SCIATIC NERVE REGENERATION USING AUTOTRANSPLANTATION OF TENDON WITH BONE MARROW IN DOGS.

A. Kh. AlJobory

Department of surgery and theriogenology. College of veterinary medicine. University of Mosul. Mosul. Iraq.

(Received 8 August 2012, Accepted 19 November 2012)

Keywords: Bone marrow, Sciatic, Tendon.

ABSTRACT

The aim of this study was to evaluate the regenerative activity of autotransplantation of superficial flexor digital tendon and bone marrow on the repair of sciatic nerve in dogs. Eight adult dogs from both sexes were used. The age ranges from 1-2 years and weight from 10-15 kgs. The animals were divided into two groups control and treatment group each group consists of four animals. Each animal in the first group was anesthetized with mixture of xylazine and ketamine at a dose of 5 mg/kg and 10 mg/kg of body weight I.M, respectively. The skin of the lateral surface of the thigh region was incised, fascia and muscle group were separated using blunt dissection, the left sciatic nerve was harvested and a 10 mm piece was transected. A segment longer than 10 mm of superficial flexor digital tendon was harvested and autografted using 5/0 nylon. In the second group animals underwent the same manner of first group as well as bone marrow applied at operative site. 60 days post operatively results exhibited improvement in activity of affected limb in treated animals with graft and bone marrow. Histologically there was an improvement at site of graft represented by formation of granulation tissue in both groups. In conclusion the local application of bone marrow

on the site of tendon graft led to enhance the regeneration of sciatic nerve resulting in functional improvement of the affected limb.

INTRODUCTION

Morphological and functional recovery after a nervous injury is seldom complete and perfect, despite of the use of modern and sophisticated treatment techniques. Uncountable factors may influence the regeneration of an injured nervous fiber, such as patient's age, kind of trauma, injury site, denervation time, kind and diameter of affected nervous fibers, the method employed for nervous repair, as well as other individual variables (1). After injury the healing process of the nervous tissue occur in a very slow manner in comparison to other tissues of the body (2) Experimental peripheral nervous injuries at the ischiatic nerve may be provoked through a number of procedures, such as: smashing by compression, trans-section, stretching and freezing. Several factors such as magnitude, duration and mechanism of compressive trauma are important to determine injury degree (3). In some traumatic nerve injuries when the gap is long autologous nerve grafting from the patient own body is the first choice for bridging the gap between the severed nerve ends (4,5). Treatment of traumatic transection of peripheral nerve is primary anastomosis either in acute phase or chronic phase. The usual anastomotic technique is suturing the nerve endings in an end-to-end fashion (6,7,8). Demyelinization and remyelinization, axonal degeneration and regeneration, focal, multifocal or diffuse nerve fiber loss and endoneural edema may be encountered due to damage of intraneuronal microcirculation. It is known that after injuries due to tissue destruction free oxygen radicals increase and cause tissue damage (9,10). There is different type of graft such as natural which may be autograft or

allograft and synthetic graft which made of different materials. Graft should be 15-25 percent longer than the gap to be spanned and its diameter should also match the diameter of the injured nerve (11). Bone marrow stromal cells injected in a lesioned peripheral nerve can survive, migrate, differentiate in Schwann cells, and promote functional recovery, they may be an important source for cellular therapy in several neurological diseases. (12) It has been shown that the transplantation of differentiated bone marrow stromal cells into silicone tube and cut ends of nerves exerts a beneficial effect on peripheral nerve regeneration (13). Autologous nerve grafting is the first choice for bridging the gap between the severed nerve ends but this therapeutic strategy has some disadvantages including permanent loss of donor function and requirement of multiple surgeries (14,15). Another way to achieve micro anastomosis of cut nerves by using cyanoacrylate-based glue appears to be effective as micro suturing the nerve ends despite more local adhesions in the glued nerve (16).

MATERIALS AND METHODS

Eight dogs of both sexes were used in this study. The age ranged from 1–2 years and weight from 10–15 kgs. The animals were divided into two groups. Each group consist of four animals. The animals were anesthetized by mixture of xylazine and ketamine at dose of 5 mg/kg of body weight and 10 mg/kg of body weight I.M respectively. Following skin incision, fascia and muscle groups were separated using blunt dissection, the left sciatic nerve was exposed, cleared of connective tissue and a 10 mm segment was removed.

Segment of superficial flexor digital tendon of the right forelimb slightly longer than the removed nerve segment was autografted by

simple interrupted technique using 5/0 nylon. In the second group the same procedure was performed with application of 2 ml of fresh bone marrow obtained from the femoral bone of the same animal on the site of grafting then the animals were followed up weekly for observation of clinical improvement. At 60 days post operatively Sciatic nerve and autografted tendon sections were collected and prepared for histopathological examinations.

RESULTS

Clinical observations

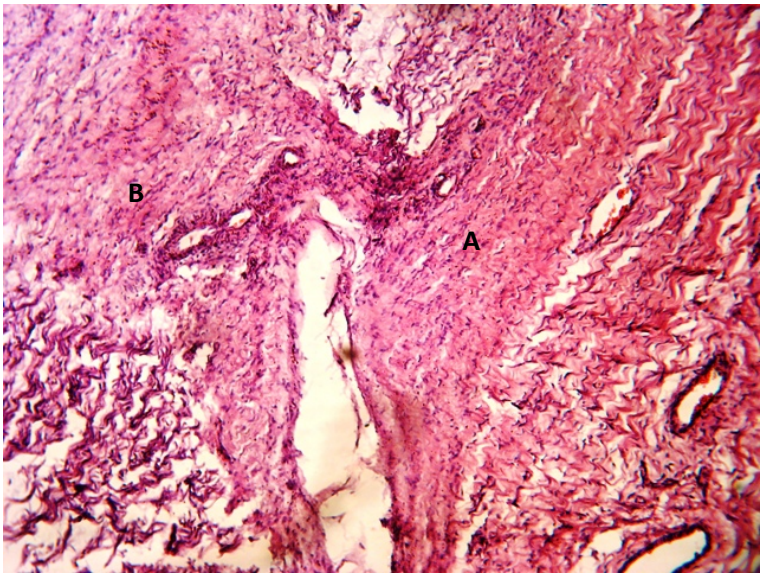
Inflammatory signs are noticed at the site of operation which include swelling, pain and redness. paralysis of the left hind limb with muscles atrophy and loss of sensation. After two week there was a slight degree of sensation and the animal try to stand on its limb but the movement was restricted. The improvement increased at the 30th day after operation represented by the ability of the animal to use his affected limb in both standing and movement state but the interrupted use manner was characteristic of this period especially in the treatment group. The sensation was good to stimuli and muscle mass of affected limb approximately returned to normal size. The gradual clinical improvement was being more prominent at 60 days post operatively specially in the second group.

Histopathological results:

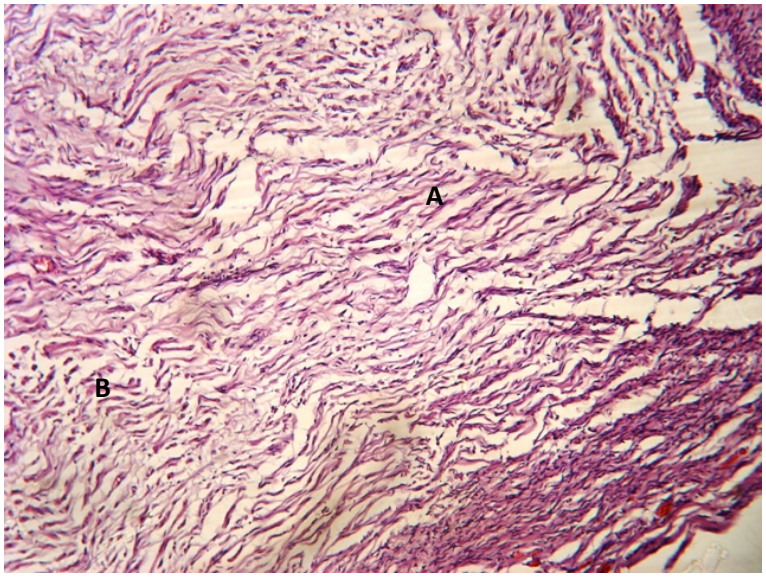
Control group: Histological sections of nerve in control group showed Proliferation of collagen and fibroblast at operative site. Mild infiltration of mononuclear cells. and vacoulation and proliferating fibrous

connective tissue (collagen fibers and newly capillaries) between nerve and tendon. Hyalinization in tendon fibers was also seen. Bridges of fibrous connective tissue between nerve and tendon. Also, proliferation of Schwann cells. Congestion of blood vessels and wallerian degeneration and demyelination have been seen (figures 1-4).

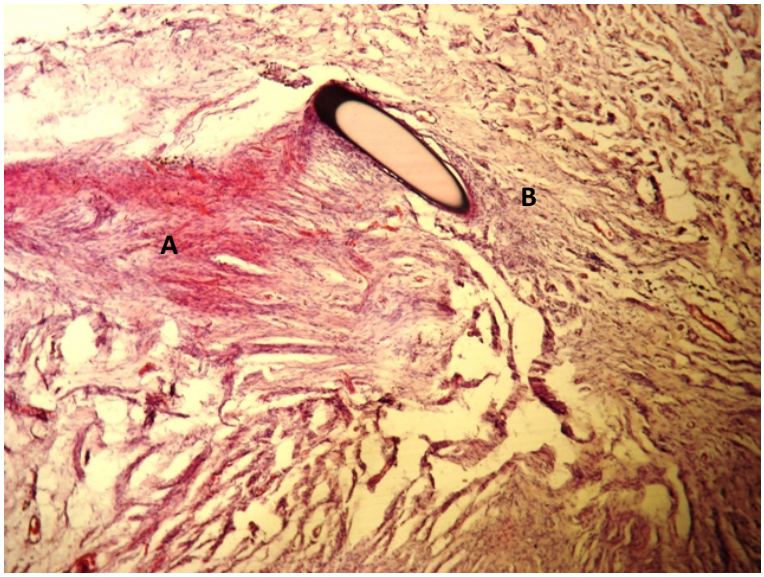
Treatment group: Histological sections of nerve in treatment group showed Mature connective tissue, glial nodule, newly capillaries. There was proliferation of Schwann cells and infiltration of mononuclear inflammatory cells. Wallerian degeneration. There was remnant of bone marrow in grafting area, proliferation of Schwann cells and fibrous connective tissue around suturing area have been seen. There were hemorrhage, vacuolation and severe demyelination have been seen (figures 5-8) .



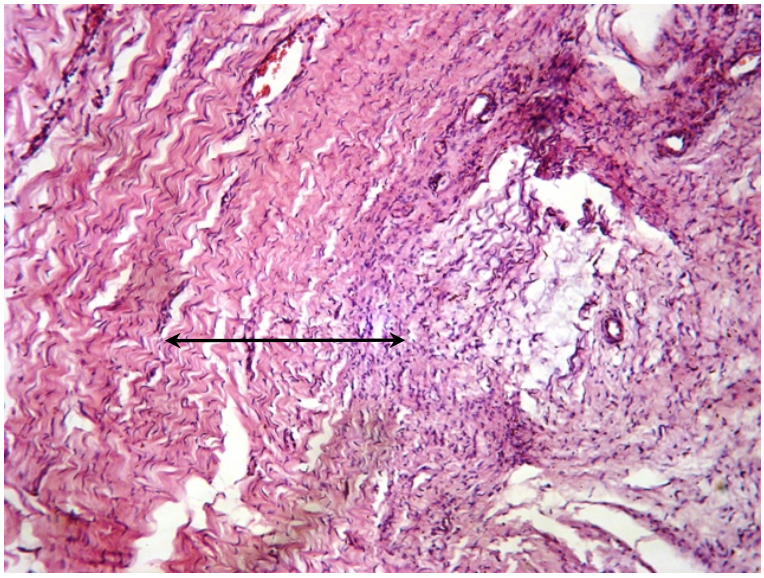
(Figure,1) photomicrograph of nerve graft segment in first group in dog 60 days postoperatively showed proliferation of collagen fibers and fibroblast (A). Infiltration of mononuclear inflammatory cells(B). (H&E 115 X).



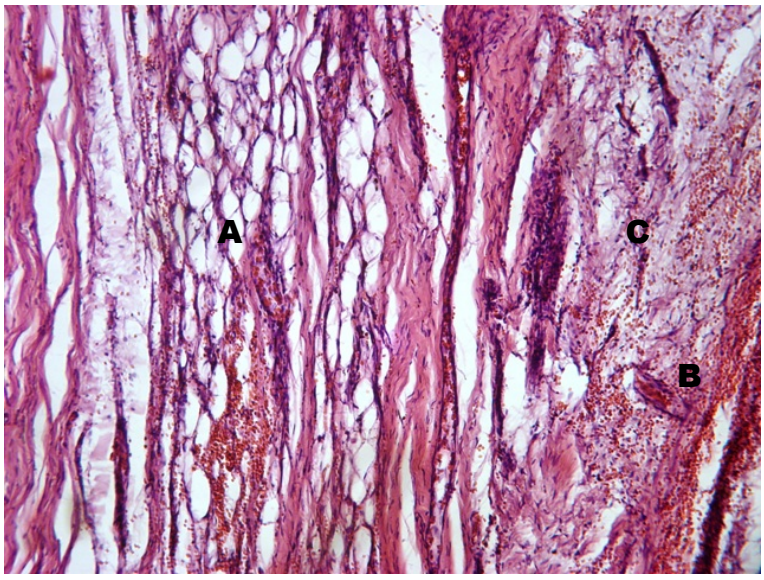
(Figure,2) photomicrograph of nerve graft segment in first group in dog 60 days postoperatively showed proliferation of fibrous connective tissue(A). Proliferation of Schwann cells(B).(H&E 115 X).



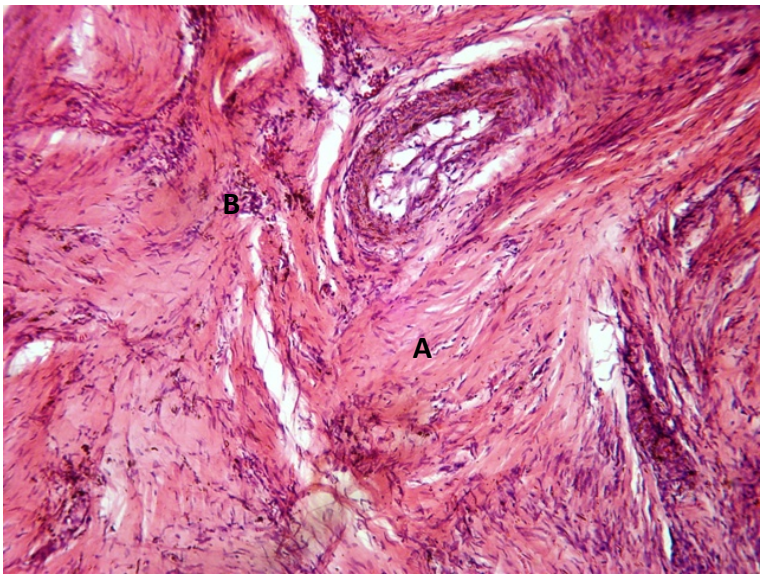
(**Figure,3**) photomicrograph of nerve graft segment in first group in dog 60 days postoperatively showed hyalinization of tendon(A). proliferation of fibrous connective tissue(B). (H&E 60 X).



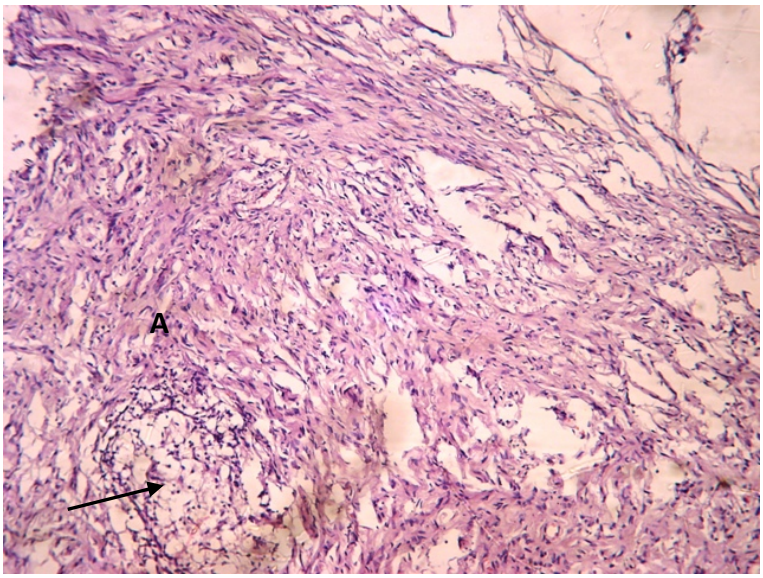
(Figure,4) photomicrograph of nerve graft segment in first group in dog 60 days postoperatively showed proliferation of fibrous connective tissue between tendon and nerve fibers (arrow). (H&E 145 X).



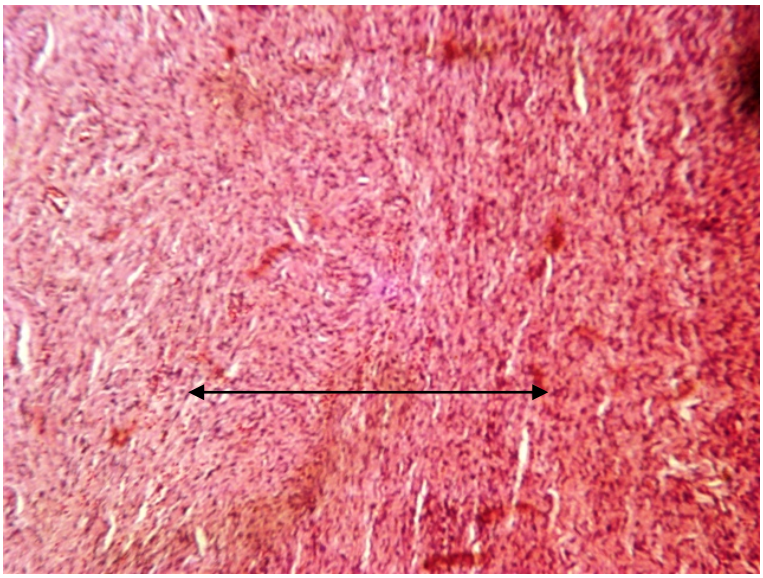
(Figure,5) photomicrograph of nerve graft segment in second group in dog 60 days postoperatively showed demyelination of nerve fibers(A). Hemorrhage (B). Infiltration of mononuclear inflammatory cells(C) . (H&E 145 X).



(Figure,6) photomicrograph of nerve graft segment in second group in dog 60 days postoperatively showed proliferation of fibrous connective tissue with newly capillaries(A). Infiltration of mononuclear inflammatory cells(C) .(H&E 145 X).



(Figure,7) photomicrograph of nerve graft segment in second group in dog 60 days postoperatively showed presence of glial nodules (arrow)associated with proliferation of collagen fibers (A). (H&E 145 X).



(Figure,8) photomicrograph of nerve graft segment in second group in dog 60 days postoperatively showed mature connective tissue(arrow). (H&E 145 X).

DISCUSSION

All animals of both groups underwent signs of paralysis and muscle atrophy as result of sciatic nerve damage induction because atrophic changes and lack of muscular function were the natural consequences of neural deprivation of muscles (17). Myelination of nerves is essential for proper function of the nervous system, predominantly by allowing faster conduction velocity of action potentials. In the peripheral nervous system, bundles of axons are assembled during embryonic development. After birth, premyelinating Schwann cells separate these bundles by extending processes between the axons. Myelinating Schwann cells establish a 1:1 ratio with axons, and envelop them in a myelin sheath. Non myelinating Schwann cells extend cytoplasmic processes between axons and separate them, but do not form a myelin sheath (18). In order to prevent any immune reaction the auto transplantation of both bone marrow and tendon was used in this study

(19). Non absorbable suture material (Nylon) was used to connect the tendon to the sciatic nerve because it causes minimal tissue reaction (20). Histologically in this study there was proliferation of Schwann cells which reflect a progress in the regeneration of sciatic nerve as the regeneration of peripheral nerves after injury is known to depend on Schwann cells in areas distal to the injury It is thought that the Schwann cells provide a guide by which re growing axons can precisely extend and re enervate the appropriate targets. In the distal stumps, axons degenerate, and so Schwann cells cannot be supported by axon-generated molecules(21) In this study 10 mm of sciatic nerve was removed because Previous studies have shown that regenerating axons were able to bridge tubular nerve conduits implanted into the rat sciatic nerve if the gap was10 mm or less, but failed in most cases with 15 mm gaps(22,23)**.**The infiltration of mononuclear inflammatory cells (macrophage) as histological results revealed in both groups indicate the regenerative process of sciatic nerve in this study because in Wallerian degeneration after peripheral nerve trauma and during primary or secondary neuropathic axonal damage, macrophages phagocytose and remove degenerating myelin in a complement-depending manner, paving the way for successful axonal regeneration(24).On the other hand presence of glial nodule in the treatment group in response to injury represent another sign of healing process as microglial cells of the central nervous system are known to proliferate rapidly in response to injury(25,26). an extremely rapid response of resident endoneurial macrophages to injury that precedes the influx of blood-derived macrophages and resembles the microglial response of the brain to injury. Much like microglial cells of the brain, resident endoneurial macrophages may be primarily involved in surveillance of the peripheral nervous system and as sensors of

pathological events, By virtue of their location and rapid response, they may act primarily as regulating cells, whereas later arriving hematogenous macrophages may take the role of effector cells of tissue destruction and repair (26). It seems that in spite of the various methods used to reproduce a nervous injury, the functional and physiological response to smashing action were the same (27).

تجدد العصب الوركي باستخدام الزرع الذاتي للوتر ونخاع العظم في الكلاب

احمد خلف الجبوري

فرع الجراحة وعلم تناسل الحيوان/كلية الطب البيطري/جامعة الموصل/ الموصل / العراق

الخلاصة

أجريت هذه التجربة لتقييم تأثير الزرع الذاتي لنخاع العظم على تجدد العصب الوركي المرقع برقائع ذاتية من الوتر الثاني الاصبعي السطحي في الكلاب. تم استخدام ثماني حيوانات من كلا الجنسين تراوحت أعمارها ما بين 1-2 سنة وأوزانها مابين 15-20 كغم. قسمت حيوانات التجربة إلى مجموعتين ضمت كل مجموعة أربع حيوانات. تم تخدير كل حيوانات التجربة بمزيج من الزيلازين 2% و الكيتامين 5% وبجرعة 5ملغم/كغم,10ملغم/كغم من وزن الجسم أعطيت عن طريق العضلة على التوالي. بعد ذلك تم إزالة قطعة بطول 10 ملم من العصب الوركي للقائمة الخلفية اليسرى لحيوانات المجموعة الأولى وبعدها تم ترقيع العصب برقعة ذاتية من الوتر الثاني الاصبعي السطحي أطول بقليل من قطعة العصب المزالة وباستخدام خيط جراحي نوع (نايلون 5\0) بعد ذلك تم خياطة العضلات والجلد. أما في المجموعة الثانية فقد تم إجراء نفس الخطوات السابقة مع إضافة نخاع العظم من نفس الحيوان موضعيا على منطقة قطع العصب. أظهرت النتائج الوظيفية حدوث تحسن في وظيفة القائمة المعالجة بنخاع العظم بعد مرور 60 يوما من إجراء العملية في حيوانات المجموعة الثانية. أما نسيجيا فأظهرت النتائج حدوث التئام لمنطقة القطع من خلال تكون نسيج حبيبي في كلتا المجموعتين. نستنتج أن إضافة نخاع العظم أدى إلى تحسن وظيفي للعصب المصاب.

REFERENCES

1-Mendonça AC, Barbieri CH, Mazzer N. (2003). Directly low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods. 129:183-90.

2- Nicolau, R. A, Martinez MS, Rigau J,Tomas J.(2004). Effect of low power 655 nm diode laser Irradiation on the neuromuscular junction of the mouse diaphragm. Laser Surg Med. 34:277-284.

3- Diao E, Andrews A, Diao J. (2004). Animal models of peripheral nerve injury. Op Techn Orthop. 14:153-62.

4- Hadlock T, Elisseeff J, Langer R, Vacanti J, Cheney M. (1998). A tissue- engineered conduit for peripheral nerve repair. Arch Otolaryngol Head Neck Surg. 124, 1081-1086.

5. Meek MF, Coert JH.(2002). Clinical use of nerve conduits in peripheral-nerve repair: Review of the literature. J Reconstr. Microsurg. 18, 97-110.

6- Birch R, Achan P. (2000). Peripheral nerve repairs and their results in children. Hand Clin 16**:**579–595.

7- Guerra WK, Baldauf J,Schroeder HW.(2007). Long-term results after microsurgical repair of traumatic nerve lesions of the upper extremities. Zentralbl Neurochir. 68**:**195–199.

8- Rochkind S, Filmar G, Kluger Y, Alon M. (2007). Microsurgical management of penetrating peripheral nerve injuries: pre, intra-and postoperative analysis and results. Acta NeurochirSuppl. 100:21–24.

9- Arslan E, Milcan A, Unal S, Demirkan F, Polat A, Bagdatoglu O, Aksoy A, Polat G. (2003). The effects of carnitine on distally-

burned dorsal skin flap: an experimental study in rats. Burns. 29: 221–227.

10- Cinel I, Avlan D, Cinel L, Polat G, Atici S, Mavioglu I, Serinol H, Aksoyek S, Oral U.(2003). Ischemic preconditioning reduces intestinal epithelial apoptosis in rats. Shock. 19: 588–592.

11-Slatter DH,(1985) Small Animal Surgery . S.B Saunder Company, Philadelphia, Vol 1: PP 1352 .

12- Cuevas P , Carceller F, Dujovny M, Garcia G,Corrochano RL,Diaz GI, Cuevas B, Gonz RD.(2002). Peripheral nerve regeneration by bone marrow stromal cells. Neurological Research.24(7):634-639.

13-Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H.(2001). Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. Eur J Neurosci.14: 1771-6.

14- Kakinoki S, Uchida S, Ehashi T, Murakami A, Yamaoka T. (2011). Surface modification of poly (L-lactic acid) nanofiber with oligo (D-lactic acid) bioactive-peptide conjugates for peripheral nerve regeneration. Polymers.3:820-832.

15- Mohammadi R, Azizi S, Delirezh N, Hobbinaghi R, Amini K.(2012). Transplantation of undifferentiated bone marrow stromal cells improves sciatic nerve regeneration and fnctional recovery through inside-out vein graft in rats. Turk J Med Sci.42(1):127-136.

16- Roth J, Shtokman J, Shamer MH, Nissan M, Shchtinkov L, Trego LL, Rochkind S.(2011). Regeneration of the transected rat sciatic nerve after suturing or adhesion with cyanoacrylate glue. J Neurosurg.114:245-252.

17- Mester E, Mester AF. (1996). The biomedical effect of laser application. Laser Surg Med.5:31-39.

18- Mirsky R, Jessen KR.(1999). The neurobiology of Schwann cells. Brain Patho. 9:293–311.

19- Kumar V, Cortan RS , Robbins SL.(2003). Robbins basic pathology. S.B Saunder Company. Philadelphia, PP 122.

20-Slatter DH,(1985) Small Animal Surgery . S.B Saunder Company, Philadelphia, Vol 1: PP 339 .

21- Fawcett JW, Keynes RJ.(1990). Peripheral nerve regeneration.Annu Rev Neurosci. 13:43–60.

22- Ceballos D, Navarro X, Dubey N, Wendelschafer-Crabb G,Kennedy WR, Tranquillo RT.(1999). Magnetically aligned collagengel filling a collagen nerve guide improves peripheral nerve regeneration. Exp Neurol.158: 290–300.

23- Evans GR, Brandt K, Katz S, Chauvin P, Otto L, Bogle M, Wang B, Meszlenyi RK, Lu L, Mikos AG, Patrick Jr CW.(2002). Bioactive poly(l-lactic) conduits seeded with Schwann cells for peripheral nerve regeneration.Biomaterials.23: 841–848.

24-Bruck W.(1997). The role of macrophages in Wallerian degeneration.Brain Pathol. 7:741–752.

25-Raivich G,Bohatschek M, Kloss CU,Werner A,Jones LL,Kreutzberg GW.(2000). Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function.Brain Res Brain Res Rev. 10:77–105.

26-Kreutzberg GW. (1996). Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19:312–318.

27- Beer GM, Steuter J, Meyer VE. (2001).Standardizing nerve crushes with a non-serrated clamp. J Reconstr Microsurg.17:531-4.