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Keywords
Mesenchymal stem cells, VEGF, Anti-inflammatory, TXNIP, Acute kidney injury, Anti-apoptotic, ASK-1

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Introduction
Background: Acute kidney injury (AKI) is a diverse set of illnesses characterized by a rapid decline in kidney function

Material and methods
Characteristics and homing of MSCs to kidney tissues were identified by flow cytometry and differentiation capability. After AKI induction by cisplatin injection in sixteen albino rats, the AKI rats were further subdivided into three subgroups. The first subgroup served as a positive control and the second one received 2 mg/kg furosemide (FUR) which served as a standard drug. The third subgroup received a single dose of 5 x 106 MSCs via tail vein injection once a week for consecutive two weeks. AKI-related biochemical parameters were assayed at 2 weeks after MSC treatment. Kidney histological changes were also evaluated. Moreover, the apoptosis of kidney cells and expression of apoptosis-related proteins were assessed by western blot.

Results
Compared with AKI rats, rats treated with MSCs showed suppressed serum levels of creatinine and blood urea nitrogen. MSC treatment alleviated the pathological abnormalities in the kidneys of AKI rats as shown by H&E staining.Furthermore, MSC treatment suppressed apoptosis of kidney cells in AKI rats via downregulation of apoptotic proteins; thioredoxin-interacting protein (TXNIP) and apoptosis signal-regulating kinase 1 (ASK1). Most importantly, MSC treatment promoted the expression of vascular endothelial growth factor (VEGF) in the kidneys of AKI rats.

Conclusions
Our results suggest that MSCs could ameliorate renal injury of AKI rats via their antiapoptotic properties. Also, the protective effects of MSCs may be mediated by their angiogenic potential effects.

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Abstract

Background: Acute kidney injury (AKI) is a diverse set of illnesses characterized by a rapid decline in kidney function However, kidney transplantation and supportive therapies still have many limitations in the alleviation of progression of kidney diseases. The effective role of MSCs in cell-based therapy and endogenous repair for AKI is still under investigation. Several studies indicated that MSCs could both repair and ameliorate kidney injury due to its anti-inflammatory and anti-apoptotic potential effects. The aim of this study is to evaluate the effects of mesenchymal stem cells (MSCs) on renal cell apoptosis in cisplatin-induced AKI rats and examine the underlying molecular mechanisms

Methods: Characteristics and homing of MSCs to kidney tissues were identified by flow cytometry and differentiation capability. After AKI induction by cisplatin injection in sixteen albino rats, the AKI rats were further subdivided into three subgroups. The first subgroup served as a positive control and the second one received 2 mg/kg furosemide (FUR) which served as a standard drug. The third subgroup received a single dose of 5 x 10^6 MSCs via tail vein injection once a week for consecutive two weeks. AKI-related biochemical parameters were assayed at 2 weeks after MSC treatment. Kidney histological changes were also evaluated. Moreover, the apoptosis of kidney cells and expression of apoptosis-related proteins were assessed by western blot.

Results: Compared with AKI rats, rats treated with MSCs showed suppressed serum levels of creatinine and blood urea nitrogen. MSC treatment alleviated the pathological abnormalities in the kidneys of AKI rats as shown by H&E staining. Furthermore, MSC treatment suppressed apoptosis of kidney cells in AKI rats via downregulation of apoptotic proteins; thioredoxin-interacting protein (TXNIP) and apoptosis signal-regulating kinase 1 (ASK1). Most importantly, MSC treatment promoted the expression of vascular endothelial growth factor (VEGF) in the kidneys of AKI rats.

Conclusion: Our results suggest that MSCs could ameliorate renal injury of AKI rats via their anti-apoptotic properties. Also, the protective effects of MSCs may be mediated by their angiogenic potential effects.

Keywords; Acute kidney injury; Mesenchymal stem cells; Anti-inflammatory; Anti-apoptotic; ASK-1; TXNIP; VEGF.

1 Introduction

Acute kidney injury (AKI) is a serious complicated disease that leads to a sudden reduction in glomerular filtration and deterioration in kidney function (Nagata *et al.* 2021). These disorders can disrupt metabolic, electrolyte, and fluid homeostasis over hours to several days. The regenerating capability of the mammalian kidney is low, which is a severe problem since kidney disease is currently a major worldwide health concern. Moreover, AKI is caused by a range of factors such as ischemic damage from sepsis, surgery, infections, toxic chemicals and trauma (Zhuang *et al.* 2021).

Early diagnosis of AKI cases is difficult causing irreversible renal damage in patients, which is treated with current ways of treatment such as dialysis or transplantation (Camarata *et al.* 2016). This limited regenerative capability of the mammalian kidney makes AKI a global public health concern and it is associated with mortality and morbidity (Chany *et al.* 2021). Despite recent understanding of the underlying pathophysiology, clinical diagnosis, and discovery of new renal biomarkers, there is currently no specific pharmaceutical treatment for AKI, and the focus has shifted to regenerative medicine as a novel therapeutic approach (Gonsalez *et al.* 2019).

The pathogenesis process of AKI includes both local impacts on the whole body and the kidney *(Makris and Spanou 2016).* Consequently, the administration of a diuretic drug like furosemide (FUR) is a widely used therapy in the hypertension management of AKI patients (Zhao *et al.* 2020). Additionally, FUR can increase Na excretion by 20%, which reduces the amount of extracellular fluid, minimizes concomitant conditions or complications, and declines kidney function (Chen *et al.* 2021). However, the expected efficiency of treatment was still not achieved due to the toxic effects of drug therapy, the inconvenience of the dialysis process, and the high cost of donors for kidney transplantation. Therefore, there new therapeutic strategies are urgently needed to suppress kidney disease progression. Recent research has focused on the discovery of new therapeutic tools to enhance the regeneration abilities of the kidney after injury caused by AKI (Mohr *et al.* 2021).

Currently, the promising treatment strategy for many disorders is cell-based replacement therapy. MSCs, as one of the essential members of the stem cell family, can be obtained from many tissues such as bone marrow, peripheral blood, adipose, and umbilical cord. MSCs have potential biological properties of anti-inflammation, immunomodulation, and tissue repair. Clinical and Preclinical studies have shown that MSCs exhibit protective and reparative effects

on renal injury (Boncon *et al.* 2019). Additionally, MSCs possess anti-apoptotic, antioxidant, anti-apoptotic, anti-inflammatory, and immunomodulatory properties via secretion of delivering extracellular vesicles (EVs) (Wang *et al.* 2021). Overall, MSC treatment is considered to be the most promising stem cell therapy for kidney disease treatment (Cao *et al.* 2021). Previous studies have indicated that MSCs are safe and effective when used for the treatment of organ injury (Monzel *et al.* 2014) (Oliva *et al.* 2019).MSCs can perform their functions by producing hepatocyte growth factor (HGF), C-C motif chemokine ligand (CCL)-2, Vascular endothelial growth factor (VEGF) and CXCL-8. Among these markers, VEGF has been proven to suppress renal injury in several experimental studies. VEGF has also been indicated to reduce glomerulosclerosis via the suppression of transforming growth factor (TGF)-β in AKI rat models (Wang *et al.* 2022). In addition, MSCs have been shown to inhibit inflammation in kidney disease models through the down-regulation of IL-1β, IL-6, and TNF-α that was mediated by the regulation of the nuclear factor kappa (NF-κB) pathway. Cellular mechanisms of administered MSCs such as transdifferentiation or endocrine effects play a potential role in the regenerative capability of the injured tissues. The protective effects of MSCs occurs via suppression of cytokines secretion and increased expression of growth factors which include HGF, IGF-I and VEGF (Xu *et al.* 2020) which can effectively ameloriate the damage of the renal cells caused by AKI. Additionally, MSCs can attenuate apoptosis via modulating the thioredoxin-interacting protein (TXNIP) pathway which induces apoptosis of β-cells by activation of the death mitochondrial pathway through the activation of Apoptosis signal-regulating kinase 1 (ASK1) (Selim *et al.* 2019). ASK1 is bound usually to thioredoxin 1 (TRX1) under basal conditions. TRX1 is a redox protein that limits cell apoptosis and reactive oxygen species (ROS) from oxidative stress, while TXNIP could suppress the antioxidant functions of TRX (Junn *et al.* 2000). TXNIP is a nucleoprotein that could be upregulated by oxidative stress. Under renal damage conditions, TXNIP binds to the TRX1 protein and the ASK1-TRX1 complex is inhibited. The TXNIP-TRX1 complex could suppress the TRX1 protein's response to ROS, which results effectively in cell apoptosis and oxidative stress (Saxena *et al.* 2010). ASK1 is a member of the family of MAPK kinase (MAP3K), it is an activator of p38 MAPK and JNK signaling pathways (Zhao *et al.* 2020). ASK1 is activated in response to apoptotic stimuli such as proinflammatory cytokines including TNF-α and interleukins. Activation of ASK1 leads to several biological responses including apoptosis, differentiation, and inflammation in different types of cells (Kawai *et al.* 2006). Many studies

indicated that MSCs are involved in several molecular mechanisms including inflammatory response, angiogenesis, ECM remodeling, and apoptosis (Liu *et al.* 2012). Despite clinical trials with either allogeneic or autologous transplantation of MSCs indicating that no tumorigenicity or severe adverse reactions were found which proved that MSCs were effectively safe in the treatment of several diseases (Hu *et al.* 2013) but still clinical applications of MSCs treatment have other limitations including tissue sources and methods of isolation that can affect MSCs differentiation and proliferation. In the present study, we have used a cisplatin-induced rat AKI model to examine the potential protective effects of MSCs on the functional and histological injury of AKI and to find out whether the therapeutic effect of MSCs is mediated via their antiapoptotic properties as well as the underlying molecular mechanisms focusing on the characterization and homing of MSCs

2 Material and methods

2.1 Chemicals

Cisplatin (*cis*-Diammineplatinum (II) dichloride), FUR (4-Chloro-*N*-furfuryl-5-sulfamoyl anthranilic acid), and other chemicals were procured from Sigma Chemicals Company (St. Louis, MO, USA). All kits were purchased from the Egyptian American company, Giza, Egypt.

2.2 Husbandry of animals

Twenty-four male albino rats (*Rattus norvegicus*) with a body weight of 180–220 g were purchased from the Nile Center for Experimental Research, Mansoura, Egypt. All rats were kept in clean polypropylene plastic cages in our laboratory's animal house under hygienic conditions, temperature (23 ± 2 °C), and humidity (50-70%) with a 12-hour dark/light cycle. Before initiating any experiment, rats were maintained for seven days for adaptation, with unrestricted access to chew and water. The experiment was done according to the animal ethical regulations and approved by the MSA University, Ethics Committee (**code: PH8/EC8/2019F).**

2.3 Bone Marrow Mesenchymal Stem Cells isolation and culture

Whole bone marrow from albino rats' tibia and femurs (six-week-old males) was flushed, cultured in the Dulbecco's Modified Eagle's medium (DMEM) with the 10% fetal calf serum, 1% penicillin/streptomycin, and 2mmol/L L-glutamine, and purified for up to five passes.. After culture for 10 to 12 days at which the cell confluence reached 80% to 90%, then each flask was

added with 1 mL pancreatin which contains 0.25% EDTA for incubation of the 30s and removed. Then, all cells were digested with the pancreatin again at 37°C for 1 min, then added with 2 to 3 mL L-DMEM supplemented with 10% FBS. (Song *et al.* 2014). Cells were identified as being bone marrow MSC by their morphology, adherence, and CD surface markers (CD44, CD90, CD105, CD14, CD45, and CD34); using suitable markers by flow cytometric analysis.

2.4 Experimental Design and cisplatin-induced Acute Kidney Injury

2.4.1 Acute kidney injury induction in rat model

The study protocol was approved by the Ethics Committee of the Faculty of Pharmacy, MSA University, Ethics Committee (**code: PH8/EC8/2019F)** in accordance with the declaration of Helsinki. The sample size of this study was calculated by G power as follows; G power: 95 %, alpha: 0.05, effect size: 0.95, and our sample size is 6 rats per group. Acute kidney injury (AKI) was induced in rats by receiving 5 mg/kg b.wt of cisplatin by an intraperitoneal injection (Sigma-Aldrich, USA). To examine the therapeutic efficacy of MSCs in the AKI cisplatin-induced animal model, the twenty-four male albino rats were divided into the following four groups (n = 6): group (1): the control group, rats received a single dose of 0.5 mL saline by intraperitoneal injection; group (2): the AKI group, rats received a single dose of cisplatin (5 mg/kg b.wt) by intraperitoneal injection (Ashour *et al.* 2016); group (3): the FUR group, rats received a single dose of cisplatin by intraperitoneal injection and 2 mg/kg FUR twice per week for two weeks after the injection of cisplatin (Prieto-García *et al.* 2020). Group (4): the MSCs group, rats received a single dose of cisplatin by intraperitoneal injection and they were administered with MSCs (5 ×106 cells suspended in 0.5ml PBS /rat) by tail vein 24 hr after the injection of cisplatin (Yao *et al.* 2015).

2.4.2 Sample collection

All animals in our study were sacrificed after 2 months of administration of MSCs, all rats were fasted during the night before being slaughtered by diethyl ether anesthesia. Blood samples were drawn from the aorta vein and placed in dry centrifuge tubes before being spun for 5 minutes at 1200 x g to separate serum. The sera were immediately frozen at -80 ◦C for kidney function testing. After scarification, one kidney per rat was removed and rinsed with saline and then stored in 10% formalin to be evaluated histopathologically and immunohistopathologically, while the other kidney was frozen at − 80 °C for analysis of gene expression.

2.4.3 Characterization and homing of MSCs

MSCs at day 14 are characterized by their fusiform or spindle shape. MSCs are expressed by flow cytometric analysis CD29, CD73, and CD105 on their cell surface, and CD44 by immunohistochemistry.

2.4.4 Evaluation of kidney function tests:

The alterations in kidney function could be used to estimate the extent of kidney damage and the prognosis. Serum creatinine (Scr) and Blood urea nitrogen (BUN) can indicate the degree of impaired glomerular filtration function to a specific extent. These kidney function tests were assayed using the Urea/BUN-spectrum diagnostic kit and Creatinine-spectrum diagnostic kit.

2.4.5 The apoptotic factors; ASK-1 and TXNIP proteins expression assay by western blot

The ReadyPrepTM protein extraction kit (Catalog 163-2086) and Bradford Protein Assay Kit (Bio Basic Inc, Markham, Ontario, Canada, Cat# SK3041) were used for protein isolation and quantification, respectively. The blot findings are presented in autoradiographs by using Bio-Rad Image software.

2.4.6 Inflammatory mediators; interleukin-6 (IL-6), IL-1β, and tumor necrosis factor (TNF-α) genes expression assay by Real-time reverse transcription PCR (qRT-PCR)

Total RNA was first extracted using the RNeasy Mini Kit (catalog no. 74104, Qiagen, Germany). The cDNA was produced using the Revert Aid Reverse Transcriptase. The SYBR Green PCR kit (catalog no. 204141, Qiagen, Germany) was used to perform RT-PCR with a total reaction volume of 25 µl using primers for each gene as shown in Table 1. (Yuan *et al.* 2006).

2.4.7 Histopathological Assessment

Hematoxylin and eosin (H&E) stain was applied to 5µm sections of paraffin-embedded renal tissues for histological investigation (Downie 1990). Using anti-VEGF rabbit polyclonal Ab [Boster Biological Technology, Pleasanton, CA, USA, Cat.# PA1080]

2.4.8 Immunohistochemical Assessment of the angiogenic factor VEGF

The immunohistochemical examinations were carried out according to the technique of Gosselin et al. (1986). HRP with streptavidin was then added after the addition of the secondary antibody to produce the brown color. The area of positive expression was measured using the light microscope (Olympus Software).

2.4.9 Statistical analysis

Graphpad Prism software was used to organize, tabulate, and analyze the acquired data statistically. The mean and standard error of the mean (SEM) were calculated. A one-way analysis of variance (ANOVA) was used to determine differences between the studied groups with the significance level of $P < 0.05$.

3 Results

3.1 Characterization and homing of MSCs in kidney tissues

MSCs were characterized by their fusiform shape, fibroblast-like cells and adhesiveness. Flow cytometric assessment of cell surface markers exhibited positive surface expression of CD73, CD105, and CD29 (Fig.1). The renal sections of the MSC treated group showed a CD44-positive immunoreactive spindle-shaped cell showing intense brown-colored granules. The positive CD44 cells are located within the cells or in between the renal tubules (Fig.1).

3.2 Kidney function damage induced by cisplatin was repaired by MSCs

Treatment with MSCs showed a significant decrease in serum creatinine and BUN levels ($P < 0.05$) as compared to AKI rats or FUR-treated rats as illustrated in Fig. 2. This data suggested that MSCs can ameliorate kidney function damage induced by cisplatin in rats.

3.3 Cisplatin-induced kidney cell apoptosis was inhibited by MSCs.

To prove the mechanism by which MSCs treatment attenuated the apoptosis process in AKI-induced rat model via targeting the ASK-1 and TXNIP pathway. The kidney apoptosis was elevated in the AKI group by the elevation of expression levels of ASK-1 and TXNIP by 3 and 2 folds, respectively, compared to the control group. On the contrary, the apoptosis process of kidney cells in the MSCs group was significantly reduced compared to the AKI group as indicated in Fig 3. This reduction indicated that MSCs exhibited antiapoptotic properties via the paracrine signaling pathway under specific pathological conditions.

3.4 Cisplatin-induced inflammation in kidneys was reduced by MSCs

AKT group showed a highly significant ($P < 0.01$) elevation in the inflammatory mediators including TNF-α, IL-6, and IL-1β levels as compared to control rats. MSC treatment of AKI rats

showed a significant suppression in IL-6, IL-1β and TNF- α ($P < 0.01$) compared with both FUR and control groups showing the potential anti-inflammatory effects of MSCs (fig.4).

3.5 Histopathological observations

H&E stained kidney sections of the control group showed normal tubules and glomeruli with normal Bowman's capsule. The AKI group stained renal sections showed that necrotic renal tubules, mononuclear inflammatory cells infiltration and dilated tubules. loss of borders was potentially observed in tubules. The FUR group was stained by H&E showing some regenerating renal tubules within the cortex. H&E sections of MScs treatment to AKI rats revealed numerous regenerating tubules in the renal cortex with mild mononuclear inflammatory cell infiltration when compared to the AKI group or FUR group. The kidney sections appeared similar to the normal control group except that there were mild mononuclear inflammatory cells as illustrated in Fig 5.

3.6 Immunohistochemical analysis of the angiogenic factor vascular endothelial growth factor (VEGF) protein expression

The control group rats revealed a positive strong reaction for VEGF protein expression in kidney sections. The AKI group revealed mild reaction for expression of VEGF expression in kidney tubules and glomeruli. Upon the MSC treatment to the AKI rats, a significant positive reaction was observed for VEGF expression in kidney sections compared to control and FUR-treated rats as shown in Fig.6. These Immunohistochemical results highlight the potential angiogenic properties of MSCs in the treatment of several diseases.

Discussion

Kidney diseases are public global health problems which affects more than 750 million of the population worldwide and lead to 6 to 10 million deaths yearly (*Rota et al.* 2019). Recently, MSCs are considered innovative tools for the treatment of Kidney diseases due to their homing, migration, and differentiation effects (Lee *et al.* 2021) (*Sherine et al.* 2022). Previous studies have indicated that MSCs are safe and effective when using MSCs for organ injury treatment (Wong *et al.* 2021). Additionally, MSC treatment enhanced the recovery of kidney function after kidney

pathogenesis via different mechanisms including anti-apoptosis, anti-inflammation and angiogenesis (Xu *et al.* 2010a). In this research, a cisplatin-induced rat AKI model was involved to examine the potential protective effects of MSCs on functional and histological injury of AKI and to find out whether the therapeutic effect of MSCs is mediated via their antiapoptotic properties as well as the underlying molecular mechanisms.

In our study, MSCs were directing themselves into the damaged kidneys of rats as observed by CD44 expression by immunohistochemistry in the renal tissue of the AKI group. These results were confirmed by (*Morigi et al. 2004*), who indicated homing of MSCs in the damaged kidneys. Our results showed that cisplatin seriously suppressed kidney function in diseased rats. This effect may result from the decrease in the glomerular filtration rate (GFR) by damaging the distal nephron, which is closely linked to an elevation in serum creatinine levels *(Pabla and Dong 2008)*. On the contrary, after injection of MSCs these levels were significantly reduced, indicating that MSCs could effectively repair the kidney function injury that can be induced by cisplatin. These results are confirmed by previous studies (Kawai *et al.* 2005) (*Ni et al. 2019)*.

Inflammation plays a significant role in the pathogenesis of AKI (Bancu *et al.* 2016). In the current study, the MSCs effectively reduced the elevated levels of IL-6, IL-1β, and TNF gene expressions in the AKI group. Renal damage induced by cisplatin contributes to the elevated level of inflammation in AKI group (Qi and Wu 2013), which plays a crucial role in formation of inflammatory mediators. In the inflammatory pathology, the TNF-α is released from damaged tissues, that leads to stimulation release of IL-β and IL-6 *(Bucher and Taeger 2002).* However, the production of cytokines in rats was significantly reduced after treatment with MSCs, suggesting that MSCs had potential anti-inflammatory properties.This significant elevation in cytokines levels in the AKI group, is similar to the observed elevation in rats with nephrotoxicity induced by cisplatin in previous studies (Filipski *et al.* 2009). This is thought to be due to the attenuation of kidney urea transporters (UTs), which elevates the pro-inflammatory factor such as TNF-α in severe inflammation circumstances (Bucher *et al.* 2003)

MSCs have been demonstrated to possess potent anti-inflammatory properties, which are mediated through several mechanisms *(Sun and Kanwar 2015)*. These mechanisms include the modulation of proinflammatory cytokine expression *(Wenjie and chen 2024)*, through the production of anti-inflammatory biomarkers including interleukin-10 (IL-10), as well as the differentiation into pericytes and endothelial cells (Chen *et al.* 2020). We tried in this study to

focus on the essential role of TNF-α in kidney diseases. It can activate apoptosis pathways, cell survival, and stimulate the renal epithelial cells to secrete cell adhesion mediators *(Woodell-May and Sommerfeld 2020)*. It has been indicated that TNF-α activates ASK1 by dissociation of ASK1 from its inhibitors 14-3-3 (a phosphoserine-binding molecule) and Trx (Orecchioni *et al.* 2019). In contrast, MSCs inhibit TNF-α–induced ASK1 activation by prevention of the release of ASK1 from the binding molecule; 14-3-3 (*Liu et al.* 2001). MSCs inhibited significantly this TNF-α apoptotic pathway by reducing TNF-α and apoptotic proteins in our current study, these results agreed with the results recorded by (*Favero et al.* 2019).

The potential mechanism by which MSCs reduced ASK-1 and TXNIP expression was observed by its suppression effects of cytokines production (Kariya *et al.* 2018). This study has shown that AKI leads to an elevation in the expression levels of these markers. This is likely due to the production of pro-inflammatory pathways via the release of reactive oxygen species (ROS) in the kidney tissue (Ibrahim *et al.* 2021). The accumulation of ROS can stimulate a pro-inflammatory state and lead to oxidative stress (Ellulu *et al.* 2017). This further exacerbates the injury process of AKI and contributes to the activation of ASK-1 and TXNIP which are key signaling mechanisms in susceptibility to AKI.

Kidney injury causes cascade reactions, the apoptosis process of kidney cells is a critical step in the kidney injury pathogenesis *(Selim et al. 2019)*. Kidney cells undergo shedding cells and apoptosis which obstructs lumen, additionally, other renal cells are subjected to oxidative stress and inflammatory reactions, which leads to tissue damage. Reduction of apoptosis of renal cells is the key main step in kidney injury repair (Chen *et al.* 2020). In this study the pathogenesis pathway of acute kidney damage caused by cisplatin upregulates apoptosis However, after treatment with MSCs, the expression of apoptotic factors; ASK-1 and TXNIP was significantly reduced in rat renal tissues. These results indicated that MSCs could significantly suppress the apoptosis of kidney cells (Bryniarski *et al.* 2021).

In order to prove the angiogenic ability of MSC, the current study demonstrated a correlation between AKI and VEGF protein expression within the renal medulla. VEGF, a protein known to promote angiogenesis, has a crucial role in the production of new blood vessels (Lee *et al.* 2016).

In this study, treatment with MSCs was observed to cause a significant elevation in VEGF expression when compared to the AKI group. These findings are confirmed by the study of (Liu and Fang, 2020). The pro-angiogenic effects of MSCs are related to the repair of these injured

tissues showing significant elevations of VEGF after treatment. Furthermore, MSCs could stimulate angiogenesis by producing factors related to the angiogenic factors such as insulin-like growth factor 1 (IGF-1)(Nie *et al.* 2020), and this could explain the upregulation of gene expression of VEGF level in MSC-treated groups. (Calcat-i-Cervera *et al.* 2021)

These results agreed with a previous study conducted by (Li *et al.* 2013) which reported elevated levels of VEGF expression in chronic renal failure after MSCs transplantation. This suggests that MSCs may enhance angiogenesis by elevation of VEGF protein levels, as previously reported in ischemic stroke (Lee *et al.* 2016), hepatic tissue *(Aravindan and Shaw 2006)*, and chronic renal failure (Jia *et al.* 2016). However, (Tögel et al 2005) also proposed that MSCs accelerate pyrosis in kidney failure by elevation of proangiogenic cytokines, such as VEGF raising questions about the effects of MSCs on kidney tissue angiogenesis in AKI. In line with our study, *(Xu et al. 2010b)* found that MSCs elevated the repair of the renal tubules, that enhanced kidney function. These findings imply that angiogenesis may be a crucial mechanism for the therapeutic effects of MSCs on AKI.

Additionally, We can explain the improvement of AKI by MSC treatment based on the enhancement of histopathological results in the current study. The kidneys of the AKI group showed distorted Bowman's space and glomeruli with clear hyalinosis. These changes agree with (*Najafian et al.2011)* who indicated that the early sign of AKI is the histological changes including the thickening of the glomerular membrane. Histological examination of the AKI group after MSC injection showed that the histology of renal tissue retained almost the normal appearance of the control ones. These results are in concomitant with the study that stated that MSCs can differentiate and regenerate, the renal cells *(Andrade-Silva et al. 2018).* Also, the renal tubules of the MSCs group retain their normal structure, and these results also agree with the studies of *(Qian et al.2008)* and *(Wu et al.2014)* which revealed the ability of MSCs to differentiate into tubular cells. As a whole, our data suggest that MSCs-based therapy appears to be an innovative intervention approach with tremendous potential for the management of AKI, however, the limitation of our research is there are still many *in vitro* and *in vivo* studies should be done before MSCs can be used for clinical treatment on a larger scale to make MSCs with more efficient targeting ability and more precise immunomodulatory function.

Conclusion

In conclusion, our data revealed that cell-based therapy using MSCs can alleviate inflammation, apoptosis, and enhance renal function in AKI, suggesting the possibility of treatment with MSCs as an effective and new therapeutic tool for AKI. However, more clinical and preclinical studies should be performed to confirm it in the future.

Declarations

Ethics approval

The study protocol was approved by the Ethics Committee of the Faculty of Pharmacy, MSA University, Ethics Committee (**code: PH8/EC8/2019F)** in accordance with the declaration of Helsinki.

Consent for publication

The signed Consent ensures that the Publisher has the Author's permission to publish the relevant Contribution.

Disclosure statement

The authors report no conflict of interest.

Authors Contributions

Amaal Abdelaziz; Resources, Data curation, Software, Investigation. **Radwa Mekky;** Software, Investigation, Validation, Visualization, Writing – Original draft. **Sherine M. Ibrahim;** Software, Investigation, Validation, Visualization.

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Availability of data and materials

The datasets used are available upon reasonable request.

List of abbreviations:

Apoptosis signal-regulating kinase 1: ASK1

Acute kidney injury: AKI

Chronic kidney disease: CKD

Furosemide: FUR

Fibroblast growth factor 2: FGF-2

Glutathione reductase: GSH-Rx

Hepatocyte growth factor: HGF

Horseradish peroxidase-conjugated: HRP

Interleukin-6: IL-6

Insulin-like growth factor 1: IGF-1

Mesenchymal stem cells: MSCs

Placental growth factor: PIGF

Real-time reverse transcription PCR: qRT-PCR

Thioredoxin-interacting protein: TXNIP

Tumor necrosis factor: TNF-α

Vascular endothelial growth factors: VEGF

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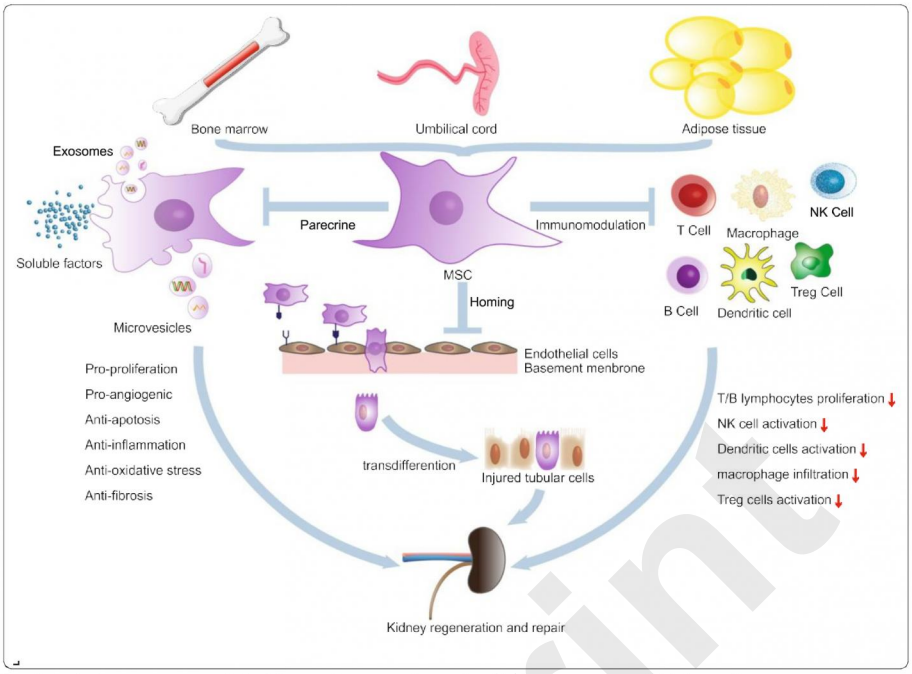
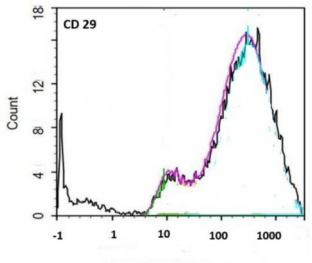


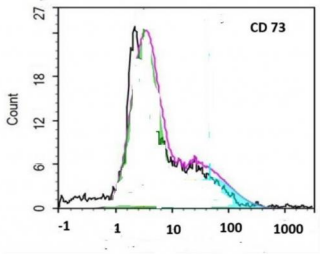
Graphical abstract: potential effects of mesenchymal cells in treatment of kidney diseases

Table (1): Oligonucleotide sequence of the forward and reverse primers of the inflammatory mediators; interleukin-6 (IL-6), IL-1β, and tumor necrosis factor (TNF-α) used for detection of gene expression by *RT*-PCR

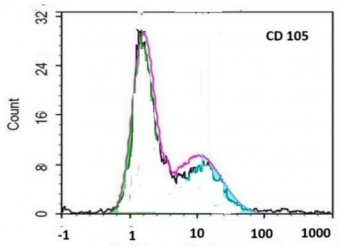
Gene	Forward	Reverse
IL-6	TCCTACCCCAACTTCCAATGCTC	TTGGATGGTCTTGGTCCTTAGCC
IL-1β	AAGACAAGCCTGTGTTGCTGAAGG	TCCCAGAAGAAAATGAGGTCGGTC
TNF-α	AAATGGGCTCCCTCTCATCAGTTC	TCTGCTTGGTGGTTTGCTACGAC
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC



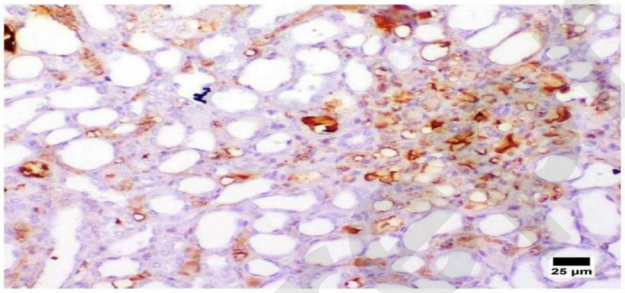
(A)



(B)



(C)



(D)

Figure 1: Histograms of flowcytometry showed positive expression of CD29 (A) and CD73 (B), and of CD105 (C). Immunohistochemical stained section of renal tubules of MSCs for CD44 at magnification × 1000 shows intensive positive reaction in the cytoplasm of spindle shaped MSC which is incorporated as brown colored granules in between renal cells (D).

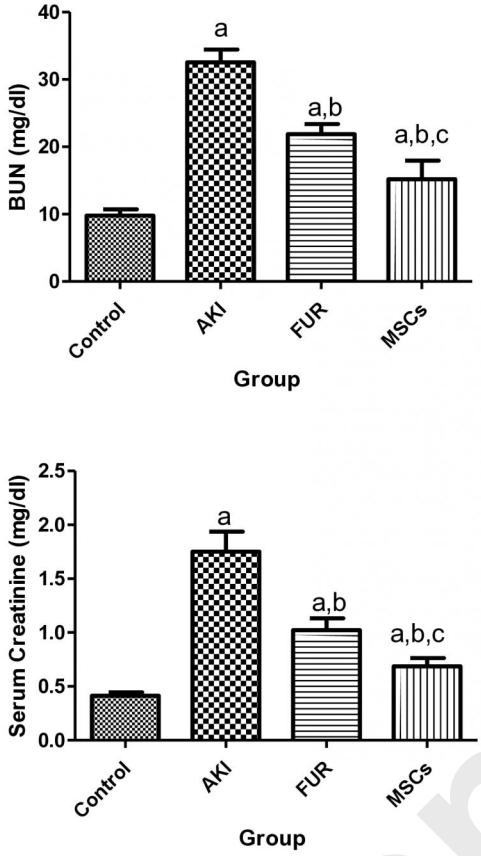
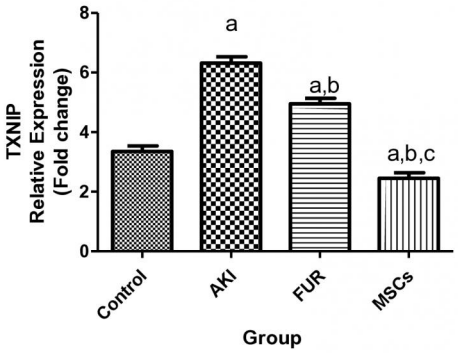
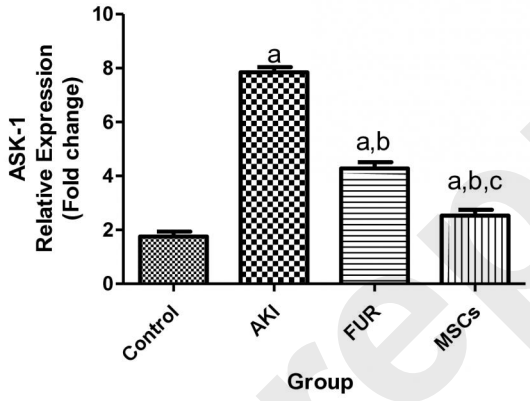
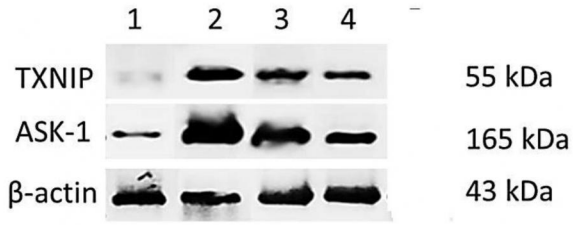


Figure 2: Role of mesenchymal stem cells in alleviating kidney injury in rats. Data were presented as means ±SEM. Each column represented the means ± SEM. Variations between the groups using Turkey's honestly significant difference test. [a]$p < 0.01$ vs. control, [b]$p < 0.01$ vs. cisplatin induced acute kidney injury cells, [c]$p < 0.01$ vs. furosemide treated cells.


TXNIP
Relative Expression
(Fold change)
8
6
4
2
0
a
a,b
a,b,c
Control
AKI
FUR
MSCs
Group


ASK-1
Relative Expression
(Fold change)
10
8
6
4
2
0
a
a,b
a,b,c
Control
AKI
FUR
MSCs
Group

(A)



(B)

Figure 3: The expression of the anti-apoptotic markers; TXNIP and ASK-1 after treatment with MSCs in AKT. (A): The kidney apoptosis was elevated in the AKI group by the elevation of expression levels of ASK-1 and TXNIP by 3 and 2 folds, respectively, compared to the control group. On the contrary, the apoptosis process of kidney cells in the MSCs group was significantly reduced compared to AKI group as indicated in Fig 3. This reduction indicated that MSCs exhibited antiapoptotic properities via paracrine signaling pathway under specific pathological conditions. Data were presented as means ±SEM. Each column represented the means ± SEM. Significant difference was measured by Turkey test; $^{a}p < 0.01$: significant difference vs. control, $^{b}p < 0.01$: significant difference vs. cisplatin induced acute kidney injury cells and $^{c}p < 0.01$: significant difference vs. furosemide treated cells. (B): the relative expression of ASK-1 and TXNIP was measured by western blot analysis; 1: control group, 2: AKI group, 3: FUR group, and 4: MSCs group.

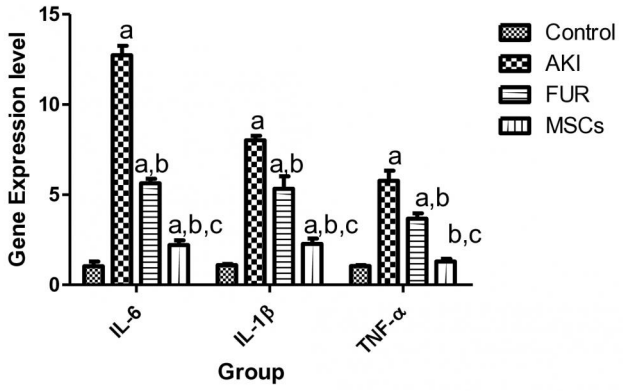


Figure 4: Anti-inflammatory effects of MSCs in AKI-induced rat model on IL-6, IL-1β, and TNF-α gene expression. AKT group showed a highly significant ($P < 0.01$) elevation in the inflammatory mediators including TNF-α, IL-6, amd IL-1β levels as compared to control rats. MSC treatment of AKI rats showed a significant supression in IL-6, IL-1β and TGF- α ($P < 0.01$) compared with both FUR and control groups showing the potential ant-inflammatory effects of MSCs. Data were presented as means ±SEM. Each column represented the means ± SEM. Means with different letters indicated the variations between the groups within the same row using Turkey's honestly significant difference ($p < 0.05$) test. $^{a}p < 0.01$ vs. control, $^{b}p < 0.01$ vs. cisplatin induced acute kidney injury cells, $^{c}p < 0.01$ vs. furosemide treated cells.

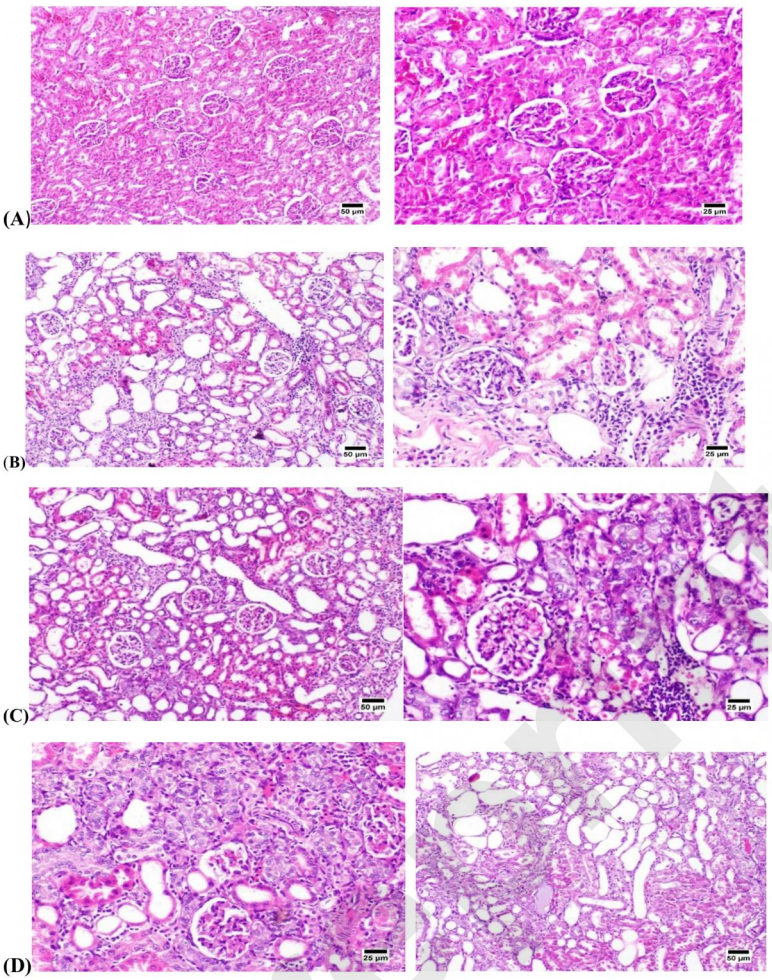
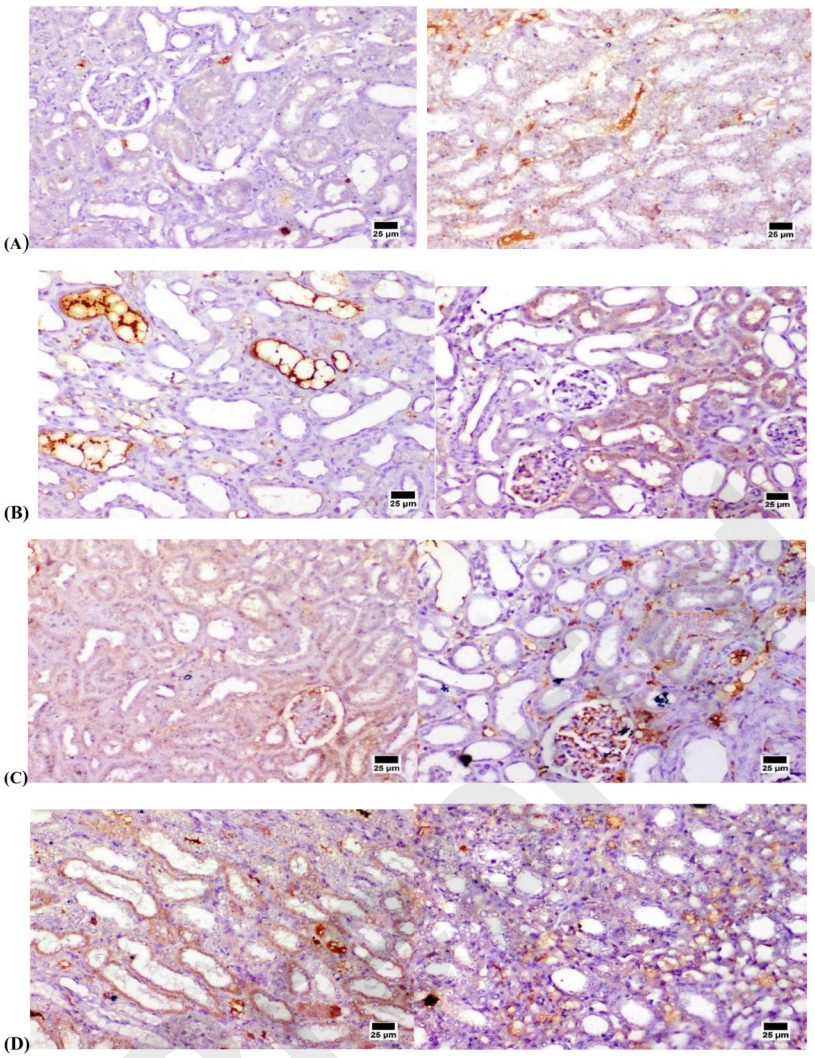
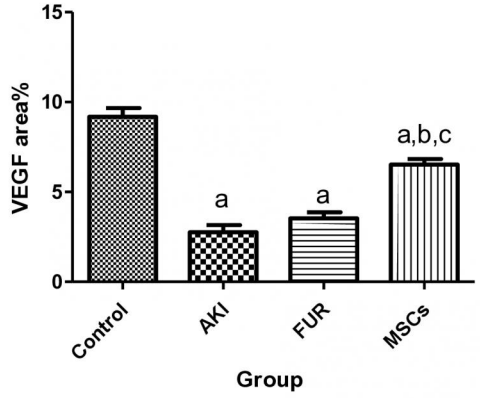


Figure 5: H&E stained renal cortex sections at magnification × 400, bar 50 µm. A: control group was stained by H&E showing normal glomeruli and tubules. B: AKI group was stained by H&E showing necrotic renal tubules, mononuclear inflammatory cells infiltration and dilated tubules C: FUR group was stained by H&E showing some regenerating renal tubules within the cortex. D: MSCs group was stained by H&E showing numerous regenerating tubules in the renal cortex with mild mononuclear inflammatory cells infiltration.


(A)
(B)
(C)
(D)
25 µm



(E)

Figure 6: Immunohistochemical staining of VEGF of renal cortex sections at magnification × 400, bar 25 µm. (A): Immunohistochemical-stained kidney sections of control group showing strong reaction of VEGF protein expression in the renal cortex. (B): Immunohistochemical-stained kidney sections of AKI group showing significant reduction in the expression of VEGF in renal medulla. (C): Immunohistochemical-stained kidney sections for VEGF protein expression of FUR group showing mild increase in the expression of VEGF in renal cortex. (D): Immunohistochemical-stained kidney sections for VEGF protein expression of MSCs group showing marked increase in the expression of VEGF in renal medulla. (E): The angiogenic effects of MSC in kidney tissue, expressed as area %. Data were presented as means ±SEM. Each column represented the means ± SEM. The mean variations between the groups using Turkey's significant difference test. $^{a}p < 0.01$ vs. control, $^{b}p < 0.01$ vs. cisplatin induced acute kidney injury cells, $^{c}p < 0.01$ vs. furosemide treated cells.