shi, (2019) found that pirfenidone has many anti-inflammatory effects, but the main one is blocking TNF-α, which is an early marker of inflammation [45].

According to this study, the ischemia group's kidney tissue had much greater levels of NF-kB and the vehicle groups compared to the sham group. However, the pretreatment PFD group had considerably lower levels of NF-kB compared to the ischemia and vehicle groups (P<0.001).

It was shown by Alsaaty & Janabi, (2024) that the induced I/R group rats' kidney tissues have a much higher level of NF-KB than the sham group [46]. A study by AL-SHIBANI et al., (2020) and Alaasam et al., (2024) demonstrated that when rats are subjected to bilateral renal ischemia for 30 minutes, followed by two hours of reperfusion, NF-kB is significantly higher in the control ischemic and vehicle groups compared to the sham group [47, 48]. It was found by Yıldırım et al., (2024) that PFD lowers NF-kB p-65 in a group of rodents that had induced (PF) [49]. The results of this study agree with those of Sharawy & Serrya, (2020), who showed that pirfenidone lessens acute kidney damage in a rat model that had RIRI [50]. In the medication leaflet, Hong et al., (2022) stated that PFD significantly inhibits the production of pro-inflammatory cytokines, such as NF-kB [31].

The results of this study showed that RIRI led to a significantly higher level of F2-isoprostane in the serum in both vehicle and ischemia groups contrasted with sham group, (P<0.001). The PFD group, on the other hand, had a significantly lower serum F2-isoprostane level than vehicle and ischemic groups, (P<0.001) after being given the drug for 30 minutes before the ischemia started. Thus, showing PFD attenuates oxidative stress and promotes an anti-oxidative effect.

According to Aksu et al., (2021) , the I/R event causes oxidative stress and inflammation to rise in kidney tissue, which is similar to what it saw in this study experiments [51]. Serteser et al., (2002) discovered that antioxidant enzymes

were reduced after 30 minutes of ischemia and 1 hour of reperfusion in rodents. These enzymes reached minimal levels and elevated oxidative stress markers after 60 minutes of ischemia and 1 hour of reperfusion [52]. Arezzini et al., (2018) showed that F2-isoprostanes increase in an induced I/R rat model, hence promoting the differentiation of myofibroblasts from lung fibroblasts in rat model [53]. The study by Granick et al., (2021) agrees with this result. They found that median serum F2-IsoPs levels were significantly higher in cats with all three stages of chronic kidney disease compared to normal cats [54]. Gaggini et al., 's (2011) study showed that PFD treatment directly suppresses redox processes, which inhibits oxidative stress caused by production of the toxic and harmful hydroxyl radicals but not superoxide radicals, in an induced PF mice model [55]. Margaritopoulos et al., (2016) and Fois et al., (2018) found that pirfenidone has well-established antioxidant properties [56, 57]. Fois et al., (2020) were the first to describe how pirfenidone affects a wide range of inflammatory and OS markers in people who have IPF [58]. According to Rincón et al., (2015), the PFD's ability to scavenge ROS and reduce inflammation can mitigate OS [59].

In this study, serum urea and serum creatinine concentration are significantly higher in the ischemia group and the vehicle groups as contracted to sham group, ($P<0.001$). In contrast, the PFD group has a significantly lower of the serum urea and serum creatinine values in contrasted with both vehicle and ischemia groups ($P<0.001$).

Azarkish et al., (2021) found that urea and serum creatinine are indicators of renal failure and are associated with the accumulation of urea, which leads to a substantial increase in blood urea levels. The atypical renal histology modifications, such as elevated kidney mass and renal injury scores, are indicative of a potential deterioration in renal functioning and structure. Consequently, these alterations reveal that RIRI directly affects kidney function [60]. Patients with AKI can be identified by checking their urea and creatinine levels, which depend on how much blood flows through their kidneys and their GFR, this was demonstrated by George et al., (2004). Higher levels of creatinine and urea are seen in some IRI-AKI models, which means that GFR goes down [61]. Sharawy & Serrya, (2020) discovered that renal function diminished in the ischemia group. This was further supported by a significant rise in serum creatinine and urea concentrations in a comparison with the control group [50]. In a study with rats, Sahu et al., (2014) found that serum urea and creatinine levels went up significantly in both the induced and vehicle groups, in contrast with the control or normal groups [62]. This study confirmed what Kocaturk et al., (2020) found: The I/R group had significantly higher levels of creatinine and urea compared to the sham and normal control groups [42].

A study's Lima-Posada et al., (2019) demonstrated that renal impairment induced by I/R results in a significant reduction in renal blood flow, as well as a significant rise in the rate of urea and creatinine compared with the sham group [14]. Higher concentrations of creatinine and urea were found in the blood of the ischemia group of mouse models in the study by Yen et al., (2015) [63]. The results of this study were in line with Gao et al., (2013), which showed that urea and creatinine levels were significantly higher as indicators of renal function in mice that were subjected to 20 min. of ischemia, which resulted in a significant renal failure [64].

The vehicle and ischemia groups had significantly more severity scores for tubular injury compared to the sham group, the score for tubular damage oh the vehicle and ischemia groups was 4, while the score for tubular damage in the sham group was 0, ($P<0.001$). Histological scanning after renal I/R

demonstrated a loss of brush borders, cellular swelling, tubular dilation, cytoplasmic eosinophilia, development of eosinophilic casts, hemorrhage, inflammation, vascular congestion, leukocyte infiltration, and cytoplasmic vacuolization. In contrast, PFD pretreatment group of mice undergoing ischemia is a significantly lower score of renal tissue injuries in histological staining examination and dropped to score-2 ($P<0.001$).

Researcher Kocaturk et al., (2020) showed that when the kidney tissue of rats was put through the I/R procedure, it exhibited significant dilation, tubular necrosis, intratubular cast formation, and apoptotic bodies. In contrast, the kidney tissue of the sham group did not exhibit any histopathological findings [42]. Havasi & Borkan, (2011) discovered the kidneys of mice that underwent sham surgery were histologically normal. However, the tubular necrosis score of the sham mice was significantly lower than that of the ischemic mice. The kidneys of mice that underwent ischemia revealed occasional dilated tubules and casts, which were indicative of a significantly greater injury, including the frequent sloughing of tubular epithelial cells and the presence of casts. Furthermore, renal epithelial cells undergo apoptosis as a consequence of ischemia [65]. A study using a mice model that had bilateral renal ischemia for 45 min. and then 24 hr. of reperfusion performed by Fu et al., (2022), which found that I/R in the kidneys result in a significant tubules injury, these signs of damage are including, tubular vacuolation, protein cast formation, dilatation, renal swelling, and necrosis [66]. In a rat model that was subjected to renal ischemia and reperfusion, Sahu et al., (2014) discovered that, in contrast to the control or normal groups, the induced and vehicle groups' serum urea and creatinine levels significantly increased [62]. This study agrees with the findings of ALawadi et al., (2020) and Ali et al., (2019), which show that the total severity of tubular injury scores was In mice with bilateral renal ischemia for 30 minutes and reperfusion for 2 hours, the induced-ischemia and vehicle groups showed significantly higher levels compared to the sham group [67, 68] .

By changing signaling pathways that aim to reduce renal fibrosis and maintain renal function, Bai et al., (2021) showed that PFD protects the renal system. This is done by maintaining the structure and function of mitochondria in the renal tubular cells, which is the main problem that comes with CKD [69]. Bassiouny et al., (2023) showed that pirfenidone is a good antifibrotic drug that restores normal lung architecture, In a rat model of PF, this lead to a substantial reduction in oxidative stress and fibrosis [70]. In canines with end-stage chronic kidney disease, Im et al., (2024) found pirfenidone serves as a therapeutic agent for addressing renal fibrosis in kidney epithelial cells [71]. This founding agrees with study of Demirkol et al., (2023) showed in bleomycin-induced lung fibrosis rat models, pirfenidone has a strong protective effect against pleuritis and fibrosis [72].

## CONCLUSION

Based on this study, it can be concluded that pirfenidone has a strong reno-protective effect against RIRI via improving kidney function and significantly lowering serum urea and creatinine levels. It also has anti-inflammatory effects in RIRI, as shown by a significant lower of pro-inflammatory markers NF-kB and TNF-α values, and it has an antioxidant impact by significant blocking oxidative stress marker, $F_2$-Isoprostane.

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**Ethical approval**

All experimental protocols in this investigation were approved by IACUC at Kufa University after submission of the required applications for ethical clearance with a number (3178), which was approved on (4/2/2025) by Kufa University/Faculty of Pharmacy. This study was directed following the guidelines submitted by the Association for Science of Laboratory Animals, as listed in the "Guide of Care and Use of Laboratory Animals". Additionally, consent from the Animals Care Committee was obtained for the ethical management and suitable handling of all mice in the study. Pain and discomfort must be minimized; anesthesia was utilized; if necessary, recurrent procedures on the same animal were avoided, and to reduce animal suffering, the mice were euthanized by handling a combination of ketamine and xylazine anesthesia.

**Statement of Permission and Conflict of Interests**

Conflict of Interests: Not found

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م / إقرار المشاريع البحثية

تحية طيبة..

استناداً إلى قرار اللجنة المركزية لأخلاقيات البحث العلمي في محضر الجلسة (الأولى) المنعقدة بتاريخ ٢٠٢٥/١/١٢، حصلت الموافقة على الجوانب الأخلاقية في مشروع البحث وأوصت بالشروع بتنفيذه للسادة الباحثين والمدرج في الجدول أدناه أسمائهم وعنوان البحث الموسوم:

| ت | الاسم الثلاثي | الكلية | اسم البحث |
|---|---|---|---|
| ١ | أ.م.د. بشائر مرتضى محمد باقر | الصيدلة | Effect of Pirfenidone on Renal Ischemia Reperfusion Injury in a Mice Model |
| ٢ | لقاء علي أحمد | | |

مع التقدير..

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