

## Efficacy of Bone Marrow Stromal Cells Implantation on Regeneration of Peripheral Nerve Injury in Dogs

Al-Timmemi H<sup>1\*</sup>, Dhurgham H<sup>1</sup> and Zaid A<sup>2</sup>

<sup>1</sup>Department of Surgery and Obstetric, College of Veterinary Medicine, Baghdad University

<sup>2</sup>National Center of Hematology, Mustansiriya University

\*Correspondence address: hameed\_altmeme@yahoo.com

### Abstract

**Objectives:** To describe the efficacy of the bone marrow stromal cells (BMSCs) implantation on peripheral nerve regeneration in a dog model.

**Methods:** Ten local breed dogs were divided into two equal group (n=5). In the epineural repair group (control group), the left sciatic nerve was skeletonized from the sciatic notch to the point of bifurcation, with the nerve been transected at the mid-shaft of the femoral bone and repaired with six epineural nerve sutures (ENS group). In the treated group, the epineural repaired nerve was implanted with BMSCs in the proximal and distal segments of the transected sciatic nerve (BMSCs group). Assessment of the nerve regeneration was based on functional recovery (motor and sensory).

**Results:** showed that the motor and sensory functions acceleration of sciatic nerve improved more rapidly in the BMSC group. The histopathological examination revealed basophilic nuclei of proliferated Schwann cells with thick myelin sheath, good orientation nerve fibers, dense axon population, large diameter of axon and myelination of nerve fibers and low fibrous tissue at the epineurium improved in the BMSCs group.

**Conclusion:** the results of the examination showed that the treated group had the best regeneration and functional recovery.

**Keyword:** Bone marrow stromal cells (BMSCs), histopathology, peripheral nerve, regeneration

{**Citation:** Al-Timmemi H., Dhurgham H., Zaid A. Efficacy of bone marrow stromal cells implantation on regeneration of peripheral nerve injury in dogs. American Journal of Research Communication, 2014, 2(8): 115-130} [www.usa-journals.com](http://www.usa-journals.com), ISSN: 2325-4076.

## Introduction

Peripheral nervous system injuries include penetrating injuries, crush, traction and ischemia compression (Robinson, 2004). Microsurgical suture repair remains the current gold standard in clinical practice (Lundborg, 2000), but obvious deficiencies remain with this technique, given that surgical repair of peripheral nerves does not result in complete functional recovery. Recent approaches have been directed towards biological factors to promote an environment conducive for growth and overcome limitations in regeneration and functional recovery. The mesenchymal stem cells (MSCs) have become one of the most interesting targets for the study of tissue and organ regeneration because of their plasticity (Al-Timmemi et al., 2013; Prokop 1997). The implantation of neural stem cells, bone marrow stromal cells (BMSCs), or fibroblasts has been shown to exert a beneficial effect on peripheral nerve regeneration (Mimura and others 2004). Thus, cell transplantation has been proposed as a method of improving peripheral nerve regeneration (Cuevas et al., 2002). The MSCs are an expression of many cytokines and cellular factors (Pittenger et al., 1999; Bhagavati and Xu 2004). This study was aimed to evaluate the effectiveness of bone marrow stromal cells (BMSCs) implantation on peripheral nerve regeneration in a dog model.

## Materials and Methods

### Experimental animals design

Ten adult local breed dogs weighing between (15-25kg) were selected. The animals were kept in separate cages and given broad-spectrum antibiotics and antihelminthic. All procedures used in this study were approved by the College of Veterinary Medicine, University of Baghdad. Dogs were randomly divided into two groups (n =5) each. Both groups comprised of animals were coaptated transected sciatic nerve with epineurial nerve suture (ENS) as the control group, and the bone marrow stromal cells implantation group was the second group. The animals from both groups were euthanized on day 112 post operations (PO).

## **Epineurial Nerve Suture (Control Group)**

### **Anesthetic Protocol**

The dogs were fasted for two hours prior to the anesthesia. Induction of anesthesia was achieved by intramuscular injection of a mixture of 15mg/kg Ketamine hydrochloride (Kepro<sup>®</sup>, Holland), 10mg/kg of Xylazine hydrochloride (Xyla<sup>®</sup>, Holland) (Flecknell 2003) and maintained with Halothane (Hikman, Jordan) and oxygen.

### **Surgical Protocol**

The left hind limb was surgically prepared. The limb was excluded from stifle joint to the end of the limb and from the surgical area, by placing a latex glove over the distal extremity and securing it to the limb with an adhesive tape. The glove was covered with sterile skin towel and secured to the limb with towel clips. The animal was placed on right lateral recumbency. The left hind limb was draped with the aperture of the fenestrated drape located at the intended operation site. The greater trochanter of the femur was palpated and the stifles used as landmarks. The skin was incised on the postero-lateral thigh, approximately 2 cm on the caudo-lateral to the greater trochanter at the level of the distal one-third of the femur, using a scalpel blade #21. Subcutaneous tissue and fascia lata were incised on the same line using scalpel blade #15. The biceps femoris muscle was separated cranially and the semitendinous muscle separated posteriorly by blunt dissection using Mayo scissors to expose the sciatic nerve and separate it from the surrounding tissues using ophthalmic scissors. A wooden tongue depressor was placed gently under the nerve and nerve was severed using scalpel blade #21 (Fig. 3.4). The nerve ends were coapted immediately and six equidistant epineurial simple interrupted sutures were applied about 1 mm from the transected edge using 6-0 nylon. The coapted nerve was replaced carefully flushed with physiological saline and the excess saline was swabbed with sterile gauze. A simple continuous suture was applied on the superficial fascia using 2-0 Vicryl with simple continuous suture and the skin was closed with using a 2-0 Vicrylsubcuticular suture pattern. All animals were given postoperative analgesia Tramadol hydrochloride (Trabar<sup>®</sup> Switzerland, 100 mg) 0.2 ml/kg intramuscular administered at 12-hour intervals for three consecutive days.

## Bone Marrow Stromal Stem Cells (Treated Group)

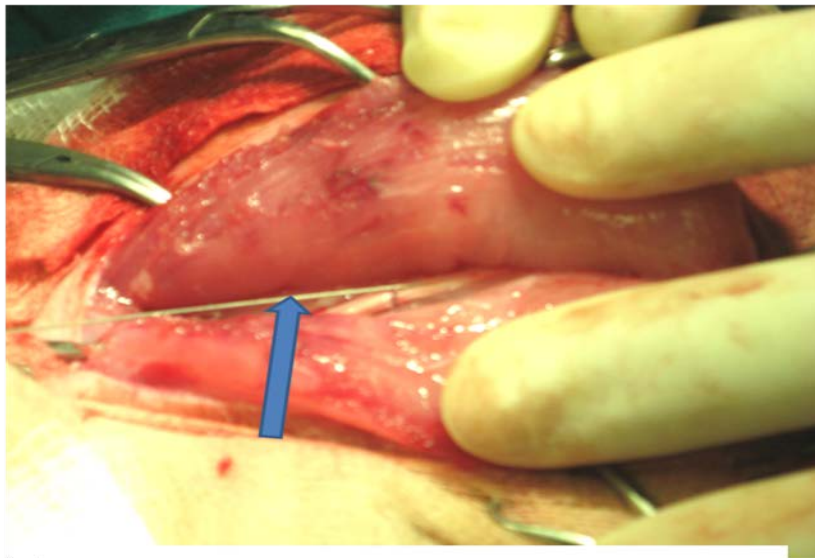
### *In Vitro*

#### Stem Cells Preparation and Identification

The BMSCs collected from the femoral bone of dogs were prepared, identified and differentiated as described by Al-Timmemi *et al.* (2013).

### *In Vivo*

Allogenic bone marrow stromal cells (BMSCs) used in this study were cultured, isolated, identified and differentiated. The coaptated transected sciatic nerves which described earlier was injected with 50  $\mu$ l of freshly prepared cultured media containing  $5 \times 10^6$  BMSCs into each of the distal and proximal nerve stumps. The culture media were administrated into the nerve segments via needle gauge # 27 (Fig. 1). Motor and sensory of sciatic nerve clinical reflexes were evaluated daily from the first day to the end of the study on day 112 PO.



**Figure1: Photograph showing the coaptated sciatic nerve injected with allograft transplanted bone marrow stromal cells in the proximal segment (arrow) in dog.**

## Clinical Signs Evaluation

### Motor Functions Evaluation

All animals were examined daily from the first day to the end of the study on day 112 PO using the grading criteria. The onset and ability to walk by the animals were recorded. The types of gait were graded as normal, crouched, or crawl on heel. Knuckling was graded based on normal, mild, moderate to severe. Muscle contraction force was graded based on weak, moderate, and strong.

### Sensory Functions Evaluation

Nerve sensory functions were recorded weekly starting from the end of the 3<sup>rd</sup> week to the end of the study on day 112 PO. Sensory functions and clinical signs scoring were evaluated using the grading criteria of toe spreading reflex, lateral aspect leg sensation, toe pinch and toe prick as a present (+) or absent (-). Foot withdrawal and vocalization tests were evaluated by lateral aspect leg sensation, toe pinch and toe prick, which were recorded as positive response, indicative of recovery.

### Neurohistopathological Procedure

The anastomosed left sciatic nerve was exposed and macroscopic observations were recorded, which involved the degree of nerve stump coaptated, dehiscence, adhesions and presence of neuroma. Both middle (coaptate site) and distal segments of the coaptated sciatic nerve were harvested from each animal and the nerves samples were fixed onto plastic plate using stay sutures to keep the nerve tissue straight (Pan and others 2006).

The nerve tissue specimens were fixed with the 10% neutral buffered formalin, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin and cut into 5- $\mu$ m thick sections and stained with hematoxylin and eosin. Light microscopic examination of nerve tissue sections were used to determine the axonal alignment, intraneural and extraneural scarring, disarrangement and presence of vacuolated degenerate nerve fibers, basophilic and density of Schwann cells

## Statistical Analysis

All data were analyzed and expressed as means and standard deviations ( $M \pm SD$ ). Statistical comparisons between groups were performed using Statistical Package for the Social Sciences (SPSS) 16.0 software (non-parametric tests), Kruskal Wallis and Mann-Witney tests. P value  $\leq 0.05$  was considered significant.

## Results

### Sciatic Nerve Motor and Sensory Functions Evaluation

#### Clinical Observation of Motor Nerve Functions

##### Epineural Nerve Sutures (ENS)

The interesting finding was that the animals regained normal gait ability on day 77 PO. Knuckling was mild, and remained until the end of the study. The muscle contraction force became strong on day 48 PO (Table 1). Skin sensation slowly progressed towards the foot, to the fetlock joint by the end of the study. Sensation of the toe spreading reflex, lateral leg sensation, toe pinch and the toe prick remained absent (Table 3).

##### Bone Marrow Stem Cells Group (BMSCs)

The interesting finding was that the animals regained normal gait ability on day 40 PO (Table 1). The motor functions were improved. The onset, walking, gait and knuckling were significant difference ( $p \leq 0.05$ ) decreased compared with control group (Table 2). Sensation of the operated left hind limb was regained and the toe spread appeared on day 70 PO accompanied by lateral aspect leg sensation, toe pinch and toe prick on days 78, 93, 95 PO respectively (Table 3). The sensory clinical signs improved in BMSCs group were significant difference ( $p \leq 0.05$ ) compared with ENS group (Table 4).

**Table (1): Mean time of motor function test observation in the ENS and BMSCs groups**

Groups mean time	Onset	Walking	Type of gait				Knuckling			Muscle contraction Force		
			Crouch	Crawl	Nor	Severe	Mod	Mild	Nor	Weak	Mod	Strong
<b>112 days</b>												
<b>ENS group</b>	9.2	12.4	50.2	54	77.2	32	43	51	N	33	41.2	48
<b>Stem Cell</b>	2.8	4.8	18.6	36.4	40.4	19.2	36.8	44.8	58.7	24.6	39	43

**Table (2): Statistical Analysis of Motor Clinical Observation in the ENS and BMSCs groups during 112 days PO**

Clinical signs	ENS	MSCs
Onset	1.2±0.2 <b>b</b>	4.2±0.3 <b>a</b>
Walk		
Crouch	1±0.0 <b>a</b>	0.2±0.2 <b>b</b>
Crawl	2±0.0 <b>a</b>	0.4±0.4 <b>b</b>
Normal	3±0.0 <b>a</b>	3±0.0 <b>a</b>
Knuckling		
Severe	1±2.8 <b>a</b>	0.2±0.2 <b>b</b>
Moderate	2±0.0 <b>a</b>	0.4±0.4 <b>b</b>
Mild	3±0.0 <b>a</b>	0.6 ±0.6 <b>b</b>
Normal	0.0±0.0 <b>b</b>	4±0.0 <b>a</b>
Muscle Contraction Force (MCF)		
Strong	3±0.0 <b>b</b>	3±0.0 <b>a</b>

<sup>a,b</sup>Values (M±SD, n=5) bearing similar superscript in the same row are not significant at (p ≤0.05)

**Table (3): Mean Time of Sensory Function Test Observation in the ENS and BMSCs groups**

Mean time Groups	Toe spread	Lateral Aspect Leg Sense	Toe Pinch	Toe prick
ENS	N	N	N	N
BMSCs	70	78	93	95

**Table (4): Statistical analysis of Sensory Clinical Observations in the ENS and BMSCs Groups on day112 PO**

Sensory Signs	ENS	MSCs
Toe spread	0±0 <sup>b</sup>	1±0 <sup>a</sup>
Lateral leg sensation	0±0 <sup>b</sup>	1±0 <sup>a</sup>
Toe pinch	0±0 <sup>b</sup>	1±0 <sup>a</sup>
Toe prick	0±0 <sup>b</sup>	1±0 <sup>a</sup>

<sup>a,b</sup>Values (M±SD, n=5) bearing similar superscript in the same row are not significant at (p ≤0.05)

## Histopathological Observations

### Epeineurial Nerve Suture (Control Group)

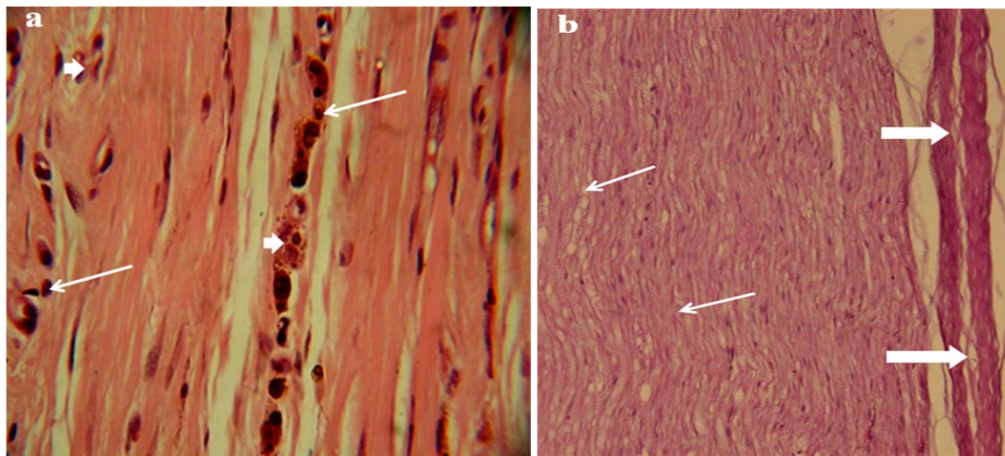
The histopathology of longitudinal sections of the coaptation nerve stump demonstrated the Wallerian degenerative of nerve fibers with scanty of Schwann cells (Fig. 2a), amorphous material represented suture materials surrounded by fibrous connective tissue and inflammatory cells particularly macrophage and lymphocytes, the nerve fiber showed congested blood vessels and fibrous connective tissue proliferation in the epineurium in addition to pyknotic of Schwann cells nuclei, disorientation of regenerative nerve fibers (Fig. 3a). The histological changed appeared at the proximal segment of congested blood vessels in the perineurium, nerve fiber expressed vacuolar degeneration, inflammatory cell infiltration a rounded congested blood vessels as well as the nerve fiber loss differentiated staining. Longitudinal section of the nerve tissue showed irregular elongated space surrounded with few Schwann cells and infiltrated with inflammatory cells. There was proliferation of collagen fibers in the nerve fascicle and degradation of Schwann cells, also it was recorded thin myelin sheath fibers and low number of axon with few pyknotic Schwann cells (Fig. 4a). The distal nerve stump sections revealed marked fibrous connective proliferation extended from perineurium to nerve fascicle that replacement most of degenerative axon, few nuclei of Schwann cells and few regenerative nerve fibers (Fig. 5a)

### Bone Marrow Stromal Cells Treatment (MSCs Group)

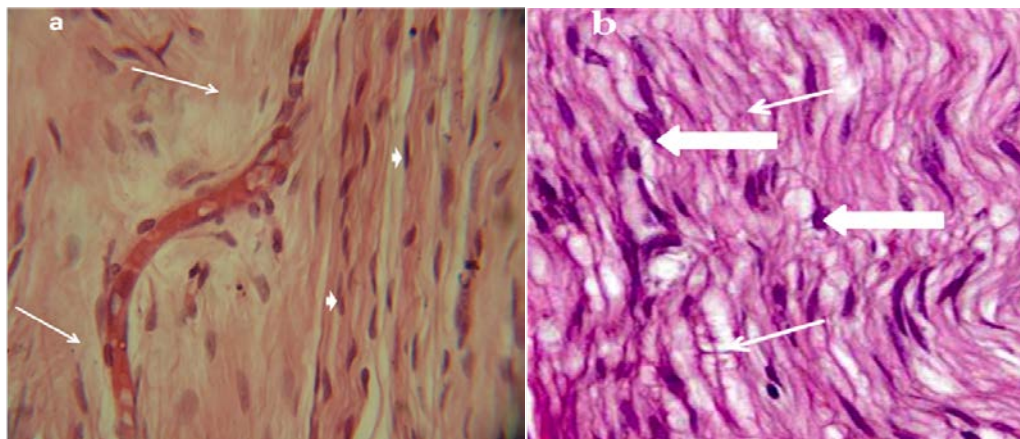
Histopathological examination of the longitudinal section of the middle segment in the BMSCs group revealed basophilic nuclei of proliferated Schwann cells with thick myelin sheath, good



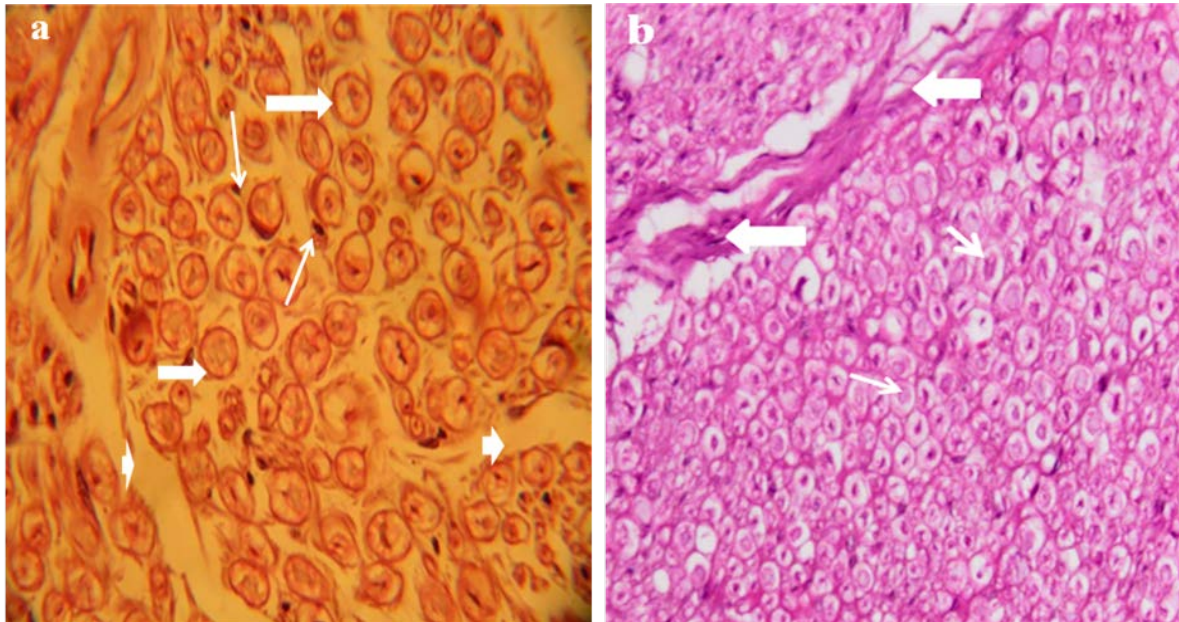
orientation and myelination of nerve fibers and low fibrous tissue at the epineurium (Fig.2b) and in other section showed congested blood vessels in the epineurium with apparently normal structure of nerve fascicle (Fig. 3b). The cross section of proximal segment of the nerve fibers demonstrated dense axon population with thick myelin sheath as well as dense basophilic nuclei of Schwann cells with large diameter of axon and thin of perineurium (Fig.4b). In the distalsegment, there are large diameter of dense axon population with thick myelin sheath and proliferation of Schwann cells in addition of highly angiogenesis (Fig.5b).



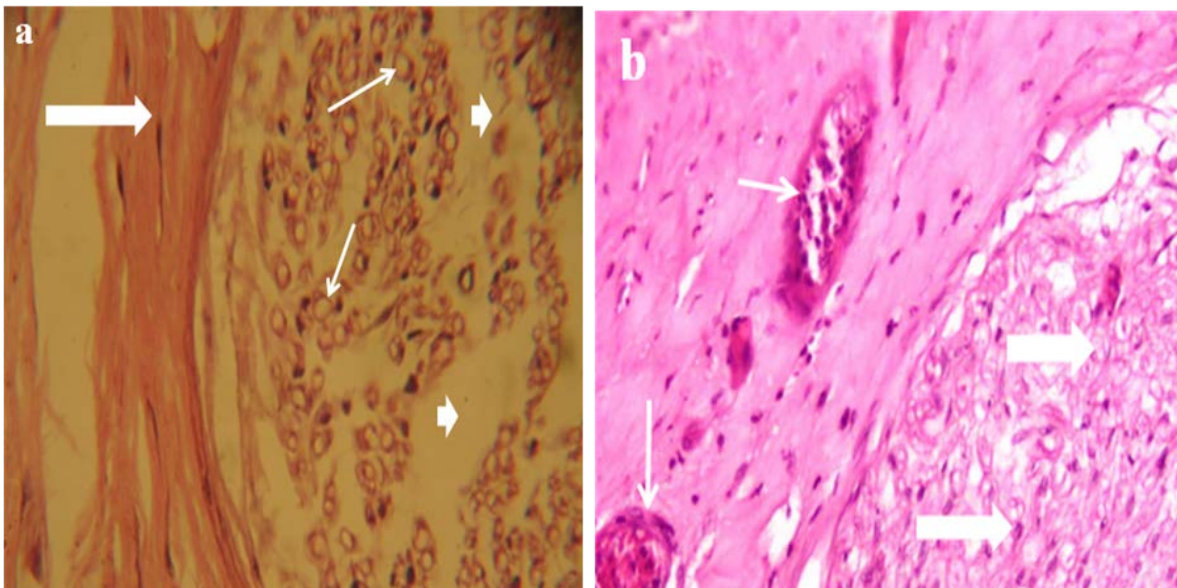
**Figure 2 : Light micrographs of coaptation site of sciatic nerve in a. ENS group shows few nuclei of Schwann cells (arrows), highly vacuolated degenerative nerve fibers, (digestive chambers) (arrow heads). b BMSCs group appear of good orientation and myelination of nerve fibers (thin arrows) and thin epineurium (thick arrows) H&EX40.**



**Figure 3: Light micrographs of coaptation site of sciatic nerve in a. ENS group shows congested blood vessels and fibrous connective tissue proliferation at the epineurium (arrows), low number of Schwann cells (arrow heads). b. BMSCs group shows good myelinated nerve fibers with Schwann cells (thick arrow), node of Ranvier (thin arrow)H&E X40.**



**Figure 4:** Light micrographs transverse section of the proximal segment of sciatic nerve in a. ENS group shows thin myelinated fiber (thick arrows) with few pyknotic Schwann cells (thin arrows) and thick perineurium (arrow heads). b. BMSCs group shows dense fiber population with thick myelinated fibers (thin arrows) in addition basophilic nuclei of dense proliferation of Schwann cells and thin epineurium (thick arrows). H&E X40



**Figure 5:** Light micrographs of transverse section of the distal segment a. ENS group shows regenerative nerve fibers (arrows), thick CT of epineurium (thick arrow) and perineurium (arrowheads). H&E. X40. b. BMSCs group demonstrated high vascogenesis (thin arrow) and many regenerative nerve fibers. H&E X40



## Discussion

Results of this study showed that the onset limb movement and walking in animals of the BMSCs was faster compared to the ENS group. The type of gait progressed to normal in all groups; however the animals in BMSCs (40days) group regained normal gait earlier compared to the ENS group (77days). There was a significant difference ( $p \leq 0.05$ ) of knuckling disappearing in the BMSCs (58days) groups while knuckling persisted in the NES group until the end of the study. Muscle contraction forces were strong in all groups but in the BMSCs (43days) groups contraction started earlier compared to ENS group (48days). Motor clinical signs which included the ability to walk on its operated left hind feet were determined based on the severity of pain which was classified into neuropathic and inflammatory pain categories. Results of this study showed that the type of gait progressed to normal in all animals in the BMSCs group, which was faster compared to those in the ENS group. This result suggested that the BMSCs have potential therapeutic effects and might participate in the injured sciatic nerve regeneration and neuropathic pain. Previous studies reported that the bone marrow stromal cells implantation decreased neuropathic pain which might be due to the secretion of neurotrophic factors, angiogenic and anti-apoptotic (Mahmood and others 2004). Another study reported the mesenchymal stem cells administration in sciatic nerve injury prevented the generation of mechanical and thermal allodynia (Coronel et al., 2009).

BMSCs acted as immunosuppressive properties which are able to modulate the function of all major immune cell populations (Sotiropoulou and Papamichail 2007). BMSCs might affect the inflammatory pain which interacts with every type of cell of the immune system, either directly or through soluble factors retarding all functions of the immune response which decreased the course of inflammatory pain.

Results from the present study showed that animals in the treated group with BMSCs rapidly regained functional recovery of the transected sciatic nerve. Al-Timmemi et al. (2011) reported that the histopathology of sciatic nerve treated with BMSCs showed an increased number of Schwann-like cells. The BMSCs are believed to act as Schwann cells in that they function to prevent neuronal cells death and promote directional axonal growth. As they proliferate to fill endoneurial sheaths, they form longitudinal columns commonly known as bands of Bungner (Scherer and Salzer 1996). Within days after injury, Schwann cells begin to divide and create a pool of dedifferentiated daughter cells without axon contact. Schwann cells

down-regulate their normal proteins such as peripheral myelin protein-22 (PMP-22), myelin basic protein (MBP), myelin associated glycoprotein (MAG), P0 and connexin-32 (Trapp et al., 1988) to convert the phenotype of premyelinating cell (Hall 2005). These dedifferentiated Schwann cells upregulate expression of the nerve growth factor (NGF), neurotrophic factors, cytokines, and other compounds that lead to Schwann cell differentiation and proliferation. The latter are important in preventing neuronal apoptosis in response to injury and potentiate the migration and adhesion of Schwann cells to axonal projections (Boyd and Gordon 2003).

It is interesting to note from this study that muscle contraction force of animals in the BMSCs groups improved rapidly compared to the ENS group. This muscle contraction force are related to muscular denervation and muscle disuse which might increase the muscle mass, indicating the progress of the motor function of the sciatic nerve (Burnett and Zager 2004). An important finding from the present study showed sensory clinical signs which are indicative of the progress of sensation in BMSCs group. The main clinical sign that helps in the sensory function assessment recovery was toe-spreading reflex. It was absent in all groups on early but it progressed and appeared on day 112 PO in the BMSCs group. A more reliable estimate of the onset and progress of recovery is the restriction of observations to the muscle group innervated specifically by the sciatic (peroneal) nerve. Movements which might be elicited by reflex are advantageous indicators of the onset of motor function. Sarikcioglu and et al. (2008) who described the spreading of three toes of the hind limbs after sciatic nerve lesions recovery and reflex movements in dogs. The muscles involved in this reflex are the peroneal II, III and IV muscles innervated by the peroneal nerve and the muscle abductor hallucis innervated by the tibial nerve, which are branches of the sciatic nerve (Craigie 1969). These peroneal muscles have a spread responsibility of the second, third and fourth toes, and constitute a reflective 'peroneal nerve function index' (PFI).

The present study confirms that the first response is a mere flicker of the first toe during the sciatic nerve regeneration. The degree of spreading increased from day to day, gradually regaining function involving the second, third and fourth toes. These results are in agreement with the report of (Schmitz and Beer 2001) who described the gradual return of peroneal nerve function. Therefore, in the present study of the sciatic nerve recovery in dogs, the reliability and

desensitization of the toe-spreading reflex were evaluated as a sign of functional recovery alongside concurrent muscle weights.

Other sensory functional tests of withdrawal reflex included the toe pinch and toe pricks which were detected through abnormal flexion of the stifle and hock joints. In the toe pinch reflex, sensitivity of the third, fourth and fifth toes were tested before sciatic nerve transection reliably induced a rapid retraction of the leg (digits 1 and 2 are innervated by the saphenous nerve). After the transection response was totally abolished in the operated limb. However, toe pinch and toe prick were absent in all groups but progressed and appeared on day 112 PO in the BMSCs groups. Progress of functional recovery of transected sciatic nerve treated by cultured fibroblast-like BMSCs constitutively expressed trophic factors and supporting substances, including nerve growth factor, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, collagen, fibronectin and laminin, thus in agreement with (Chen and others 2007). Other studies by Cuevas and et al. (2002) and Cuevas and others (2004) demonstrated significant functional improvement, via walking track analysis and sciatic nerve index (SFI) when undifferentiated BMSCs were injected directly into the primary repair site in a rat sciatic nerve injury.

Progress in the development of neuromotor function parameters, including the onset, ability to walk, type of gait, regression of knuckling, strength of muscle force contraction and regain of muscle mass developed better and faster than the development of nerve sensory function parameters. These results are consistent with previous studies on mixed nerve, suggesting preferential motor reinnervation, which means the motor axons preferentially reinnervate the motor pathways while the sensory axons may be more random (Brushart 1993). This phenomenon is termed preferential motor reinnervation and its occurrence has been linked to the expression of specific adhesion molecules by Schwann cells of the motor endoneurial tubes, which preferentially attract regenerating motor axons (Martini 1994).

In this study, the histopathological observations in the BMSC group showed a significant improvement as compared to the ENS group. The histopathological results of BMSC implantation showed the acceleration of nerve tissue regeneration. The Schwann cells might egress in the proximal and distal segments; these cells were provisions of trophic (feeding) and tropic (guidance) factors for regenerating axons (Osbourne, 2007). Histopathological observations in this study confirmed that the BMSC group was more progressive in healing than

the ENS group due to the accuracy of reinnervation, which depended largely on the nerve reconnection with end-organs. Regenerating motor growth cones will enter their original fascicles in the distal nerve stump and connect with the target organ (Brushart 1993; Yun et al., 2010). In the ENS group, the histology of the transection injured sciatic nerve fibers challenges both histopathology and regeneration because of the disruption of the endoneurial sheath with the loss of the axon alignment. The continuous Wallerian degeneration and deposition of collagen retarded the myelination process. This influences the functional recovery and these histological findings are supported by the results of function observation, which showed delayed progress of motor and sensory functions in the ENS group. This result is consistent with the findings of Dilley et al. (2003) who reported that extraneural fibrosis and wound-bed adhesions may tether the suture site and adjacent nerve bed. In conclusion, this study showed that the motor and sensory functions and acceleration of sciatic nerve regeneration in the BMSC group improved more rapidly when compared with the ENS group.

#### ACKNOWLEDGEMENTS

Authors would like to thank the National Center of Hematology staff for collaboration in the isolation of bone marrow stromal cells.

#### REFERENCES

- Al-Timmemi, H., Dhurgham H. and Zaid A. (2013) Identification of adipogenesis and osteogenesis pathway of differentiated canine bone marrow stem cells in vitro (Submitted for publication).
- Al-Timmemi, H., Ibrahim, R., Zuki, Abz. Azmi, Ti & Al-Jashamy Karim. (2011) Clinical and pathological observations of autologous bone marrow stromal cells implantation on the regeneration of sciatic nerve neurotmesis in rabbit. *African Journal of Biotechnology*10, 6310-6318.
- Bhagavati, S & Xu W. (2004) Isolation and enrichment of skeletal muscle progenitor cells from mouse bone marrow. *Biochem. Biophys. Res. Commun*318, 119-124.
- Boyd, J.G. & Gordon, T. (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Molecular Neurobiology*,27277-323.
- Brushart, T (1993) Motor axons preferential reinnervate motor pathways. *J. Neurosci*13, 2730-2738.

- Burnett, M. & Zager E (2004) Pathophysiology of peripheral nerve injury: a brief review. *Neurosurg. Focus* 16, 1-7.
- Chen CJ, Ou YC, Liao SL, Chen WY, Chen SY, Wu CW., Wang CC, Wang WY, Huang YS. & Hsu SH (2007) Transplantation of bone marrow stromal cells for peripheral nerve repair. *Exp. Neurol* 204, 43-453.
- Coronel, M.F., Musolino, P.L., Brumovsky, P.R., Hofelt, T. & Villar, M.J. (2009) Bone marrow stromal cells attenuate injury-induced changes in galanin, NPY and Y1-receptor expression after a sciatic nerve constriction. *Neuropeptides* 43, 125-132.
- Cuevas, P., Carceller, F., Dujovny, M., Garcia-Gomez, I., Cuevas, B, Gonzalez-Corrochano, R., Diaz-Gonzalez, D. & Reimers, D (2002) Peripheral nerve regeneration by bone marrow stromal cells. *Neurol. Res* 24, 634-638.
- Cuevas, P., Carceller, F., Garcia-Gomez, I., Yan M. & Dujovny, M. (2004) Bone marrow stromal cell implantation for peripheral nerve repair. *Neurology Research* 26, 230-232.
- Dilley, A., Lynn, B., Greening, J. & DeLeon N (2003) Quantitative in vivo studies of median nerve sliding in response to wrist, elbow, shoulder and neck movements. *Clin. Biomech* 18, 899-907.
- Flecknell, P. (2003). Anesthesia of rodent. In *Veterinary anesthesia and analgesia*. D. McKelvey and K. Hollingshead (Eds.), 3<sup>rd</sup> (ed). St. Louis, Missouri U.S.A. pp. 350-386.
- Craigie, E.H. (1969). *Bensley's Practical Anatomy of the Rabbit*. 8<sup>th</sup>. (ed). Toronto University of Toronto Press pp. 334-339. .
- Hall, S. (2005) Mechanisms of repair after traumatic injury *Journal of Bone Joint Surgery* 87, 1309-1319.
- Lundborg G (2000) A 25-year perspective of peripheral nerve surgery: evolving euroscientific concepts and clinical significance. *J. Hand Surg. Am* 25, 391-414.
- Mimura, T., Dezawa, M., Kanno, H., Sawada, H. & Yamamoto, I. (2004) Peripheral nerve regeneration by transplantation of bone marrow stromal cell-derived Schwann cells in adult rats. *J. Neurosurg* 101, 806-812.
- Mahmood, A., Lu, D. & Chopp, M. (2004) Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. *Journal Neurotrauma* 21, 37-57.
- Martini, R. (1994) Expression and functional roles of neural cell surface molecules and extracellular matrix components during development and regeneration of peripheral nerve. *Journal Neurocytology* 23, 1-28.
- Osbourne A. (2007) Peripheral Nerve Injury & Repair. *T S M J* 8, 29-31.
- Pan, H., Yang, D., Chiu, Y., Lai, S., Wang, Y., Chang, M. and Cheng, F. (2006) Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell. *Journal of Clinical Neurosciences* 13, 570-575.
- Pittenger, MF., MacKay, AM., Beck, SC., Jaiswal, RK., Douglas, R., Mosca, JD., Moorman MA., Simonetti, DW., Craig, S. & Marshak, DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143-147.
- Prokop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissue. *Science* 276, 71-76.
- Robinson, LR. (2004) Traumatic injury to peripheral nerves. *Suppl. Clin. Neurophysiol* 57, 173-86.
- Sarikcioglu, L., Demirel, B.M. & Utuk, A. (2008). Walking track analysis: an assessment method for functional recovery after sciatic nerve injury in the rat. *Folia Morphology* 68, 1-7.

- Scherer, S.S. & Salzer, J.L. (1996) Axon-Schwann cell interactions during peripheral nerve degeneration and regeneration. In *Glial cell development*, K.R. Jessen and W.D. Richardson (Eds.). Bios. Scientific, Oxford, UK, pp. 169–196..
- Schmitz, H. & Beer, G (2001). The toe-spreading reflex of the rabbit revisited functional evaluation of complete peroneal nerve lesions. *Lab. Animals* 35, 340-345.
- Sotiropoulou, P.A. & Papamichail, M. (2007). Immune properties of mesenchymal stem cells. *Methods Molecular Biology* 407, 225-43.
- Trapp, B.D., Hauer, P. & Lemke, G. (1988) Axonal regulation of myelin protein mRNA levels in actively myelinating Schwann cells. *Journal of Neuroscience* 8, 3515-3521.
- Yun, Y., Won, J., Jeon E., Lee, S., Kang, W., Jo, H., Jang, J., Shin, U. & Kim, H. (2010). Fibroblast growth factors: biology, function, and application for tissue regeneration. *J. Tissue Eng.* pp. 1-18.