Investigate the therapeutic effect of autologous adipose-derived stem cells (ADSCs) on ischemic renal failure in dogs.

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Abstract

Background/ aim: The kidney excretes waste materials and regulates important metabolic functions, and renal disorders constitute a significant medical problem and can result in fatalities. In the present study, mesenchymal stem cells derived from canine adipose tissue were isolated and evaluated for their ability to improve renal function in a canine model of ischemic renal failure. **Materials and Methods**: The canine ischemic renal failure model was developed by a traumatic occlusion of the renal artery in 10 dogs. ASCs were administered directly into the renal artery following ischemic renal failure induction. Blood analysis and histological parameters were analyzed. **Results**: The group treated with ASCs had decreased blood urea nitrogen and creatinine levels, and showed an improving histological manifestation. ASCs were detected around the tubules of these kidneys at the histological level. **Conclusion:** our findings suggest that ASCs could be an alternative therapeutic agent for canine ischemic renal failure.

Key words: *dog, ischemic renal failure, autologous adipose-derived stem cells*

Introduction

Acute renal failure (ARF) is a type of kidney disorder where epithelial cells of the renal proximal tubule in the nephron undergo necrosis as a result of ischemia or toxic damage. This leads to sudden decrease in glomerular filtration rate (GFR), which is caused by loss of auto-regulation, tubular obstruction, and increased renal vasoconstriction [(1)]. In kidney failure, the therapeutic strategies of renal replacement are still more sufficient than those of current hemodialysis [(2)]. The adipose tissue has recently been shown to be involved in the pathophysiology of renal disease and kidney failure [(3)]. The main cause of renal failure in dogs is renal ischemia. Usually renal failure caused by renal nephrotoxic substances, infection, renal ischemia, inflammation, shock or hypovolaemia and obstruction of urinary tract by calculi, strictures or tumors [(4, 5)]. Adipose-derived stem cells (ASCs) are confirmed as a source of multipotent stem cells that can be differentiated into osteogenic, chondrogenic, myogenic, and adipogenic cells in the presence of lineage-specific induction factors in vitro [(6)]. The major cause is tubular necrosis as a consequence of ischemic renal injury after episodes of hypotension or surgical vascular clamping [(7)]. The pathogenic incidents in ischemia/reperfusion damage include apoptosis and acute tubular necrosis, glomerular damage, and inflammation. Healing of inflammatory and ischemic renal injury include mesangial, epithelial and endothelial, regeneration [(8)].

Mesenchymal stem cells have the ability to divide, generate and differentiate, which at last is part of either an adult or embryonic tissue [(9)]. It has tremendous potential for the evolution of therapies at the future [(10)]. Mesenchymal stem cells can secrete growth factors and

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cytokines such as vascular endothelial growth factor (VEGF), insulin like growth factor-1 (IGF-1), hepatocyte growth factor (HGF) and anti-apoptotic cytokines which have capability to diminish the fibrosis of tissues through regeneration and play a role in tissue repair [11, 12]. MSCs have capability to differentiate into three types of cells such as chondrocyte, osteocytes, and adipocytes. Mesenchymal stem cells are immunosuppressive and non-immunogenic, and have the capability to migrate to sites of damaged tissue and inflammation to share in tissue healing [10, 13].

Adipose tissue is a more eligible source because of its plenty and rapid expansion rate in culture. Subcutaneous adipose tissue usually composed of mature adipocytes and a heterogeneous stromal vascular fraction which involve fibroblasts, pre-adipocytes, endothelial cells, vascular smooth muscle cells, monocytes, lymphocytes and adipose stem cells. Adipose stem cells are promising candidates for cellular therapy in regenerative medicine due to its properties such as anti-inflammatory, anti-apoptotic, proangiogenic, anti- scarring and immunomodulatory effects [14]. Angiogenesis based on the pathology of ischemic diseases, it is reasonable to believe that angiogenesis and vasculogenesis are the indispensable premises for tissue regeneration [15]. The purpose of the study reported here was to investigate the effects of autologous adipose stem cells on renal function and renal structural changes, in dogs.

Materials and Methods

Ten healthy dogs age range, 1 to 1.5 years; weight ranges, 15 to 18 kg were used in the study. It divided into two equal groups. Prior to the experiment, all dogs were acclimated for at least two weeks and screened for underlying diseases on the basis of results of a complete blood count CBC and serum biochemical analysis. During the experiment, dogs were housed separately in cages and fed dry food and water.

Evaluation of renal function: Renal function was evaluated based on blood urea and creatinine levels. Blood samples were collected from cephalic vein from each dog three days before surgery, three days after surgery and same period after treatment with ASCs to determine the serum chemistry profile. The reference range of serum BUN was 8-30 mg/dl and that of serum creatinine was 0.5-1.5 mg/dl, Serum BUN and creatinine levels above the reference range were considered abnormal.

Collection adipose tissues: Briefly, adipose tissue was obtained from subcutaneous, inguinal fat depots of dogs, using standard surgical procedures. At obtaining, adipose tissue was placed into a sterile 50 mL conical tube containing 15 mL of phosphate-buffered saline (PBS) and brought quickly to stem cells laboratory.

Cell culture and characterization:

Collected adipose tissue was washed three times with phosphate-buffered saline containing 100 IU/mL penicillin and 100 g/mL streptomycin, then chopping sample with scalpel blade size 15 and surgical forceps and digested for 1 hr. at 37 ˚C with collagenase type IA. The enzymatic activity was inhibited with (DMEM) Dulbecco's Modified Eagle's Medium containing 10% (FBS) fetal bovine serum. Following centrifugation at 1200 x g for 5 min, the pellet was filtered through a 70 µm falcon cell strainer to remove debris, then incubated in DMEM containing 10% FBS at 37˚C in a humidified atmosphere of 5% CO_2 after 48 hrs. cultures were washed with PBS to remove non-adherent cells and incubated with fresh medium, which was changed every 48hrs. until cells reached 70% to 80% confluence. The cells were then repeatedly subculture under standard conditions [4]. Cells were brought to a final volume of 2 mL with PBS and loaded into a sterile syringe for injection.

Induction of unilateral renal ischemia reperfusion (I/R):

After standard surgical preparation performing, Dogs were anesthetized by intramuscular injection mixture of ketamine HCL (15 mg/kg) and 2% xylazine (5 mg/kg). A midline abdominal incision was made to expose the left kidney. Blood supply to the kidney was interrupted by clamping the left renal artery using an artery clamp for 1 hour. After 1 hour the clamp was removed and reperfusion was confirmed visually. Then the catheter was inserted into the renal artery, while its proximal end inserted through a tunnel created in the dorsal wall of the abdominal cavity and fixed to the skin out the body with silk stitch suture and closed its opening permanently. The wound layers were then closed routinely and the animals were allowed to recover

with free access to food and water [16].

Intra-arterial ASCs infusion:

At day 3, after inducing ischemic renal failure, all dogs placed under general anesthesia, and ASC_S were injected directly into the left renal artery via a catheterization approach. A total of 10^6 ASCs suspended in 2 ml phosphate-buffered saline was slowly infused through the arterial catheter into the kidney over 1– 2 minutes in treated group while control group injected with normal saline.

Histopathological examination:

At day 10 of ischemic renal failure induction (day 7 of treatment with normal saline and ASCs infusion), the kidneys were harvested and fixed with 10% buffered formalin. The fixed tissues were embedded in paraffin wax and 4-µm sections were stained with hematoxylin and eosin (H&E) by standard procedures for histological examination.

Statistical analysis: Renal function analyzed using SPSS Statistics. Statistical analysis of serum BUN and creatinine was performed by one way ANOVA for comparisons among groups. P values of $p < 0.05$ were considered to record statistical significance. All data were expressed as means ± standard error (SE).

Results

Evaluation of renal function:

The results showed that the levels of serum blood urea nitrogen (22.70 ± 0.54 and 14.40 ± 1.67) and creatinine (0.93 ± 0.10 and 0.77 ± 0.10) in both groups at 3 days before induction ischemic renal failure was with in normal reference range. At 3 days after induction ischemic renal failure the results showed increase significant differences ($P < 0.05$) in the levels of BUN (36.80 ± 0.63 and 33.90 ± 1.27 in control and treated groups respectively) and creatinine (2.70 ± 0.10 and 2.71 ± 0.03 in control and treated groups respectively) compared with the levels at 3 days before surgery. While the levels of BUN and creatinine at 7 days after treatment showed continuous increasing in the group which treated with normal saline, but significantly decreased in the ASCs treated group. (Tab.1, Tab.2)

Table (1): Shows Mean Values of Creatinine mg/dl.

Time/days / Groups	3 days before surgery	3 days after surgery	10 days after surgery (7 days after treated)
Control	0.93 ± 0.10 A a	2.70 ± 0.10 B b	3.86 ± 0.09 C c
Treated	0.77 ± 0.10 A a	2.71 ± 0.03 B b	1.78 ± 0.03 C d

Similar capital letters horizontally denote no differences $P<0.05$ among periods.

Different small letters vertically refers to the existence of significant differences $P< 0.05$ among groups.

Table-2: Shows Mean Values of blood urea nitrogen (BUN) mg/dl.

Time/days / Groups	3 days before surgery	3 days after surgery	10 days after surgery (7 days after treated)
Control	22.70 ± 0.54 A a	36.80 ± 0.63 B b	39.87 ± 0.39 B c
Treated	14.40 ± 1.67 A b	33.90 ± 1.27 B b	30.20 ± 0.50 B d

Similar capital letters horizontally denote no differences P<0.05 among periods.

Different small letters vertically refers to the existence of significant differences P< 0.05 among groups.

Histopathological examination:

Control group at day 7 of treatment with normal saline infusion showed, atrophied glomeruli as well to peri-glomerular necrosis of renal tubules, in addition to marked dilation of some renal tubules. Hyperplasia of mesengial cells, as well to vacuolation of mesengial cells of the glomerulus, in addition to necrosis of peri-glomerular renal tubules with infiltration of inflammatory cells (Fig.1).

Treated group at day 7 of treatment with ASCs, revealed infiltration of polymorphic stem cells in the surrounding area of the renal arteriole, also there was an elongated fibroblast-like cells deposition in the necrotic renal tubules (Fig. 2) and in the peri-glomerular region (Fig. 3) as a replacement state.

Marked fibrin deposition in the peri-renal tubules area, and there was a marked area of fibroblasts proliferation in the renal parenchyma (Fig. 4).

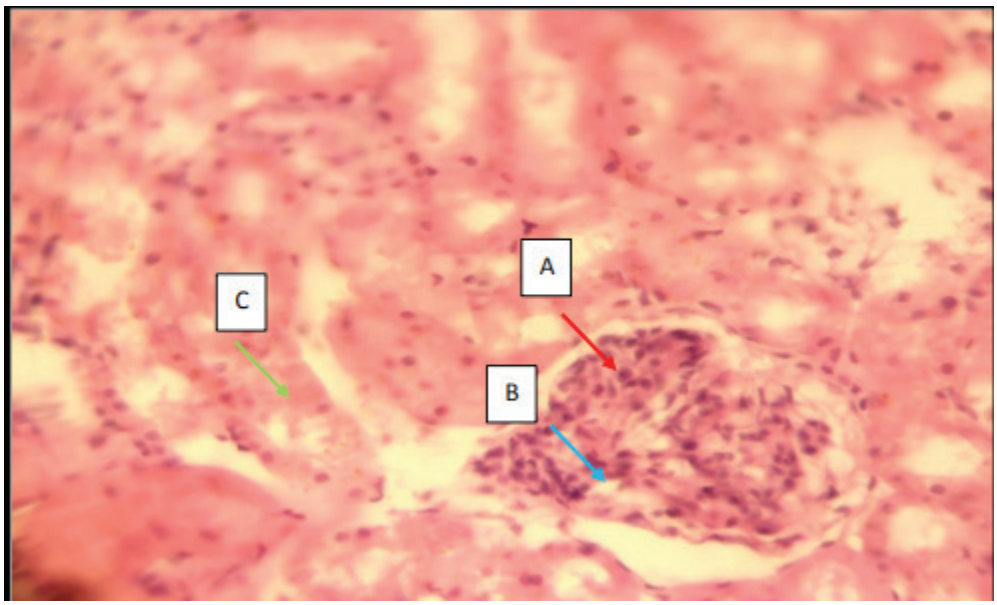


Figure (1): Control group at day 7 of treatment with normal saline infusion showed, hyperplasia of mesengial cells (A), as well to vacuolation of mesengial cells of glomerulus (B), in addition to necrosis of peri-glomerular renal tubules with infiltration of inflammatory cells (C). H&E. Stain. 40X

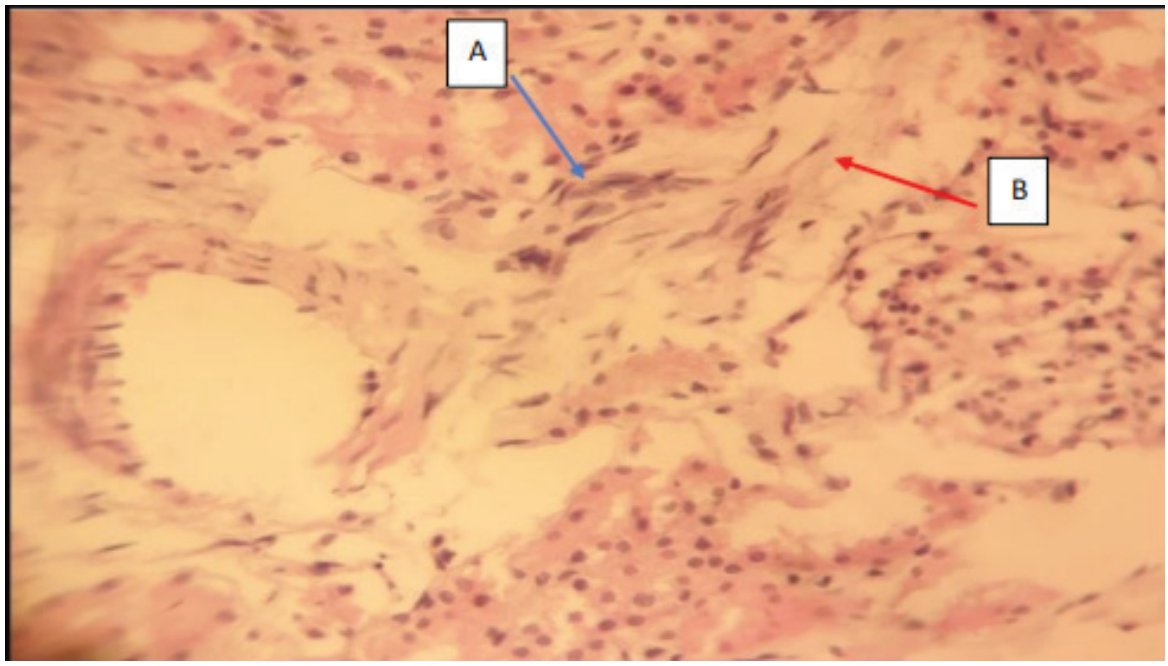


Figure (2): Histopathological section of kidney of treated group at day 7 of treatment with ASC_S showed infiltration of polymorphic stem cells in the surrounding area of renal arteriole (A), also there are an elongated fibroblast like cells deposition in the necrotic renal tubules as a replacement state (B). H&E Stain 40X.

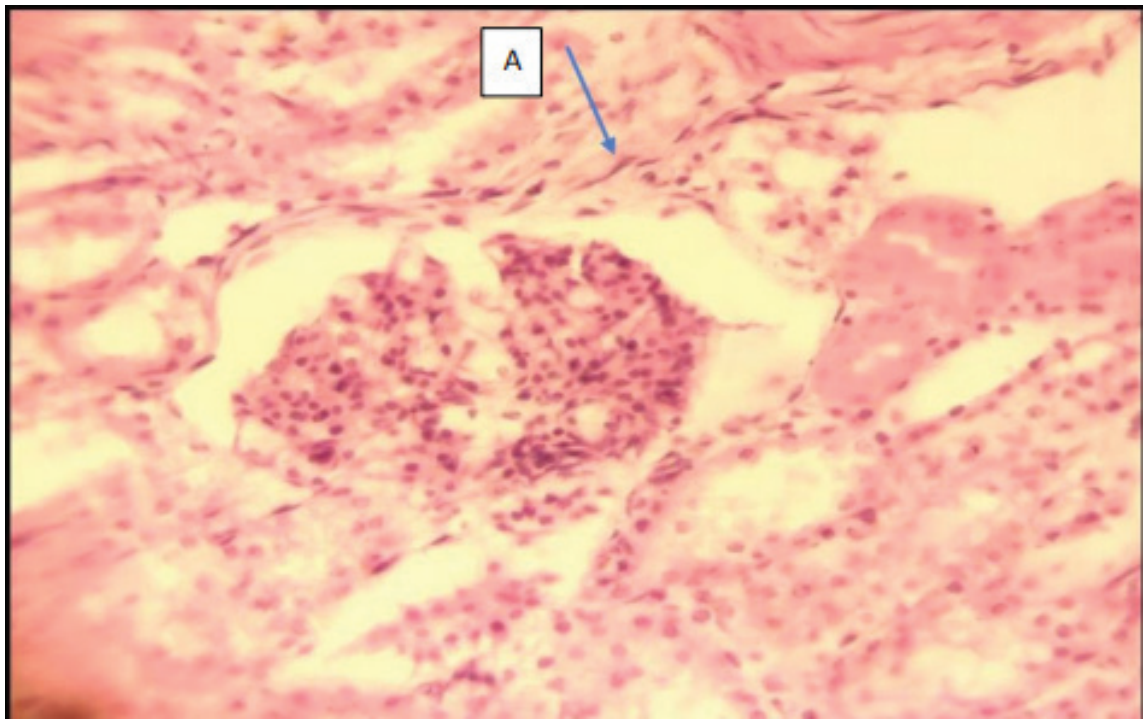
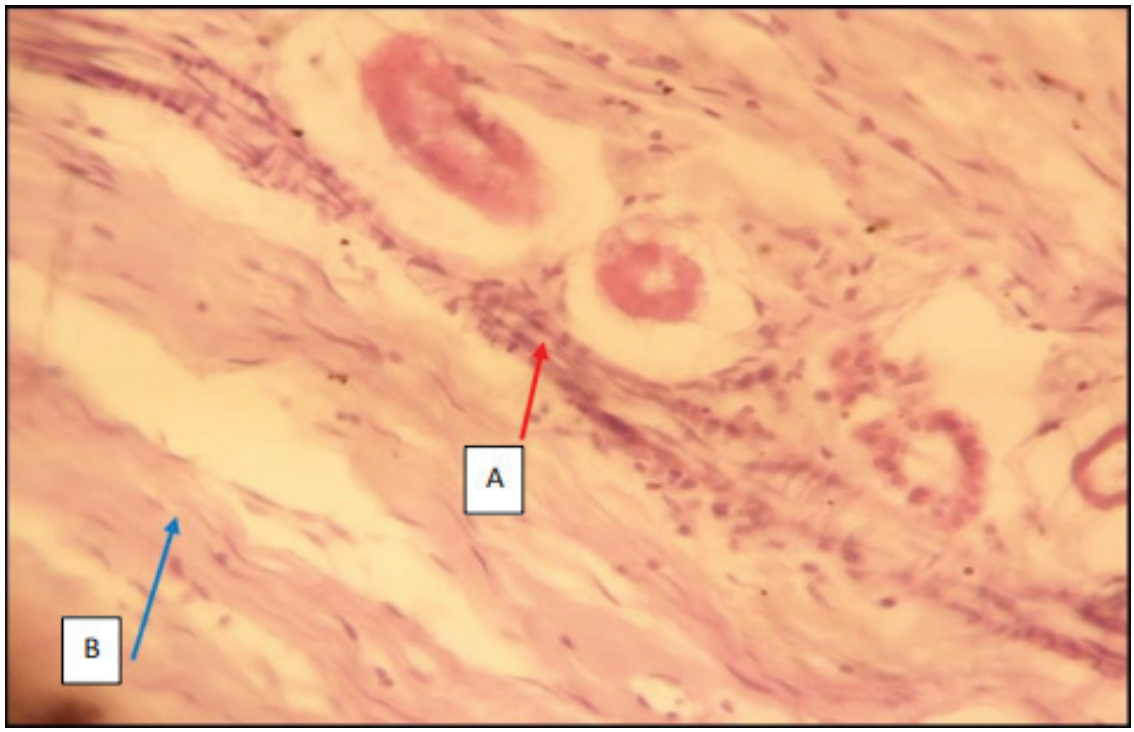


Figure (3): Histopathological section of kidney of treated group at day 7 of treatment with ASC_S showed elongated fibroblast like cells deposition in the peri-glomerular region as a replacement state (A). H&E. Stain 40X.



Discussion

Artificial ischemic renal failure is induced by mechanical or medical method [4]. In our research we used mechanical procedure and the induction time was 60 minutes. Monitoring serum BUN and creatinine levels alone are quite an accurate measure of renal function and a meaningful method in a clinical setting [17]. Creatinine is a muscular metabolic product. As a more precise indicator of renal function than BUN, creatinine is a significant criterion of the severity of renal failure [18]. Recently, the potential for renal repair by using stem cells has been clarified for various renal diseases. Among several sources of stem cells, adipose stem cells have attracted attention because of its ease of access [19]. Effects of adipose stem cells on the damaged organs occur through mesenchymal stem cells fusion with existing host cells rather than via true differentiation [20]. In our study, creatinine and blood urea nitrogen serum levels of the ASCs-treated ischemic group decreased after ASCs infusion compared with those observed in the ischemic renal failure injected with normal saline group this findings agreement with other workers [4]. Treating animals with mesenchymal stem cells will result in a reduction of creatinine and urea plasma [8]. MSCs may not only secrete cytokines within the injured kidney but also participate in endothelial cell proliferation or angiogenesis to facilitate renal regeneration [21].

To evaluate the therapeutic effects of ASCs in the ischemic renal failure group, we performed histopathological analysis which revealed infiltration of polymorphic stem cells in the surrounding area of the renal arteriole, also there was an elongated fibroblast-like cells deposition in the necrotic renal tubules and peri-glomerular region as a replacement state, in addition to marked fibrin deposition in the peri-renal tubules area, and there was a marked area of fibroblasts proliferation in the renal parenchyma, these finding reduce the damage in ASC_S group. Many studies have revealed that after local damage to the tissue, MSCs replace their damaged counterparts in the bone, fat, liver, heart, brain, heart and skeletal muscle [22]. MSCs therapy is effective, safe and reduced the rate of renal damage for patients with acute renal failure [20] and hastens the regenerative process [23].

The route of MSCs administration is more significant; in the other reports, intra carotid, jugular vein; cephalic vein, femoral artery and lateral caudal veins injection was used [22, 24, and 25]. Many reports have shown that systemically administered MSCs move through the blood circulation and are ultimately retained at the liver, lungs or spleen [24, 26]. Whereas we directly injected ASCs into target organ (kidney), as it could also

hold the cells. Migration of injected ASCs to damaged tissues can accelerate renal healing. Local vasculature procedure in cell therapy is an attractive approach in which select cells can be introduced into an aimed area and that will result in achieving cell localization with infusion little number of stem cells as well as it could minimize superfluous adverse effects and this considered an advantage in stem cells therapy as reported recently [19].

Conclusions

The administration of ASCs trans catheterization lead to improve renal excretory function, which was verified by the levels of serum BUN and creatinine in the canine ischemic renal failure model also play important role in renal tissue reconstitution there for, our findings suggest that ASCs could be an alternative therapeutic agent for canine ischemic renal failure.

Conflict of Interest: None

Funding: Self-funding

Ethical Clearance: The present study was approved by the Animal Welfare and Ethics Committee and Faculty of Veterinary Medicine/ University of Basrah. Copy Enclosed

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