Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Efficacy of Adipose Tissue Mesenchymal Stem Cells Implantation on Regeneration of Acute Spinal Cord Injury in Dogs Model

Ahmed Kadhim Munahi * Hameed A. Al-Timmemi **
***Department of surgery and obstetrics, college of veterinary medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.**
****Department of surgery and obstetrics, college of veterinary medicine, University of Baghdad, Baghdad, Iraq.**
E.mail: hamed.a@covm.uobaghdad.edu.iq
Phone: +9747706762455
ORCID: 0000-0002-2263-3410

Abstract

Spinal cord injury is the most prevalent cause of permanent neurological disorders, which can result in full palsy in severe cases. Unfortunately, most spinal cord injury treatments are ineffectual. The current study aims to assess the effect of adipose-derived mesenchymal stem cells on spinal cord regeneration in dogs. Sixteen healthy mongrel dogs were employed for this purpose. They were randomly separated into two equal groups (n=8). Dorsal laminectomy and left lateral hemisection at the level of the second lumbar vertebra were performed on all dogs. The hemisections in the control group were treated with 0.2 mL of phosphate buffer saline. The stem cell group with the hemisection cavity was treated with adipose-derived mesenchymal stem cells 50 µl ($5x10^6$), for bridging lesion sites after the operation. Each group was subsequently subdivided into two equal subgroups (n=4) based on the post-operative periods, which were the eighth and sixteenth weeks for clinical follow-up and histological tests. Clinical testing of motor and sensory spinal cord functions indicated statistically significant ($p < 0.05$) differences between the stem cell and control groups. Histopathological examinations of the stem cell group revealed reduced cavitation, orientation of regenerative nerve fibers in white matter, increased number of regenerative neuron cells in grey matter, increased angiogenesis, and minimal scar tissue formation at the injured spinal cord site. In conclusion, the current study demonstrated that the adipose-derived mesenchymal stem cells accelerated and improved regeneration of the damaged spinal cord based on clinical and histological findings.

Keywords: Adipose tissue, stem cells, spinal cord injury, dog.

Introduction

Spinal cord injury (SCI) can produce a sudden and devastating impact on quality of life due to severe motor, sensory, and autonomic dysfunction, comprising bowel, bladder, and sexual impairment (1). Traumatic spinal injury results from injury to bony, ligamentous and/or neurological structures of the spinal column and acute intervertebral disc extrusion can cause significant morbidity and mortality (2). Spinal cord injury can be treated by five major modalities; neuroprotective and neuroregenerative pharmaceuticals, neuromodulation, stem cell-based therapies, and various external prosthetic devices. (3).Cell therapies exhibit neuroprotective and nerve regeneration potential in SCI with different targets and responses to stimuli, such as regulating inflammatory responses, providing nutritional support, and improving plasticity (4). Although excessive potential mechanisms, various cells from different tissue sources, including bone marrow mesenchymal stem cells (BM-MSCs) which have secrete neurotrophic factors, promote axonal regeneration reduce inflammatory

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

reactions and reduce astroglial scarring density (5 , 6).Bone marrow mesenchymal stem cell scaffold improved somatosensory parameters and hind limb performance after SCI in dogs. Adipose-derived MSCs (AD-MSC) transplantation demonstrated satisfactory effects in chronic and acute SCI (7). Intravenous administration of AD-MSCs activates angiogenesis and upregulates extracellular signal-regulated kinase (ERK) which improves hind limb motor function (8,9). Adipose-derived MSCs (AD-MSCs) also promote cell survival and tissue repair by improving motor function following SCI in animal models, when used in the acute phase of injury (10).There are few documents on the study of stromal cells used in treatment of spinal cord injury in dogs model so the objectives of this research will be focused on isolation and identification of adipose-derived mesenchymal stem cells and assessment the efficiency of the adipose tissue mesenchymal stem cells implantation on repair of the injured spinal cord.

Materials and Methods

Sixteen healthy mongrel male dogs weighing 15-20 kg and aged 8-12 months were employed. The dogs were kept in individual cages and supplied commercial food and water. The animals were given Ceftriaxone (22 mg/kg) broad-spectrum antibiotic injection intramuscularly twice a day for five days and anthelmintic injection of 0.2 mg/kg Ivermectin (Ivomec, Holland) subcutaneously was given .The animals were randomly assigned into two equal groups (n=8) for dorsal laminectomy and left lateral hemisection cordectomy at the second lumbar vertebra.The control group (n=8) the hemisection was treated with 0.2 ml phosphate buffer saline and stem cell group (n=8) was treated with adipose-derived mesenchymal stem cells $5x10^6$ (7) were implanted locally at the site of injured spinal cord. All animals were followed up clinically included motor and sensory reflex weekly beginning with the first week of the study and ending with the 16th week post operation (PO). After the eighth and sixteenth weeks, the animals in each group were euthanized for histological examination.

Adipose Derived Mesenchymal Stem Cells

Adipose Tissue Sampling of Dog

Following anesthesia, the subcutaneous abdominal region had been clipped and disinfected. Adipose tissue was collected aseptically and processed to isolate and identify adipose-derived mesenchymal stem cells for use in this work.

In Vitro Protocol

Isolation of Adipose-MSCs

Isolation of AD-MSCs from adipose tissue aspirate was described by (11,12), AD-MSCs were created in the (Stem Cells Lab, College of Veterinary Medicine, University of Baghdad). Canine adipose tissue was washed with sterile PBS, minced, adding 1% (v/v) antibiotic-antimycotic and placed in a 0.2% collagenase type I solution for digestion for 50 minutes at 37 degrees Celsius, 5% CO2, and 95% humidity, with brief stirring every 10 minutes. FBS (10%) was added to the digested tissue; the suspension was filtered through a cell strainer 70 µm and centrifuged for 5 minutes at 2000 rpm (1400 g).The cell pellet was resuspended in 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) Low Glucose and centrifuged under the same conditions. Finally, the pellet was resuspended in 79% DMEM Low Glucose + 20% FBS (Santa Cruz, USA) + 1% antibiotic-antimycotic (basal media) that had been pre-warmed. Tissue culture flasks were labeled and incubated at 37 degrees Celsius, 5% CO2, and 95% humidity. The media was changed 24 hours later, and cell growth was monitored daily until the cell sheets were confluent. The cells splitting are repeated for four consecutive sub-cultures and all non-adherent cells were removed. Confluent, adherent cells were designated P0. Passaging was carried out in a T50 cell culture flask with basal medium. A haemocytometer (Neubaur, Hemocytometer, Hawksleyand son. Ltd, England) was used to count the quantity of cells in each culture flask. After discarding the media, the cells were rinsed with PBS and

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

trypsin was added to detach them. After that, 10 ml of fresh DMEM was added to replace the trypsin. The medium and cells were collected in a test tube and centrifuged at 2000 rpm for five minutes at refrigerated centrifuge 40C (Hettich, Germany). The pellet was re-suspended in 1 ml DMEM after the supernatant was decanted. In a sterile manner, 0.1 ml of the cell suspension was withdrawn and put to a dilution tube containing 0.8 ml of DMEM and 0.1 ml of 0.4% Trypan Blue stain. The mixture was gently mixed and incubated at room temperature for 5 minutes before the stained cell suspension was transferred to a haemocytometer, the latter was covered with a cover slip. A little drop of the cell suspension was aseptically extracted with a pasteur pipette and deposited on both sides of the haemocytometer before being observed using an inverted microscope (Optika, Japan). The total number of viable cells in each of the haemocytometer's four corners was counted. The total number of cells taken from the tissue culture flasks was calculated using the formula below (13). NCxDx104 /#Q NC=number of count vital cells (non-vital cells are dyed blue), D=sample dilution (10) and Q=number of haemocytometer squares used.

Differentiation of AD-MSCs

To assess the "stemness" of formed cultures, cells were stimulated to differentiate toward adipogenic and osteogenic differentiation using suitable cultures (14).

Adipogenesis of AD-MSCs

Cells show lipid droplets deposition in the cytoplasm of adipocytes during adipogenic differentiation, which can be stained with Oil Red O. On passage four, mesenchymal stem cells were collected following complete confluence. The cell suspension was plated into DMEM-based mesenchymal stem cell expansion media at a density of 105 cells per cm2 of a 25cm2 tissue culture flask (SPL®, Korea) with 5 ml per flask. At 37°C, 5% CO2, and 90% humidity were used to incubate the flask. After the cells had reached 100% confluence, 1.0 ml of adipogenesis induction medium (DMEM-low glucose, fetal bovine serum 10%, 10 mM Dexamethasone, 0.5 M 3-isobutyl-1-methylxanthine, recombinant human insulin 10 mg/ml, 10 mM Indomethacin, penicillin and streptomycin) (PAA, Austria) was added. During the 21-day culture period, the adipocytes' induction media was replaced every 2-3 days.

Osteogenesis of AD-MSCs

During osteogenic differentiation, the cells expressed increased amounts of alkaline phosphatase and generated an intracytoplasmic mineralized osteocyte matrix that can be stained with Alizarian Red S. In 1ml of DMEM, mesenchymal stem cells were isolated. The cell suspension was injected in DMEM at a density of 105 cells per cm2 of a 25 cm2 tissue culture flask and incubated at 37°C in 5% CO2 and 90% humidity. After achieving 100% confluence, the media was aspirated and 1ml osteogenesis induction medium (DMEM-low glucose, fetal bovine serum 10%, 10 mM Dexamethasone solution, ascorbic acid 2-Phosphate solution, L-glutamine, penicillin and streptomycin) (PAA, Austria) was added to each well. During the 21-day culture period, the osteogenic induction medium was replaced every 2-3 days.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

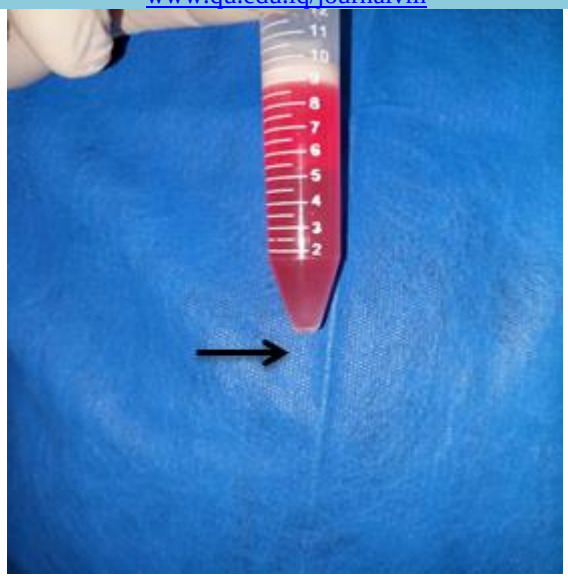
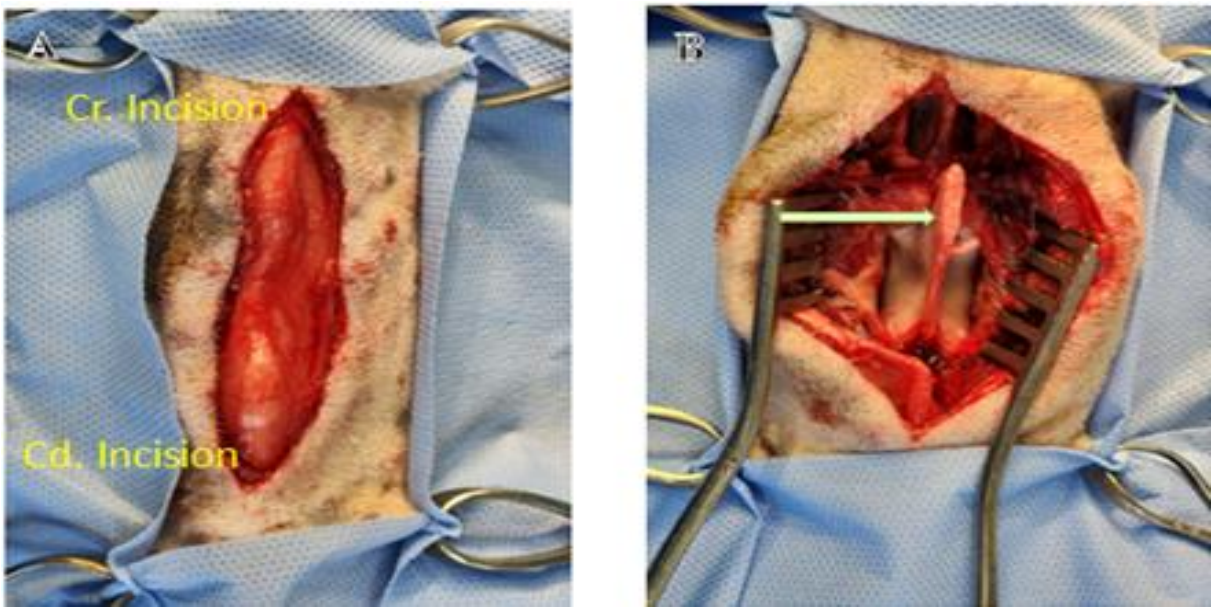


Figure (1). Shows harvested AD-MSCs on Passage 4 (arrow).

Holland) and 15mg/kg Ketamine hydrochloride (Kepro®, Holland) intramuscularly (15,16,17). The surgical dorsal laminectomy procedure on which the current investigation was based as illustrated by (18). (Fig. 2. A,B) and (Fig. 3. A,B).

Experimental Animals

Prior to anesthesia, the dogs were fasted for six hours. Dogs were premedicated with 0.03 mg/kg atropine sulfate (Kepro®, Holland), then anaesthetized with a mixture of 5 mg/kg Xylazine hydrochloride (Xyla®,



Figure(2). Photograph showing the initial steps of hemicordectomy. A. Shows the surgical incision over dorsal midline from L1 to L3. B. Elevated epaxial muscles from dorsal spinous processes, laminae, articular facets, and pedicles of L2 (arrow).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

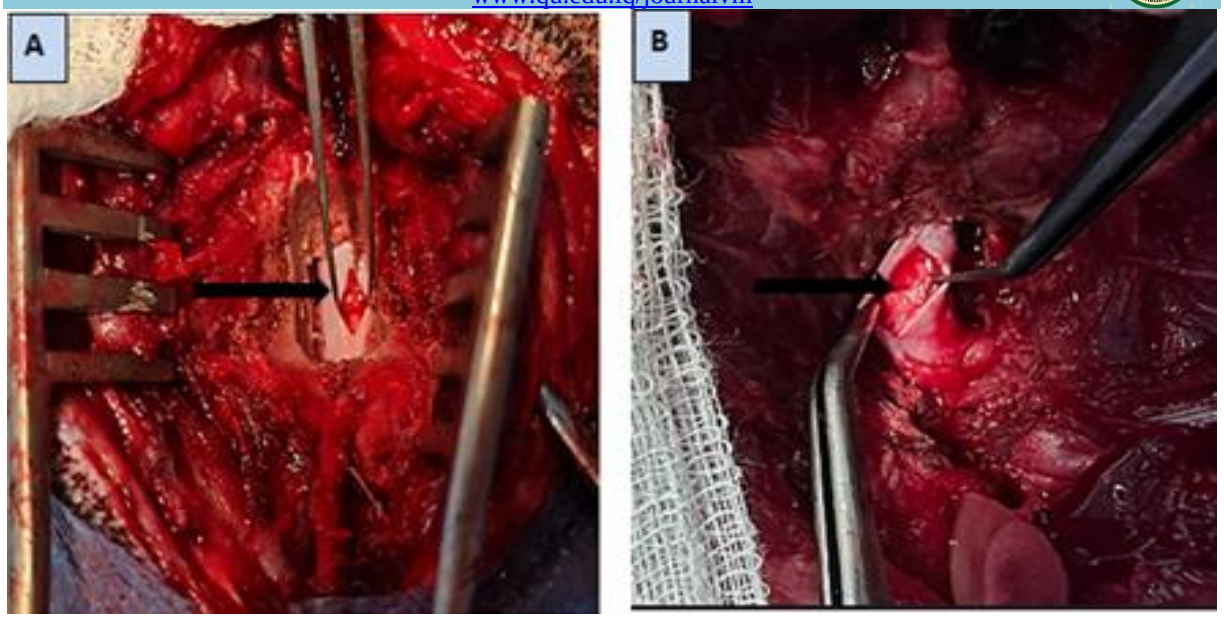


Figure (3). Photograph showing. A. A longitudinal incision is made through the meninges (arrow) B. Left lateral hemisection of the spinal cord (arrow).

The stem cells group carried out by implantation the hemisection site with a ($5x10^6$) AD-MSCs, the dura mater was closed with 4/0 Vicryl (Fig. 4. A, B).

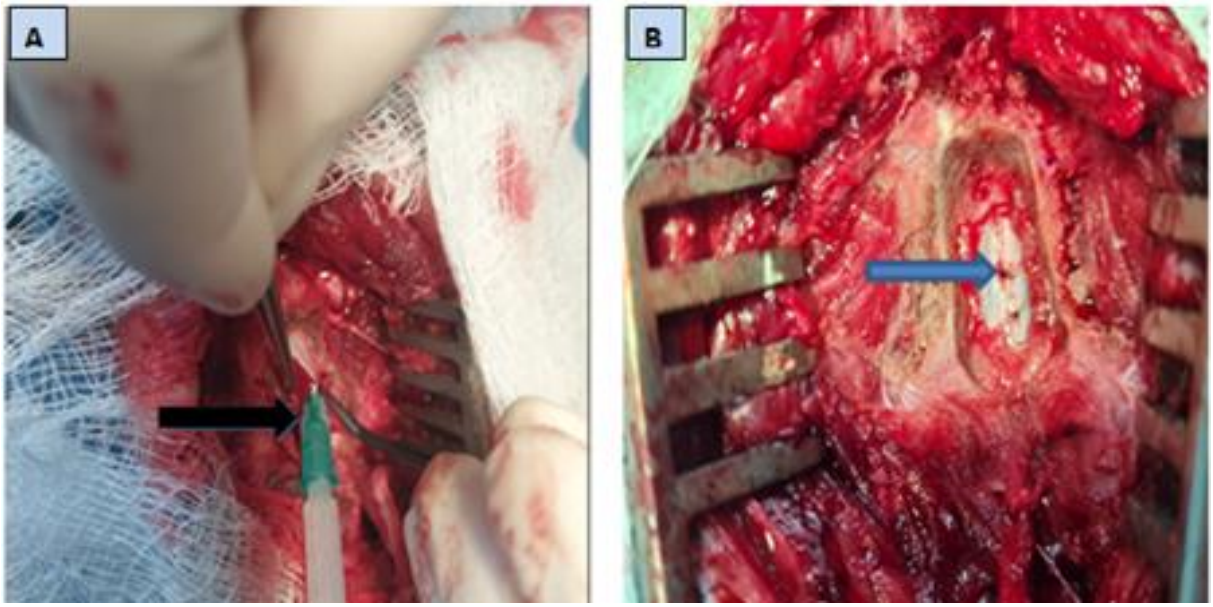


Figure (4). Photographs show. A. Transmplantation AD-MSCs at the injured spinal cord (arrow) B. Closed Dura matter (arrow).

Evaluation of the Motor Functions

All of the animals survived surgery and were later used for analysis. Tarlov Scale Modified (19) and Texas Spinal Cord Injury Scale (TSCIS) (20). The locomotor recovery was evaluated using a behavioral evaluation system. Knuckling function improvement was scored on a scale of normal, mild, moderate, and severe (Tab. 1).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Table 1. Modified clinical signs grading system for motor recovery (19,20).

Clinical observation	Grade	Description
Gate	**6**	**complete motor activity**
	5	**Normal gate but inability to leap**
	4	**Ability to walk with minor difficulty**
	3	**Ability to walk with minor difficulty**
	2	**Ability to push upon hind leg and take few steps**
	1	**Ability to push upon hind leg but no take steps**
	0	**Total paralysis of hind leg**

Sensory Functions Evaluations

The superficial nociception (soft tissue pain) was tested by pricking the lateral aspect of the leg and planter surface of the foot with needle, Deep nociception (bone or joint pain) was assessed by pinching the most distal region of a digit with forceps squeezing (Tab. 2).

Table (2). Texas Spinal Cord Injury score (TSCIS) Modified Scoring for Evaluation the Sensory Clinical Signs by (208).

Clinical Observation	Description	Score
1.Superficial nociception a.(lateral aspect leg sensation)	**Induced by pricking the lateral aspect of leg with needle**	
Absent deep and superficial Present superficial noci. Present deep noci. Present deep and superficial		**0** **1** **2** **3**
b.(Toe Prick)	**Reflex induced by pricking the planter surface of foot with needle**	
Absent deep and superficial Present superficial noci. Present deep noci. Present deep and superficial		**0** **1** **2** **3**
2.Deep nociception (Toe pinch)	**Reflex induced by pinching the most distal portion of digit with forceps**	
Absent deep and superficial Present superficial noci. Present deep noci. Present deep and superficial		**0** **1** **2** **3**

Histopathological Examination

The neurohistopathological investigation was carried out at the 8th and 16th weeks postoperatively (four dogs per period) were anesthetized with xylazine and Ketamine before being euthanized with a 10% intracardial injection of formaline.

Ethical approval:

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, reviewed and approved all procedures. involved in the current study.

Statistical Analysis

The Statistical Analysis System- SAS (2009) program was utilized to evaluate the impact of various factors on research parameters. In this study, the least significant difference -LSD test (ANOVA) was used to compare means (21).

Results

Identifications and Characterization of Adipose Derived Mesenchymal Stem Cells

The culture of adipose mesenchymal stem cells (AD-MSCs) on special media revealed that on the first day of primary culture, the majority of cells were floating round shape in culture medium, while the remaining cells began to stick to the plastic surface of the

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

culture flask (Fig. 5. A). After ten days, colonies gradually grow in size and link to create a monolayer of adherent cells (Fig. 5. B). After 12 days of culture, these cells had small rounded, spindle-shaped, or large flattened morphology (Fig. 6. A). Adipose-derived mesenchymal stem cells demonstrated high confluence of spindle-shaped cells on second passage (P2) at 2 days post culture, and the cells oriented themselves along their longitudinal axis. (Fig. 6. B). After 5 days of culturing, the ADMSCs at P3 revealed a variety of big, flat, spindle-shaped, round, and polygonal-shaped cells (Fig.7. A). At P4, the ADMSCs morphologically changed into homogeneous fibroblastic-like cells. (Fig. 7. B).

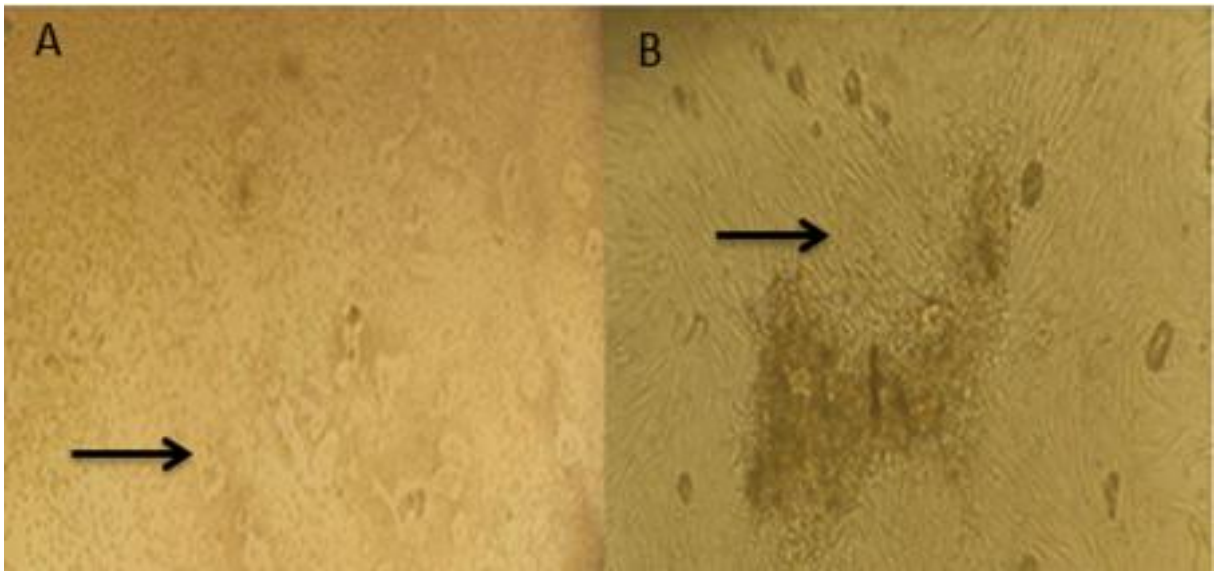
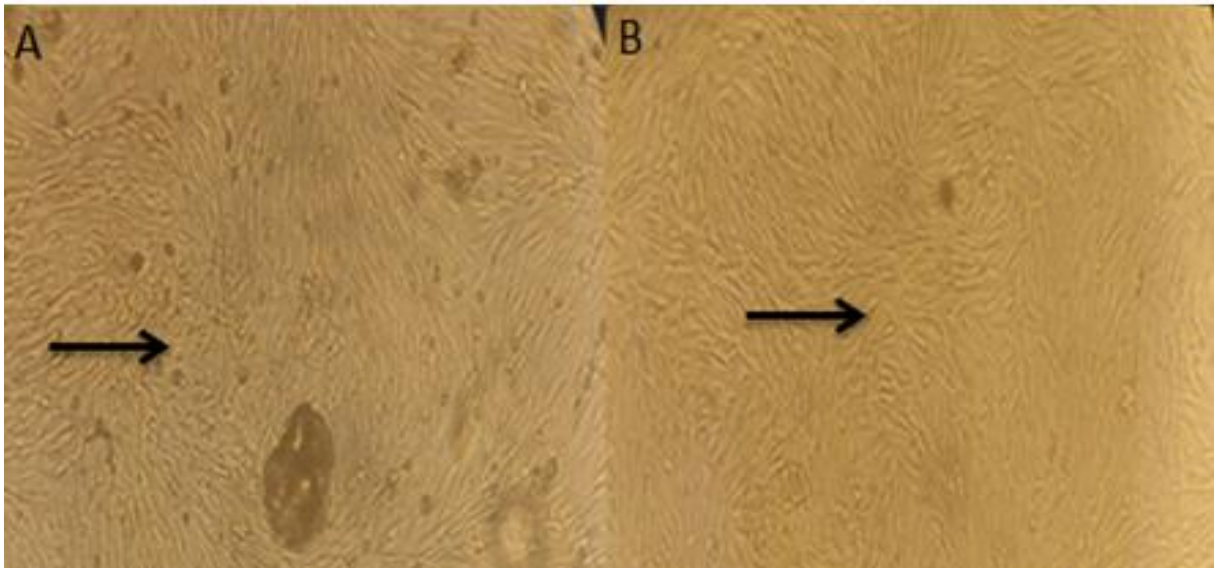


Figure (5). Photograph depicting the growth of ADMSCs A. On the first day of primary culture, the majority of cells are floating round in culture fluid (arrow), with the remainder cells adhering to the plastic surface of the culture flask. B. After ten days in culture, colonies grew in size and linked to form a monolayer of adherent cells. (arrow) X100



Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Figure (6). A. Light micrograph of Adipose derived mesenchymal stem cells of dog on passage 1 shows spindle polymorphic cells with round and polygonal cells (arrow). B. fibroblastic-like AD-MSCs colonies grew to confluence at second-passage culture (P2) (arrow) X100.

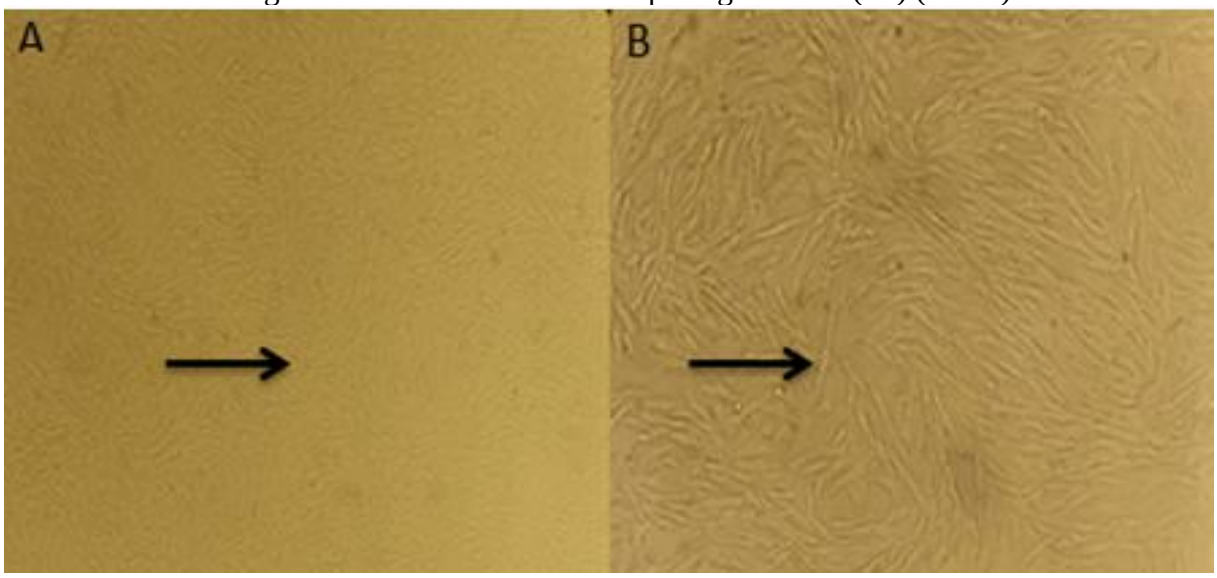


Figure (7). A. Light micrograph of Adipose derived mesenchymal stem cells of dog on passage 3 displays a variety of big, flat, spindle-shaped cells, round cells, and polygonal-shaped cells. (arrow) B. AD-MSCs are fibroblast-like cells (P4) shows spindle cells (arrow) X100.

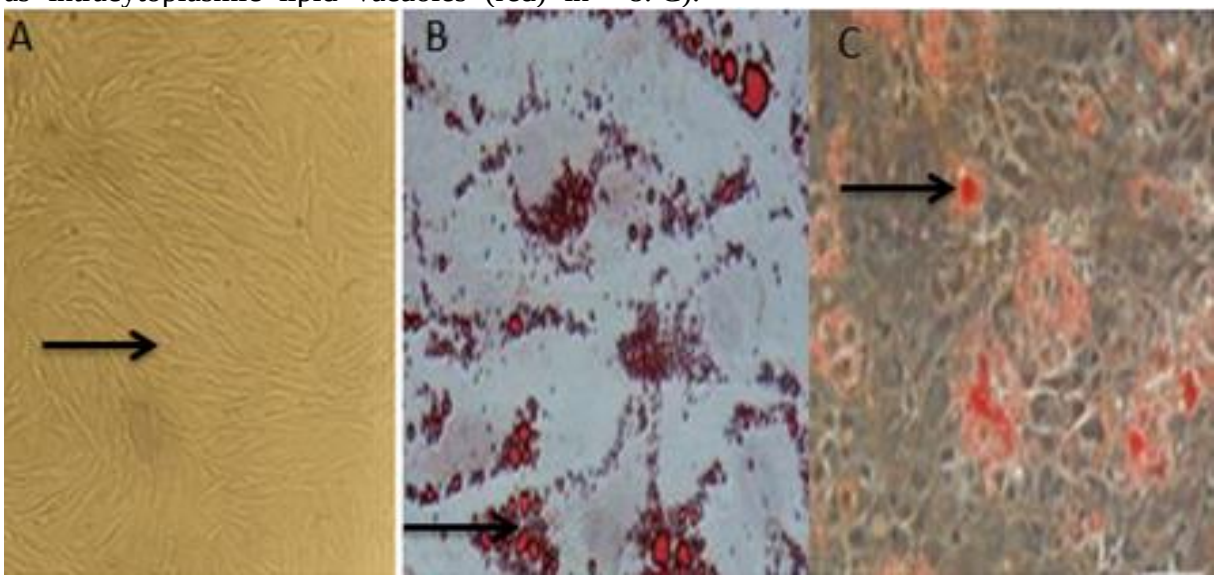
Differentiation of Adipose Derived Mesenchymal Stem Cells

Adipogenesis of Adipose Derived Mesenchymal Stem Cells

Morphologically, the adipose-derived mesenchymal stem cells were homogeneous fibroblast-like cells at P4 (Fig. 8. A) AD-MSCs were differentiated into adipogenic cell after 21 days and became mature adipocytes. The intracellular buildup of lipid droplets seen as intracytoplasmic lipid vacuoles (red) in adipocytes was stained red with Oil Red O solution, while the cells' nucleus was stained black with hematoxylin. (Fig. 8. B).

Osteogenesis of the Bone Marrow Stem Cell

Adipose derived mesenchymal stem cells differentiated into osteogenic cell after 21 days, resulting in mature star-shaped osteocytes with calcium deposits that stained orange red with Alizarin Red Solution. (Fig. 8. C).



Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Figure 8. Light micrograph shows AD-MSCs of dog A. AD-MSCs are fibroblast-like cells at P4 show spindle-shape cells (control). B. Differentiated adipocytes show accumulation of intracellular lipid droplets detected by Oil Red O stain (arrow) Oil Red O stain. X100. C. Differentiated osteogenic cells display deposition of calcium (arrow) determined by Alizarin Red S staining. X100

Clinical Evaluation

Control Group

From the first to the second week after surgery, all animals displayed significant dysfunction characterised by full paralysis of pelvic limbs with dragging of the caudal half of the body during walking (Tab. 3) and severe knuckling (Tab. 6). However, there were no deep or superficial pain sensations in the hind leg recorded (Tab. 4). The clinical examination revealed that total paralysis of the hind limb was obvious four weeks after surgery, and some animals suffered from skin erosion on the dorsum of the hind limbs three weeks after surgery as a result of the animals crawling on the hind limbs (Tab. 3). All of the animals, however, developed severe knuckling (Tab. 6). There were no feelings of the hind limb recorded (Tab. 4). Two animals in this group had total paralysis of their hind limbs that lasted until the end of the trial, and six animals were able to push up on their rear leg but were unable to take a few steps at the end of the eight week after surgery period (Tab. 3). However, knuckling was severe eight weeks after the operation (Tab. 6). There were no feelings of the hind limb recorded. (Tab. 4). Clinical assessments at the conclusion of the investigation demonstrated no improvement in normal gait after sixteen weeks (Tab. 3). Knuckling was moderate and persisted throughout the trial (Tab. 6). Sensation was still missing (Tab. 4).

Stem Cell Group

The clinical assessment for this group revealed entire paralysis of the hind limbs beginning on the first post-treatment day and lasting until the end of the second week (Tab. 3), as well as severe knuckling at the end of the second week (Tab. 6). However, there were no feelings of the hind limb noticed (Tab. 4). All animals in this group demonstrated the ability to push on the hind limb without taking a few steps beginning at the third week post treatment (Tab. 3) and knuckling remained severe until the end of the fourth week post treatment (Tab. 6) but there were no sensations (Tab. 4).All animals were able to walk with major difficulty from the beginning of the seventh week until the completion of the eighth week post treatment (Tab. 3), and the knuckling became moderate on the eight week post-treatment (Tab. 6) but the sensation was still gone at the end of the eighth-week post-treatment (Tab. 4).The animals resumed normal pelvic gait motion from thirteen weeks after therapy until the completion of the experiment (Tab. 3), and the knuckling was mild at sixteen weeks (Tab. 6). However, by the end of the study, the sensation had gradually advanced towards the foot. Lateral aspect leg and toe prick responses were observed on days 95 and 99 post-treatment, respectively, while toe pinch response was shown on day 83 (Tab. 4).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Table (3). Neurologic Status as Measured by the Modified Tarlov Neurologic Recovery Scale in All Groups (Subgroup N=4).

Time Week	Control group	Stem cells group
1Wk PO	Total paralysis of hind leg	Total paralysis of hind leg
2Wk PO	Total paralysis of hind leg	Total paralysis of hind leg
3Wk PO	Total paralysis of hind leg	Ability to push upon hind leg but don't take steps
4Wk PO	Total paralysis of hind leg	Ability to push upon hind leg but don't take steps
5Wk PO	Total paralysis of hind leg	Ability to push upon hind leg and take few steps
6Wk PO	Ability to push upon hind leg but don't take steps	Ability to push upon hind leg and take few steps
7Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with major difficulty
8Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with major difficulty
9Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with minor difficulty
10Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with minor difficulty
11Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with minor difficulty
12Wk PO	Ability to push up hind leg and take few steps	Ability to walk with minor difficulty
13Wk PO	Ability to push up hind leg and take few steps	Normal gait but inability to leap
14Wk PO	Ability to push up hind leg and take few steps	Normal gait to leap
15Wk PO	Ability to push up hind leg and take few steps	Normal gait to leap
16Wk PO	Ability to push up hind leg and take few steps	Normal gait to leap

Table (4). The Mean Time (Weeks) of Sensory Clinical Observations in All Groups During the Study Period (Subgroup n=4).

Control	**(Sup. nociception) Lat. Aspect Leg Sense**	**(Sup. nociception) Toe Prick**	**(Deep nociception) Toe Pinch**
2 Wks	-	-	-
4 Wks	-	-	-
8 Wks	-	-	-
16 Wks	-	-	-
Stem cells			
2 Wks	-	-	-
4 Wks	-	-	-
8 Wks	-	-	-

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

16 Wks	+ (95 Day)	+ (99 Day)	+ (83 Days)

In the control group, neurologic testing indicated no change in gait score and no hind leg posture reactions for proprioceptive positioning. The total Modified Tarlov scores and Texas Spinal Cord Injury Scale score improved after AD-MSC implantation. However, on the second week after treatment, the animals in Tarlov score in the stem cell group achieved a higher average was significant ($p < 0.05$) (0.62 ± 0.18) than control group (0.00 ± 0.00). On four weeks after treatment the stem cell group (1.50 ± 0.26) was significantly ($p < 0.05$) higher than and control group. (0.00 ± 0.00) (Tab 5). Furthermore, eight weeks after treatment, stem cell group (4.5 ± 0.18) was highly significant ($p< 0.05$) than the control group (0.87± 0.12). At sixteen weeks post-treatment, the stem cell group (6.0± 0.0) was significantly higher ($p< 0.05$) than the control group (1.75 ± 0.16). At the 8th and 16th weeks, the significant differences still between the treatment and control groups. (Tab 5). However, the dogs in the stem cell group restored normal pelvic gait movement with no return of neurological problems at thirteen weeks.

Table (5). Statistical Analysis of Motor Clinical Observations on Weeks of the Study Period in all Groups (Subgroup n=4).

Weeks	Control	Stem cell
2	0±0Cc	0.62±0.18Db
4	0±0Cc	1.50±0.26Cb
8	0.87±0.12Bb	4.5±0.18Ba
16	1.75±0.16Ab	6±0Aa
LSD(P<0.05)		

Means with different capital letters in the same column and small letters in the same row are significantly different at p <0.05.

While proprioceptive posture was detected, severe knuckling was observed in the stem cell group (1.75± 0.16) ($P< 0.05$) as compared to the control group (1.0 ±0.0). Furthermore, on the fourth week after treatment, moderate knuckling was severe in the stem cell (1.87± 0.12) which were significant ($p <0.05$) than the control group (1.0 ± 0.0) (Tab. 6). Nevertheless, eight weeks following treatment, the knuckling was moderate in the stem cell group (2.5 ± 0.18) and severe in the control group (1.75± 0.16). There was a normal response (Planter surface of the foot facing the ground) sixteen weeks after treatment in the stem cell group (3.87 ± 0.12) which were highly significant ($p< 0.05$) than the control group (2.37 ± 0.18) (Tab 6).

Table (6). Statistical Analysis of Knuckling Function Tests on Weeks of the Study Period in All Groups (Subgroup n=4)

Weeks	control	Stem cell
2	1±0Cb	1.75±0.16Ca
4	1±0Cc	1.87±0.12Cab
8	1.75±0.16Bc	2.5±0.18Bb
16	2.37±0.18Ab	3.87±0.12Aa
LSD(P<0.05)		

Means ± SE with different capital letters in the same column and small letters in the same row are significantly different at p <0.05.

Sensory reflexes, including superficial and deep nociception, were absent at weeks 2, 4, and 8 after therapy. On sixteen weeks after treatment, animals in the stem cell had higher significant sensory reflexes ($p <0.05$) than the control group (Tab 7). However, the toe pinch reaction came on day 83 post-treatment in the stem cell group (Tab 4).

Table 7. Statistical Analysis of Sensory Clinical Observations at the End of Experimental Study in All Groups (Subgroup n=4).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Signs	Control	Stem cell
Lat aspect leg sense	0±0Ab	1±0Aa
Toe Pinch	0±0Ab	1±0Aa
Toe Prick	0±0Ab	1±0Aa
LSD(P<0.05)		

Means ± SE with different capital letters in the same column and small letters in the same row are significantly different at p <0.05.

Histopathological Examination

The histopathological examination at the site of the spinal cord injury in control group at 8th week post operation revealed multiple cystic cavities containing granular cellular debris surrounded by reactive gliosis with marked vacuolation in the white matter which indicating Wallerian degeneration (Fig. 9. A). while at 16th week post operation revealed large cystic cavity (thick arrows) surrounded by glial scar tissue and vacuolated nerve fibers (Fig. 9. B).

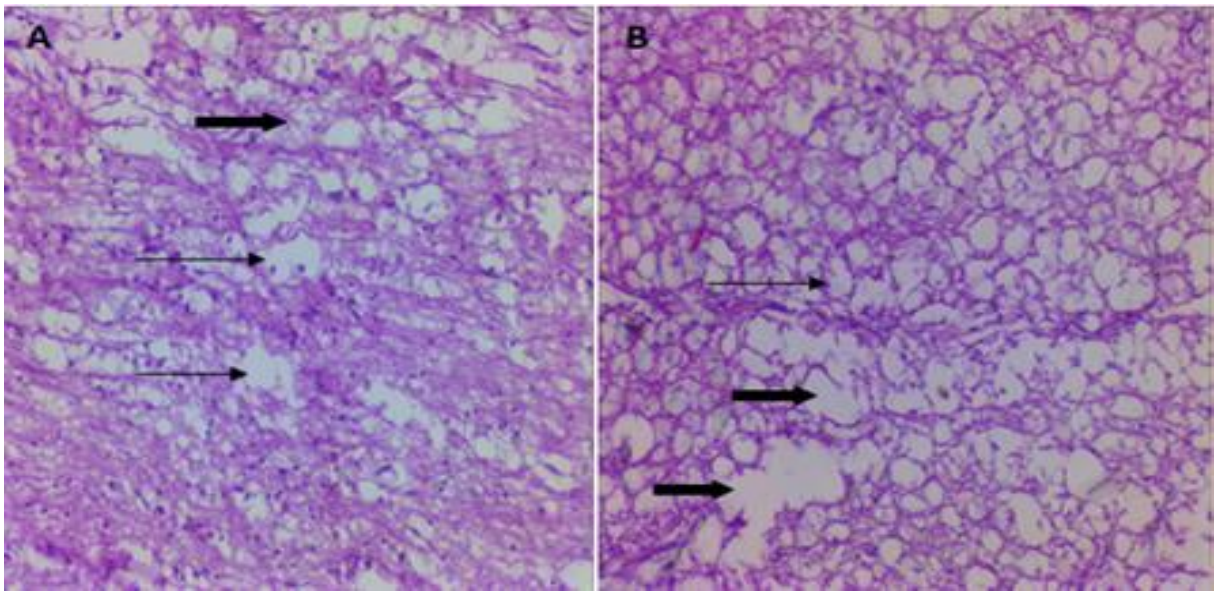


Figure 9. Micrograph of the longitudinal section of control group at the site of the spinal cord injuries **A.** 8 weeks PO shows multiple cystic cavity, containing granular cellular debris (thin arrows) surrounded by reactive gliosis and presented debris of necrotic with prominent vacuolization in white matter (thick arrow). **B**. 16 wks shows large cystic cavity (thick arrows) surrounded by glial scar tissue and vacuolated nerve fibers H&EX10.

The histopathological examination at the location of the spinal cord injury of stem cell group at 8 weeks PO displayed reduced the number of cavities near the central canal with remyelination regenerative nerve fibers in white matter and spheroid body in spaces of axons (Fig. 10. A). While at 16weeks stem cell group illustrated normal orientation of nerves fiber with remyelinated nerve fibers with few vacuolated nerve fibers (Fig. 10. B).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

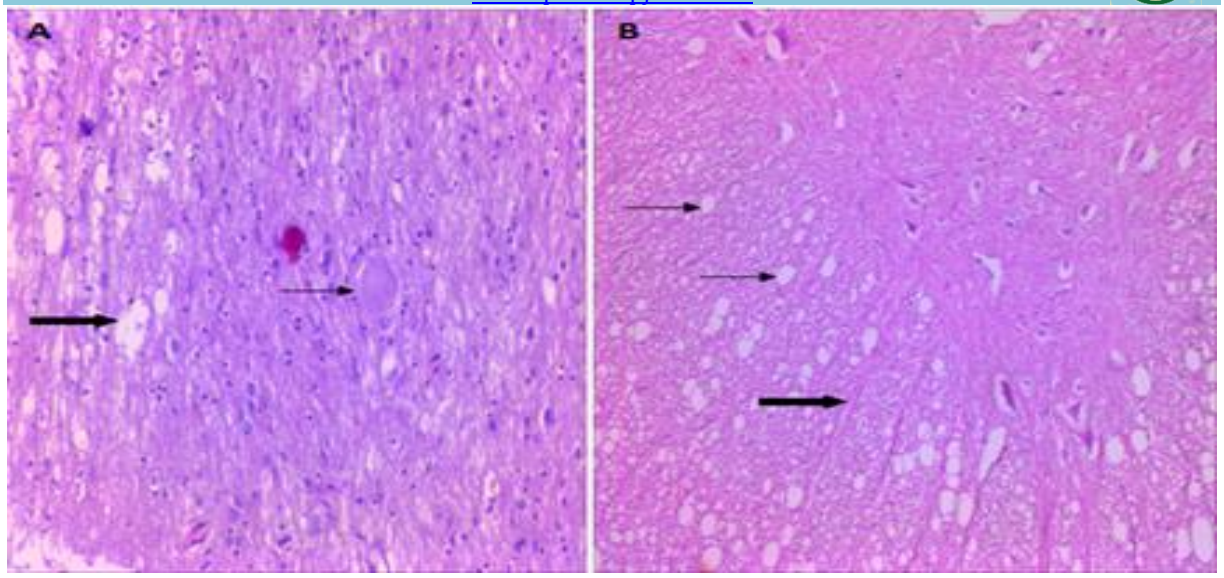


Figure (10). Micrograph of the longitudinal section of stem cell group at the site of the spinal cord injuries. **A**. 8 weeks PO shows narrow cavity near the central canal (thick arrow) with remyelination regenerative nerve fibers in white matter and spheroid body in spaces of axons (thin arrow). H&E X10 **B**. 16wks stem cell group reveals normal orientation of nerves fiber with remyelinated nerve fibers with few vacuolated nerve fibers (thin arrows) H&E X10.

Discussion

One of the most challenging ailments to treat is a neurological injury, because neurons have relatively limited regeneration capacities, the majority of the deficits caused by lesions are irreversible and permanent (22). In the current research, the modified Tarlov scale and the Texas Spinal Cord Injury Scale were used for quantitative evaluation of neurological status following spinal cord injury. This score was developed based on the stages of motor and sensory recovery that occur after spinal cord injury; increasing scores reflect less significant disability (20). The intriguing conclusion from this study is that after 16 weeks PO, the motor reflex in the stem cell group was significantly better ($p<0.05$) compared to the control group. The dogs in the control group had paraplegia and were not expected to regain a normal gait without treatment. Tarlov, on the other hand, does not score above three until 16 weeks after SCI. These findings are consistent with (23) findings of significant spinal cord injury and the creation of cavities surrounded by scar tissue with high quantities of collagen, resulting in irreversible paraplegia after SCI. However, the gait returned to normal in the stem cell (98 days). Motor clinical symptoms, such as the capacity to walk, were classified depending on the severity of pain, which was divided into neuropathic and inflammatory pain.The current study found that the gait progressed to normal faster in the stem cell group. This result suggested that the potential therapeutic effects of the synergistic treatment on improving functional recovery of the injured spinal cord by releasing a wide range of neurotrophic factors that promote myelin sheath formation, neovascularization, and reduced inflammatory reaction and edema as a result of relief pain caused by spinal cord injury.All of the experimental animals in the treatment group had no knuckling. However, it vanished in the stem cell (13-week) group. The control group, on the other hand, continued to knuckle until the completion of the trial. This could be due to the effectiveness of AD-MSCs on functional recovery at the site of SCI hemisection defects via improved early innervation of the extensor and flexor muscles, which control normal limb locomotion and promote myelin sheath

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

formation and neovascularization. This improvement could be attributed to AD-MSCs' ability to expedite axon rebuilding and regeneration, as well as decrease neuropathic pain creation, which could be attributed to the secretion of neurotrophic, angiogenic, and anti-apoptotic substances (24). According to (25), the implantation of mesenchymal stem cells in spinal cord damage will inhibit the development of mechanical and thermal allodynia.Furthermore, AD-MSCs have immunosuppressive features that can influence the function of all major immune cell populations. AD-MSCs may alter inflammatory pain by interacting with every type of immune system cell, either directly or through soluble substances that inhibit all immune response functions that reduce the course of inflammatory pain (26). Furthermore, AD-MSCs establish a micrtoenvironment favourable for neural regeneration by counteracting several detrimental processes such as inflammation and apoptosis (27). AD-MSCs can promote axonal regeneration via two distinct mechanisms: a paracrine effect by releasing a wide range of trophic factors such as factor (BDNF), (NGF), (VEGF), (FGF)-2, (TGF)- and (IGF)-1 or differentiating or promoting trans differentiation into neurons or glial cells, AD-MSCs can promote axonal regeneration via two distinct mechanisms: a paracrine effect by releasing a wide range of trophic factors such as factor (BDNF), (NGF), (VEGF), (FGF)-2, (TGF)- and (IGF)-1 or differentiating or promoting trans differentiation into neurons or glial cells (28,29). According to (30), the therapeutic effects of AD-MSCs in SCI were suppression of macrophage infiltration and lower expression of inflammatory cytokines following SCI. Extracellular matrix (ECM) can, on the other hand, lower inflammation while boosting functional tissue remodelling. Furthermore, ECM can influence M1/M2 phenotypes in invading macrophages and resident microglia, which differ significantly from monocyte-derived macrophages (31). (32) revealed that spinal cord damage reduced astrocyte activation, which in turn reduced M1-like macrophage infiltration via a possible feedback mechanism.On 16 weeks PO, the sensory reflex in the stem cell group was significantly better ($p<0.05$) compared to the control group. However, the absence of sensation in the presence of proprioceptive positioning, particularly when severe, causes the animal to walk on the dorsum of the foot, resulting in a worse state during the healing process. The key clinical indicator that aids in sensory function evaluation recovery is the withdrawal reflex test, which includes toe pinches and pricks. However, after hemisection of the spinal cord, toe pinch and toe prick were absent in two groups at 2, 4, and 8 weeks PO, but progressed and appeared at 16 weeks PO in the stem cell group. Dogs in the in the stem cell group, toe prick and pinch responses were evident on days 95 and 99, respectively, whereas toe pinch response occurred on day 83, the animals in the control group had lost sensation.This research demonstrated that transplanted AD-MSCs could result in functional recovery of SCI, as shown by higher scores on the Texas SCI scale following transplantation.However, AD-MSCs can produce various growth factors, neuroprotective cytokines and chemokines, including HGF, VEGF, fibroblast growth factor (FGF), BDNF, and NGF, which could indeed underlie functional benefits associated with MSC transplantation and demonstrated that MSCs are an efficient source of HGF which is the therapeutic effects of MSC transplantation are partly mediated by HGF secreted by these cells (33). The current research found that AD-MSCs can reduce the dispersion of blood-derived immune cells around the SCI, promoting functional recovery and this accord with (34). In this research, motor capabilities were restored earlier than sensory functions in treated animals, these may be due to the extent of wallerian degeneration, the axonal environment, and the fiber composition of particular nerves all playing key roles in nerve regeneration. These findings corroborate earlier research. (35,36) proposed preferential

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

motor reinnervation, which suggests that motor axons preferentially re-innervate motor routes while sensory axons may be more random. According to (37), functional recovery after axonal degeneration is likely dependent on the number, accuracy, and rate of growth of axons, as well as re-innervation of target organs, and these factors may be linked to the progressive atrophy and loss of Schwann cell tubes in the distal nerve stump and target organs. As a result, the pace of development of motor and sensory axons may have a direct effect on functional recovery.At 8 weeks, neuro-histopathological examination of the hemisection spinal cord in the stem cell revealed significant improvement and acceleration of injured spinal cord compared to the control group. The histology of the hemisection spinal cord in the control group exhibited severe vacuolation of the nerve fibres in the white matter, which was linked to pro-inflammatory cytokines which result in secondary cascades of events that occur after several hours to days of spinal cord injury, including mitochondrial dysfunction, failure of aerobic energy metabolism, and eventually the production of free oxygen radicals, which lead to lipid peroxidation and increased vascular permeability, local ischemia, intraneuronal edoema, and degenerate axons. (38,39,40) have all found similar results.In the eighth week, vacuolation in the grey matter is caused by degenerated and necrotized neurons, while neuronal cell necrosis is caused by direct trauma, which happens after spinal cord secondary injury. Ischemia, caused by insufficient blood supply to the tissue, results in hypoxia and a decrease in perivscular pH due to the accumulation of acid metabolites such as lactate. According to (39,40), this tissue perfusion may promote cellular damage by boosting the entry of free radicals and other harmful byproducts.Moreover, cavitation was observed in the control group at the region of the injured spinal cord. This phenomenon consequences from the complexity of regenerative failure, A number of studies have suggested that this secondary process of cavitation is associated with ischemia (43), haemorrhage (44), lysozyme activity (45) or macrophage infiltration (46), and inflammation (47). As a result, any treatment intervention that can prevent the secondary cascades if applied early and stimulates axonal regeneration can preserve the anatomical structure of the spinal cord (48). Whereas AD-MSCs revealed a narrow cavity with a high number of regenerating axons at the site of hemisection, white matter revealed minimal vacuolated nerve fibres, and grey matter showed significant decreases in the number of atrophied neurons with proliferation of glial cells that produce matrix materials to support axon regeneration with angiogenesis, Both treated groups could expedite SCI repair at this stage due to the function of AD-MSCs in regeneration of injured spinal cord, as reported by (49). The current research's findings indicated that using AD-MSCs is an effective method for the control of inflammation and the restoration of structural continuity made the growth of nerve fiber regeneration in the spinal cord after injury. AD-MSCs revealed reduced lesion cavity, implying that the AD-MSCs inhibited inflammatory cell migration. This behaviour could be explained by AD-MSCs expressing low levels of histocompatibility complex antigens (class II) and releasing soluble factors and cytokines to modulate the inflammatory process in response to SCI. This finding is consistent with the findings of (50), who discovered that transplanted AD-MSCs may regulate macrophage, astrocyte, and T lymphocyte-mediated neuroinflammation and help generate a microenvironment that promotes tissue repair and regeneration.When compared to the control group, histopathological analysis of hemisection spinal cord at 16 weeks PO demonstrated considerable improvement in the treatment groups. The control group had significant vacculation in the white matter due to continuous nerve fibre degeneration, glial scar formation, and neurons appeared atrophied in the grey matter due to reactive astrocyte proliferation, and there is still a gap between

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

the pre and post transection area due to hyperatrophy and proliferation of astrocytes gemicytes, apoptosis of oligodentrocytes, and proliferation of microglia cells. These implications on functional recovery and histology findings are consistent with clinical reports of delayed motor and sensory function progression. These findings are congruent with those of (51).The present study demonstrated that animals treated with AD-MSCs quickly recovered functional recovery of the injured spinal cord, with a substantial improvement compared to the control group in the 16 weeks. The histopathological analysis of the stem cell group revealed normal orientation of nerve fibres with re-myelinated nerve fibres and the presence of a few vacuolated nerve fibres. Axonal regeneration at the site of hemisection begins more prominently in the stem cell group as a single therapy, in white matter there were regenerate axons arranged in transverse and longitudinal directions, and in grey matter there was apparently normal regenerative neuron. These alterations were observed in both the 8th and 16th weeks. Furthermore, some researchers have found that AD-MSCs have anti-inflammatory or immunosuppressive capabilities after being implanted into injured spinal cord (52). These features are thought to diminish the immediate inflammatory process following SCI, hence decreasing cavity formation and astrocyte and microglia/macrophage reactivity (53,54).

Conclusion

Implantation of AD-MSCs at the site of acute spinal cord injury is able to alleviate secondary and extended inflammation to the lesion site, thereby contributing to SCI repair, this encouraged functional recovery via the early regulation of inflammatory cell recruiting with inhibition of apoptosis and secondary inflammation.

Conflict of Interest: The authors declare that there is no conflict of interest

References

1 .Layer RT, Ulich TR, Coric D, Arnold PM, Guest JD, Heary RH, Hsieh PC, Jenkins AL, Kim KD, Lee KS, Masuoka LK, Neff KM, Ray WZ, Theodore N, Fehlings MG. New Clinical-Pathological Classification of Intraspinal Injury Following Traumatic Acute Complete Thoracic Spinal Cord Injury: Postdurotomy/Myelotomy Observations From the INSPIRE Trial. Clin Neurosurg. 2017;64(1):105-109.https://doi.org/10.1093/neuros/nyx204

2 .Kumar R, Dewan MC, Park KB. Traumatic spinal injury: global epidemiology and worldwide volume. World Neurosurg. 2018;2(33):1-29.https://doi.org/10.1016/j.wneu.2018.02.033

3 .Shah M, Peterson C, Yelmaz E, Halameh DR, Moisi M. Current advancements in the management of spinal cord injury: A comprehensive review of literature. Surg Neurol Int. 2020;11(2):1-7.https://doi.org/10.25259/SNI_568_2019

4 .Huang L, Fu C, Xiong F, He C, Wei Q. Stem Cell Therapy for Spinal Cord Injury. Cell Transplant. 2021;30:1-16.https://doi.org/10.1177/0963689721989266

5 .Han D, Wu C, Xiong Q, Zhou L, Tian Y. Anti-inflammatory Mechanism of Bone Marrow Mesenchymal Stem Cell Transplantation in Rat Model of Spinal Cord Injury. Cell Biochem Biophys. 2014;10:1-7.https://doi.org/10.1007/s12013-014-0354-1

6 .Matyas JJ, Stewart AN, Goldsmith A, Nan Z, Skeel RL, Rossignol J, Dunbar GL. Effects of Bone-Marrow-Derived MSC Transplantation on Functional Recovery in a Rat Model of Spinal Cord Injury: Comparisons of Transplant Locations and Cell Concentrations. Cell Transplant. 2017;26(8):1472-1482.https://doi.org/10.1177/0963689717721214

7 .AL-Ameri SH, AL-Timmemi HA. Effect of bone marrow mesenchymal stem cell scaffold on functional recovery from acute spinal cord injury in dogs. Online J Vet Res. 2018;22(8):692-702.

8 .Al-Timmemi H, Dhurgham H, Zaid A. Efficacy of bone marrow stromal cells implantation on regeneration of peripheral nerve injury in dogs. Am J Res Commun. 2014;2(8):115-30.

9 .Fakhri S, Dargahi L, Abbaszadeh F, Jorjani M. Effects of astaxanthin on sensory-motor function in a compression model of spinal cord injury: Involvement of ERK and AKT signalling pathway. Eur J Pain. 2019;23:750-764.https://doi.org/10.1002/ejp.1342

10 .Yousefifard M, Maleki SN, Askarian-Amiri S, Vaccaro AR, Chapman JR, Fehlings MG, Hosseini M, Rahimi-Movaghar V. A combination of mesenchymal stem cells and scaffolds promotes motor functional recovery in spinal cord injury: a systematic review and meta-analysis. J Neurosurg Spine. 2019;1:1-16.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

11 .Kisiel AH, McDuffee LA, Masaoud E, Bailey TR, Esparza Gonzalez BP, Nino-Fong R. Isolation, characterization, and in vitro proliferation of canine mesenchymal stem cells derived from bone marrow, adipose tissue, muscle, and periosteum. Am J Vet Res. 2012;73(8):1305-1317.https://doi.org/10.2460/ajvr.73.8.1305

12 .Neupane M, Chang CC, Kiupel M, Yuzbasiyan-Gurkan V. Isolation and Characterization of Canine Adipose-Derived Mesenchymal Stem Cells. Tissue Eng Part A. 2008;14(6):1007-1015.https://doi.org/10.1089/ten.tea.2007.0207

Apologies for not covering all the references in my previous response. Let's continue arranging the remaining references into the Vancouver style and without the DOIs:

13 .Mahdi AK, Al-Falahi NH, Nahi HH. Effects of chitosan and hyaluronic acid in healing of chemically induced oral ulcer in rabbits. Kufa J Vet Med Sci. 2016;7(2).https://doi.org/10.36326/kjvs/2016/v7i24331

14 .Kresic N, Prišlin M, Vlahović D, Kostešić P, Ljolje I, Brnić D, Habrun B. The expression pattern of surface markers in canine adipose-derived mesenchymal stem cells. Int J Mol Sci. 2021;22(14):7476.https://doi.org/10.3390/ijms22147476

15 .Lutvikadic I, Maksimovic A. Use of ketamine and xylazine anesthesia in dogs: A retrospective cohort study of 3,413 cases. Indian J Anim Sci. 2022;92(11):1280-1284.https://doi.org/10.56093/ijans.v92i11.123310

16 .Eesa MJ. Evaluation of general anaesthesia by using Propionylpromazine, Xylazine and Ketamine in rabbits. Iraqi J Vet Med. 2010;34(1):208-17.https://doi.org/10.30539/iraqijvm.v34i1.681

17 .Ali AF. Evaluation of Midazolam and Ketamine Preceding by Xylazine as General Anesthesia in Rabbits. Iraqi J Vet Med. 2013;37(2):144-148.https://doi.org/10.30539/iraqijvm.v37i2.274

18 .Fossum TW. Small animal surgery of the thoracolumbar spine. St Louis, United States: Elsevier-Health Sciences Division; 2019. pp. 1460-1469.

19 .Tarlov IM, Kinger H, Vitale S. Spinal cord compression studies. I. Experimental technique to produce acute and gradual compression. AMA Arch Neurol Psychiatry. 1953;70:813-9.https://doi.org/10.1001/archneurpsyc.1953.02320360128010

20 .Levine GJ, Levine JM, Budke CM, Kerwin SC, Au J, Slater MR. Description and repeatability of a newly developed spinal cord injury scale for dogs. Prev Vet Med. 2009;89(1-2):121-127.https://doi.org/10.1016/j.prevetmed.2009.02.016

21 .Daniel W. Biostatistics: A foundation of analysis in the health science. 9th edition. USA: John Wiley and Sons, Inc.; 2009.

22 .Cooke P, Janowitz H, Dougherty SE. Neuronal redevelopment and the regeneration of neuromodulatory axons in the adult mammalian central nervous system. Front Cell Neurosci. 2022;16:872501.https://doi.org/10.3389/fncel.2022.872501

23 .Salih SI, E'atelaf A, Mahdi AK, RA S. Role of Chitosan Application in Postoperative Abdominal Adhesions in Rabbits. Iraqi J Vet Med. 2015;39(1):105-11.https://doi.org/10.30539/iraqijvm.v39i1.206

24 .Al-Timmemi H, Hussein H, Ammal B. Acellular Xenograft Bovine Urinary Bladder Submucosa a Novel Conduit for Regeneration of Sciatic Nerve Defect in Rabbit Model. Adv Ani Vet Sci. 2017;5(1):1-10.

25 .Forouzanfar F, Amin B, Ghorbani A, Ghazavi H, Ghasemi F, Sadri K, Hosseinzadeh H. New approach for the treatment of neuropathic pain: Fibroblast growth factor 1 gene-transfected adipose-derived mesenchymal stem cells. Eur J Pain. 2018;22(2):295-310.https://doi.org/10.1002/ejp.1119

26 .Ceccarelli S, Pontecorvi P, Anastasiadou E, Napoli C, Marchese C. Immunomodulatory effect of adipose-derived stem cells: the cutting edge of clinical application. Front Cell Dev Biol. 2020;8:236.https://doi.org/10.3389/fcell.2020.00236

27 .Mukhamedshina YO, Gracheva OA, Mukhutdinova DM, Chelyshev YA, Rizvanov AA. Mesenchymal stem cells and the neuronal microenvironment in the area of spinal cord injury. Neural Regen Res. 2019;14(2):227.https://doi.org/10.4103/1673-5374.244778

28 .Sowa Y, Mazda O, Tsuge I, Inafuku N, Kishida T, Morimoto N. Roles of adipose-derived stem cells in cell-based therapy: current status and future scope-a narrative review. Dig Med Res. 2022;5.https://doi.org/10.21037/dmr-22-32

29 .Xia Y, Zhu J, Yang R, Wang H, Li Y, Fu C. Mesenchymal stem cells in the treatment of spinal cord injury: Mechanisms, current advances and future challenges. Front Immunol. 2023;14:1141601.https://doi.org/10.3389/fimmu.2023.1141601

30 .Zhou Z, Tian X, Mo B, Xu H, Zhang L, Huang L, Zhang H. Adipose mesenchymal stem cell transplantation alleviates spinal cord injury-induced neuroinflammation partly by suppressing the Jagged1/Notch pathway. Stem Cell Res Ther. 2020;11(1):1-17.https://doi.org/10.1186/s13287-020-01724-5

31 .An N, Yang J, Wang H, Sun S, Wu H, Li L, Li M. Mechanism of mesenchymal stem cells in spinal cord injury repair through macrophage polarization.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Cell Biosci. 2021;11:1-14.https://doi.org/10.1186/s13578-021-00554-z
32 .Xia Y, Yang R, Wang H, Hou Y, Li Y, Zhu J, Fu C. Biomaterials delivery strategies to repair spinal cord injury by modulating macrophage phenotypes. J Tissue Eng. 2022;13:20417314221143059.https://doi.org/10.1177/20417314221143059
33 .Essa HH, Jasim HS, Kadhim HA. Immunological and Hematological Response to Local Transplantation of Stem Cells in Injured Radial Nerve of Dogs. Iraqi J Vet Med. 2020 Dec 28;44(2):45-55.https://doi.org/10.30539/ijvm.v44i2.976
34 .Hong IS. Enhancing stem cell-based therapeutic potential by combining various bioengineering technologies. Front Cell Dev Biol. 2022;10:901661.https://doi.org/10.3389/fcell.2022.901661
35 .Brushart T, Kebaish F, Wolinsky R, Skolasky R, Li Z, Barker N. Sensory axons inhibit motor axon regeneration in vitro. Exp Neurol. 2020;323:113073.https://doi.org/10.1016/j.expneurol.2019.113073
36 .Bolívar S, Udina E. Preferential regeneration and collateral dynamics of motor and sensory neurons after nerve injury in mice. Exp Neurol. 2022;358:114227.https://doi.org/10.1016/j.expneurol.2022.114227
37 .Akram R, Anwar H, Javed MS, Rasul A, Imran A, Malik SA, Hussain G. Axonal regeneration: Underlying molecular mechanisms and potential therapeutic targets. Biomedicines. 2022;10(12):3186.https://doi.org/10.3390/biomedicines10123186
38 .Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. Acta Neurobiol Exp (Wars). 2011;71(2):281-99.https://doi.org/10.55782/ane-2011-1848
39 .Rabchevsky AG, Michael FM, Patel SP. Mitochondria focused neurotherapeutics for spinal cord injury. Exp Neurol. 2020;330:113332.https://doi.org/10.1016/j.expneurol.2020.113332
40 .Fan B, Wei Z, Feng S. Progression in translational research on spinal cord injury based on microenvironment imbalance. Bone Res. 2022;10(1):35.https://doi.org/10.1038/s41413-022-00199-9
41 .Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic spinal cord injury: an overview of pathophysiology, models and acute injury mechanisms. Front Neurol. 2019;10:282.https://doi.org/10.3389/fneur.2019.00282
42 .Li S, Dinh HTP, Matsuyama Y, Sato K, Yamagishi S. Molecular Mechanisms in the Vascular and Nervous Systems following Traumatic Spinal Cord Injury. Life. 2022;13(1):9.https://doi.org/10.3390/life13010009
43 .Tran AP, Warren PM, Silver J. The biology of regeneration failure and success after spinal cord injury. Physiol Rev. 2018;98(2):881-917.https://doi.org/10.1152/physrev.00017.2017
44 .Malomo T, Allard Brown A, Bale K, Yung A, Kozlowski P, Heran M, Kwon BK. Quantifying Intraparenchymal Hemorrhage after Traumatic Spinal Cord Injury: A Review of Methodology. J Neurotrauma. 2022;39(23-24):1603-1635.https://doi.org/10.1089/neu.2021.0317
45 .Hu X, Xu W, Ren Y, Wang Z, He X, Huang R, Cheng L. Spinal cord injury: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther. 2023;8(1):245.https://doi.org/10.1038/s41392-023-01477-6
46 .Thanoon MG, Eesa MJ, Alkenanny ER. Histopathological evaluation of the platelets rich fibrin and bone marrow on healing of experimental induced distal radial fracture in local dogs. Iraqi J Vet Med. 2019;43(1):11-20.https://doi.org/10.30539/iraqijvm.v43i1.465
47 .Hellenbrand DJ, Quinn CM, Piper ZJ, Morehouse CN, Fixel JA, Hanna AS. Inflammation after spinal cord injury: a review of the critical timeline of signaling cues and cellular infiltration. J Neuroinflammation. 2021;18(1):1-16.https://doi.org/10.1186/s12974-021-02337-2
48 .Schmidt J, Quintá HR. Mitochondrial dysfunction as a target in spinal cord injury: intimate correlation between pathological processes and therapeutic approaches. Neural Regen Res. 2023;18(10):2161-2166.https://doi.org/10.4103/1673-5374.369094
49 .Wang D, Wang K, Liu Z, Wang Z, Wu H. Valproic acid labeled chitosan nanoparticles promote the proliferation and differentiation of neural stem cells after spinal cord injury. Neurotoxicity Res. 2021;39:456-466.https://doi.org/10.1007/s12640-020-00304-y
50 .Pang QM, Chen SY, Fu SP, Zhou H, Zhang Q, Ao J, Zhang T. Regulatory role of mesenchymal stem cells on secondary inflammation in spinal cord injury. J Inflamm Res. 2022;573-593.https://doi.org/10.2147/JIR.S349572
51 .Al-Timmemi HA, Ibrahim R, Zuki AZ, Azmi TI, Al-Jashamy K. Bone marrow stromal cells implantation and suture repair of peripheral nerve: A comparative study of functional, histopathological, morphometric and relative gastrocnemius muscle weight in rabbits. Afr J Biotechnol. 2011 Aug 15;10(44):8976-83.https://doi.org/10.5897/AJB11.1279
52 .Hussein Abed H, Hameed Fathullah AL-Bayati A. Clinical and Histopathological Study of the Effect of Adipose-Derived Mesenchymal Stem Cells on Corneal Neovascularization following Alkali Burn

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

in a Rabbit Model. Archives of Razi Institute. 2022 Oct 1;77(5):1715-21.

53 .Al-Timmemi H, Ibrahim R, Al-Jashamy K, Zuki A, Azmi T, Ramassamy R. Identification of adipogenesis and osteogenesis pathway of differentiated bone marrow stem cells in vitro in rabbit. Ann Microsc. 2011;11:23-9.

54. Essa HH, Jasim HS, Kadhim HA. Immunological and Hematological Response to Local Transplantation of Stem Cells in Injured Radial Nerve of Dogs. Iraqi J Vet Med. 2020 Dec 28;44(2):45-55.https://doi.org/10.30539/ijvm.v44i2.976