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Reno-Protective Effects of Platelet-Rich Plasma and Exosome on a Model of Renal Ischemia/ Reperfusion in Rats

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Abstract :Renal Ischemia/Reperfusion (I/R) injury is one of the factors contributing to acute kidney injury (AKI), which is brought on by a rapid, and sudden stoppage of blood flow followed by a massive influx of blood, and reoxygenation. Methods: This study is designed to evaluate the potential renal protective effect of subcapsular injection of both exosome and PRP before the reperfusion stage on the renal tissues following renal I/R injury by assessing both histopathological, and biochemical alternations in kidney tissue. Forty mature male rats were utilized furthermore, subdivided into control, I/R, PRP, exosome, and PRP+Ex. The left renal cortex and medulla were processed from all groups for histological examination, and biochemical evaluations of serum Na, K, urine creatinine, and urea were performed. Results: Our finding proved that PRP injection restored normal renal histomorphology. Significant enhancement in serum and urine indicators relates to kidney functions. Conclusions: We determined the significant superior effect of subscapular injection of PRP before the reperfusion phase on the kidney following the renal I/R injury model.

Keywords: **Acute kidney injury, Renal ischemia, Exosomes, Platelet-rich plasma, *In vivo***

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1. Introduction

Acute kidney injury (AKI) is an eminent cause of high rates of morbidity and mortality during major surgeries such as partial nephrectomy, kidney transplantation, vascular surgery, hemorrhagic shock, and other urological problems **(Güvenç et al., 2019)**. Renal ischemia/reperfusion (I/R) injury is considered one of the main causes that lead to AKI. Ischemia is the sudden stoppage of blood flow, nutrients, and oxygen to the kidney after clamping the renal pedicles. Also, characterized by a lowering in P^H that ultimately leads to the accumulation of toxic nitrogenous wastes as creatinine and urea intracellular sodium and calcium, excessive generation of reactive oxygen species (ROS) that subsequently leads to damage parenchyma cells as tubular epithelial cells, and endothelial cells that adversely affect the rate of excretion of urine creatinine and urea**(Ahmadvand et al., 2019)**. The subsequent stage of renal ischaemia, known as reperfusion, is marked by a massive reinflux of blood and oxygen as well as an increase in pH, which negatively damages tissue and results in inflammation, oxidative stress, impaired energy metabolism, apoptosis, and tubular necrosis. **(Z. Liu et al., 2020)**

Platelet-rich plasma (PRP) therapy is a technique based on the centrifugation of fresh blood samples to extract a huge number of platelets including essential autologous growth factors (Salem, Helmi, & Assaf, 2018). These concentrated growth factors such as hepatocyte growth factor (HGF), insulin-like growth factor-1(IGF-1), epidermal growth factor (EGF), Transforming growth factors(TGF-β) , and vascular endothelial growth factor (VEGF) have been reported their superior effective roles in regeneration medicine, wound healing, and carrying unique characters such as repair of renal tubules, proliferation of endothelial cells, anti-inflammatory, tissue regeneration, and curbing tubular necrosis, and renal healing after ischemia (Salem et al., 2018).

Exosomes, which have an average diameter of 30-150 nm **(Camussi, Dominguez, Dominguez, Xie, & Kelly, 2018),** are subtypes of extracellular vesicles (ECVs). Exosomes are found naturally in urine, saliva, and breast milk, among other bodily fluids. Exosomes can also be produced by normal cells, such as mesenchymal stem

cells, T-cells, and natural killer cells (NKC). Their payload includes a variety of biomolecules, including proteins, lipids, and miRNA, which makes them a desirable tool for treating renal damage. **(Zahran, Ghozy, Elkholy, El-Taweel, & El-Magd, 2020)**, However, MSC-derived exosomes are thought to be a more appealing biological tool for treating renal damage than mesenchymal stem cell therapy alone because of their anti-inflammatory, anti-apoptotic, and pro-angiogenic properties **(Li et al., 2019)**. Therefore, the purpose of this study is to assess the amelioration impact of PRP and exosome injection on the kidney after renal ischemia/reperfusion prior to the reperfusion phase.

2. Material and Method

2.1. Ethical statement and sample size

The Institutional Animal Care and Use Committee of Zagazig University, Zagazig, Egypt, with registration number ZU-IACUC/1/F/126/2023, approved all experimental protocols used in this work.

2.2. Experimental design

The present study was conducted on 48 pubertal male Sprague–Dawley rats, weighted (mean ± SD) 205.95 ± 12.266 g. Rats were housed under constant standard conditions of 22 ± 2 °C and a relative humidity of 65 -70% at a 12-h light/12-h dark cycle. Animals were with free access to water and food. A period of two weeks of acclimatization on the new housing conditions was considered before the surgery. Rats (n=48) were divided mainly into 3 groups; Donor rats (n=8) that were used for PRP preparation, control rats (n=8); normal un-operated rats, and I/R Rats (n=32): The operated rats, that were further subdivided into 4 groups n=8 depending on the treatment method.

1. Renal ischemia/ Reperfusion (I/R) group: received no treatment.
2. Platelet-rich plasma (PRP) group: 15 minutes before renal reperfusion, rats received one subcapsular injection of 300µl PRP.
3. Exosome (Ex.) group: 15 minutes before renal reperfusion, rats received one subcapsular injection of 300µl exosome.
4. Platelet-Rich plasma and Exosome (PRP+Ex.): 15 minutes before renal reperfusion, rats received one subcapsular injection of 150 µl PRP and 150 µl exosome mixture.

2.3. Preparation of exosome

Exosome ® was purchased from Stem Vie®, Al Haram, Giza, Egypt. It was diluted with its excipient vial that containing 2 ml Garamycin and thus, each 1ml contains 3x106 Mesenchymal stem cells exosome extract.

2.4. Platelet-Rich Plasma (PRP) Preparation

The PRP was ready from the donor rats (n=8) according to Okuda, et al and others **(Okuda et al., 2003),(Ehrenfest, Rasmusson, & Albrektsson, 2009),(Mohammadi et al., 2016)**. Briefly, the whole blood was collected via a cardiac puncture, the immediately the whole blood was drawn into tubes with anticoagulant (4% sodium citrate) and centrifuged at 160Xg for 20 minutes resulting in two layers; an upper straw-yellow plasma part and a distal red blood cell part. The upper part was moved to another plain tube that was later on centrifuged at 400Xg for almost 15 minutes. The upper two-thirds of the supernatant in the plain tube that considered as a platelet-poor plasma was discarded and the lower part that considered as PRP was retained

2.5. Surgical procedures

Surgical procedures were operated by the same surgeons at the same time 10 AM to avoid the influence of both circadian rhythm and surgery variables on the results **(McCulloch, Taylor, Sasako, Lovett, & Griffin, 2002),(Hibberd et al., 2023)**.

Anaesthesia was achieved using intra-peritoneal injection of both 5 mg kg-1 Xylazine Hcl (20 mg/ml, XYLAJECT, ADWIA CO, Egypt) and 75 mg kg-1 Ketamine Hcl (50mg/ml, KETAMINE, SIGMA TECH CO, Egypt) **(Samy et al., 2020).** Rats were prepared by clipping, shaving and a disinfecting the whole abdominal area from xiphoid to the pubis. A fenestrated drape was fixed and centred on the mid line abdominal area. A midline laparotomy incision of 3 cm length was performed in the skin, subcutaneous tissue and the linea-alba, then the left kidney was approached after siding the abdominal organs. A micro-vascular bulldog clamp was used to clamp the left renal pedicle after it had been separated from the surrounding renal fat using small artery forceps. The clamp was then repositioned in the retroperitoneal cavity, filled with a warm 40° C 0.9% NaCl

saline solution, the abdominal skin incision was closed with small towel clamps, and the abdominal incision was covered with sterile gauze to induce left renal ischaemia for 45 minutes. (Fig. 1).

Fifteen minutes before renal reperfusion, a single dose of PRP and/or exosomes was injected subcapsularly in the ischemic kidney in treatment groups by a dose of 0.3 ml PRP, 0.3 ml Exosome or PRP(0.15 ml)/Exosome(0.15 ml). Five minutes before left renal de-clamping, a right nephrectomy was performed through the same incision line by approaching the right renal pedicle, isolation, double ligation using 3/0 polyglycolide sutures (MAXON, COVIDIEN CO, USA) and careful cutting in between. Left renal reperfusion was achieved by approaching the left clamped kidney and careful de-clamping of the clamped pedicle. Finally, the laparotomy incision was closed routinely by continuous patterns .

Postoperative care

Rats received 50 mg/kg Amoxicillin (Flumox, EIPICO, Egypt) and 5 mg/kg meloxicam (Anti-cox II, 15mg/3ml, ADWIA, Egypt) intramuscularly once daily for the next three days. The wounds were dressed daily with povidone-iodine

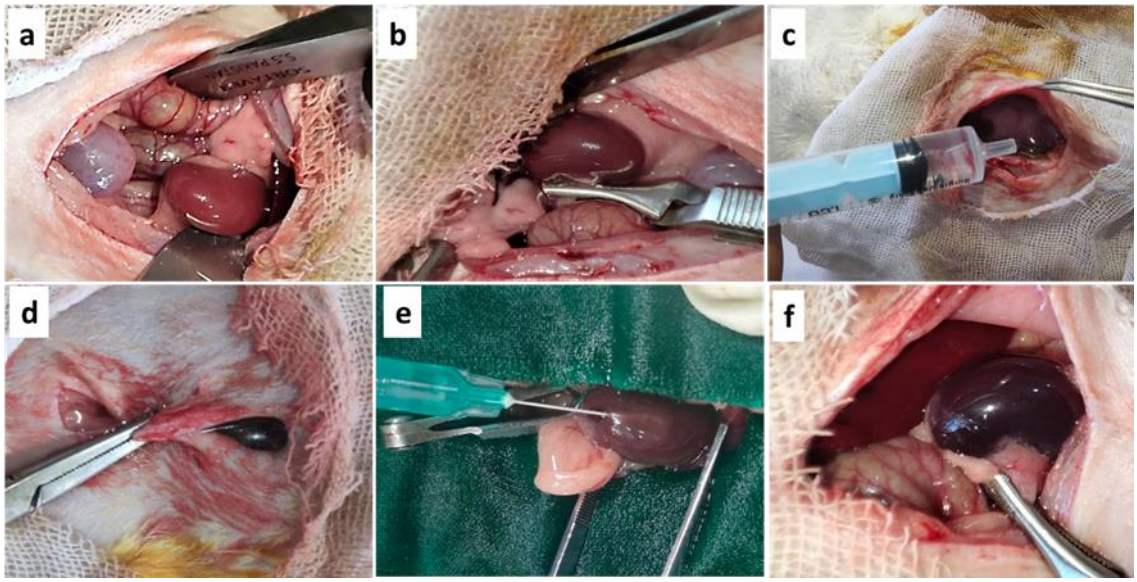

Figure (1): step by step shows the surgical procedures for induction of left renal I/R injury. **(a):** Approaching the left kidney through the laparotomy incision. **(b):** The left renal pedicle was clamped used a vascular bulldog. (**c):** The peritoneal cavity was flushed and filled with saline. **(d)** Temporary closing the incisional line after reinsertion of the clamped pedicle into its original situation. **(e): c**areful subcapsular injection of the left ischemic kidney. **(f):** The congested kidney just before reperfusion.

Collection of biological samples

Experimental rats were placed in metabolic cages after 3 days of reperfusion, and 24-hour urine samples were collected by sterile technique to estimate urinary parameter levels. Euthanasia was performed by intraperitoneal injection of thiopental sodium at a dose of 7.5 mg /100g BW **(Close et al., 1996)**. The whole blood of rats was obtained via cardiac puncture in a plain tube, and centrifuged after clotting at 3000 rpm/minute for 5 minutes then serum was harvested and stored at -80℃ for further biochemical analysis. The left kidney was collected and cut in half; one was fixed in the buffered 10% formalin for further histological examination and the other half was washed and, homogenized in 20mL of 1× PBS then stored overnight at ≤ -20℃ and after two freeze-thaw cycles, the homogenate was centrifuged at 5000×g for 5 minutes for further biochemical analysis.

Histological examination

According to standard procedures, the formalin fixed kidney halves was embedded in paraffin, and sliced into sections of 3µm thickness. Gradually the sections deparaffinized, hydrated, stained with hematoxylin

and eosin (H&E) and finally, examined by an experienced pathologist using a light microscope at ×400 magnification power.

2.6. Measurement of kidney function indicators.

Assessment of Na+ and K+ was performed using a colorimetric method at wavelength 410 nm and 380 nm respectively using commercial kits (SPINREACT, Girona, Spain). The level of urine creatinine and urea using commercial kits (SPINREACT, Girona, Spain) by spectrophotometry colorimetric method at wavelength 492nm, and 340nm respectively according to the manufacturer's protocol.

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) software, version 23.0 (Chicago, Illinois, USA). Comparison of three or more groups was performed using a One-way analysis of variance (ANOVA) followed by the Tucky test as a post hoc analysis. Whereas a difference with a chance probability of $P < 0.05$ was accepted as statistically significant while $P < 0.0001$ was considered highly significant. All graphs were carried out using GraphPad Prism software, version 8.0 (CA, USA).

3. Results

Biochemical results

Table (1): The effect of platelet-rich plasma and exosome administration on serum sodium and potassium levels.

Groups	Na^+ (mmol/L)	K^+ (mmol/L)
Control	139.81±1.856	3.79±0.42
I/R	147.80±2.29[a]	6.81±0.86[a]
Exosome	143.81±1.18[ab]	4.55±0.233[ab]
PRP	140.87±2.42[b]	4.17±0.15[b]
Ex.+PRP	143.45±1.97[ab]	4.41±0.216[b]

As depicted in **Fig.2 (A &B)**, and **Table (1)**, Significant increase in serum levels of Na^+ and K^+ in ischemic operated rats compared with control groups. Our finding showed that rats that received **PRP,** exhibited the greatest significant reduction in both levels of serum potassium and sodium ($p \leq 0.0001$) compared to the ischemic control rats. Additionally, serum potassium significantly decreased in rats injected with both groups **PRP and exosome** and **exosome only** ($p \leq 0.0001$) compared to the ischemic control group.

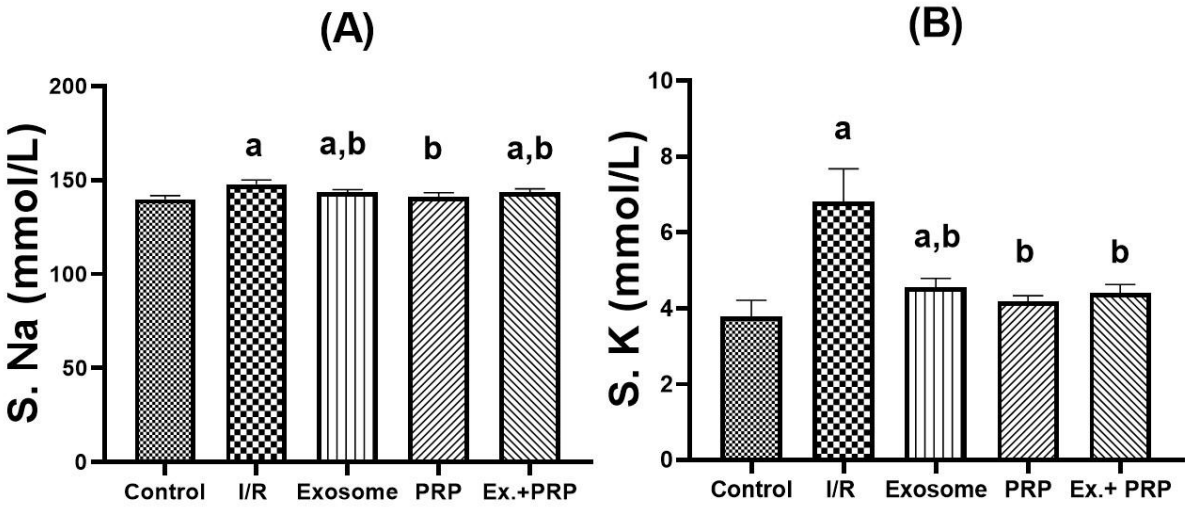

Figure (2): The effect of platelet-rich plasma and exosome administration on the levels of serum sodium and potassium. (d) Variation of serum sodium level among all experimental groups. (e) Variation of serum potassium level among all experimental groups. Statistical analysis using one-way ANOVA followed by Tukey's post-test. Variables were presented as mean ± standard deviation (SD). (n=8). [a] significant *vs* control group (P< 0.0001). [b] significant *vs* renal I/R group (P< 0.0001).

Table (2): The effect of platelet-rich plasma and exosome administration on levels of urine creatinine and urine urea.

Groups	Urine creatinine (mg /24h)	Urine urea (gm/24h)
Control	11.40±1.56	21.66±2.93
I/R	29.64±2.72[a]	35.30±3.82[a]
Exosome	16.30±2.03[ab]	26.64±3.19[ab]
PRP	13.85±0.883[ab]	22.78±1.93[b]
Ex.+PRP	15.93±1.326[ab]	24.87±2.17[b]

As illustrated in **Fig.3 (A, B)**, and **Table (2)**; Both levels of urine creatinine and urea were increased in I/R untreated rats compared with normal control groups. Compared to the renal ischemic rats, the rats who were injected with **PRP** experienced a significant decrease in urine creatinine and urea ($p \leq 0.0001$). Similarly, the rats that received **PRP and exosome** caused a significant reduction in urine creatinine and urea ($p \leq 0.0001$) compared to the renal ischemic group. In addition, it was shown that in rats injected with **exosome only**, the level of urinary creatinine and urea significantly reduced ($p \leq 0.0001$) compared to renal ischemic untreated rats.

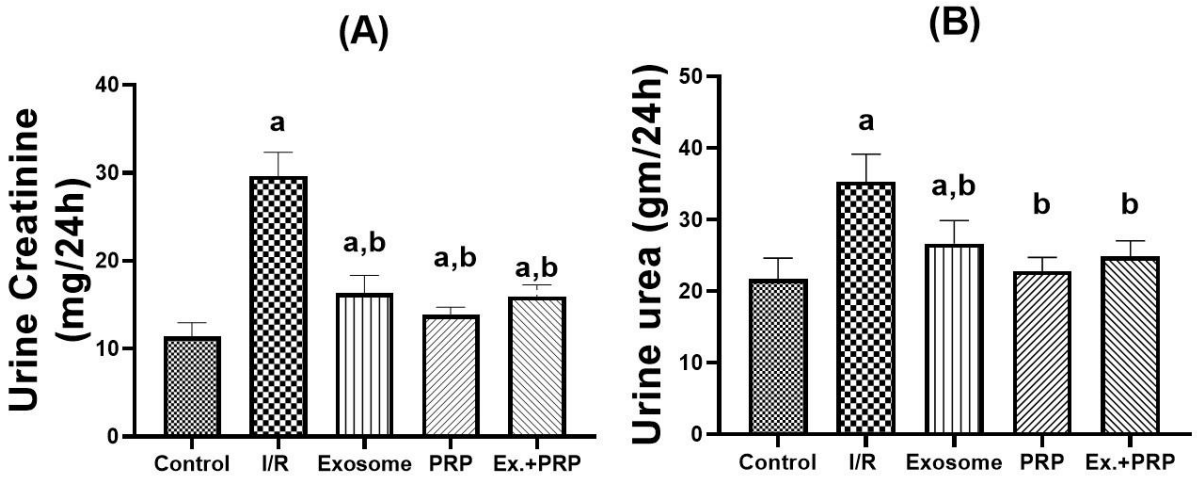

Figure (3): The effect of platelet-rich plasma and exosome administration on the levels of urine creatinine and urea. (a) Variation of urine creatinine level among all experimental groups. (b) Variation of urine urea level among all experimental groups. Statistical analysis using one-way ANOVA followed by Tukey's post-test. Variables were presented as mean ± standard deviation (SD). (n=8). [a] significant *vs* control group (P< 0.0001). [b] significant *vs* renal I/R group (P< 0.0001).

Histopathological results

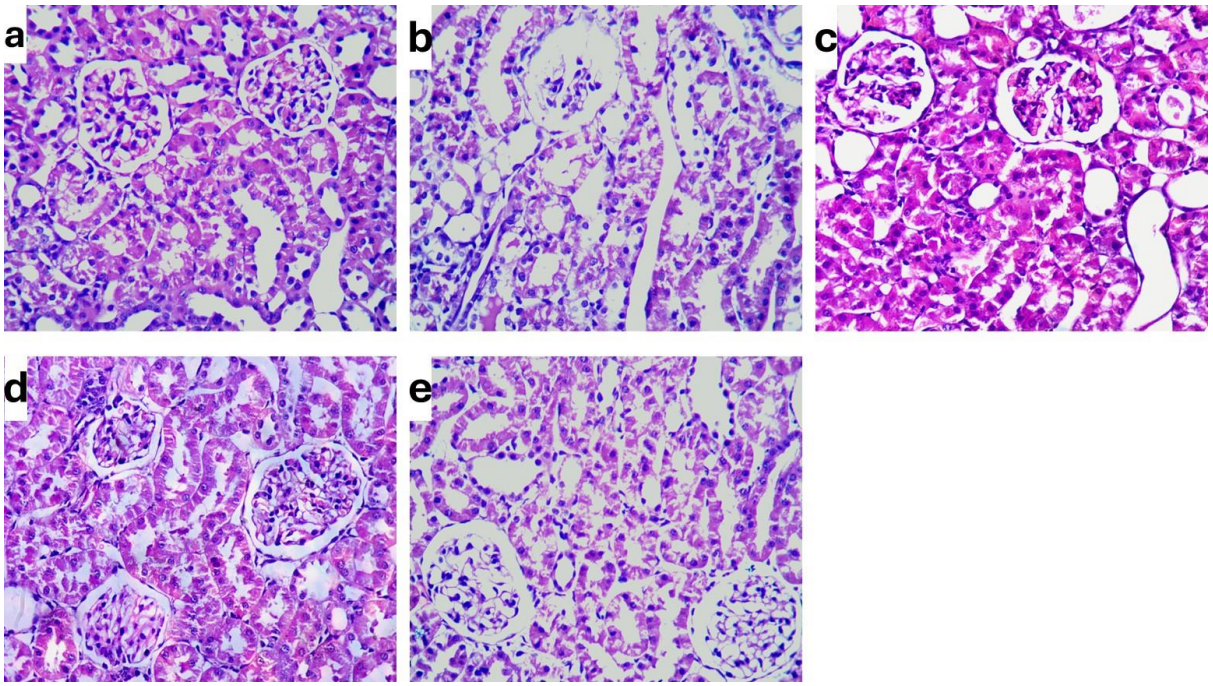

Figure (4): A photomicrograph of left renal cortex sections of adult male rats of different groups: **(a): control group. (b): I/R group. (c): exosome group. (d): PRP group. (e): PRP+Ex. group (H&E, x400).**

A rat from the control group's renal cortex displays (Fig. 4a) Malpighian corpuscles with a glomerulus that is packed with capillaries and encircled by debris-free Bowman's space. The vesicular nuclei of the proximal and distal convoluted tubules are rounded. Bowman's capsule displays the flat squamous cells that make up its parietal layer. The renal cortex of the I/R group has enlarged Malpighian corpuscles with big

segmented glomeruli, engorged capillaries, and a broad Bowman's gap that is full with debris (Fig. 4b). Flat cells can be seen in the Bowman's capsules' parietal layer. Few tubules contain rounded vesicular nuclei, but the majority of renal tubules have darkly pigmented nuclei. Large casts or acidophilic materials are seen to fill their lumens. With reference to the Ex. group, the renal cortex shows (Fig. 4c) the Malpighian corpuscle, which has a glomerulus with numerous capillaries and is encircled by a large, debris-filled Bowman's gap. Flat cells can be seen in the Bowman's capsules' parietal layer. While some proximal and distal tubules have darkly pigmented nuclei and vacuolated cytoplasm, others have rounder, pale nuclei. The renal cortex in relation to the PRP group (Fig. 4d). shows slightly damaged tissue that is nearly normal. Malpighian corpuscle having a normal Bowman's space and a glomerulus with many capillaries. Bowman's capsules have flat squamous cells in their parietal layer. Some tubules have luminal exfoliated cells and darkly pigmented nuclei, while others have rounder nuclei that are almost twisted. There are tiny capillaries found. Lastly, the combined PRP+Exosome group's renal cortex displays (Fig. 4e) mostly healthy renal tissue with minor damage. A large Bowman's space with minimal detritus surrounds the Malpighian corpuscle, which contains a glomerulus with numerous capillaries. The cells in the Bowman's capsules' parietal layer range in shape from cuboidal to flat. In contrast to those with darkly stained nuclei, luminal exfoliated cells, casts, and large, crowded capillaries, the convoluted tubule had rounded nuclei.

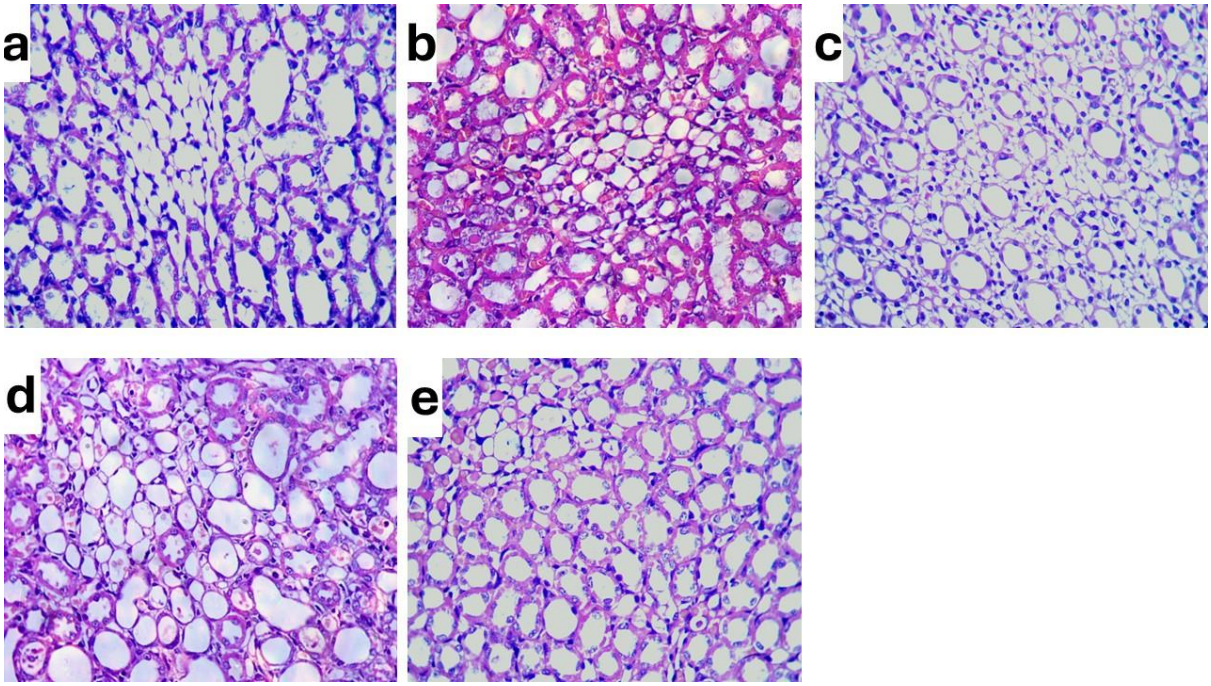

Fig. 5 A photomicrograph of left renal medulla sections of adult male rats of different groups: **(a): control group. (b): I/R group. (c): exosome group. (d): PRP group. (e): PRP+Ex. group (H&E, x400).**

The renal medulla (Fig. 5a) in the control group shows a few loops of Henle with flat squamous lining cells. The lining cells of collecting tubules or ducts are often cuboidal in shape. There are hardly many capillaries visible. A few loops of Henle with flat squamous lining cells may be seen in the renal medulla (Fig. 5b) of the I/R group. Cuboidal lining cells can be seen in nearly all collecting tubules or ducts. Nearly all of the tubules have casts and nuclei that are deeply stained. Colloidal materials and clogged capillaries are observed between tubules.

The renal medulla (Fig. 5c) of the Ex. group shows a few loops of Henle with flat squamous lining cells. Cuboidal lining cells are found in the majority of collecting tubules or ducts. The cytoplasm of some tubules is vacuolated. There are several casts and clogged capillaries visible. There are a few loops of Henle (LH) with flat-lining cells in the renal medulla of the PRP group (Fig. 5d). Cuboidal lining cells are found in nearly all collecting tubules. The nuclei of a few tubules are darkly pigmented. There are tiny capillaries and casts visible.

Lastly, the PRP +Ex. group's renal medulla displays a few loops of Henle with flat squamous lining cells (Fig. 5e). The lining cells of nearly all collecting tubules or ducts are cuboidal in shape. The nuclei of several tubules were deeply pigmented and exfoliated. There are visible casts and clogged capillaries. It is possible to see tiny colloidal materials between tubules.

4. Discussion

Renal ischemia/reperfusion sets off a complex chain of events that begins with a lack of oxygen and tissue nutrients during the ischemic stage, which is followed by the cessation of aerobic cellular metabolism, which results in anaerobic conditions, which lowers the rate of ATP production, dysfunction of the ATPase pump and Na+/K+ ATPase, which causes Na+,K accumulation in the cytoplasm, and finally hypertension **(Lima et al., 2021)** , In addition, renal I/R damage has a negative impact on the proximal tubules in the renal cortex and medulla because of their high oxygen demand, which in turn prevents the extraction of urine creatinine and urea **(Han et al., 2020)**. Our kidney function data from this investigation showed that induction renal I/R significantly raised serum Na, K, urine creatinine, and urea levels. Our results, which are in line with earlier research discussed above, demonstrate that rats in both groups received injections of platelet-rich plasma and a mixture of PRP and Ex before the reperfusion phase showed greater improvement in all biochemical parameters and histological tissues because PRP has special properties compared to exosomes alone. In contrast to untreated renal ischemia rats, rats given PRP injections prior to reperfusion showed a superior and substantial improvement in serum kidney function measures by lowering serum levels of Na and K. Additionally, PRP alleviates hypertension by lowering elevated levels of both Na and K. Rats given a combination of platelet-rich plasma and exosomes prior to reperfusion showed a gradual improvement, albeit one that was not as noticeable as that of the rats given PRP prior to reperfusion. Serum kidney function measures, urine creatinine, urea, and serum Na and K levels were all lower. Since just exosomes were administered to the rats in question, the effects of the injected exosomes were hindered by their quick clearance, poor retention, and low encapsulation ability. **(Imai et al., 2015)**,**(Y. Liu et al., 2020)** Before reperfusion showed the least impact on both kidney function metrics, the rats were given a single dosage of exosomes.

When renal histological deterioration occurs, the Malpighian corpuscles enlarge with large, segmented glomeruli, congested capillaries, and surrounded by a wide Bowman's space. This results in a decrease in glomerular filtration rate, which causes uremic toxins to accumulate and damages renal proximal TECs due to their high demand for oxygen supply in the cortex and medulla. **(Gholampour et al., 2018)**,**(Han et al., 2020)**. Due to the greater regeneration ability of its growth factors, it was demonstrated in our study that renal subcapsular injection of PRP improved renal histological deteriorations and returned the control group's rats to normal. Furthermore, in ischemia untreated rats, the morphometry examination was an essential confirmatory method for determining the quantity of deteriorated tubules and casts in the renal cortex and medulla following their sharply increasing level.

Conclusion

In conclusion, we found that because of the unique remarkable characteristics of PRP growth factors, injections of PRP alone and PRP + EX in combination before the reperfusion phase have a significantly better effect on kidney biochemical parameters and improve kidney tissue regeneration that is almost identical to that of normal rats.

Abbreviations:

I/R (Ischemia/Reperfusion)**, PRP** (Platelet-Rich Plasma)**, Ex.** (Exosome), **AKI** (Acute Kidney Injury), **TECs** (Tubular Epithelial Cells)**.**

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical approval and consent to participate.

All applicable international, national, and/ or institutional guidelines for the care and use of animals were followed.

Availability of data materials

'No applicable'

Funding

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Authors contributions

A.S. H.A. M.S. and E.A. designed the experiment and performed all surgical procedures. H.A., M.S., and E.A. carried out the biochemical, and physiological studies, contributed to the sequence alignment, and drafted the manuscript.

Consent of publication

'No applicable'

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