CLINICAL AND HISTOPATHOLOGICAL STUDY OF THE EFFECT OF THE PULSED MAGNETIC FIELD AND LOW LEVEL LASER THERAPY ON THE REGENERATION OF THE SCIATIC NERVE IN RABBITS

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ABSTRACT

The aims of this study was to compare between the effect of low level laser therapy and the magnetic field on regeneration of sciatic nerve injury and selected a good method for treating through comparing several methods of treatment ,and study the effect of Laser ,magnetic treatment on blood liver enzymes such as [Glutamic Oxaloacetate transaminase(GOT), Alkaline phosphatas (ALP) and Glutamic Pyruvic Transminase (GPT)].

Twelve mature male rabbits were used in experiment. Animals divided into three groups. Each group contain four rabbits control group, electromagnetic field(EMF) group and laser group.

Sciatic nerve has been transected in all animals of experiment ,after surgical procedure in animal, in the (EMF) group, the wounds were exposed to pulsed magnetic field (high tension= 310 gauss) and in laser group ,the wounds were exposed to first wave length(632.8 nm) for 15 minutes daily for 15 days except control group, they healed with conventional treatment. Animals were followed up clinically for 14 days, and histologically for 60 days after surgery.

The results either clinically, or histopathologically indicated that the magnetic field accelerate the healing. However, there are differences among groups in faster healing response. The laser and EMF groups have somewhat similar results.

INTRODUCTION

The sciatic nerve is the longest and largest nerve in the body and it is arising from the lumbo-sacral plexus. The sciatic nerve supplies the caudal thigh muscle and the caudal hip muscle except the external obturator (1).

The electromagnetic field has been used for treatment of various types of wounds and other conditions during the last 10 years (2). The electromagnetic field (EMF) have encouraged healing of fractured bones and benefited reanastomosis of peripheral nerves after transaction (3). Magnetic field therapy is safe, has multiple uses, simplified using, and significant results . There are many effects of physical agents like electricity, ultrasound, low power laser and magnetic field on the outcome of the healing of peripheral nerve, soft tissue and bones (4,5).

The use of low levels of visible or near infrared light for reducing pain, inflammation and edema, promoting healing of wounds, deeper tissues and nerves, and preventing tissue damage has been known for almost forty years since the invention of lasers (6). The major advantages of laser welded nerve coaptation are, under optimal circumstances, the decreased surgical time, the extreme precision, the minor damage to the nerve tissue, and the prevention of foreign-body reactions (7,8). Low power laser has been used in medicine for over three decades in many medical centers of the world to treat wide variety of diseases(9). Many Studies that evaluated the effects of magnetic irradiation on fractures (10) and wound (11,12) tendon (13) and (14) showed positive results.

In the present study used the electromagnetic field and laser therapy as astrategy for treatment of sciatic nerve transection in rabbit and comparative between two methodologies of treatment.

MATERATS AND METHODS

Twelve adult healthy males rabbits were chosen in this study from local market in Basra city, weight range between (1.5-2)kg.

Animal Preparations

The animal was given a mixture of Ketamine- Xylazine (15mg-5mg)/ kg B.W I\M (PANAPHARMA S.A France). The rabbits were randomly divided into three equal groups: Control, EMF and laser group

Surgical Procedures

Firstly, prepare the animals to surgery, the left thigh was prepare under aseptic technique, skin incision about 4 cm in length performed parallel and behind the femur bone ,the sciatic nerve was exteriorized to the wound surface(Fig 1),after that, the nerve was transect by using a surgical blade, then the two ends of nerve suture using 5-0 nylon suture, are placed equidistantly around the nerve in the epineurium by using simple interrupted suture (15)(Fig 2), . The muscles and subcutaneous fascia closed with 3-0 cut gut suture (China) simple continuous. and then the skin closed with 3-0 silk suture (Silkam BIBRAUM AESCULAP, China). The animals are given systemic antibiotics intramuscular daily for 7 days postoperatively by use intra muscular injection of procaine penicillin 800.000 IU/kg(ZMC Import GmbH Germany), silk suture was removed after 7 days. The animals were fixed on coach and prevent them from the motion during exposure period, then after 24 hrs from surgical operation the rabbits in magnetic field groups should be treated with magnetic field, treatment was applied by exposure the site of operation to magnetic field for 15 minute / daily for 2 weeks (16).

Similar procedure was done as in laser group, the site of nerve injury was exposed to 632.8 nm wave length of this laser after put them on distance 10 cm between the light source of laser and target tissues of the wound.

Magnetic Field Devices

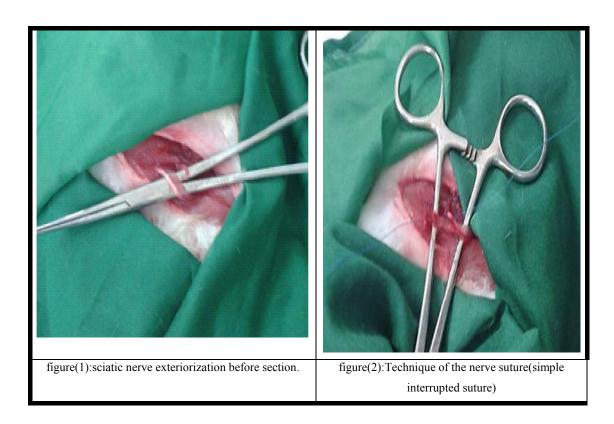
This apparatus consist of five parts: power supply, magnetic coil, analogue or digital ammeter, compass, modified wood and plastic bed Fig(3).

Laser Devices

He-Ne(632.8nm) laser unit emitting red light with 1mw maximum power was radiated with a manual k1 probe in direct and continues wave(Cw)from (Griffin &George-London).

The clinical signs were recorded until the end of the experiment ,the macroscopic findings and neurohistopathological examination were done at the

16 week , post operation after killed the animals in all group and before taking the specimens the following macroscopic observation were obtained presence of adhesions, degree of nerve coaptation, and presence of thickening).



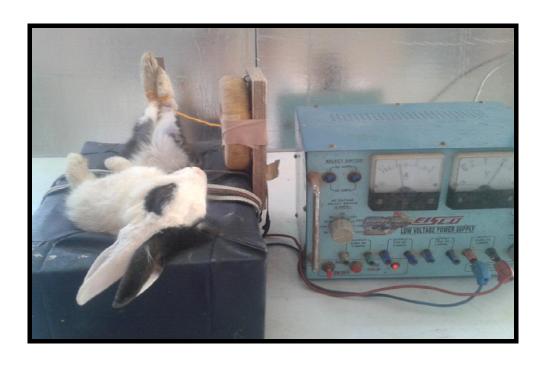


figure (3)The apparatuses of magnetic fields generation and animal exposure to magnetic field

Biochemical parameters:

The blood samples(5 ml) were collected from heart and kept in tubes and analyzed by using spectrophotometer (P D 303S serial No.301101, Japan), England, and diagnostics kits.

Histopathological examination

At the end of all period ,the specimens were taken from target tissue and fixed immediately in 10% neutral buffered Formalin then routinely processed and embedded in paraffin. The paraffin embedded blocks were cut into 5 microns (17) and stained by hematoxyline and eosin stains, and they were examined under light microscope. The histopathological examination was taken from animals at 60 days after operation .

Statistical analysis

Data were reported as mean SEM. statistical significance of the difference in was assessed by students t-test according to (18).the level of significance was set at (p 0.05).

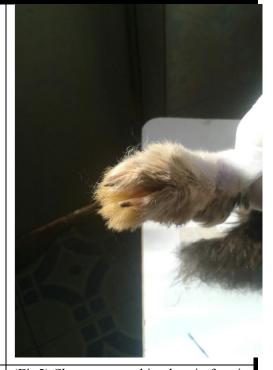
RESULTS

In control group the signs which appear on the animals were paralysis of leg, weak of muscle force and atrophy of thigh muscle, severe knuckling, sore in the hock joint, tropic ulcer at the dorsal surface of the left foot (fig 4). In laser group the signs were similar to magnetic group, the pain was relief after exposure to EMF and laser therapy and the animals have ability to return the affected leg to right position post operation in the 7 days, the tropic ulcer of foot occurs in 2 animal in laser group and in the other animal doesn't occur (fig 5), paralysis of left hind limb which is clear, early development of mild sore of hock joint in all animals. The sore began to reduce in size to became moderate and then mild - size at the end of experimental to form scabies in the end. The macroscopic findings in control group explain present the coaptation the sciatic nerve injury in 3 animals but the one animal didn't have coaptation between the ends, presence of adhesion with the tissue (fig 7). In laser group the coaptation of the

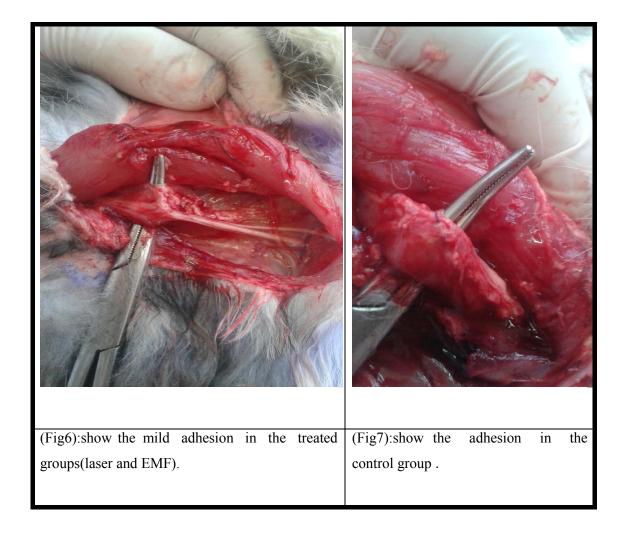
two ends of the nerve injury was seen and no thickness and mild adhesion in the most animals. In magnetic group show the coaptation of the ends was seen very good and no thickness and mild adhesion(fig 6) .



(Fig4):show atrophic ulcer in foot (large size)in control group



(Fig5):Show no atrophic ulcer in foot in treated group (laser and EMF).



Regarding the GOT and ALP enzymes there was no effect of magnetic field or laser therapy on GOT enzyme Table (1), while there was an increase of ALP enzyme after exposal to magnetic field after the operation Table (2) ,also show significant (p 0.05) increase in GPT enzyme after exposal to magnetic field and laser therapy Table (3)

Table (1):The effect of magnetic field and laser therapy on GOT in the rabbit (Mean±S.D.)

Time Group	Zero Day	After Week
magnetic field	20.5 ± 2.06	23.64 ± 2.11
laser therapy	20.16 ± 1.38	23.7 ±1.21

Table (2):The effect of magnetic field and laser therapy on ALP in the rabbit (Mean±S.D.)

Time Group	Zero Day	After Week
magnetic field	70.30 ± 3.79	86.45 ± 2.11
laser therapy	68.4 ± 1.69	68.7 ± 1.03

Table (3): The effect of magnetic field and laser therapy on GPT in the rabbit (Mean±S.D.)

Time Group	Zero Day	After Week
magnetic field	5.1 ± 0.7	12.1 ± 1.8
laser therapy	3.8 ± 0.3	5.1 ± 0.3

After 16 week killing the animals and opening the muscles to take biopsy of sciatic nerve. The neurohistopathological and macroscopic examination appears that accelerated the regeneration of sciatic nerve and improvement the motor and sensory function of the nerve were best in the magnetic field group and then in the laser group, explains by present of The coaptation of the two ends of the nerve injury seen no thickness and no adhesion with other tissues, but in laser group show mild adhesion in the most animals. The section of the sciatic nerve in magnetic field group shows completed regeneration of nerve fibers with granulation tissue and congested blood vessels Fig(8 and 9). The section of the sciatic nerve in laser group show mild regeneration of nerve fibers with excessive vacuolated of neurocytes with hemorrhagic area in healing site Fig(10 and 11), While in control group the section of the sciatic nerve explain proliferation of fibrocyte with oedematous fluid with vacuolation in healing site Fig(12 and 13).

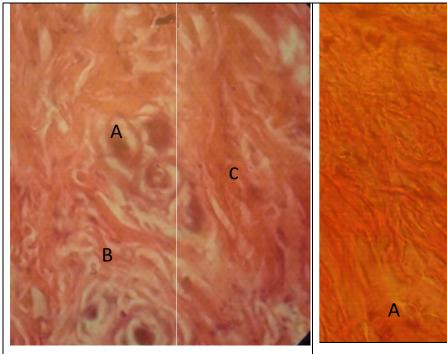


figure (8): Histopathlogical section in nerve of magnatic field group at 16 week post surgery(A) new blood vessels,(G) granulation tissue ,increase fibrocyte (H&E stain) 40 X.

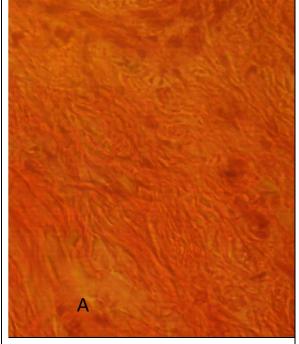


figure (9): Histopathlogical section in nerve of magnatic field group at 16 week post surgery(A) proliferation nerve fiber (H&E stain) 40 X

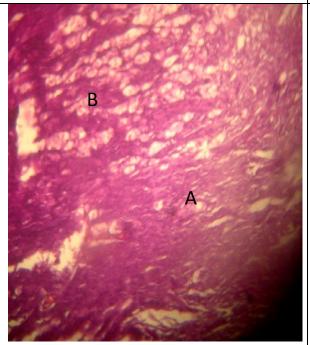


figure (10): Histopathlogical section in nerve of laser group at 16 week post surgery(A) excessive vacuolated of neurocytes (B) fibrinous exudates in healing site(H&E stain) 40x

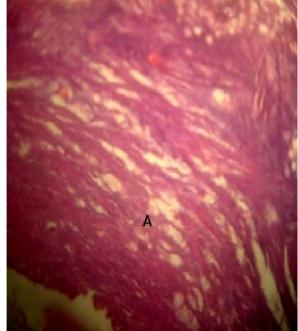
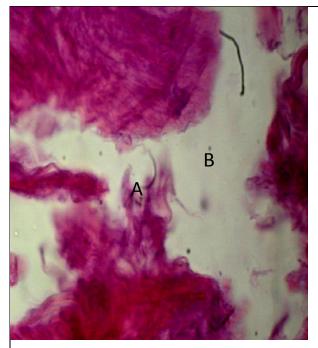


figure (11): Histopathlogical section in nerve of laser group at 16 week post surgery show (A) excessive vacuolated of neurocytes (B)hemorrhagic area in healing site(H&E stain) 40 X.



C A B

figure (12): Histopathlogical section in nerve of control group at 16 week post surgery show(A) proliferation of fibrocyte in healing site, (B) oedema.(H&E stain) 40 X

figure (13): Histopathlogical section in nerve of control group at 16 week post surgery(A)proliferation of fibrocyte (B) edematous fluid (c)congestion in healing site (H&E stain) 40x.

DISCUSSION

The present study evaluated the electromagnetic field and laser therapy as a strategy for treatment of sciatic nerve damage in rabbits as comparative between tow methodologies of treatment. The duration of application of magnetic field and laser therapy for 15 minute/daily persist to two weeks and there were proper to heal or regenerate of nerve injury. Injury of a peripheral nerve frequently results in considerable disability. In an extremity, such lesions may be associated with loss of sensory and motor functions, which leads to severe occupational and social consequences. Surgical repair is the preferred modality of treatment for severe peripheral nerve injury (9). The pain was relief and analgesic, this result agrees with (16, 19). Pain is transmitted along nerves cells as an electric signal, a pain signal depolarizes a cell. Magnetic appear to raise the depolarizes potential of the cell so that the signal is blocked from depolarized in effect, (blocking the pain). The ability of nerve to send pain is slowed by magnetic field. These phenomena can aid in the relief of pain throughout the body (20), the duration

of analgesic effect of magnetic field has not been found but the mechanism by which the magnetic field can induce pain relief is by depolarization, repolarization and hyper polarization of neurons (21). The paralysis of the left hind limb post operation dysfunction of peripheral nerves result from damage to the manifestated by neuron, to the Schwann cells, or to the myelin sheath. The damage nerves cannot transmit impulse in normal fashion this result agreement with (22). Thigh muscle atrophy was noticed in all animals of control and treated groups with variable degrees of severity, in control group, it was very severe this agreed with (16,23). The histopathological and macroscopic examination appear that accelerated the regeneration of sciatic nerve and improvement the motor and sensory function of the nerve was best in the magnetic field group, then laser group .The positive effects of applied electromagnetic field on the nerve regeneration include increase metabolism and tissue repair because these things are associated with increased blood flow, the blood flow carries more oxygen and nutrients to the exposure area and thus promoting healing (12). In Addition to The magnetic therapy can help to improve and enhances the absorption of nutrients (24). The effects of applied electromagnetic field on the nerve regeneration, include increase cell differentiation, increase axon sprouting and increase speed of regeneration (25). The regeneration of nerve fibers marked with decrease of swollen vacuolated degeneration nerve fibers in the magnetic field groups. This result agrees with (16).

The decision to include studying GOT, ALP and GPT enzymes in this study in spite of our prior knowledge that these enzymes are present in the liver, red and white blood cells, heart cells, muscle tissue (12), these organs could be affected by magnetic field although they were not a target of magnetic field therapy.

The present study used electromagnetic field and laser therapy and found that there were synergistic effects of this method to improve neurological recovery after sciatic nerve injury in clinical, macroscopic and microscopic considerations.

دراسة سريريه ونسجية مرضية لتأثير المجال المغناطيسي المتناوب والليزر على تجدد العصب ألوركي في الأرانب

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الخلاصة

الهدف من هذه الدراسة ,المقارنة بين استخدام الليزر والمجال المغناطيسي على تجدد العصب ألوركي المجروح. بالاضافه إلى دراسة تأثير العلاج على اللانزيمات .قيمت التجربة سريريا ونسيجا , بالاضافه إلى قياس مستويات خمائر الكبد مثل خميرة ال GOT) Glutamic Oxaloacetate transaminase) .استخدم اثنا (Alkaline phosphatas (ALP. وخميرة .GPT) Glutamic Pyruvic Transminase) .استخدم اثنا عشر أرنبا ذكور في التجربة وقسمت حيوانات التجربة إلى ثلاث مجاميع وكل مجموعة تحتوي أربعة أرانب والتي تضمنت مجموعة السيطرة ,مجموعة معالجة بالمجال الكهرومغناطيسي ,مجموعة معالجة بالليزر .تم قطع العصب ألوركي في جميع حيوانات التجربة وعرضت الجروح إلى المجال المغناطيسي الثابت بجرعة (310 كاوس والليزر (632,8)) مره في اليوم 15دقيقه ولفترة 15يوم عدا مجموعه السيطرة التي شفيت بصوره طبيعيه .تمت متابعة حيوانات المجاميع المختلفة سريريا لمدة 14 يوما بعد العملية ونسيجيا ٦٠٠٠ يوما بعد العملية .

أظهرت النتائج تعجيل سرعة عملية الالتئام بالإضافة إلى ذلك هناك اختلافات بين المجاميع المعالجة في سرعة استجابة التئام فقد أظهرت مجموعتي المعالجة الليزر والمجال المغناطيسي تشابها نوعا ما في النتيجة الفحص النسيجي والعياني يبين أن سرعة تجدد العصب ألوركي وتحسن الوظيفة الحركية والحسية للعصب تكن أفضل في مجموعة المجال المغناطيس وبعدها مجموعة الليزر.

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