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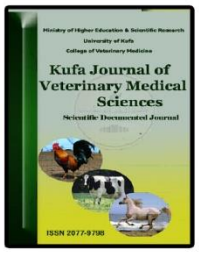

A Comparative Study of The Effect of Red Laser Wavelength and Green Laser Wavelength on The Healing of Achilles Tendon Injury In Rabbits

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Abstract

This study was designed to compare the effect of laser wavelength treatment on an injured Achilles tendon; between two laser types red laser(650nm-10w) and green laser(532nm-10w). 18 rabbits, male and mature were used in the present study, the animals were divided into three groups, a control group, red laser group and green laser group. Each group was tenoctomized the Achilles tendon, two groups were treated with one joule red laser and one joule green laser for 10 minutes daily for 14 days, laser beam applied vertically on the tendon dorsally, while the control group leaves to conventional recovery. The red laser group was showing significant values at P≤0.05 in most items or parameters while green laser group was showed convergence with a control group except few items at P≤0.05, as well as a red laser group show better healing sonographically while histopathologically , images reveal no different in all groups at microscopic observation. **In conclusions:** laser therapy mainly depends on wavelength more than density and red laser better than green laser in treatment of Achilles tendon injury.

Keyword: Red laser, Green laser, Laser therapy, Achilles tendon

دراسة مقارنة لتأثير الطول الموجي لليزر الاحمر والطول الموجي لليزر الاخضر على التئام جرح وتر العرقوب في الارانب

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الخلاصة:

قد تم تصميم هذه الدراسة لمقارنة تأثير الطول الموجي لليزر على جرح وتر العرقوب. بين نوعين من الليزرات من الليزر الأحمر (650nm-10mw) والليزر الأخضر (532nm-10mw).استخدمت 18 ارنب من الذكور الناضجة في هذه الدراسة، تم تقسيم الحيوانات إلى ثلاث مجموعات، مجموعة سيطرة، مجموعة ليزر الاحمر ومجموعة ليزر الاخضر. تم قطع الوتر لكل مجموعة وتر اكيلس ، تم علاج مجموعتين مع واحدة ليزر أحمر جول واحد ليزر أخضر جول لمدة 10 دقيقة يوميا لمدة 14 يوما، شعاع الليزر تطبق عموديا على ظهريا

وتر، في حين يترك السيطرة على المجموعة إلى الانتعاش التقليدي. مجموعة ليزر أحمر كان يظهر القيم الهامة في $P\leq0.05$ في معظم الفقرات بينما أظهرت مجموعة ليزر الاخضر التقارب مع المجموعة السيطرة باستثناء بعض الامور في $P\leq0.05$، وكذلك الأحمر وتظهر مجموعة ليزر أفضل الشفاء بينما نسيجيا ، لا يختلف في جميع الفئات في الاستنتاجات العلاج بالليزر يعتمد بشكل رئيسي على الطول الموجي أكثر من الكثافة والليزر الأحمر أفضل من الليزر الأخضر في علاج وتر اكيلس

Introduction

Low level laser therapy is one of important alternative medicine. Low level laser therapy (L LL T)is a light source treatment that generates light of a single wave length. It is emits no heat ,no sound, no vibration. Laser radiation alter calls and tissues, experimental studies suggest that irradiation stimulate collagen production alters DNA synthesis ,therefore; the authorizes have been suggested these theory: increases ATP production by mitochondria and increases O_2 consumption on the cellular level , increases serotonin and endorphins, has anti-inflammatory effects, improved blood circulation to the skin, decrease permeability of membrane of nerve cells for Na^+ _K^+ channel causing hyper polarization, increase lymphatic flow and decrease edam[1]. Red laser and green laser are semiconductor laser (A_P_N diode),it's used to determined optical characteristic[2].Semiconductor diode laser was predicted in 1959,and are generated light single delivered over optical fibers ,telephone communication, TV and computers services[3]. Semiconductor laser are used in medical application, it is most in cancer treatment by photo-dynamic therapy, muscle spasm relaxant ,muscles degeneration, and diabetic retinopathy[4].Semiconductor laser was applicants in tendon injuries ,tendiopathy by stimulate fibro-blast regeneration ,collage formation ,increase blood important and inhibit prostaglandins[5].Achilles tendon constitutes the distal insertion of gastrocnemius–soleus musculototendinous muscles unit (Triceps surae muscles)[6].Achilles tendon is surrounded throughout its length by the paratenon, paratenon function as an elastic sleeve and permits free movement of tendon with surrounding tissue[7] .Under paratenon Achilles tendon is surrounding by a fine smooth connective tissue sheath called epitenon, together paratenon and epitenon are called peritenon[8]. Major calls which consist of tendon are fibroblast have elaborate spindle-like shape[9].The unit of tendon contain special fluid called Synovial Sheath, fluid are surround the Achilles tendon, it is allow the tendon to glide freely[10]. Blood supply that provide tendon are come from three sources; intrinsic muscular system at musculo-tendon junction ,extrinsic segmental muscular system at musculo-tendon junction and osteotendinous junction[11]. Blood supply increase in tendon with exercise and during injured tendon healing[12].Achilles tendon is innervated by small fascicule nerve from coetaneous nerve ,particularly sural nerve[13] .There are many receptors spereated on the surface of tendon sheeth such as mechanoreptors and nociceptors which are responsible .to transfer stimuli from outer surface of skin converted to the tendon to convert mechanical response[14]. Tendon injury or rupture define as a rupture that occurs during movement and activity ,Achilles ruptures commonly occur in mid-substance of tendon, therefore ;many etiological factors cause rupture

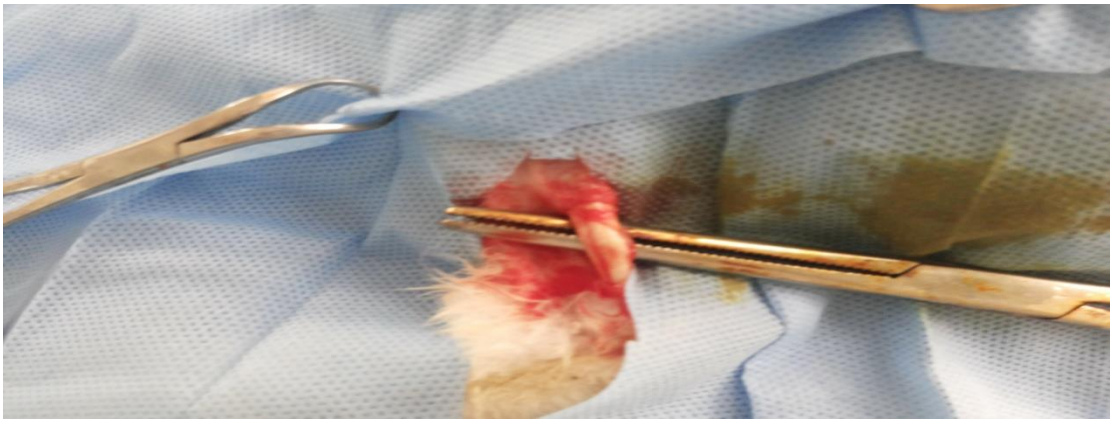
of tendon such as over load ,choric degeneration or sudden jumped[15].

Material and Methods

Animals and Administration Procedure

At the present study, twelve rabbis were used (*Lepus cuniculus*),same genera (male),age 7±2 months, one in similar condition and fed with bread and hay ,which were divided into three groups,1st group was control ,while 2nd and 3rd group were treated by low level laser therapy (L LL T) figure(1).All group were increase rabbits tendon completely(tenoctomized tendon) and suture by nylon suture. (figure2)

Figure(1) Red Laser and Green Laser



Figure(2) the site of Achilles tendon incision

Carefully, under general anesthesia(12 mg , kg/BwKetamine+3 mg, Kg/Bw)[16], and aseptic technique ,the Achilles was appeared and incised near the distal and sutured by Nylon suture with single arrow of special suture was called Looking loop pattern .Looking loop pattern a special tendon suture describe as a pair of double loop made on the side of tendon edges and ties together tightly[17] .

Figure(3) the method of suture

Skin was suture with blanket suture by silk suture and remove after 7 days post surgery. The animal weight were measure before and after surgery and repeated interval 3 days until 3 weeks.

The second group was exposed to red laser (645 nm-10 mw)* after surgery by range (10 min/day)daily for 14 days .the third group was exposed to green laser (532-10 mw)** after surgery by range (10 min/day)daily for 14 days.(*Class II products with CFR 21 CHINA*) figure(4)

Figure(4) laser therapy of tendon

The intensive of laser were measured by *Alkofahi et al*[18] .

Intensity(I)=Power (P)/area(A)……1

Power(P)=Joules (J)/Time (T)……2

$I=0.1W/0.5Cm^2$

$=0.2W/ Cm^2$

J=P*t

=0.1W*10 min

=1Joul

Clinical Views

To estimate the walk behavior of rabbits by eye vision, and view the lumping of rabbits after surgery.

Physiological Biomechanical Tests

Jumped test, is a sport to measured the ability of man to reach highest point with special column, but in present study were used to injured tendon

recovery by measured the relationship between and patient which attempt to jump to highest point at column[19] . Algorithm equation of the relationship is

Jumped (J) = Mass(M)*G (gratify) / Height(H)

J=M*G /H , **G**=9.8k/m^2

J=jumped power, M=body weight, G= gravity, H=highest point (when animal jumped to eat it's food which put in it).

Load Test

Physical test to knowledge the relationship between the ability of injured tendon to standing on hind legs , how long time period when eat its food which was put on high point vertically at rabbits mouth. Algorithm equation[20] of the relationship is

Load (L)= Mass(M) * Gravity (G) / Time (T)

L=M*G/T , G=9.8k/m^2

Slope Test

Physical test to measure the vehicle power run on slope surface, in present study was evidenced the relationship between recovery the injured tendon and ascending the slope surface, per time period or without. Algorithm equation[21] of the relationship is

F=MG sinΦ+ μ cos Φ……..(1)

F=force, **M**= mass body weight , **G**= 9.8k/m^2 (Gravity),

Φ= 60° = slope angle , **μ**=0.5= fraction factor (between rabbit and wood slab)

I=F/T……...(2)

I=intension , **F**=force, **T**= time of running

Physical Tests were taken at period 3,6,9,12,15 days post surgery.

Ultrasonographical Images[22]

To estimate the tendon recovery and affiliate the stage of healing by ultrasonographical images were taken at 3,9,15 days after surgery. Ultrasound machine is *BIOMED/BMD-3000 PALMTOP ULTRASOUND SCANNER, USA.*

Histopathological Sample and Slices[23]

Histopathology where examined post 15 days and slices were stained by H and E stain.

Statistical Analysis[24]

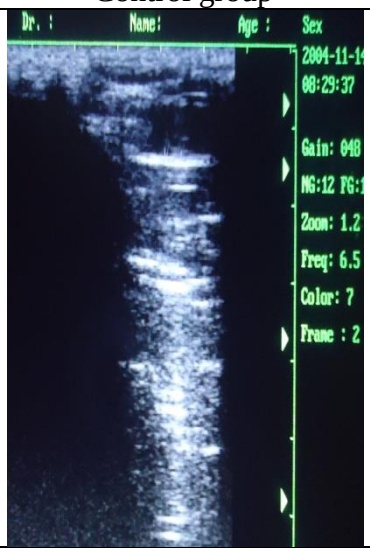
The data were collected and analyzed by used Microsoft excel 2007 and SPSS 17.00. Data were express as mean+ standard deviation(SD), one way ANOVA test was used to compare means among the different groups, significant values at $P \leq 0.05$.

Results

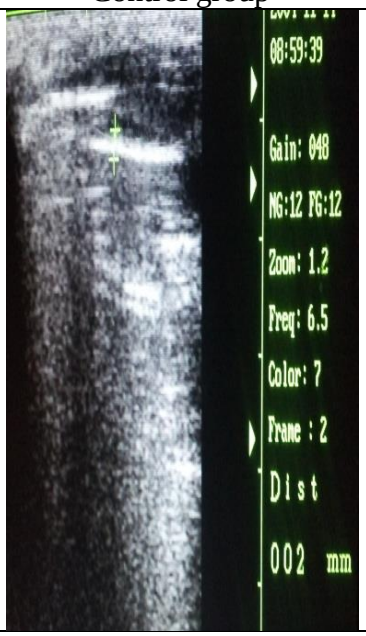
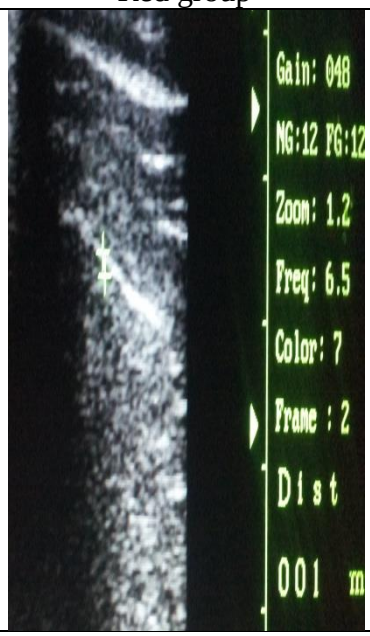
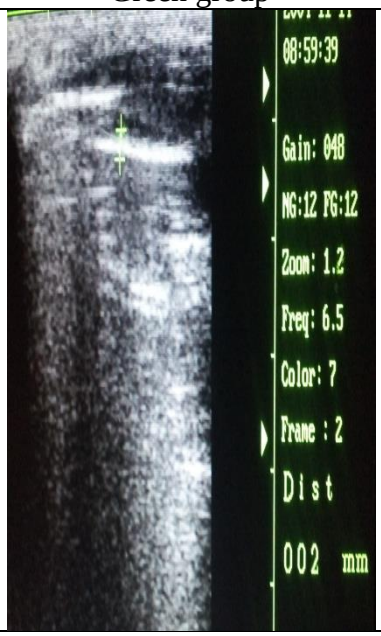
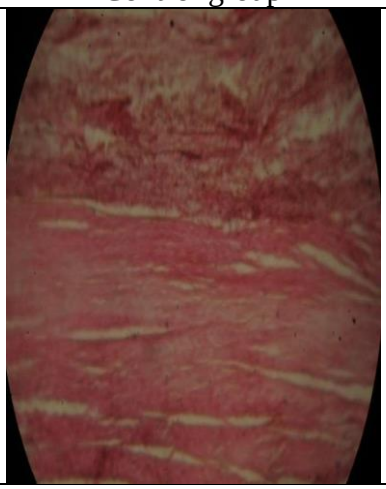
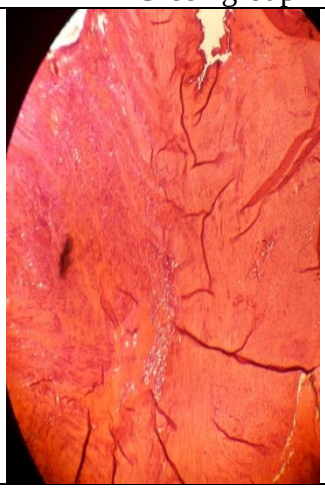
All groups after surgery take medicine (procaine penicillin 400 IU) , edema is small in size and gradually disappeared after 3-5 days after surgery. Lameness was disappeared after one day post surgery in all groups, (-)edema , (+)no edema, table (1)

Table (1) edema post surgery

Days/group	control	Red laser	Green laser
3days	-	-	+
4days	-	-	-
5days	+	+	-

US images at 3 day		
Control group	Red group	Green group
		
Sagittal-plane scan, show transverse AT* section at superficial tendon ,2D** distance =4mm -6 mm, Homogeneity with deep tendon *AT Achilles tendon **2D two dimension	Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =4mm -6 mm, Homogeneity with deep tendon	Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =5mm -6 mm, Homogeneity with deep tendon

US images at 9 day		
Control group	Red group	Green group
		
Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =4mm -6 mm, Homogeneity with deep tendon	Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =1mm -2 mm, Homogeneity with deep tendon	Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =4mm -5 mm, Homogeneity deep tendon with

<table>
<tr><td colspan="3">US images at 15 day</td></tr>
<tr><td>Control group</td><td>Red group</td><td>Green group</td></tr>
<tr><td> 
</td><td> 
</td><td> 
</td></tr>
<tr><td>Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =2mm -4mm, Homogeneity with deep tendon</td><td>Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =0mm -1 mm, Homogeneity with deep tendon</td><td>Sagittal-plane scan, show transverse AT section at superficial tendon ,2D distance =2mm -4 mm, Homogeneity with deep tendon</td></tr>
<tr><td colspan="3">Histopathological images at 15 day</td></tr>
<tr><td>Control group</td><td>Red group</td><td>Green group</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Histopathology image of AT after 15 days, show blood spotted, and fibers, vacuoles , stained by H&E , 40X</td><td>Histopathology image of AT after 15 days, show blood spotted, and fibers, vacuoles ,line of healing and spotted by suture stained by H&E , 10X</td><td>Histopathology image of AT after 15 days, show blood spotted, RBCs, and fibers, vacuoles ,line of healing and spotted by suture stained by H&E , 10X</td></tr>
</table>

Discussion

Tendon laser therapy was deeply look for[25,26] , but present study was interested to knowledge the wavelength effect, whenever the density is constant on the healing of Achilles tendon injury repair, there aren't similar study discuss this object , but similar in some views[27,28]. Clinical signs as edema and lameness were disappeared due to intensive care and take medicine regularly, as well as no sever wound was induce[29] and laser therapy properties[1]. The present study was unique assay to know injured tendon recovery, therefore the results wasn't accuracy perfect. Mechanism of laser therapy and factors which were effecting on healing of injured tendon played main role in progress healing, the mechanism of LLLT are two important biological responses for beneficial clinical effect, first is stimulation of fibroblast metabolism and collagen deposition[30,31] , second is reduce inflammation through reduction of prostaglandin E2 concentration and inhibition cyclo-oxygnase2[32,33] . when the laser power is constant in red laser (10mw-645nm) and green laser (10mw-532nm) ; the main difference effect regarded to laser wavelength as well as laser power in spite of few density in present study but had Superficial effect, and physical tests (jumped test, loading test and slope test) had correlated with wavelength more than power, to evident this correlation, the result of red laser group in most periods of tests were significant at $P\leq 0.05$ compare with green laser group and control group, as well as significant results between 3 day period and 15 day in same group of red laser group, the results were impacted negatively by fear factor that was increase adrenaline in animal body accompanied with tests, the adrenaline play negative role in muscle contraction which were effect in results[34,35,36,37] . Clinical observation of red laser654nm group similar to other result in develop the tendon, they observe collage deposition at injured area, less edema, and no pain , but they neglect laser power effect, therefore they observed excessive collage in gross section, scale sometime and hyperemia above skin[38,39,40,41]. While there weren't researchers used green laser for injured tendon because no benefit in tendon healing to stimulate pro-collagen proliferation, therefore green laser group weren't take significant values at $P\leq 0.05$ in most period tests, their result were asymptotic to control results, and most application of green laser therapy in soft tissue surger[42, 43]. The method of immobilization and suture model of injured tendon was effected on the healing and collagen deposition, in present study tendons of the animals were completely or semi-completely healing either red laser group or other group, while Pneumaticos *et. Al*. and Raeddy *et. Al*. in their experimental study they showed delay injured tendon healing because they used simple interrupted suture while this study were used Looking loop pattern [44,45,17] . Early exercises were negative effect on the result because the movement edges of the harvested tendon and may be those were better when delay 7 days after incision[46] . Ultrasonic examination were observed red laser group better healing compare other groups due to red laser therapy proprties[30,31] . Histopathological examination showed similar different due to immature sectioning, and most researchers were made slid sectioning at 30-35 days post surgery of tendon while this study the slide section at 15 day after suergery[44,47] .

Conclusion

The laser wavelengths effect on the healing of Achilles tendon injury and the long laser wavelength have ability to penetrate to the tissues more than short laser wavelength

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