RESEARCH PAPER | Biochemistry | Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Biochemical Evaluation to Mesenchymal Stem Cells Therapy of Renal Tubulointerstitial Injury

KEYWORDS: Mesenchymal Stem Cells (MSCs)-Acute kidney Injury (AKI)-ischemia/reperfusion (I/R)-Acute renal failure (ARF)

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ABSTRACT *Background Acute renal failure (ARF) remains a frequent clinical complication, Ischemia is the major cause of acute kidney injury (AKI), associated with high mortality and morbidity. Mesenchymal stem cells (MSCs) have multilineage differentiation potential and can be a potent therapeutic option for the cure of AKI. The aim of this study was, therefore, to evaluate the therapeutic effectiveness of bone marrow–derived mesenchymal stem cells in a rat model of ischemia/reperfusion (I/R) ARF.*

Materials and Methods MSCs were cultured and rats were used as a common I/R model to induce ARF by clamping both renal pedicles for 45 minutes then stem cells were transplanted. Ki-67 was used as marker for regeneration of renal tubulointerstitial injury.

Results This study reports that Rat MSCs decreased cell apoptosis and increased proliferation and reduced fibrosis in common I/R model in rats.

Conclusion MSCs may be useful in regeneration of renal tubulointerstitial injury.

INTRODUCTION

The kidney is comprised of heterogeneous cell populations that function together to perform a number of tightly controlled and complex processes. Renal ischemia\ reperfusion (I\R) injury is a common cause of acute renal failure and contributed to consider able morbidity associated with surgery and anesthesia **(Chander *et al.*, 2006)**. One cause of acute kidney injury (AKI) is ischemia which can occur for a number of reasons, for example with the use of the vasoconstrictive drugs or radiocontrast agents; hypotension linked to loss of blood after surgery **(Asif and Bruce, 2011)**.

Stem cells play fundamental roles in the self-renewal of adult tissues throughout life. Bone marrow–derived hematopoietic stem cells have been discovered to transdifferentiate into cells of different germ layers **(Krause *et al.*, 2001)**. Physiologically, mesenchymal stem cells give rise to osteocytes, chondrocytes, and adipocytes, but were recently found to differentiate into endothelial, myocardial **(Nagaya *et al.*, 2004)**, liver **(Shu *et al.*, 2004)**, renal **(Morigi *et al.*, 2004)**, and pulmonary epithelial cells **(Ortiz *et al.*, 2003)**. Based on this background, the objective of the present study was to test the therapeutic potential of mesenchymal stem cells, administered to rats following induction of ARF by ischemia/reperfusion (I/R) and showed the regeneration of renal tubulointerstitial injury that marked by the pathological change and uses of marker Ki-67.

Materials and Methods:

Animals were approved by the animal house, faculty of science, Zagazig University, Egypt. I/R (ARF) were induced in anesthetized (ketamine/valpam) adult male Sprague Dawley rats, weighing 200 to 250 g by clamping both renal pedicles for 45 minutes. Then animals were injected with 4 ml warmed normal saline gave intraperitoneally before abdominal closure. **(Claudia *et al.*, 2005)**

Cell culture and transplantation

Rat mesenchymal stem cells were generated from the bone marrow of adult Sprague-Dawley rats by standard procedures **(Javazon *et al.*, 2001, Lange *et al.*, 2003)**. Mesenchymal stem cells used in this study were cultured for more than 3 passages, which practically excludes hematopoietic cell contamination.

After 3 days of isolation non-adherent cells were removed by two to three washes with PBS and adherent cells further cultured in complete medium. The medium was changed every 3 days until the monolayer of adherent cells reach 70-80% confluence. Trypsinization was made for cell splitting by Trypsin/EDTA solution (0. 25%, lonza, USA) for passage 1 .Number of cells were evaluated by Homocytometer and cellular viability by the Trypan Blue exclusion test. Each 250-300 $\times 10^3$ cells were inoculated in 75 Cm2 culture flask that were incubated at 37°C and 5% CO_2. Cell cultivation was maintained up to the 3rd passage. After reflow, 2×10^6 mesenchymal stem cells in 0.5 mL of complete medium were infused into the renal vein. **(Uta *et al.*, 2007).**

Biochemical study:

Renal function was monitored by measurement of serum creatinine according to the method of **(Young., 2001)** and blood urea nitrogen (BUN) according to the method of **(Henry *et al.*, 1974)**

Histopathology study:

a) Histology

The kidneys were fixed in a 10% neutral-buffered formalin solution, embedded in paraffin and 5µ thick sections were stained with Hematoxylin and Eosin for injury scoring according to a previously published guideline. **(Solezk *et al.*, 1979)**

b) Imunohistochemstry

Other sections were stained with Ki-67 imunohistochemstry

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marker to show the regeneration in renal tubulointerstitial cells according to a previously published guideline (Hall et al; 1988). And the staining protocol according a previously published guideline **(Scholzen, Gerdes., 2000)**.

Results:
Cell culture

Attachment of spindle-shaped cells to tissue culture flask was observed after 1 day of culture BMSCs. After 9 days, spindle shaped cells reached 80% confluency. Morphology of cells changed gradually with passage number. Cells become more flat-shape with increasing in passage number. BMSCs showed the ability to form multilayer after confluent. **(Fig.1).**

Biochemical study
Forty five minutes of ischemia led to severe renal damage in control ARF animals, causing significant increase in serum creatinine levels in positive control animals **(Fig. 2)** from a common baseline for both groups of 0.4 ± 0.13 mg/dL to 1.25± 0.3 mg/dL, 0.85±0.3 mg/dL, and 0.75±0.3 mg/dL at days 3, 5, and 7, respectively **(Fig. 2).** But animals with ARF that received mesenchymal stem cells immediately post-reflow had a significantly better renal function on days 3, 5 and 7 after ARF **(Fig. 2).** Serum creatinine on day 3 was found to be 0.81±0.3 mg/dL, on day 5, 0.7±0.1mg/dL, and on day 7, 0.56± 0.2 mg/dL). Obtained serum creatinine data were qualitatively paralleled by changes in BUN levels which showed increase in serum BUN levels of positive control animal **(Fig. 3)** from a common baseline for both groups of 18.0±2.0 mg/dL to 37.5±2.0 mg/dL, 31.3±4.0 mg/dL, and 27.0±3.0 mg/dL at days 3, 5, and 7, respectively **(Fig.3)**. But animals with ARF that received mesenchymal stem cells immediately post-reflow had a significant better renal function on days 3, 5 and 7 after ARF **(Fig. 3).** Serum BUN on day 3 was 28.0±2.0 mg/dL, on day 5, 24±2.0 mg/dL, and on day 7, 20.5±4.0 mg/dl).

Histopathology study
Histology

Forty five minutes of ischemia led to severe renal damage in control ARF animals, causing glomeruli shrinkage of vascular tuft and increase in acute tubular necrosis (ATN) and tubular atrophy. **(Fig. 4, 5and 6)**Animals with ARF that received mesenchymal stem cells immediately post-reflow had a significant decrease in ATN and glomeruli may be returned to its normal shape gradually with increasing in regeneration in renal tubulointerstitial.**(Fig. 7, 8and 9).**

b) Imunohistochemstry
using Ki-67 imunohistochemstry marker show increased in regeneration in renal tubulointerstitial in treated animals more than untreated animals (control +ve).**(Fig. 10, 11 and 12).**

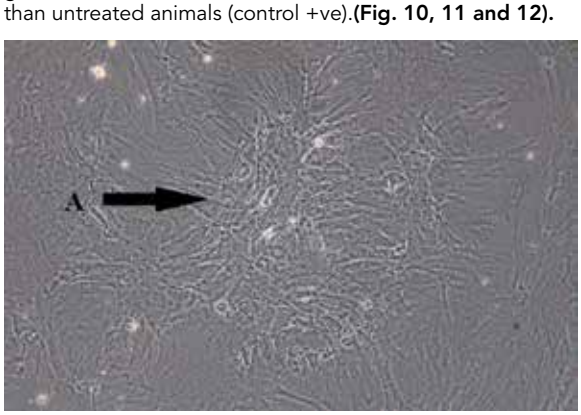

Fig.1. (A) shows Attachment of BMSCs spindle-shaped cells to tissue culture flask in passage 3 Scale bar=100 µm

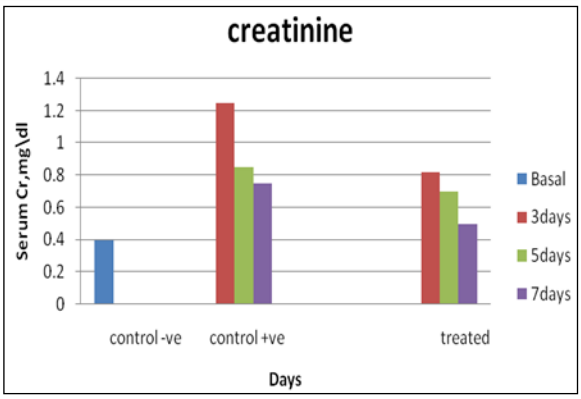

Fig.2.Shows the difference in creatinine levels between treated animals and untreated animals (control +ve).

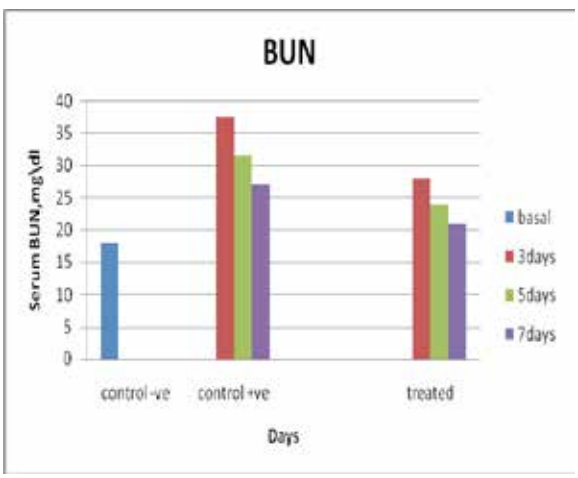

Fig.3. Shows the difference in BUN levels between treated animals and untreated animals (control +ve).

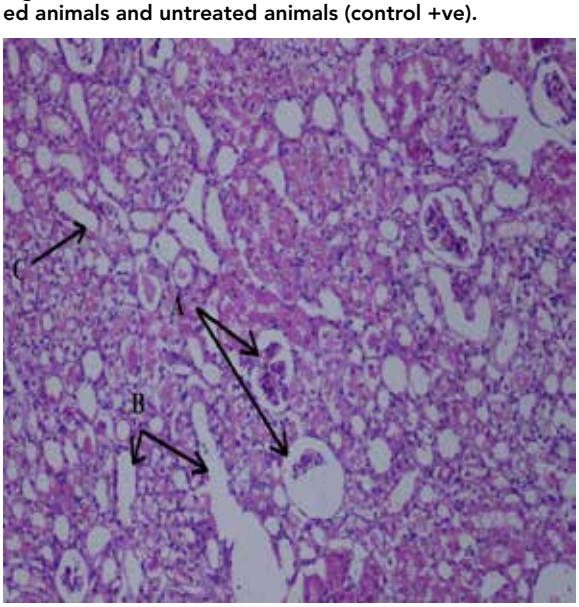

Fig.4. (A) Shows –glomeruli shrinkage of vascular tuft, (B) Tubules moderate tubular atrophy and ATN and (C) regeneration about 10% in untreated (control +ve) animals after3days of ARF Scale bar=100 µm.

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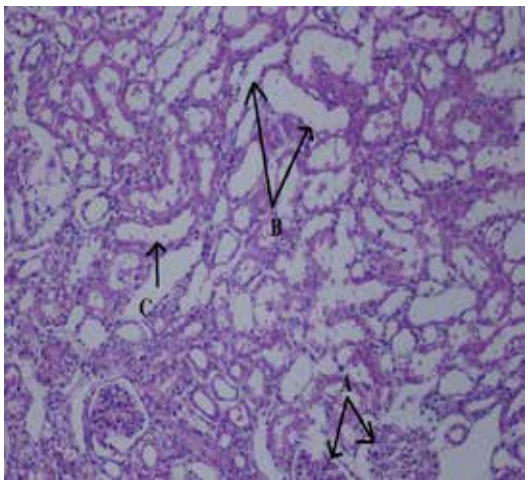


Fig.5. (A) Shows glomeruli congested capillaries, (B) Tubules marked ATN and increase tubules injury and (C) regeneration about 30%in untreated animals (control +ve) after 5 days of ARF Scale bar=100 µm.

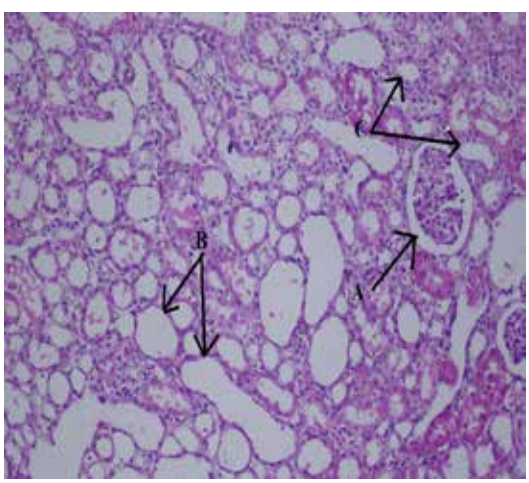


Fig.6. (A) Shows glomeruli normal, (B) Tubules marked ATN, tubules atrophy and marked dilatation and (C) regeneration about 40% untreated animals (control +ve) after 7 days of ARF Scale bar=100 µm.

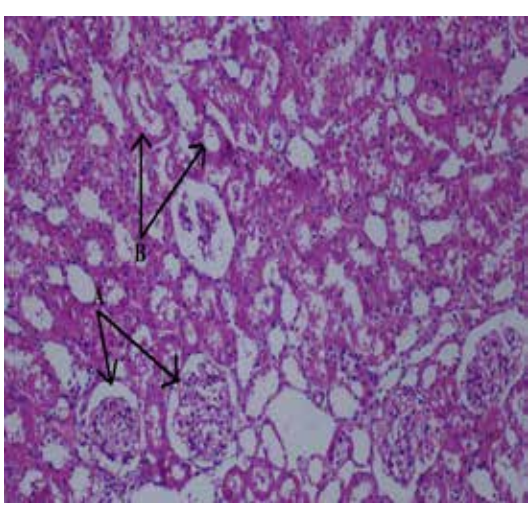


Fig.7. (A) Shows glomeruli mostly normal, (B) Tubules decrease in ATN and increase in regeneration and regeneration about 25% treated animals after 3 days of ARF Scale bar=100 µm.

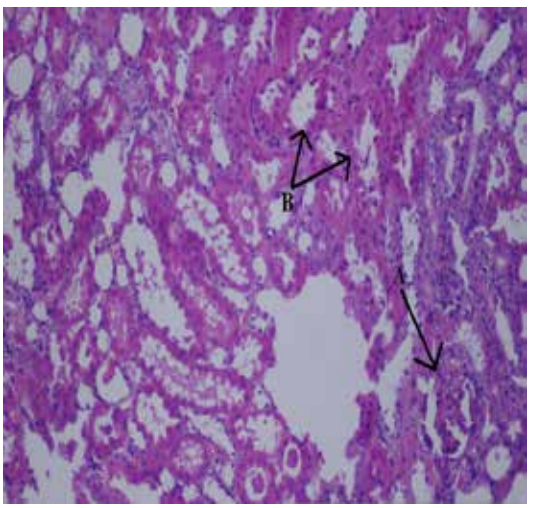


Fig.8. (A) Shows glomeruli congested capillaries, (B) more increase in regeneration and decrease ATN and regeneration about 50%in treated animals after 5 days of ARF Scale bar=200 µm.

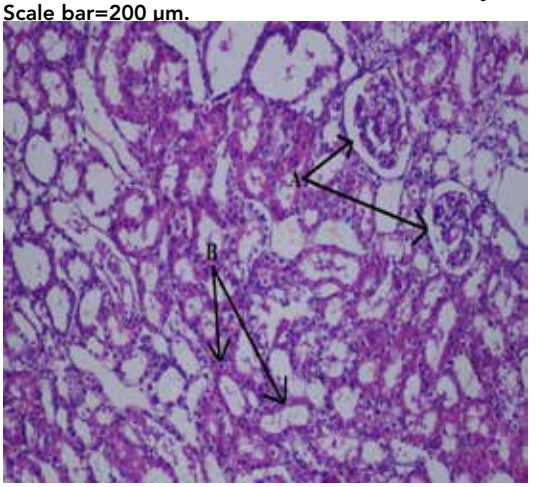
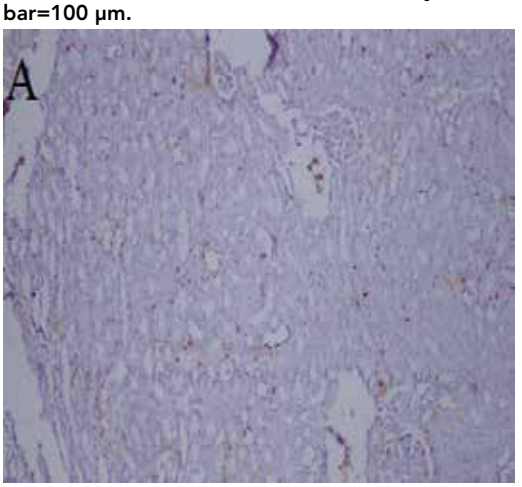


Fig.9. (A) shows glomeruli normal, (B) more increase in regeneration and decrease mild ATN in and regeneration about 75% treated animals after 7 days of ARF Scale bar=100 µm.



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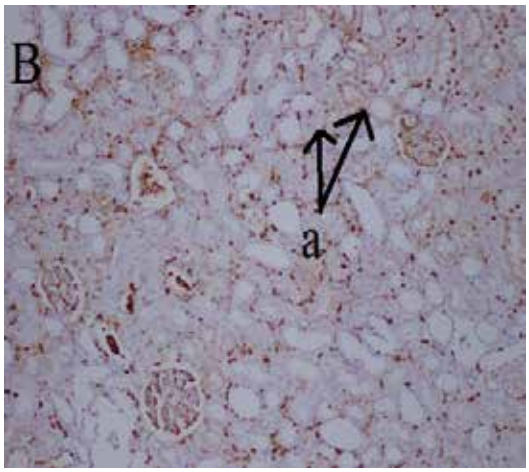
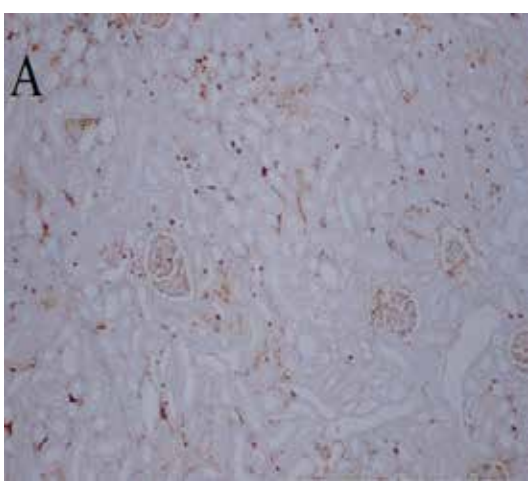


Fig.10. shows the difference between (A) untreated animals (control +ve) and (B) treated animals after 3 days of ARF with Ki-67 marker which showed increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs Scale bar=100 µm.



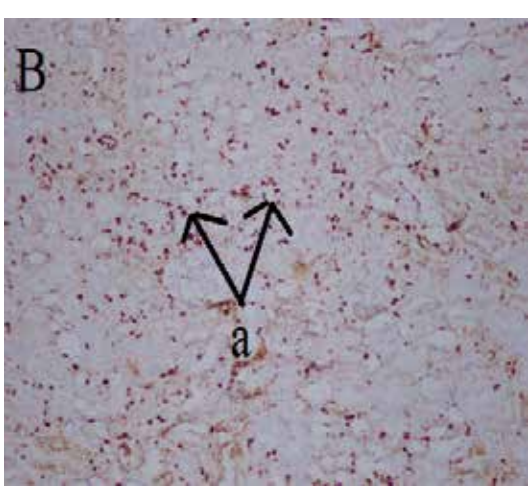
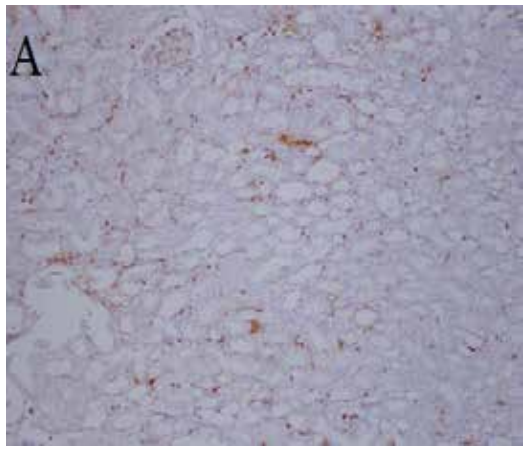


Fig.11. shows the difference between (A) untreated animals (control +ve) and (B) treated animals after 5 days of ARF with Ki-67 marker which showed more increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs Scale bar=100 µm.



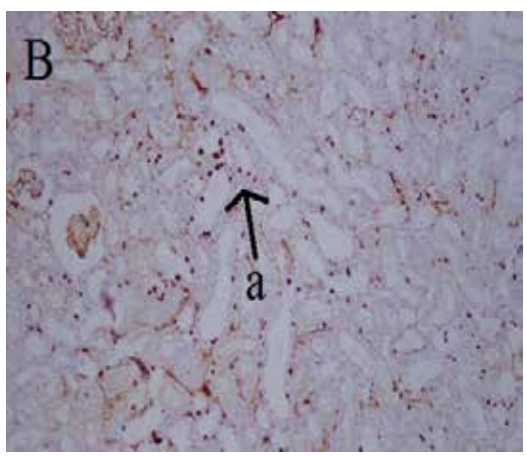


Fig.12. shows the difference between (A) untreated animals (control +ve) and (B) treated animals after 7 days of ARF with Ki-67 maker which showed more increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs Scale bar=100 µm

Discussion:

In the present study, bone marrow was isolated from rats from femurs and tibiae by flushing with DMED containing 10% FBS and 1% penicillin/ streptomycin, and then was cultured for 24 hour after that the product was washed with FBS to remove adherent cells. The cells adhered to the flask and constituted a rapidly expanding into spindle shaped cells and fibroblast like cells and this agree with **(Zahran *et al.*, 2013)**.

Injected with 2×10^6 mesenchymal stem cells in 0.5mL of complete medium were infused into the renal vein this gave a great result in treatment and this agree with**(Uta *et al.*, 2007)**.but **(Claudia *et al.*, 2005)** study use saline instead of complete medium and injected with 1.5×10^6 mesenchymal stem cells and it is not different in results. So using saline or complete medium, also injected with 2×10^6 mesenchymal stem cells or 1.5×10^6 mesenchymal stem cells gave approximately the same results.

Renal function was monitored by measurement of serum creatinine which showed decrease in level in treated animals to reach normal level gradually by time as the time of days increased the creatinine level decreased, Also BUN level was exactly likes creatinine and this agree with **(Claudia *et al.*, 2005)**

Histopathology study showed that sections were stained with Hematoxylin and Eosin and recorded that severe renal damage in positive control ARF animals, causing glomeruli

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shrinkage of vascular tuft and increase in acute tubular necrosis (ATN) and tubular atrophy but animals with ARF who received mesenchymal stem cells immediately post-reflow had a significantly decrease in ATN and glomeruli returned to its normal shape gradually with increasing in regeneration in renal tubulointerstitial cells, as time increase the regeneration increase, and this agree with **(Claudia *et al.*, 2005)** but in the study they stained with PAS stain.

Staining with Ki-67 imunohistochmistry maker show increased in regeneration in renal tubulointerstitial cells in treated animals more than untreated animals and this agree with **(Masoud *et al.*, 2012)**

Conclusion

Our results demonstrate the effects of mesenchymal stem cells on enhanced recovery of renal function in renal ischemia and provide the basis for a new therapeutic concept for the treatment of ARF.

Acknowledgments

The authors are grateful to Dr/ Mohammed Abd-Elhameed, Urology and Nephrology Center, Mansoura University, Mansoura, Egypt, for his great experience in histopathology in this study.

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