RESEARCH PAPER	Biochemistry	Volume : 4 Issue : 4 Apr 2014 ISSN - 2249-555X			
Store Ranifed Reverse	Evaluation of Renal Tubulointerstitial Injury after Mesenchymal Stem Cells Treatment.				
KEYWORDS	Mesenchymal stem cell; obstruction; epithelial-mesenchymal transition; fibrosis; kidney				
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ABSTRACT Purpose - Mesenchymal stem cells (MSCs) hold apromise for the treatment of renal disease, While MSCs have been shown to accelerate renal recovery and prevent acute renal failure in multiple disease models, the effect					

been shown to accelerate real recovery and prevent acute renal failure in multiple disease models, the effect of MSC therapy on chronic obstruction-induced renal fibrosis has not previously been evaluated. Materials and Methods -60 C57BI/6 male mice underwent injection of bone marrow-derived stem cells (MSCs) immediately prior to sham operation or induction of left ureteral obstruction (UUO). One or 2 weeks later, the kidneys were harvested, fixed in 10% buffered formalin, and embedded in paraffin for morphological studies. Serum creatinine, Blood urea nitrogen (BUN) and uric acid were measured in all mice involved in this study. Results - There was a significant decrease in serum creatinine, Blood urea nitrogen (BUN) and uric acid in all MSC groups compared to all control groups (p < 0.001). Kidney specimens obtained from mice treated with MSC before operation showed regeneration of the renal tubular cells, less tubular atrophy, very mild interstitial fibrosis and normal blood vessels. While kidney specimens obtained from mice treated with MSC (1Week)and (2 Weeks) after induction of UUO showed mild shrinkage of vascular tuft with normal basement membrane and cellularity, marked tubular atrophy with cast formation, mild interstitial fibrosis and normal blood vessels. Conclusions - Bone marrowderived MSCs provide protection against renal tubulointerstitial injury induced by ureteral obstruction.

Introduction

Inflammation of the tubulointerstitial compartment, leading to fibrosis, is a major factor in the progressive loss of renal function in patients with a wide variety of kidney diseases. About 80% of total kidney volume is composed of tubular epithelial cells and cells within the interstitial space. Most of the non-epithelial cells are associated with the rich vascular network of the kidney. There are also a small number of resident mononuclear cells and fibroblasts (Manucha & valles, 2008).

It is widely recognized that progressive renal disease is accompanied by tubulointerstitial changes characterized by tubular atrophy, increased number of interstitial fibroblasts, phenotypic change of interstitial cells, accumulation of matrix proteins, and interstitial infiltrate of mononuclear cells. Deterioration of renal function is determined to a large extent by the degree of tubulointerstitial changes rather than by the extent of histologic changes in the glomeruli in many forms of glomerulonephritis (Strutz, 2009).

However, the pathogenic mechanisms of tubulointerstitial changes have not yet been elucidated fully. Common pathogenic mechanisms exist in the pathogenesis of tubulointerstitial changes. However, there is little detailed description of the molecular mechanism of renal fibrosis, and moreover, an effective treatment procedure has not been established (Gao, Aqie et al., 2014).

Ureteric obstruction causes impedance to the flow of urine in the ureter. The obstruction can be partial or complete, unilateral, or bilateral. The etiology is variable and wide ranging. Classification is according to cause, duration and degree. Unilateral ureteral obstruction (UUO) induces after a few hours cellular infiltration in the tubulointerstitium.

These infiltrating cells (mainly macrophages) secrete growth factors and cytokines inducing disequilibrium between apoptosis and proliferation of tubular cells, as well as inducing fibroblast activation and proliferation. Fibroblasts infiltrate from the circulation into the interstitium, appear by epithelial-mesenchymal transition (EMT) or appear by proliferation of the few resident fibroblasts (Bascands & Schanstra, 2005).

Activated fibroblasts secrete the extracellular matrix (ECM) that is starting to accumulate into the interstitium as soon as myofibroblasts appear. As the obstruction continuous, ECM deposition becomes massive and uncontrolled apoptosis of tubular cells results in tubular atrophy (Bascands & Schanstra, 2005).

Some of the most promising and frequent research in the field of regenerative medicine has focused on the use of stem cells. These cells, by definition, are undifferentiated cells with significant self-renewal capabilities. Additionally, stem cells are able to proliferate and establish daughter cell lines for tissue generation. This reparative or regenerative medicine is currently used in the care of hematological and neoplastic diseases, but promising results have been obtained in the care of other diseases involving heart, arteries, liver and brain (Richichi, Brescia et al., 2013).

Today, multipotent bone marrows stem cells, which are the precursors of various types of blood cells, are routinely used. The bone marrow is the source of mesenchymal stem cells (MSC) from which many tissues may be obtained. The ability of adult MSC to "transdifferentiate" could revolutionize regenerative medicine. (Al-Nbaheen, Vishnubalaji et al., 2013). (MSC are of great interest to both clinicians and researchers for their great potential to enhance tissue engineering. Their ease of isolation, manipulability and potential for differentiation are specifically what has made them so attractive. These multipotent cells have been found to differentiate into cartilage, bone, fat, muscle, tendon, skin, hematopoietic-supporting stroma and neural tissue. Their diverse in vivo distribution includes bone marrow, adipose, periosteum, synovial membrane, skeletal muscle, dermis, pericytes, blood, trabecular bone, human umbilical cord, lung, dental pulp and periodontal ligament (Lotfinegad, Shamsasenjan et al., 2014).

Materials and Methods Experimental animals

A total number of 60 C57Bl/6 male mice, 12-14 weeks old and weighing 25-30 gm were used in the experimental investigation of this study. Mice were obtained from the Research Institutes of Ophthalmology, Giza, Egypt. Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libtium through specific nipple. Mice were kept at a constant environmental and nutritional condition throughout the period of the experiment.

Methods of obtaining bone marrow (BM) specimens for isolation and culture of bone marrow mesenchymal stem cells (BM-MSCs) (Mcfarlin et al., 2006):

- The animals were killed by cervical dislocation, then the skin was sterilized with 70% ethyl alcohol before cutting the skin.
- The femurs and tibia were carefully dissected from adherent soft tissues then they were placed into sterilized beaker containing 70% ethyl alcohol for 1-2 min.
- The bones were put in Petri dish contain PBS for wash.
- The bones were taken to laminar air flow to extract the bone marrow, the two ends of the bones were removed using sterile scissors.
- Bones were flushed with 3-5ml of complete media from one end, the marrow plugs were expelled from the opposite end of bone into sterile 15ml tube.
- The marrow plugs were cultured in 20 ml complete media.

Culturing of bone marrow (Mcfarlin et. al., 2006):

– The cells were cultured in 75cm² tissue culture flask containing 10-15 ml complete media in humidified incubator at 37°C in 5% CO₂ and 95% air (by volume).

- The cultured cells were examined daily using the inverted microscope to follow up the growth of the cells.
- After 24h the old media were removed by aspiration using sterile pipette, the cells were then washed with 5ml PBS, then 15ml complete media was added to the flask, MSCs were distinguished from other bone marrow cells by their ability to adhere to tissue culture polystyrene flask.
- The second exchange for media was done after 3-4 days.
- The cells take 4 weeks to be confluent and be ready for Passaging.
- Passaging was done for the cells till passage 3 were we had a suitable number of cells.
- The media changed twice a week.

Counting cells

Stem cells were resuspensed in 1 ml of appropriate media then from this cell suspension, $10 \,\mu$ l was removed for counting depending on the (using a microscope) cell number, a dilution factor between two and ten was used to count cells, test the cell viability $10 \,\mu$ l of cells was add to $10 \,\mu$ l of Trypan blue 0.4% (lonza, USA) and mix them well and take $10 \,\mu$ l of the mixture and put it on hemocytometer (Neubauer, Germany) and count cell under Ordinary microscope (Olympus CX31, USA). Then use this equation. NO of cells / ml. = average of count cells x dilution factor x 104 (Takahashi, Tanabe et al., 2007).

Study design

Mice were subdivided into 6 main groups (10 each) as follow;

- Group I (Sham group): mice subjected to sham operation without left ureteral obstruction (UUO).
- Group II (+ve control 1 Week): mice were subjected to left ureteral obstruction (UUO) without Mesenchymal stem cells (MSCS).
- Group III (+ve control 2 Weeks): mice were subjected to left ureteral obstruction (UUO) and treated without Mesenchymal stem cells (MSCS).
- Group IV (UUO+ MSCS) before Operation: mice were subjected to left ureteral obstruction (UUO) and treated

with Mesenchymal stem cells (MSCS) just before the UUO operation.

- Group V (UUO+ MSCS) 1 Week after Operation: mice were subjected to left ureteral obstruction (UUO) and treated with (MSCS) 1 week after the UUO operation.
- Group VI (UUO+ MSCS) 2 Weeks after Operation: mice were subjected to left ureteral obstruction (UUO) and treated with (MSCS) 2 week after the UUO operation.

Mesenchymal stem cell dose injection

Mesenchymal stem cells were injected in mice through caudal veins 1×106 for each mouse according to the protocol. Mice were killed under pentobarbital anesthesia 14 days after (MSCS) treatment (Chen and Xiang et al., 2010).

Experimental model (UUO):

Unilateral ureteral obstruction (UUO) was done as follow: with the mice under pentobarbital anesthesia (12 mg/100 gm Body Weight), then the abdomen was entered through midline laparotomy and the left ureter was ligated with 4-0 silk at two locations and cut between the ligatures to prevent retrograde urinary tract infection at the ureteropelvic junction. The abdominal incision was sutured by 4/0 silk sutures (Satoh et al., 2001).

Sham operation

Sham operation was done as follow: with the mice under pentobarbital anesthesia (12 mg/100 gm Body Weight), then the abdomen was entered through midline laparotomy and the left ureter was manipulated then the abdominal incision was sutured by 4/0 silk sutures (Satoh et al., 2001).

Investigations Provided to Measure Renal Injury:

Mice Sacrifice and Kidney Removal: Mice will be sacrificed to evaluate the severity of injury in each kidney, at the end point all mice will be sacrificed under anesthesia induced with phentobarbital sodium injection (50 mg/kg body weight intraperitoneal) then Kidneys will be removed, cut transversely and will be fixed in 10% buffered formalin, and embedded in paraffin for morphological study (Yamagishi H et al., 2001).

Biochemical examination in blood: Serum creatinin, BUN and uric acid will be measured in all mice involved in this study according to (Murray, 1984; Tietz et al., 1995; Schultz, 1984).

Results

A) Laboratory kidney Function Tests

The present study involved 6 groups and the results of studied renal functions parameters of these groups were analyzed and presented in tables (1-3) and in figures (1-3).

Serum creatinine in all studied groups:

The sham group showed no significant change in serum creatinine at the different periods (day 0, 1 and 14) of follow up. While, the control groups (1week and 2 weeks) showed significant increase in serum creatinine at days 1 and 14 compared to day 0 (p < 0.001). Also, they showed significant increase in serum creatinine at day 14 compared to day 1 (p < 0.001). Moreover, all groups of UUO+ MSC showed significant increase in serum creatinine at days 1 and 14 compared to day 0 (p < 0.001) except UUO+ before operation and UUO+ MSC (1Week) groups the increase in serum creatinine was non-significant at day 14. Compared to day 1 serum creatinine in all groups of MSC was significantly decreased at day 14 (p < 0.001).

At day 0, serum creatinine levels were comparable in all studied groups (sham, control and UUO + MSC). Compared to sham group, serum creatinine was significantly increased in all control groups and MSC groups at day 1 (p < 0.001). However, MSC before operation group showed significant decrease in serum creatinine compared to other MSC and all control groups (p < 0.001). Compared to sham group, all groups showed significant increase in serum creatinine in all control and MSC groups except MSC before operation and 1 Week at day 14 (p < 0.001). Also, there was a significant decrease in serum creatinine in all MSC groups compared to all control groups (p < 0.001). Moreover, MSC (before operation and 1 Week) groups showed significant

Decrease in serum creatinine compared to MSC (2 Weeks) at day 14 (p < 0.001).

• Serum uric acid in all studied groups:

The sham group showed no significant change in serum uric acid at the different periods (day 0, 1 and 14) of follow up. While, the control groups (1week and 2 weeks) showed significant increase in serum uric acid at days 1 and 14 compared to day 0 (p < 0.001). Also, they showed significant increase in serum uric acid at day 14 compared to day 1 (p < 0.001). Moreover, all groups of UUUO+ MSC showed significant increase in serum BUN at days 1 and 14 compared to day 0 (p \leq 0.005) except UUO+ before operation and UUO+ MSC (1Week) groups the increase in serum uric acid in all groups of MSC was significantly decreased at day 14 (p < 0.001) except MSC before operation.

At day 0, serum uric acid levels were comparable in all studied groups (sham, control and UUO + MSC). Compared to sham group, serum uric acid was significantly increased in all control groups and MSC groups at days 1 and 14 ($p \le 0.002$) except MSC group before operation at day 14. However, MSC before operation group showed significant decrease in serum uric acid compared to other MSC and all control groups (p < 0.001). Compared to sham group, all groups showed significant increase in serum uric acid in all control and MSC groups except MSC before operation and 1 W at day 14 (p < 0.001). Also, there was a significant decrease in serum uric acid in all MSC groups (p < 0.001). Moreover, MSC (before operation and 1 W at Week) groups showed significant

decrease in serum uric acid compared to MSC (2 Weeks) at day 14 (p < 0.001).

• Serum blood urea nitrogen (BUN) in all studied groups: The sham group showed no significant change in serum BUN at the different periods (day 0, 1 and 14) of follow up. While, the control groups (1week and 2 weeks) showed significant increase in serum BUN at days 1 and 14 compared to day 0 (p < 0.001). Also, they showed significant increase in serum BUN at day 1 (p < 0.001). Moreover, all groups of UUO+ MSC showed significant increase in serum BUN at days 1 and 14 compared to day 0 (p < 0.001). Compared to day 1 serum BUN in all groups of MSC was significant judgereased at day 14 (p < 0.001) except MSC group before operation which showed non-significant decrease in BUN.

At day 0, serum BUN levels were comparable in all studied groups (sham, control and UUO + MSC). Compared to sham group, serum BUN was significantly increased in all control groups and MSC groups at day 1 (p < 0.001). However, MSC before operation group showed significant decrease in serum BUN compared to other MSC and all control groups (p < 0.001). Compared to sham group, all groups showed significant increase in serum BUN in all control and MSC groups at day 14 (p < 0.001). Also, there was a significant decrease in serum BUN in all MSC groups compared to all control groups (p < 0.001). Moreover, MSC (before operation and 1 Week) groups showed significant decrease in serum BUN is all more serum BUN in all Control groups (p < 0.001). Moreover, MSC (before operation and 1 Week) groups showed significant decrease in serum BUN compared to MSC (2 Weeks) at day 14 (p < 0.001).

B) Results of histopathological examination

At day 14, the kidney specimens obtained from mice in sham (negative control) group showed normal kidney architecture without any abnormality in renal tubules and glomeruli (fig.4a and 4b). Kidney specimens obtained from mice in positive control groups treated with saline 1 and 2 weeks after induction of UUO showed marked disturbed kidney architecture in the form of shrinkage of vascular tuft, thrombosis of glomerular capillaries, disrupted glomerular basement membrane (glomerular necrosis), marked tubular atrophy with tubular necrosis, interstitial haemorhage, fibrosis and inflammation with normal blood vessels. Fig.5a and b are representive samples from positive control group (1Week), while Fig.6a and b are representive samples from positive control group (2Weeks).

Kidney specimens obtained from mice treated with MSC before operation showed regeneration of the renal tubular cells, less tubular atrophy, very mild interstitial fibrosis and normal blood vessels (fig. 7). While kidney specimens obtained from mice treated with MSC (1Week)and (2 Weeks) after induction of UUO showed mild shrinkage of vascular tuft with normal basement membrane and cellularity, marked tubular atrophy with cast formation, mild interstitial fibrosis and normal blood vessels. Fig.8 is a representive sample from MSC group (1Week), while Fig.9 is a representive sample from MSC group (2Weeks).

Discussion

End-stage renal disease is one of the most prevalent complications of hypertension, diabetes, and intrinsic renal diseases (Frolich, 2001). Obstructive nephropathy is a major cause of renal failure and end-stage renal disease in adults and children, it is characterized by the progressive accumulation of extracellular matrix (ECM) in the glomeruli (glomerulosclerosis) and between tubules (tubulo-interstitial fibrosis), many studies suggest that it is the severity of tubulo-interstitial fibrosis that best correlates with the degree of renal impairment and the risk for renal failure progression (Klein and Gonzalez et al., 2011).

Interstitial fibrosis is a complex pathophysiological process involving inflammatory cell infiltration, fibroblast proliferation, and an imbalance in extracellular matrix (ECM) synthesis and degradation (Pulskens, Butter et al., 2014). Activated fibroblasts are the principal effector cells responsible for extracellular matrix deposition and the development of tubulointersitital fibrosis, and growing evidence suggests that growth factors, such as Transforming growth factor beta 1 (TGF- β 1), can induce renal tubular epithelial cells to undergo phenotypic transformation into matrix producing myofibroblasts (EMT) under pathological conditions (Strutz et al., 1995; Zeisberg et al., 2001). Renal cortical TGF-β1 levels increase significantly in response to obstruction (Huang, Shen et al., 2014). And evidence indicates that TGF-β1 is a major regulator of fibrosis via stimulation of EMT, fibroblast proliferation (Rastaldi et al., 2006; Roberts et al., 1992).and extracellular matrix synthesis (Eddy, 1996; Miyajima et al., 2000; Roberts et al., 1992).

Bone marrow-derived stem cells (BMSC) such as haematopoietic stem cells (HSC) possess a higher degree of plasticity than previously recognized, indicated by the fact that they contribute to the restoration of injured peripheral tissue (Grove et al., 2004; Lakshmipathy and Verfaillie, 2005). The supposed mechanisms underlying this regenerative response are that BMSC transdifferentiate into or fuse with the principal cells of the injured tissue (Rodic et al., 2004; Leri et al., 2005). Although there are also indications that a more paracrine fashion of support may arise by providing relevant growth factors (Togel et al., 2005; Hess et al., 2003). Among the non-haematopoietic tissues targeted by BMSC also the kidney can be found (Lin et al., 2003; Kale et al., 2003). Moreover, BM-derived cells have the capability to migrate through the glomerular basement membrane and can thus end up in the luminary space of Bowman' capsule, thereby crossing the obstacle set up by the basement membrane. So, in the present study we investigated the effect of therapy with BM derived stem cells on the regenerative process in a mice model of UUO. (Sugimoto et al., 2006).

Complete ureteric obstruction is characterized by an interstitial infiltration of mononuclear cells, release of cytokines, fibroblast activation, tubular proliferation, death and atrophy, and imbalance of extracellular matrix synthesis, and degradation (Hewitson et al., 2010). Also, UUO is associated with progressive renal fibrosis and scarring and a decline in renal function. In the present study, the obstructed kidney demonstrated significant increase in renal function parameters as evidenced by significant increase in serum creatinine (table 1 and fig.1), BUN (table 2 and fig.2) and uric acid (table 3 and fig.3) in control groups treated with saline 1 week and 2 weeks, at one and 14 days after induction of unilateral ureteral obstruction.

The deterioration in renal function became more marked at day 14 after UUO. These findings might be due to kidney scarring and renal interstitial fibrosis and glomerular damage.

Inflammatory cell infiltration occurs in renal interstitium shortly after ureteral obstruction, releasing cytokines and Transforming growth factor (TGF), including relatively well- known TGF- β 1 and Tumor necrosis factor alpha (TNF- α), which promote extracellular matrix synthesis and proliferation of fibroblast (Chevalier et al., 2009). Inconsistence with this, in the present study, kidney specimens obtained from mice exposed to UUO and treated with saline only showed marked shrinkage of vascular tuft, thrombosis of glomerular capillaries, disrupted glomerular basement membrane, marked tubular atrophy with tubular necrosis, interstitial haemorhage, fibrosis and inflammation.

Collagen accumulation in fibrosis is a balance between synthesis and degradation; with most collagen degradation in the kidney controlled by MMPs. Collectively they are capable of degrading all extracellular matrix (ECM) proteins, with their ability to remodel collagen an important counterbalance to synthesis (Ronco et al., 2007). The role of Matrix metalloproteinases (MMPs) may therefore vary from one model or disease to another and may change temporally (Ronco et al., 2007; Ho et al., 2007; Kapila, 2003). Regardless, there are few in vivo data about MMP activity over time, and even fewer studies have accounted for the activity of their inhibitors, the TIMPs. The observed up-regulation of MMP-2 at d 9 after UUO is most likely a compensatory response, with the increase in MMP-2 potentially being a reaction to increased TGF- (Wick et al., 2001). and/or collagen (Olaso et al., 2001). production. Nevertheless, this seems to have been overwhelmed by the rapid fibrogenesis.

However, it was noted that degradation of basement membranes may also promote epithelial-mesenchymal transition in kidney disease (Hewitson et al., 2007).

Extensive studies on MSC therapy in various acute and chronic renal diseases, mostly with a rodent animal model and different degrees of therapeutic effects, could be found at present so, the main of this study was to investigate the effect of treatment with stem cells before and 1 week and 2 weeks after induction of unilateral ureteral obstruction. We found in the present study highly significant improvement in markers of renal function (serum creatinine, BUN, uric acid) in mice exposed to UUO and treated with MSC before induction of obstruction. Also, mice exposed to UUO and treated with MSC 1 week and 2 weeks after induction of UUO showed significant reduction in the serum levels of creatinine, BUN and uric acid. However, the effect of treatment before induction of UUO was marked than treatment after induction by 1 Week and 2 Weeks. These findings are in agreement with several studies demonstrated that the administration of bone marrow-derived MSC may protect or reverse both acute kidney injury and chronic kidney injury, as well as in other experimental models (Striker, 2011; Alexandre et al., 2009; Lindoso et al., 2011; Ninichuk et al., 2006; Perico et al., 2011; . Tögel and Westenfelder, 2010; Tögel et al., 2009; Herrera et al., 2007; Behr et al., 2009; Zhi-ming et al., 2013).

Demonstration in a mice model of unilateral ureteral obstruction (UUO) that MSCs had intensified signals in left kidney region on the 3rd day after administration of it. (Zhi-ming et al., 2013).

This was caused by proliferation of MSCs in renal microvasculature and those migrated to interstitium and renal Between 4–10 days after transplantation of fluorescently labeled MSCs, around 20%–50% of glomerulus were found fuorescence positive, indicating that MSCs preferentially home to sites of tissue injury, which was consistent with our experimental results, fluorescence signal declined from Day 7 and disappeared on the Day 14, resulting from MSC apoptosis due to microenvironment changes on the one hand; and on the other hand, progressive atrophy occurred even after obstruction relief, thus the distance between kidney and body surface increased and parts of signals were absorbed in tissues and could not be detected consequently (Zhiming et al., 2013).

On the other hand, (Stokman et al., 2008). Demonstrated that effective stem cell mobilization does not alter renal fibrosis in a mice model of UUO.

The possible role for MSC in protection against renal injury in case of obstructive uropathy was previously investigated. This study investigated its effect on renal fibrosis and pathology. It was found in the present study that BM-derived cells contribute to restoration of renal function and tissue repair, but may also give rise to generation of non-functional tissue and fibrosis as evidenced by significant decrease in kidney hydroxyproline content especially when given before induction of obstructive uropathy. Renal fibrosis started by invasion of kidney tissues by myofibroblasts. Renal myofibroblasts are reported to be derived from different sources: from proliferating interstitial fibroblasts, from the transition of tubular epithelial cells (TEC) into myofibroblasts and from the bone marrow (Kalluri et al., 2003). Fibrocytes are circulating bloodborne cells displaying leukocyte surface markers and which produce extracellular matrix proteins.

Tubule region, which was consistent with opinions held by some researchers (Kunter et al., 2006).

Fibrocytes are involved in wound repair, upon TGFb1 exposure may express alpha-SMA (Quan et al., 2004). Are thought to be important in mediating pulmonary fibrosis (Phillips et al., 2004).

And recently have been implicated in renal fibrosis (Sakai et al., 2006).

(Stokman et al., 2008). found that the total number of alpha-SMA expressing cells after UUO did not differ between both treatments groups, suggesting that if fibrocytes are the potential source of bone marrow-derived myofibroblasts in the kidney, their contribution to fibrosis was not altered by cytokine induced mobilization in our study.

In accordance, collagen type I deposition by BM derived (myo) fibroblasts in UUO injury was found to be insignificant compared to that of fibroblasts of renal origin (Roufosse et al., 2006).

Table (1): Effect of mesenchymal stem cell therapy on serum creatinine (mg/dl) in mice model of unilateral ureteral obstruction.

	Daya		
Sham group	0.58 ± 0.034		
Control group (1 W)	0.59± 0.039		
Custrol group (2 W)	0.58 ± 0.038		
UUO + MSC (Before	0.57± 0.056		

All data are expressed as Mean \pm SD. UUO= unilateral ureteral obstruction, MSC = mesenchymal stem cells. One way ANOVA test with posthoc Tukey's test. a significant vs day 0 of the same group, b significant vs day 1 of the same group, c significant vs sham group, d significant vs control group (1 W), e significant vs control group (2 W), f significant vs UUO (before OP), g significant vs UUO (1 W) (p \leq 0.05).



Fig. (1): Effects of bone marrow-derived stem cell therapy on serum creatinine (mg/dl). UUO= unilateral ureteral obstruction, MSC = mesenchymal stem cells. a significant vs day 0 of the same group, b significant vs day 1 of the same group, c significant vs sham group, d significant vs control group (1 W), e significant vs control group (2 W), f significant vs UUO (before OP), g significant vs UUO (1 W) ($p \le 0.05$).

Table (2): Effect of mesenchymal stem cell therapy on serum blood urea nitrogen (BUN) (mg/dl) in mice model of unilateral ureteral obstruction.

	Day 0	Day 1	Day 14
Sham group	15.03 ± 3.22	15.06 ± 3.31	14.81± 3.30
Control group (1 W)	13.98± 3.46	59.63± 3.44	73.93± 2.91
		ac	abc
Control group (2 W)	13.51 ± 3.01	59.97± 3.99	83.80 ± 2.59
		ac	abed
UUO + MSC (Before	14.59± 2.72	$27.30\ \pm 0.90$	23.05 ± 0.93
OP)		acde	acde
UUO + MSC (1W)	14.36± 2.46	61.88± 3.78	33.88 ± 2.63
		acf	abcdef
UUO + MSC (2 W)	13.55 ± 2.51	61.82± 2.96	42.68± 3.30
		acf	abcdefg

All data are expressed as Mean \pm SD. UUO= unilateral ureteral obstruction, MSC = mesenchymal stem cells. One way ANOVA test with posthoc Tukey's test. a significant vs day 0 of the same group, b significant vs day 1 of the same group, c significant vs sham group, d significant vs control group (1 W), e significant vs control group (2 W),), f significant vs UUO (before OP), g significant vs UUO (1 W) (p \leq 0.05).

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Fig. (2): Effects of bone marrow-derived stem cell therapy on serum BUN (mg/dl). UUO= unilateral ureteral obstruction, MSC = mesenchymal stem cells. One way ANOVA test with posthoc Tukey's test. a significant vs day 0 of the same group, b significant vs day 1 of the same group, c significant vs sham group, d significant vs control group (1 W), e significant vs control group (2 W), f significant vs UUO (before OP), g significant vs UUO (1 W) ($p \le 0.05$).

Table (3): Effect of mesenchymal stem cell therapy on serum uric acid (mg/dl) in unilateral ureteral obstruction mice model

	Day 0	Day 1	Day 14
Sham group	1.50 ± 0.116	1.50 ± 0.082	1.50± 0.094
Control group (1 W)	1.49 ± 0.145	$3.47 \pm$	4.74 ± 0.350
		0.481 ac	abc
Control group (2 W)	1.48 ± 0.187	$3.65 \pm$	6.72 ± 0.282
		0.344 ac	abcd
UUO + SC (Before OP)	1.53 ± 0.095	2.06 ±	1.81 ± 0.099
		0.127 acd	abde
UUO + SC (1W)	1.55 ± 0.158	3.69± 0.307	2.36 ± 0.284
		acf	abcde
UUO + SC (2 W)	1.57 ± 0.134	3.65 ±	2.85± 0.579
		0.430 acf	abcdefg

All data are expressed as Mean \pm SD. One way ANOVA test with posthoc Tukey's test. UUO= unilateral ureteral obstruction, MSC = mesenchymal stem cells. One way ANOVA test with posthoc Tukey's test. a significant vs day 0 of the same group, b significant vs day 1 of the same group, c significant vs sham group, d significant vs control group (1 W), e significant vs control group (2 W), f significant vs UUO (before OP), g significant vs UUO (1 W) (p< 0.05).



Fig. (3): Effects of bone marrow-derived stem cell therapy on serum uric acid (mg/dl). UUO= unilateral ureteral obstruction, MSC = mesenchymal stem cells. One way ANO-VA test with posthoc Tukey's test. a significant vs day 0 of the same group, b significant vs day 1 of the same group, c significant vs sham group, d significant vs control group (1 W), e significant vs control group (2 W), f significant vs UUO (before OP), g significant vs UUO (1 W) ($p \le 0.05$).



Fig. (4a): kidney specimens with normal architecture (normal glomeruli and renal tubules (sham group) (magnification 100x).



Fig. (4b): kidney specimens with normal architecture (normal glomeruli and renal tubules (sham group) (magnification 100x).



Fig. (5a): kidney specimens showing shrinkage of vascular tuft with thrombosis of glomerular capillaries, marked tubular atrophy with tubular necrosis, interstitial haemorhage and normal blood vessels. Control group (1 Week) Magnification a= 100x.



Fig. (5b): kidney specimens showing shrinkage of vascular tuft with thrombosis of glomerular capillaries,



Fig. (6a): kidney specimens showing marked thrombosis in glomerular capillaries with disrupted glomerular basement membrane, Leukocytic infiltration (glomerular necrosis), marked tubular necrosis, interstitial haemorhage and inflammation and normal blood vessels. Control group (2 W) Magnification a= 100x.



Fig. (6b): kidney specimens showing marked thrombosis in glomerular capillaries with disrupted glomerular basement membrane, Leukocytic infiltration (glomerular necrosis), marked tubular necrosis, interstitial haemorhage and inflammation and normal blood vessels. Control group (2 W) Magnification b= 400x.



Fig. (7): kidney specimens showing regeneration of the lumen, less tubular atrophy, very mild interstitial fibrosis and normal blood vessels. MSC group (before OP) Magnification a= 100x.



Fig. (8): kidney specimens showing normal regarding messangium and vascular tuft, mild interstitial fibrosis and normal blood vessels. MSC group (1W) Magnification a= 100x



Fig. (9): kidney specimens showing showed mild shrinkage of vascular tuft with normal basement membrane and cellularity, marked tubular atrophy with cast formation, mild interstitial fibrosis and normal blood vessels. MSC group (2W) Magnification a= 100x.

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