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RESEARCH ARTICLE

Breast Milk-Derived Stem Cells Diminished Cisplatin-Induced Acute Kidney Injury in Male Rats Via Modulating Renal Growth Factor Receptors, Autophagy, and Oxidative Stress

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ABSTRACT

One of the main causes of renal impairment and failure is acute kidney injury (AKI), which has few treatment options that can preserve kidney function. Stem cells represent a promising regenerative tool, among these are stem cells derived from breast milk (Br-dSCs), which have demonstrated greater potential for regenerative capacity and plasticity in several diseases than other types of stem cells (SCs). However, their application in kidney injury disorders still needs to be fully studied, and their underlying regenerative mechanisms still need to be discovered. Thus, the current investigation aimed to examine the Br-dSCs' capacity for regeneration in acute renal injury and address the possible molecular mechanisms implicated in the reparative modalities of these cells regarding cellular autophagy. Eighteen adult male Sprague Dawley rats were assigned into 3 equal groups 10 rats each: control, AKI, and AKI + Br-dSCs groups. The kidney function tests' serum level, oxidative stress in the kidneys, histopathological examination, immunohistochemical expression of the renal receptor of insulin growth factor 1 (IGF-1r), epidermal growth factor 1 (EGF-1r), autophagy promoting factor beclin-1, and renal collagen deposition were performed. The results illustrated that Br-dSCs improved the serum level of blood urea nitrogen, uric acid, as well as creatinine rendering them to the ordinary physiological tone of the group of control. Moreover, the administration of Br-dSCs markedly increased the IGF-1r, EGF-1r, beclin-1, and downregulated collagen synthesis and deposition. We could conclude that Br-dSCs regenerate the injured kidney tissue via a modulating growth factor receptor that potentially promote the renal autophagy process.

***Keywords*:** Acute renal injury, Breast milk-derived stem cells, Autophagy; Cisplatin, and IGF-1.

Introduction

Acute renal injury (AKI) is a phenomenon wherein a variety of causes cause the renal function to rapidly decrease over hours or days. Renal tubular damage, tubulointerstitial inflammation, decreased glomerular filtration rate and reduced renal perfusion are the primary pathophysiological alterations [1]. AKI is a worldwide public health issue that affects around 14 million people and results in 1.7 million fatalities annually [2]. Furthermore, half of the inpatients required renal replacement treatment and 20% had complicated AKI [3]. Even if minor and treatable, can have fatal outcomes [4, 5]. AKI's long-term effects include the onset and exacerbation of renal disease at its latter stage and chronic renal disease[6,7]. Despite our

progress in understanding the pathophysiology of AKI, there are still few clinical therapy options available. Numerous therapeutic approaches, including the use of diuretics, antioxidants, dopamine, in addition to lowering the exposure to nephrotoxic medications, do not alter disease's trajectory [8].

Autophagy is intracellular mechanism of degradation and recycling long-lived proteins and cytoplasmic organelles to control growth and maintain homeostasis. Defective autophagy is describing as underlying cause of several clinical ailments, including vacuolar myopathies, neurodegenerative illnesses, liver disease, and several types of cancer [9]. In order to preserve cellular homeostasis and survive the AKI caused by cisplatin, autophagy is crucial. Increases in autophagy and apoptosis were both decreased in NRK-52E cells treated with cisplatin following beclin-1 knockdown, demonstrating that autophagy mediates cell damage [10]. Another study, however, demonstrated the protective role of autophagy in cisplatin-induced kidney injury by showing that autophagy suppression accelerated apoptosis [11]. Additionally, autophagy can stop cisplatin-induced proximal tubule apoptosis and AKI [12]. Autophagy regulator beclin-1 expression in renal tubules is kidney protective via promotion of cell proliferation, and inhibition of kidney fibrosis, consequently improving kidney recovery post-AKI [13]

Epidermal growth factor receptor (EGFR) is expressed in normal and diseased kidneys. Progression of kidney disease as defined by a decline in EGFR is associated with tubular necrosis and nephron loss [14]. Therefore, epidermal growth factor EGF plays an essential role in maintaining uroepithelial integrity after acute tubular necrosis [15]. Moreover, EGFR activation leads to generation of eicosanoids as well as growth of epithelial cells [16]. The insulin-like growth factor (IGF) is expressed in the kidney with several autocrine, endocrine, and paracrine actions. Moreover, IGF-1 receptor (IGF-1R) can regulate vascular homeostasis and endothelial function. Decreased expression of IGF-1R impairs the endothelial function and increases the fibrosis of kidney disease [17]. Therefore, alterations of IGF-1/IGF-1R signaling recorded in a number of kidney diseases [18, 19].

Renal replacement therapy, a last resort for terminal illness, comes with a number of high-cost side effects that inflict a heavy emotional and financial strain on patients, and it is not always more effective in improving kidney function recovery [20].Recent advances in regenerative medicine have opened the door to promising therapeutic methods for the prevention or treatment of AKI. Multidirectional differentiable and self-renewing stem cells, known as mesenchymal stem cells (MSCs), are derived from mesoderm [21, 22].

They are a hotspot for research because of their abundance of sources, ease of isolation, high pace of migration and proliferation, low rate of immunological rejection, and lack of ethical debate [21, 22].The primary mechanisms by which MSCs protect the kidneys include anti-inflammatory, angiogenesis-promoting, endogenous stem cell mobilization, anti-fibrosis, anti-oxidation, anti-apoptosis, and cell reprogramming [23]. Mesangial cells, capillary endothelial cells, renal tubular epithelial cells, and podocytes are only a few of the intrinsic kidney components that can be generated from bone marrow-derived stem cells, according to studies [24, 25].

Animal models with AKI have been used to study the therapeutic impact of stem cells (SCs) due to their capacity for regeneration and their propensity to migrate towards damaged tissue in various disorders. Preclinical studies have revealed that MSCs can secrete cytokines such as IL-6, IL-10, TGF-β, and other cytokines to counteract the early

inflammatory milieu of AKI [26, 27]. Additionally, MSCs can develop into cells resembling pericytes and secrete hepatocyte growth factor, insulin-like growth factor-1, and vascular endothelial growth factor to promote angiogenesis and renal vascular perfusion [28-31]. They also can help the injured renal cells to regenerate by moving to the kidney and giving rise to renal parenchyma cells [32].

Even though MSCs have demonstrated a lot of promise in the animal model of AKI, several problems still exist. Firstly, the research has shown that MSCs fail to migrate to the site of injury to perform repair because of organ obstruction, such as the liver and lung, and instead encourage an increased number of granulocytes, aggravating kidney injury resulting from immune response [33, 34]. Secondly, the bulk of studies indicates that MSC can differentiate into renal parenchyma cells in specific situations, including glomerular cells, glomerular mesangial cells, and renal tubular epithelial cells. Therefore, MSC are believed to be able to repair the kidney by differentiating into renal parenchyma cells [35, 36]. Nevertheless, despite advances in our knowledge of MSCs, some research has failed to find evidence of MSC migration and differentiation in the kidney [37, 38].

Thirdly, the clinical investigation on MSC treatment did not yield the expected results. A multicenter randomized, double-blind, placebo-controlled clinical trial of intra-arterial infusion of allogeneic MSCs in 156 patients with AKI after heart surgery found that MSCs did not reduce the rate of death, the amount of time needed for renal function to recover, or the need for dialysis [39].Ultimately, it has been demonstrated that the transplant method and dosage of MSCs are the primary determinants influencing their efficacy; nevertheless, the various routes and dosages of MSCs have not been compared in the present animal research [40, 41]. Thus, the current investigation was created to investigate the potential therapeutic implications of breast milk derived stem cells (Br-dSCs) in AKI as well as address the underlying regeneration process concerning the control of the renal autophagy signaling pathway.

Materials and methods

Samples

Under aseptic conditions, twenty milk samples were obtained from the Zagazig University of pediatric medical facility with the mother donor's ready written agreement.

Laboratory animals

Eighteen adult male Sprague-Dawley rats, weighting between 200 and 250 g and being between 6 and 8 weeks old, were obtained from the Animal House, Faculty of Veterinary Medicine at Zagazig University, Egypt. The rats were given an unlimited supply of water, housed in a 12/12 light/dark cycle, and fed a chewable meal every day for seven days. All experimental procedures were carried out in accordance with the arrival guidelines provided by the institutional Animal Care and Use Committee of the Badr University in Cairo (BUC) School of Veterinary Medicine (Approval No. BUC-IACUC/VET/149/A/2024).

Induction of nephropathy or kidney injury

Rats were given 5 mg/kg body weight of cisplatin (Mylan, USA) single intraperitoneal injection in order to develop nephropathy [42, 43]

Design experimentation

Eighteen mature male three equal sets of ten Sprague-Dawley rats each were randomly divided. Groups G1 and G2 were control and nephropathy, respectively. G3 was treated single intraperitoneal injection of Br-MSCs 2 x 10^7 suspended in 0.25 mL phosphate buffer saline (PBS). Blood samples were drawn from the median eye canthus after 12 weeks post treatment with breast milk derived stem cells (Br-MSCs). The kidney

tissues were then quickly separated into three parts: the first one, was wrapped in foil made of aluminum and kept at -80 ℃ for oxidant/antioxidant activity; on neutral formalin buffer 10%, the second and third ones were put for histopathology and immunohistochemistry.

Breast milk derived stem cells isolation, and identification

Using Patki et al. method [44], breast milk derived stem cells were isolated, In a nutshell, the milk samples were centrifuged at 285 g for 10 minutes after being made diluted 1:2 using Dulbecco modified Eagles media (DMEM) with fetal bovine serum 10% (Invitrogen, USA) containing 1% penicillin, streptomycin, and amphotericin and the supernatant was collected, the cells were sown in tissue culture flasks. The cell pellets were then collected and twice washed with PBS (Invitrogen, USA). Subsequently, DMEM medium with fetal bovine serum 10% (Invitrogen, USA), 1% penicillin and streptomycin, and amphotericin (Invitrogen, USA) was used to seed the cells in tissue culture flasks.

The tissue culture flask was set up in a CO_2 incubator at 37 ^oC and 5% CO_2. After the confluence reached 80%, the adherent cells were harvested using trypsin-EDTA and passaged three times consecutively. The medium was changed every 48 hours. After the third passage, the cells were identified using flow cytometry and the following positive surface markers: cluster of differentiation 90 (CD90), CD106, and CD105 and for the negative surface markers, use CD34, CD45, and Human leukocyte antigen - DR isotype (HLA-DR); the antibodies that used in the flow cytometrical analysis were obtained from Minneapolis, USA. The Br-MSCs were labeled with PKH-26 red fluorescent cell linker kit (Sigma Aldrich, USA) in compliance with the manufacturer's instructions in order to follow the transplanted cells [44, 45].

Determination of kidney function tests

Measurements were made of serum creatinine, serum uric acid, and blood urea nitrogen following manufacturer's instructions (SPINREACT, Spain).

Determination of the oxidant/antioxidant activity

For use in the oxidant/antioxidant assay, tissue from kidney of each experimental group was homogenized, centrifuged for 10 minutes at 3000 rpm, and the supernatant was kept at -80 following the manufacturer's instructions. The total antioxidant capacity (TAC)Vwas determined using a colorimetric test kit (ABTS, Enzyme technique, MyBioSource, San Diego, USA). Using the sandwich ELISA technique, levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx) in the kidney were determined.

Histopathological examination

Light microscopy for routine staining

Rats of all groups were used to dissect the chosen kidney specimens, which were then immediately preserved in 10% neutral buffered formalin. Next, the kidney specimens were water washed, subjected to ethanol dehydration in increasing grades, benzene cleared, soft paraffin infiltration (50–55 °C), and finally hard paraffin embedding (56–68°C). Harris's Hematoxylin and Eosin stain (H&E) was used to deparaffinize, rehydrate, and stain paraffin slices with a thickness of 4-5 µm in order to identify overall structure. Additional deparaffinized and rehydrated sections will be stained using Crossman's trichrome stains and the Periodic Acid Schiff (PAS) technique, respectively, in order to detect collagen and neutral mucopolysaccharide, respectively [46]

Immunohistochemical analysis

The Avidin Biotin Peroxidase method was employed according to Hsu *et al*.

[47].To sum up, 3 µm-thick paraffin slices were deparaffinized, rehydrated, and cleaned with distilled water before being exposed to antigen retrieval in sodium citrate buffer for 25 min at 100 °C, and then rinsed in PBS for 5 min. After that, slices were incubated for 25 minutes in a 3% H_2O_2/methanol solution to quench endogenous peroxidase. After a PBS wash, 10% normal blocking serum was applied and allowed to sit at room temperature for an hour in order to inhibit nonspecific antibody binding. Then, using rabbit polyclonal anti- insulin like growth factor 1 receptor (anti-IGF1R) (bs-0227R, Bioss), EGFR (Cat. No. A11351, Abclonal) and Beclin-1 (Cat. No. A7353, Abclonal) antibodies at 1:200 dilutions, slices were incubated for an entire night at 4°C to demonstrate insulin-like growth factor receptors, epidermal growth factor receptors and autophagy, respectively.

Sections were treated with corresponding secondary antibodies that were biotin-conjugated after being cleaned in PBS. Sections were cleaned with phosphate-buffered saline solution with a low-concentration detergent (PBST) and then incubated for 60 minutes with streptavidin-peroxidase conjugate, and then for 3 minutes with 3,3-diaminobenzidine tetrahydrochloride (DAB)-H_2O_2 solution to observe the streptavidin-biotin complex. Mayer's hematoxylin was used as a counterstain for the sections at the end. All of the stained sections were inspected using an OPTIKA ITALY standard light microscope that was attached to a digital camera in the central lab of the Zagazig University Faculty of Veterinary Medicine.

Histoplanimetry

The morphometric investigation (Fiji image J; 1.51 n, NIH, USA) was carried out on 3 fields per rat (6 rats from each group) using Image J analytic software. Using a semiquantitative scale [48], the tubular injury score was assessed in H&E-stained sections at X40 magnification. Each score represented the proportion of damaged cortical tubules and ranged from 0 = normal to 4 = >75%. Additionally, at 400x magnification, the area percentage of collagen fibers was determined in sections stained with Crossman's trichrome. In immunostained sections containing anti-EGFR, anti-Beclin-1, and anti-IGF1R antibodies, respectively, the area percentage of EGFR, IGF1R, and Beclin-1 immunoreactivities was also determined.

Data analysis and statistics

Statistical analysis was performed by GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA, United States). Data expressed as mean ± standard error mean (SEM). Statistical comparisons were performed using a one-way analysis of variance (ANOVA) test followed by a post hoc Tukey test. The results indicate a statistical significance when P value < 0.05.

Results

Identification and homing of Br-dSCs in the kidney

Using the flow cytometry the isolated cell populations were favorable for CD106, CD105, and CD90 (Figure 1 A – C). Even though, they were favorable negative for surface marker CD45, CD34 in addition to HLA-DR (Figure 1D – F). The outcome demonstrated that the isolated cells often expressed the traits common to stem cells (Figure 1A-G).

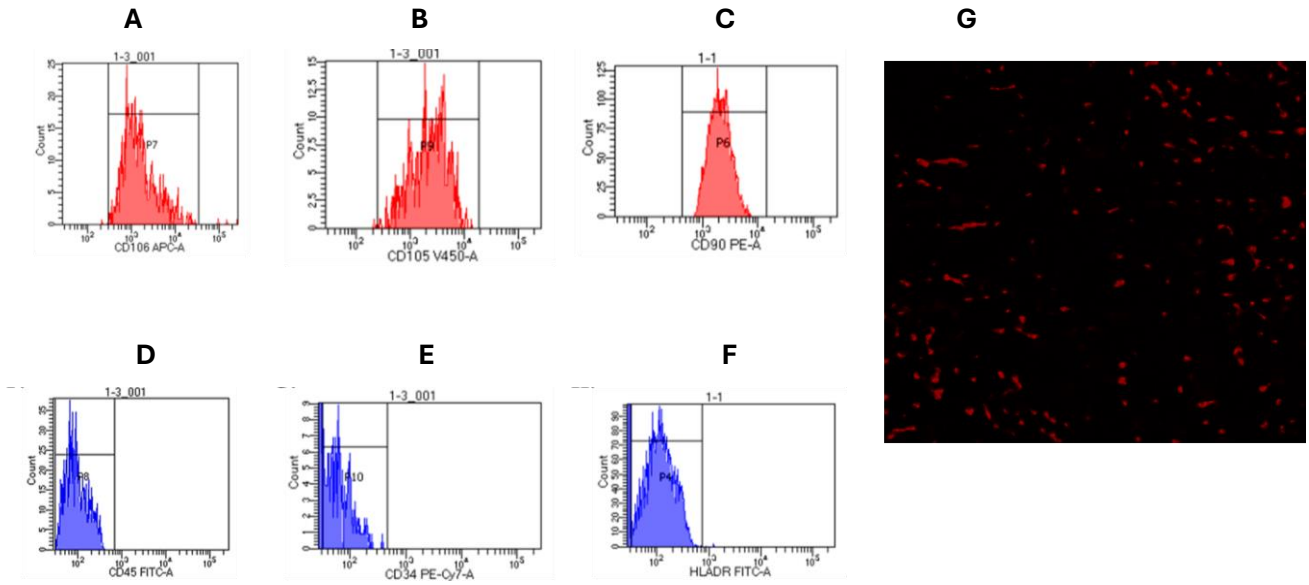

Figure 1. Identification and detection of homing of Br-dSCs were detected in the kidney tissue of rats (A–G). Flow cytometrical identification of Br-dSCs, cell populations of gated Br-dSCS were positive to CD106 (A), CD105 (B), CD90 (C), populations of gated Br-dSCS were negative to CD45 (D), CD34 (E), HLA-DR (F), and G. Homing of the Br-dSCs in the kidney tissue of Br-dSCs- treated AKI rats.

The outcome of administering Br-dSCs on the serum level of uric acid, creatinine and urea

According to the current data, the levels of uric acid, creatinine, and blood urea nitrogen in serum were significantly ($P < 0.0001$) greater in the cisplatin-induced AKI rats than in the group of control. (Figure 2 A – C). Remarkably, compared to the AKI group, the treatment of Br-dSCs markedly improved the serum level of the previously described measures (Figure 2 A – C). In the same context, Br-dSCs brought the serum level of uric acid, creatinine, and blood urea nitrogen to the normal physiological tone of the control group (Figure 2 A – C).

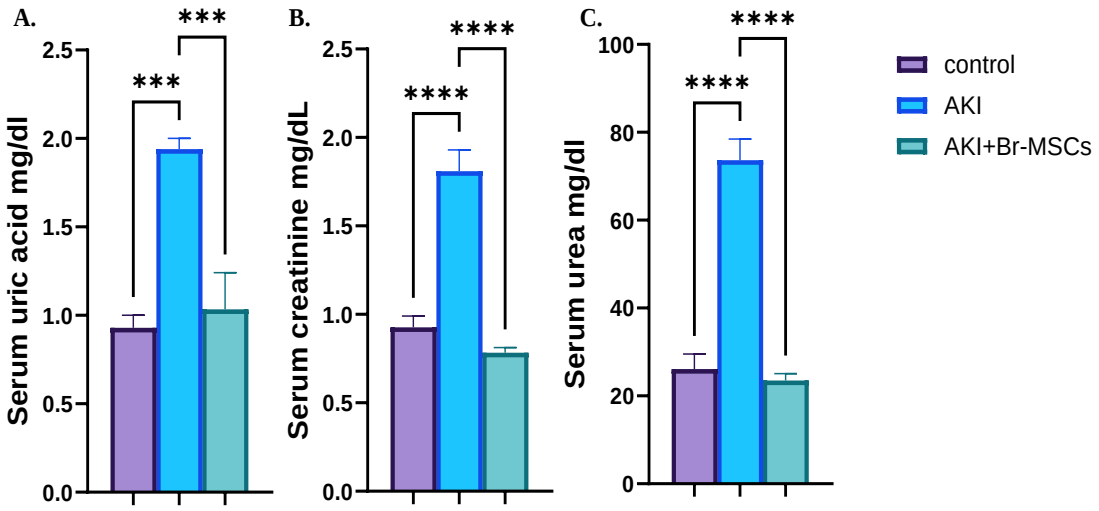

Figure 2 (A-C): The outcome of administering Br-dSCs on the serum level of uric acid, creatinine and urea. A. uric acid Serum level mg/dL, B. creatinine Serum level mg/dL, and C. Urea Serum level mg/dL. Six rats on average per group ± S.E.M. is displayed in the data, with ****p < 0.0001, ***p < 0.001, **p < 0.01and *p < 0.05.

How renal oxidant/antioxidant activity is affected by breast milk derived stem cells treatment

The investigation's current findings showed that the AKI group displayed a sharp ($p < 0.0001$) elevation in the MDA, a marker for lipid peroxidation, furthermore marked ($p < 0.0001$) decline in the antioxidant activity of renal SOD, GPx, and CAT (Figure 3 A – D). However, Br-dSCs significantly improved the renal oxidative stress which reflected in a marked decrease in the MDA and increase in the antioxidant activity of renal SOD, CAT, and GPx (Figure 3 A.–D.). Conversely, the group that received Br-dSC treatment showed a noteworthy ($p < 0.01$) rise in in the renal peroxidation markers furthermore fall in the average value of the antioxidant activity of the enzymes than the group of control (Figure 3 A–D).

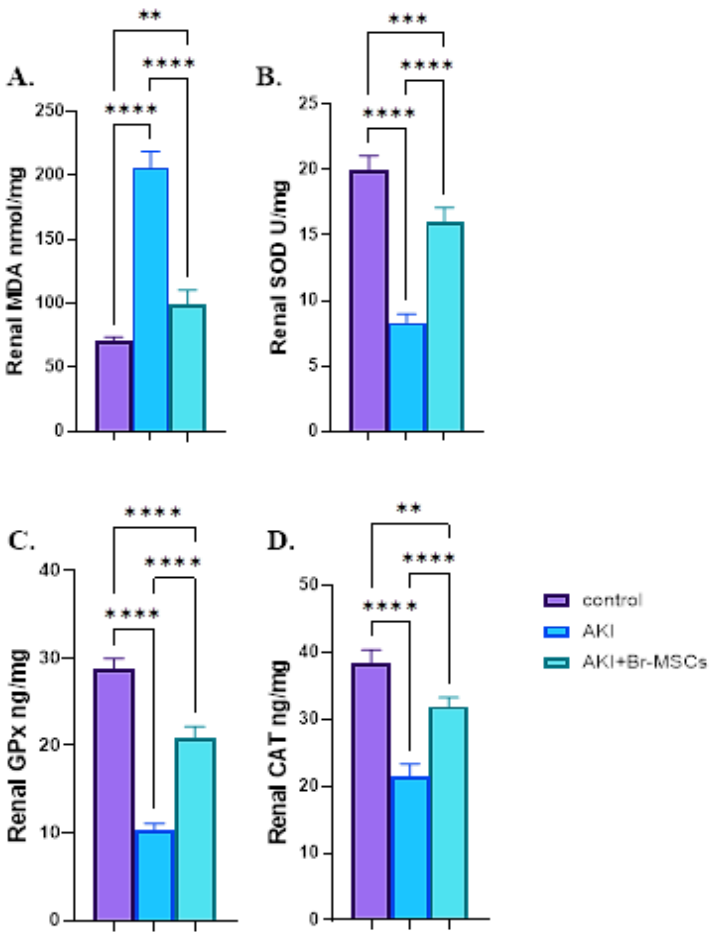

Figure 3: Outcome of administration of breast milk derived SCs on the renal oxidant/antioxidant activity (A – D). A: MDA in rat kidney nmol/mg. B: SOD (superoxide dismutase) U/mg, and Gpx (glutathione peroxidase) ng/mg, and CAT (Catalase) ng/mg. Six rats on average per group ± S.E.M. is displayed in the data, with ****p < 0.0001, ***p < 0.001, **p < 0.01and *p < 0.05.

Human breast milk-derived stem cells (hBr-dSCs) restore renal cortical tissues structure in CP-induced AKI

As shown in Figure 4, the tissue cuts stained with H&E in the renal cortex of control group reflected typical histological structure of its renal corpuscles, proximal and distal convoluted tubules and outer cortical collecting ducts with little interstitial tissues (Figure 4A). In the AKI group, crucial renal alterations were revealed as glomerular necrosis with tubulointerstitial nephritis represented by necrotic renal tubules, cystic dilatation of the renal tubules, presence of eosinophilic debris in the lumens of some renal tubules and numerous numbers of interstitial mononuclear inflammatory cells (Figure 4B).The renal structural changes of the AKI +hBr-dSCs group were minimal as they displayed almost normal glomeruli, many regenerated tubules of basophilic cellular aggregates, then forming lumens and finally elongate into mature tubules. Few injured renal tubules with moderate numbers of interstitial mononuclear inflammatory cells were detected (Figure 4C). Statistically, semiquantitative assessment of tubular injured scores significantly increased in the AKI group in contrast with control group. Conversely, when compared to the AKI group, they were significantly lower in the AKI +hBr-dSCs group (Table 1).

Examination of Periodic Acid-Schiff (PAS)-stained cuts of control group reveled well-defined apical brush borders in proximal convoluted tubules (PCT). Thin PAS positive basement membranes were observed surrounding the glomerular capillaries, parietal layer of Bowman's capsule and renal tubules (Figure 5A). In AKI group, focal and completely loss of the apical PAS+ brush border of many proximal convoluted tubules was noticed. Marked thickening in the basement membrane of outer layer of Bowman's capsule, tubules and glomerular capillaries was detected (Figure 5B). In AKI +hBr-dSCs group, moderately PAS+ brush border and corrugated basement membrane of glomerular capillaries and parietal layer of Bowman's capsule and tubules were mirrored regeneration (Figure 5C).

Concerning the fibrotic features, Crossman's trichrome stained sections of the control group displayed normal distribution of little collagen fibers within glomeruli, outer layer of Bowman's capsule and tubular basement membranes (Figure 5D). However, AKI group showed marked fibrosis within glomeruli, periglomerular, peritubular, perivascular and interstitial areas (Figure 5E). After hBr-dSCs administration, moderate deposition of collagen fibers within glomeruli, periglomerular, peritubular, perivascular and interstitial areas were noticed (Figure 5F). Statistically, collagen fiber area percentage increased significantly in the AKI group relative to the CON group, but it reduced significantly in the AKI+hBr-dSCs group when compared to the AKI group. (Table 1).

Table (1) Effect of hBr-dSCs on the kidney injury score and area % of immunohistochemical parameters in AKI rat model.

Criteria	Control	AKI	AKI + hBr-dSCs
kidney injury score	0.14±0.004^{c}	3.9±0.16^{a}	1.63±0.29^{b}
Area% collagen	3.4±0.27 ^{c}	15.37±0.57 ^{a}	7.81±0.509 ^{b}
Area% beclin-1	13.25±0.55 ^{b}	4.47±0.28 ^{c}	22.29±0.41 ^{a}
Area% EGF-r1	7.01±0.34 ^{b}	1.62±0.07 ^{c}	12.98±0.189 ^{a}
Area% IGF-r1	3.67±0.13 ^{b}	0.87±0.086 ^{c}	8.39±0.21^{a}

AKI: Acute kidney injury, hBr-dSCs: human breast milk-derived stem cells

a, b, and c as distinct superscript letters denoted values with significant differences ($p < 0.05$).

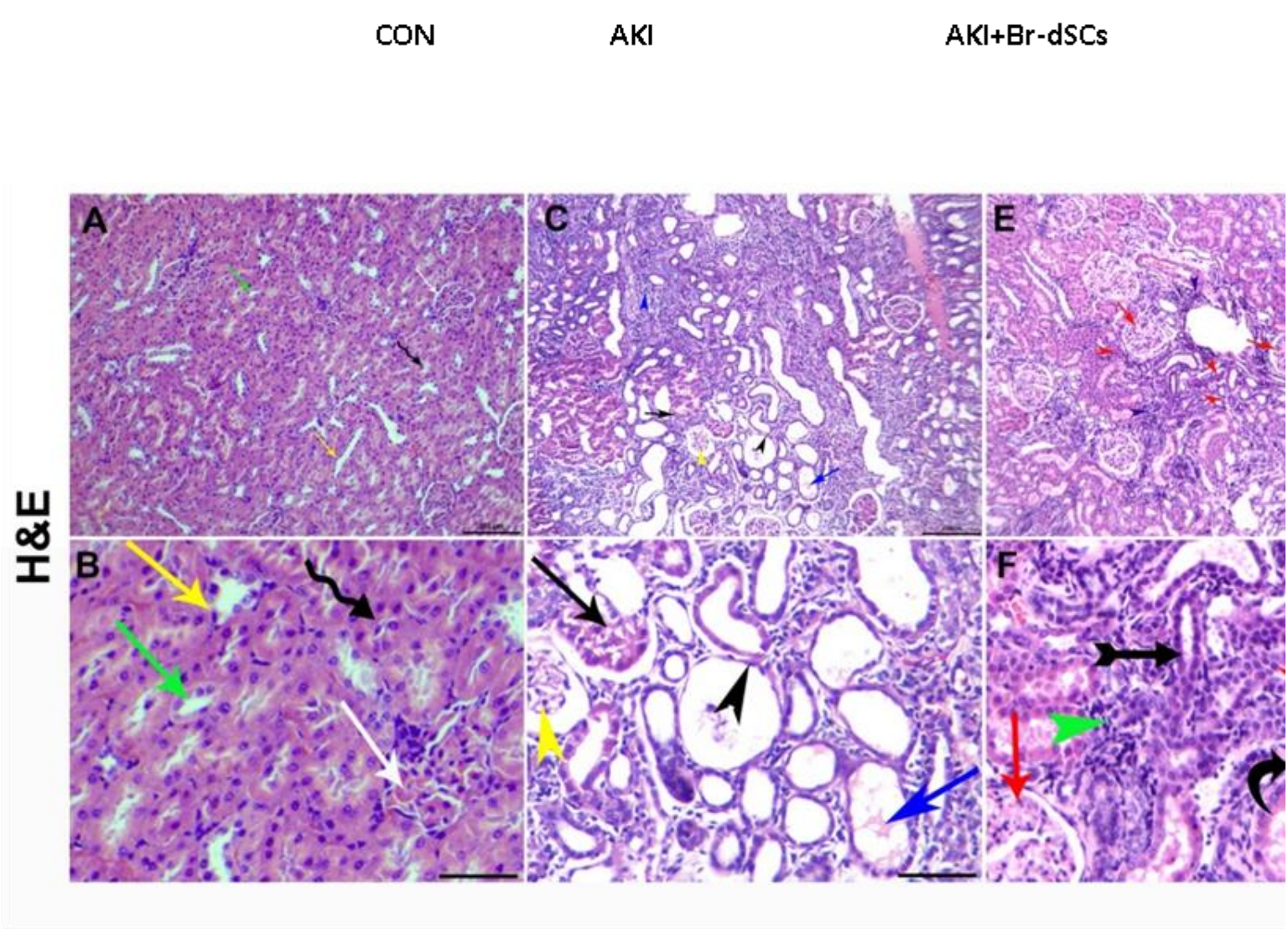

Figure 4 Representative photomicrographs of the H&E-stained tissue sections of the renal cortex of control (A), AKI (C) and AKI +Br-dSCs (E) groups and their respective higher magnifications (B, D, and F). A, B: normal renal corpuscle of vascular glomeruli and Bowman's capsule (white arrow), proximal convoluted tubule (zigzag arrow), distal convoluted tubule (green arrow), outer cortical collecting duct (yellow arrow). C,D: glomerular necrosis (yellow arrowhead), with tubulointerstitial nephritis represented by necrotic renal tubules (black arrow), numerous numbers of interstitial mononuclear inflammatory cells (blue arrowhead), cystic dilatation of the renal tubules (black arrowhead), and presence of eosinophilic debris in the lumens of some renal tubules (blue arrow). E,F: moderate numbers of interstitial mononuclear inflammatory cells (green arrowheads), almost normal glomeruli (red arrow), and numerous regenerated renal tubules of basophilic cellular aggregates (red arrowheads), then forming lumens (biforked arrow) and finally elongate into mature tubules (closed arrow). Scale bars for A, C and E= 200 µm ; B, D, and F = 50 µm

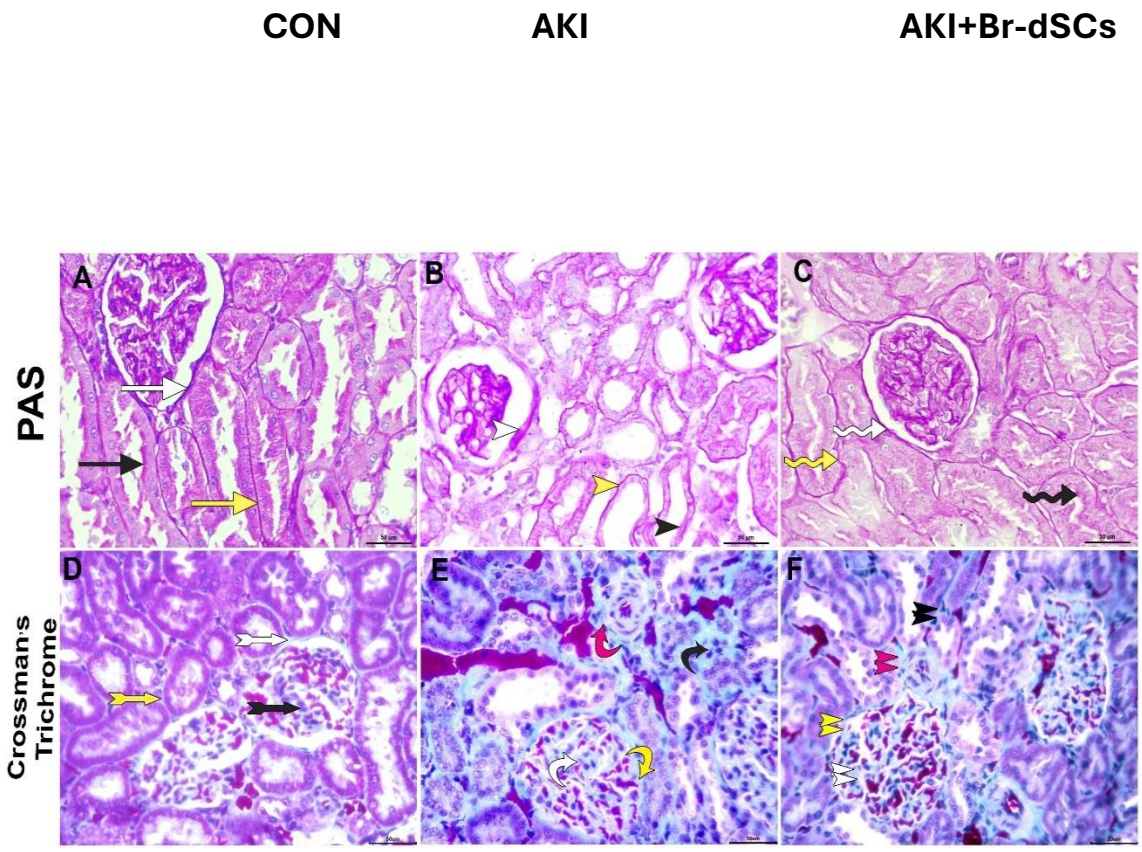

Figure 5 Representative photomicrographs of the PAS (A-C) & Crossman's trichrome (D-F) stained tissue sections of the kidney cortex in the control (A, D), AKI (B, E) and AKI + hBr-dSCs (C, F) groups displaying normal PAS^+ apical brush border (black arrow), thin basement membrane of the outer layer of Bowman's capsule (white arrow), and tubules (yellow arrow) (A), loss and hardly detected PAS^+ apical brush border (black arrowhead), thicken basement membrane of the outer layer of Bowman's capsule (white arrowhead) and tubules (yellow arrowhead) (B), moderately PAS^+ brush border (black zigzag arrow) and corrugated basement membrane of the of Bowman's capsule outer layer (white zigzag arrow) and tubules (yellow zigzag arrow) (C). Notice normal distributed few collagen fibers within glomeruli (black biforked arrow), outer layer of Bowman's capsule (white biforked arrow) and tubular (yellow biforked arrow) basement membranes (D), fibrosis of interstitium (black curved arrow), and glomeruli (white curved arrow), periglomerular (yellow curved arrow), perivascular (red curved arrow) (E), few collagen fibers within glomeruli (double white arrowheads),periglomerular (double yellow arrowheads), perivascular(double red arrowheads) and interstitium (double black arrowheads) (F). Scale bars= 50 µm.

Human breast milk-derived stem cells enhance growth factor receptors immunoreactivities in CP-induced AKI

With anti-IGF1R and EGFR the immunohistochemical staining was carried out for immunoreactivities detection of growth factor receptors. Both IGF1R and EGFR were mainly localized along the cytoplasmic membranes of glomerular endothelium, podocytes (inner layer of Bowman's capsule), proximal convoluted tubules and cortical collecting ducts, showing a moderately, hardly detected and strongly cytoplasmic

immunostaining in the control, AKI, and AKI+ hBr-dSCs, respectively (Figure 6, 7). Statistically, when comparing the AKI group to the control group, there was a significant reduction in the area percentage of both IGF1R and EGFR. In the meantime, when compared to the AKI group, the administration of hBr-dSCs demonstrated a considerable rise in these parameters (Table 1).

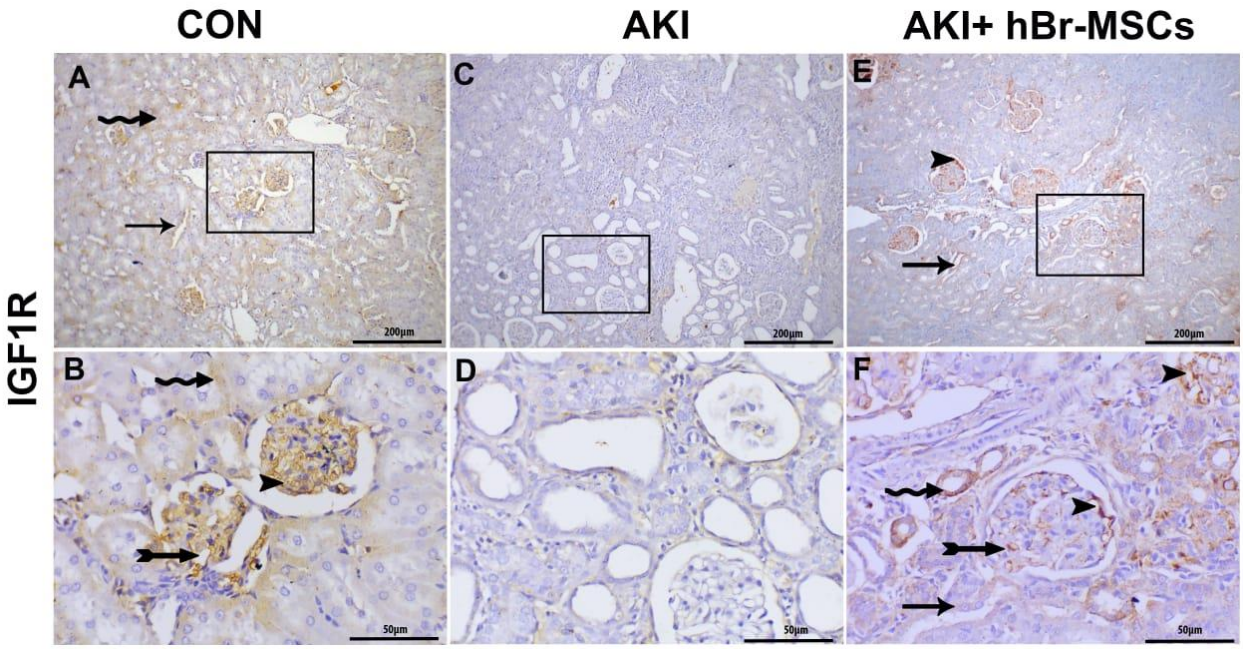

Figure 6: Representative photomicrographs of the IGF1R immunohistochemical stained renal cortical tissue sections of the control (A), AKI (C), and AKI+ hBr-dSCs groups (E) and their respective higher magnifications (B, D, F) showing glomerular endothelium (biforked arrows), podocytes (arrowheads), proximal convoluted tubules (zigzag arrows) and cortical collecting ducts (arrows) with moderately, hardly detected, and strongly cytoplasmic immunostaining in the control, AKI, and AKI+ hBr-dSCs, respectively. Scale bars: A, C, E =200 µm; B, D, F=50 µm.

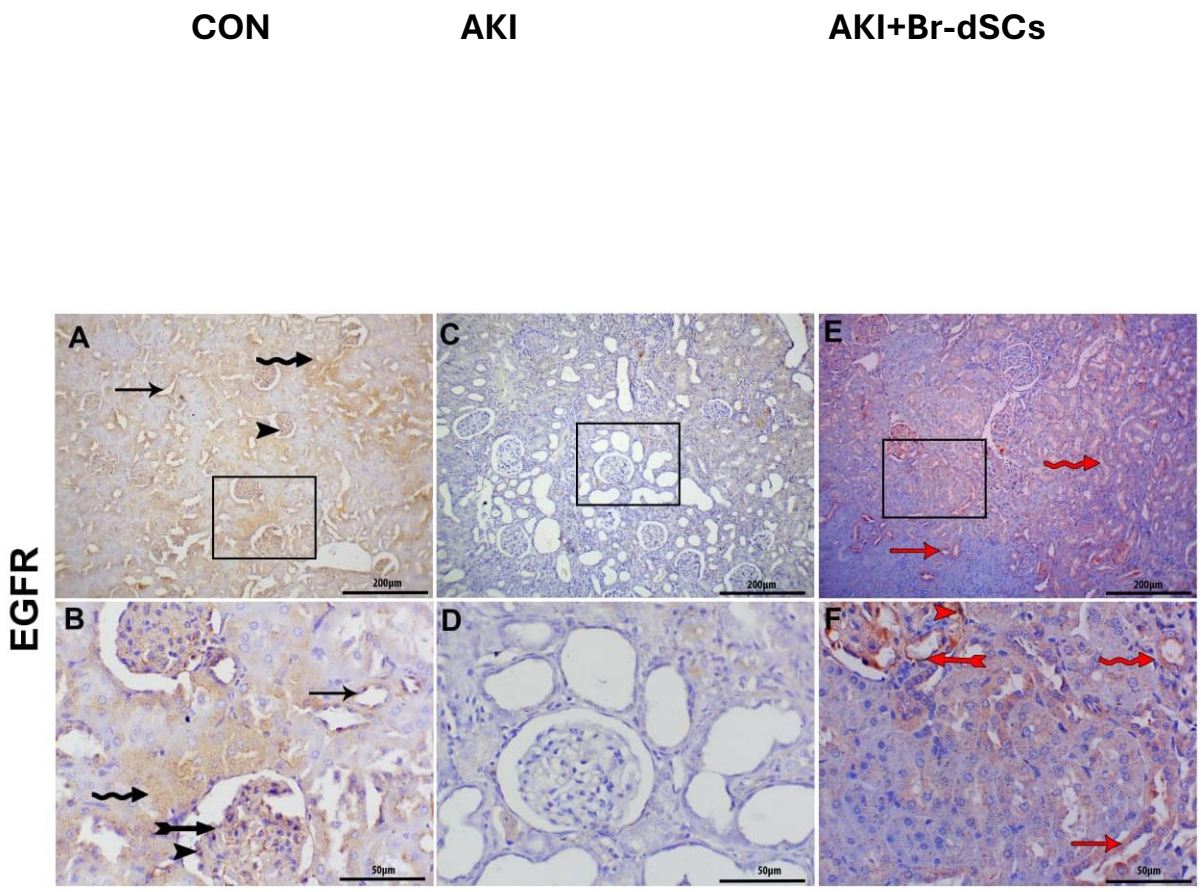

Figure 7: Representative photomicrographs of the EGFR immunohistochemical stained renal cortical tissue sections of the control (A), AKI (C), and AKI+ hBr-dSCs groups (E) and their respective higher magnifications (B, D, F) showing glomerular endothelium (biforked arrows), podocytes (arrowheads), proximal convoluted tubules (zigzag arrows) and cortical collecting ducts (arrows) with moderately, hardly detected, and strongly cytoplasmic immunostaining in the control, AKI, and AKI+ hBr-dSCs, respectively. Scale bars: 200 µm for A, C, and E; 50 µm for B, D, and F.

Human breast milk-derived stem cells enhance autophagy in Cisplatin-induced AKI

Using immunohistochemical staining, the localization of the Beclin1 autophagy protein in renal cortical tissues was investigated. In control group, the moderate Beclin1^{+} reactions localized in the cytoplasm of glomerular endothelium, podocyts, proximal convoluted tubules and cortical collecting ducts. On the other hand, these reactions were hardly detected in AKI group. Interestingly, the intense Beclin1^{+} reactions in glomerular endothelium, podocytes and almost of renal tubules appeared in AKI + hBr-dSCs group (Figure 8). Notably, the area % of Beclin1^{+} glomerular endothelium, podocytes and renal tubules was significantly decreased in AKI group compared to that of control group. On the other hand, this reaction was significantly elevated in the AKI + hBr-dSCs group compared to the AKI group (Table 1).

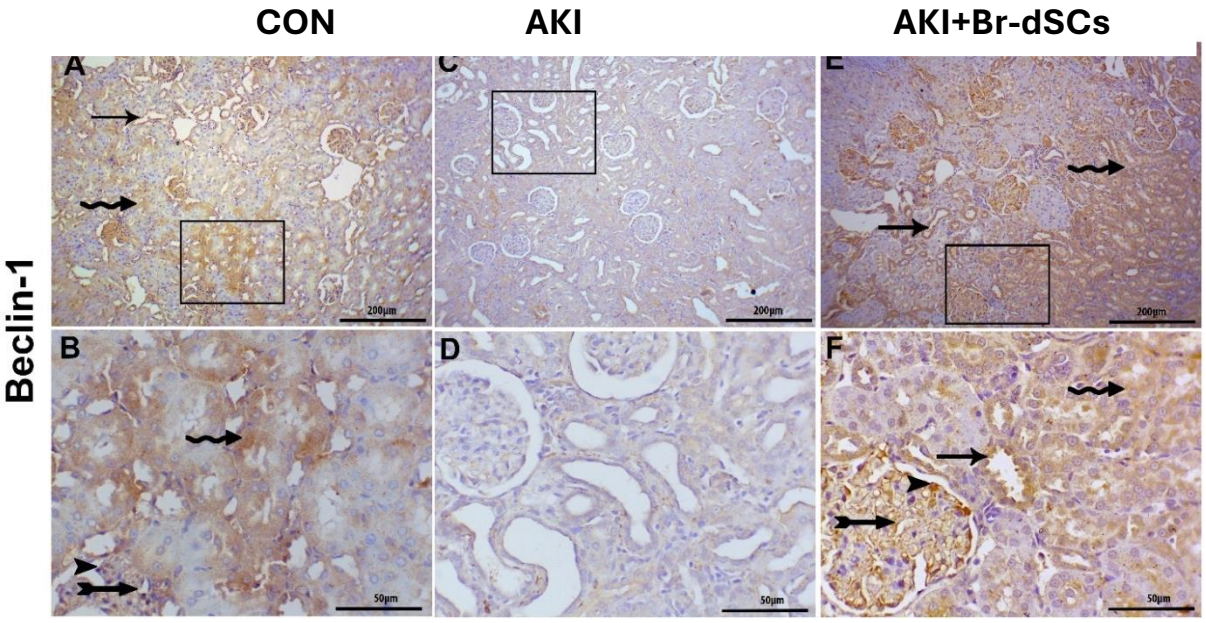

Figure 8: Representative photomicrographs of the Beclin-1 immunohistochemical stained renal cortical tissue sections of the control (A), AKI (C), and AKI+ hBr-dSCs groups (E) and their respective higher magnifications (B, D, F) showing glomerular endothelium (biforked arrows), podocytes (arrowheads), proximal convoluted tubules (zigzag arrows) and cortical collecting ducts (arrows) with moderately, hardly detected, and strongly cytoplasmic immunostaining in the CON, AKI, and AKI+ hBr-dSCs, respectively. Scale bars: 200 µm for A, C, and E; 50 µm for B, D, and F.

Discussion

Kidney illnesses have been more common over the past few decades despite the lack of effective treatment options. Furthermore, rather than curing the condition, practically all of current treatment modalities just halt its course and associated consequences [49].

MSCs meaning mesenchymal stem cells are considered to be as one of the promising therapeutic approaches that can stop the progression of kidney disease by using a variety of repairing mechanisms. Nevertheless, the use of MSCs is fraught with difficulties, including restricted tissue bioavailability as a result of lung trapping after IV injection [50, 51]. However, exosomal vesicles secreted by mesenchymal stem cells are rich in genetic material such as miRNA and mRNA, trophic factors, and soluble growth factors. These vesicles have the potential to both exert a tissue reparative process and mitigate increasing tissue damage [49]. Consequently, the current study was designed to examine the possible contribution of mesenchymal stem cells from breast milk to cisplatin-induced nephropathy, taking into account the underlying mechanism of epigenetic control of renal fibrosis and autophagy signaling pathways. The current study's findings demonstrated that the separated cells were characteristically fibroblastic in appearance, and were favorable for CD106, CD105 and CD90, while not favorable for HLA-DR, CD45, and CD34.

These data suggested that the isolated cells demonstrated mesenchymal stem cell characteristics [45, 52]. The onset and progression of both AKI and kidney disease (KD) are influenced by the Oxidative stress [53]. Numerous studies indicate that oxidative stress contributes to tubular kidney fibrosis, inflammation, and renal tubular epithelial cell death, all of which accelerate the progression of kidney disease [54, 55]. According to the current study's findings, the group with nephropathy had a significantly higher mean value for lipid peroxidation markers and kidney function tests, and a significantly lower mean value for antioxidant activity (CAT, SOD, and GPx), which is consistent with Ali *et al.* [56]. In recent years, MSCs have been employed as an antioxidant therapy to treat KD as numerous studies have demonstrated [41, 42]. Interestingly, administering Br-MSCs improves both the renal function test and the level of antioxidants and oxidants, respectively. Because of their antioxidant qualities, MSCs increase Calbindin-1 expression, this protein sequesters excess calcium, reduces the generation of reactive oxygen species ROS, and delays the programmed cell death [57]. Additionally, MSCs have the ability to reverse the effects of oxidative stress by stimulating the Nrf-2/Keap-1 pathway and enhancing activity of mitochondria through a paracrine impact brought on by the release of various soluble growth and trophic factors; heme oxygenase-1 [(HO-1), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and indoleamine 2,3-dioxygenase (IDO)] [58]. Moreover, as the system that regulates and repairs the quality of cells, autophagy is in charge of recycling damaged organelles and preserving energy during stressful situations. To get rid of hazardous proteins that lead to inflammation and cell death, it may also cooperate with the ubiquitin system [59]. It's interesting to note that the current study's results are consistent with the previously mentioned outcome because the AKI group demonstrated a clear decline in the beclin-1 immunohistochemical protein expression. This may cause the autophagy system to be actively blocked, increasing kidney cell death and inflammation [60]. On the other hand, Beclin-1 was dramatically elevated and the autophagy pathway—which preserves renal cellular energy and reduces inflammation and death in renal cells—was dynamically activated upon administration of Br-MSCs. The observed phenomenon may be explained by the capacity of Br-MSCs to enhance the expression of Small nucleolar RNA host gene 7 (SNHG-7), thereby upregulating the autophagy process. This is supported by the notable upregulation of Beclin-1's mRNA and protein expression, which are in charge of autophagic membrane nucleation and elongation [61, 62].

As documented previously [63], renal autophagy was activated by MSC transplantation, which reduced cardiac fibrosis. Since autophagy activation increases CD4+ lymphocytes and induces macrophages to exhibit an anti-inflammatory phenotype, it helps create a local anti-inflammatory microenvironment. Furthermore, it stimulates the expression of transcription factor EB (TFEB) /PGC-1α (peroxisome-proliferator-activated receptor-γ coactivator-1alpha)-mediated lysosome-autophagy, which cooperates with the ubiquitin system to break down harmful proteins that serve as auto-antigens [64]. preventing further tissue edema and inflammation, while autophagy suppression results in a rise in the production process of prostaglandin E2, an immunosuppressive substance [65]. Furthermore, A typical pathophysiological sign of the progression of renal damage, Which ultimately leads to renal disease at the end stage, is renal interstitial fibrosis [66]. As an epithelial-mesenchymal transition (EMT) is a critical process in renal tubular cells, inhibiting renal tubular EMT is a possible treatment approach for AKI. Renal

interstitial fibrosis is characterized by fibroblast proliferation and an imbalance between the production and degradation of ECM (extracellular matrix) [67]. This outcome made the antifibrotic qualities of Br-MSCs more evident [68, 69].

Interestingly, In AKI or chronic kidney disease (CKD), EGFR and IGF-1r activation has two effects: it reduces renal damage in experimental AKI by promoting tubular cell migration and proliferation, which triggers the regenerative phase that happens after acute renal damage. the findings of the present investigation corroborated with great strength the previously cited literature as the IGF-1r protein expression displayed a sharp decline in the positive immunostained cells in the AKI rat groups than the control one. However, Br-MSCs transplantation markedly upregulated the expression of those proteins than the AKI group, which could be owed to the MSCs' secretory effect for a number of growth and soluble factors among of them is IGF-1 and EGF-1r that in turn activated the expression of its receptors

Conclusion

Based on the previous finding it could speculated that Br-dSCs regenerate the injured kidney tissue via a modulating growth factor receptors expression and subsiding the renal oxidative state which diminished the renal fibrosis and initiate renal autophagy. Further, future experimental work will be required to fully address the exact epigenetic mechanism beyond this regenerative potency.

Conflict of interest:

All authors have reviewed and approved the paper for publication, and they all state that they have no conflicting financial or scientific interests.

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الملخص العربى

الخلايا الجذعية المشتقة من حليب الثدي تقلل من إصابة الكلى الحادة الناجمة عن السيسبلاتين في ذكور الفئران من خلال تعديل مستقبلات عامل نمو الكلى، والبلعمة الذاتية، والإجهاد التأكسدي

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يعد تلف الكلى الحاد أحد الأسباب الرئيسية لضعف وفشل الكلى، والذي لا تتوفر له سوى خيارات علاجية قليلة يمكنها الحفاظ على وظائف الكلى. تمثل الخلايا الجذعية أداة تجديدية واعدة، ومن بينها الخلايا الجذعية المشتقة من حليب الثدي (Br-dSCs)، والتي أظهرت إمكانات أكبر للقدرة التجديدية والمرونة في العديد من الأمراض مقارنة بأنواع أخرى من الخلايا الجذعية (SCs). ومع ذلك، لا يزال تطبيقها في اضطرابات إصابة الكلى بحاجة إلى دراسة كاملة، ولا يزال يتعين اكتشاف آلياتها التجديدية الأساسية. وبالتالي، يهدف البحث الحالي إلى فحص قدرة خلايا Br-dSCs على التجديد في إصابة الكلى الحادة ومعالجة الآليات الجزيئية المحتملة المتورطة في الوسائل الإصلاحية لهذه الخلايا فيما يتعلق بالالتهام الذاتي الخلوي. تم توزيع ثمانية عشر ذكر بالغ من جرذان سبراج داولي في ثلاث مجموعات متساوية، كل مجموعة تتكون من عشرة جرذان: المجموعة الضابطة، ومجموعة الفشل الكلوي الحاد، ومجموعة الفشل الكلوي الحاد + الخلايا الجذعية المشتقة من حليب الثدي. تم إجراء اختبارات وظائف الكلى على مستوى المصل، والإجهاد التأكسدي في الكلى، والفحص النسيجي المرضي، والتعبير المناعي الكيميائي لمستقبلات الأنسولين الكلوية لعامل النمو 1 (IGF-1r)، وعامل النمو البشري 1 (EGF-1r)، وعامل تعزيز الالتهام الذاتي بيكلين-1، وترسيب الكولاجين الكلوي. أظهرت النتائج أن Br-dSCs تعمل على تحسين مستوى نيتروجين اليوريا في الدم وحمض البوليك وكذلك الكرياتينين في المصل مما يجعلها في المستوى الفسيولوجى الطبيعى للمجموعة الضابطة. علاوة على ذلك، أدى إعطاء Br-dSCs إلى زيادة ملحوظة في IGF-1rوEGF-1rو beclin-1 وخفض تخليق الكولاجين وترسيبه. يمكننا أن نستنتج أن Br-dSCs تعمل على تجديد أنسجة الكلى المصابة من خلال مستقبل عامل النمو المنظم الذي يعزز عملية الالتهام الذاتي الكلوي.