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Evaluation the Effect of Low Power Laser Irradiation on Healing of Induced Tendon Injuries in Rabbits

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A B S T R A C T

This study aimed to evaluate the efficacy of diode laser in accelerating the healing process of injured tendons and to determine the best irradiation doses for impulse and continuous laser irradiation. The semimembranosus muscle tendon of forty mature local breed rabbits (Oryctolagus cuniculus) of both sexes was partially injured under general anesthesia. The rabbits were randomized into five groups and treated on the first day postoperatively. Group C served as a control and received no treatment, while groups A, B, and D were subjected to diode impulse laser with a power of 2×10^{-3} watts and a wavelength of 904 nm for 15, 25, and 35 min per session, respectively. Group E received continuous diode laser for 30 min per session with a power of 3×10^{-3} watts and a wavelength of 904 nm. The treated groups received irradiation for 5, 8, 15, and 21 days postoperatively. Subsequent healing processes were assessed macroscopically and microscopically at each time point. In treated groups versus the control group, epitenon thickness increased from day 5, inflammatory and fibroblast cell responses were more evident, and collagen fibers were clearer and more differentiated. On day 15, when the remodeling stage began, group B healed best. The impulse diode laser was found to be more effective than the continuous diode laser in promoting the healing of surgical defects of the tendons at varying degrees. In the continuous diode laser group, there was a sustained high cellular response until day 21 with the appearance of unorganized and irregular collagen fibers. This study demonstrated that diode laser can accelerate the healing process of injured tendons and that impulse diode laser is more effective than continuous diode laser.

Keywords: laser, low power laser, tendon, rabbit

INTRODUCTION

Tendon injuries are a common in both animals and humans. These injuries vary in type and severity, including acute and chronic injuries. Tendon injuries may result in partial or complete cutting of tendons, especially those in superficial sites. These injuries can lead to significant economic losses, such as in racehorses, as well as reduced functionality of the affected animal. The severity of the injury determines the extent to which normal movement is affected (1). Treatments vary depending on the type and severity of the injury, with the goal being to achieve the best degree of healing by forming tissue approximately similar to that of the normal. One treatment option is low-energy diode laser therapy, which has been used in various branches of medicine. Diode laser therapy can correct disorders in patient immunity, stimulate healing in external wounds and internal anastomosis, prevent adhesions, provide pain relief, and prevent functional and organic complications post-operation (2).

However, the absence of standard protocol is a major problem. Determining the appropriate wavelengths, power, energy density, and other factors for different therapeutic treatments can be difficult (3, 4).

This study aims to evaluate the effect of diode laser therapy on accelerating the healing process of injured tendons and to determine the best irradiation doses for impulse and continuous laser irradiation.

MATERIALS AND METHODS

Ethics and Experimental Animals

All procedures used in this study were reviewed and approved following animal welfare ethical standards by the Scientific Committee of the Department of Veterinary Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, and the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

A total of forty mature, local breed rabbits (*Oryctolagus cuniculus*), with about 1.5-2 kg in weight of both sexes were used in this study. Animals were housed in standard cages at the animal house, College of Veterinary Medicine, University of Baghdad and managed under the same conditions of temperature (20±2°C), light-dark cycle (12:12 h), standard ventilation system and humidity (50±5%). For a two-week acclimation period, animals were fed *ad libitum* on a concentrated diet (pellets) and green forage.

Surgical Operation and Tendon Injury

All operations were performed under general anesthesia, where the animals were intramuscularly injected with a combination of 10% ketamine hydrochloride (35 mg/kg BW, Alfasan, Holland) and 2% xylazine (5 mg/kg BW, VMD, Belgium) (5). This protocol efficiently maintained the animals under anesthesia during the operations. The operation site was prepared using routine aseptic techniques. During the operations, each rabbit was positioned on its left side, while recumbent to use the right hind limb for all animals. The prepared area was covered with a thin layer of cotton saturated with 70% alcohol. The skin was incised by scalpel about 2 cm parallel to the Achilles tendon. The tendon sheath was incised to show the tendon of the semimembranosus muscle, and then the tendon was caught with the tip of artery forceps, and this part was cut around the tip by scalpel (Figure 1). All tendons were injured with the same type of injury. The tendon sheath was not sutured, and the skin was sutured by the simple interrupted pattern with the 3.0 silk. After the injury, none of the limbs were immobilized. The sticks were removed on the 7th postoperative day, except for those from which specimens were removed on the fifth postoperative day.

Treatments

After a partial injury to semimembranosus muscle tendon was induced, injured rabbits were divided at random into 5 groups (A, B, D, and E) with 8 in each, the fifth group C was left without treatment (irradiation) and considered as a control. The treatment was started on day one postoperative day. The periods of irradiation (sessions) continued daily in all treated groups for 5, 8, 15, and 21 postoperative days. The groups A, B, and D were irradiated with diode impulse laser with wavelength of 904 nm and 2×10^{-3} watt power as follows: 15 min/session for group A, 25 min/session for group B, and 35 min/session for group D, thus the doses were 1800, 3000, and 4200 rad, respectively. For group E, the continuous diode laser was applied with a power of 3×10^{-3} watt for 30 min/session with a dose of 5400 rad.

Figure 1. A representative photograph showing the site of tendon injury (white arrow)

Macroscopic and Microscopic Assessments

Healing tendons and changes to surrounding tissues were evaluated across all groups using macroscopic and microscopic assessments. Each session involved the euthanization of two rabbits and the removal of 1.5 cm of injured tendon using stitches as a visual guide. The specimens were fixed with 10% formalin and stained with hematoxylins and eosin (H&E) and some slides with Masson's Trichrome to stain the collagen fibers. Histopathological study was performed under light microscope (Olympus) in histopathological laboratory of College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

RESULTS

Macroscopic Findings

After euthanasia, the tendons were visually examined for macroscopic changes. In the control group on the fifth postoperative day, adhesion was observed between the tendon and its sheath, as well as between the sheath itself and the surrounding tissue. These signs persisted until the 8th postoperative day. The injured area was dark in color and easily distinguishable from the tendon. On the 15th postoperative day, there was further development of tendon edema, and adhesion was still present, along with the presence of a white substance that covered the injured area, suspected to be fibrin (Figure 2A).

In the 15-minute diode impulse laser irradiated group (group A), mild adhesion and some hemorrhages were observed on the 15th postoperative day. By the 21st

postoperative day, the area appeared more healed and could not be distinguished from the surrounding tendon area. The tendon sheath also showed signs of healing. In the 25-minute diode impulse laser irradiated group (group B), adhesions were observed on the 8th postoperative day, but there was no hemorrhage. Good healing was observed on the 15th postoperative day (Figure 2B). In the 35-minute diode impulse laser irradiated group (group D), there was no hemorrhage at the injured tendon, and it appeared healed on the 15th postoperative day, but it was still distinguishable on the 21st postoperative day due to its dark color. In the 30-minute diode continuous laser irradiated group (group E) with continuous diode laser, there was healing with hemorrhage on the 15th postoperative day, and the injured area appeared dark in color, but there were no adhesions. On the 21st postoperative day, the injured area still appeared dark in color despite healing.

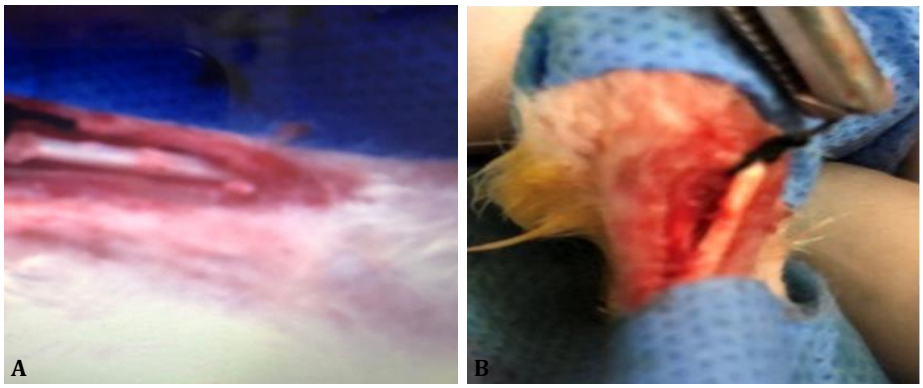

Figure 2. Representative photographs of injured area in the local rabbit **(A)** Control group on 15th postoperative day tendon edema, adhesion, white substance (suspected to be fibrin) covers injured area, **(B)** 25-min diode impulse laser irradiated group on 15th postoperative day showing good healing with no adhesions

Microscopic Findings

Fifth postoperative day

In the control group, the results showed diffuse hemorrhage with the presence of fibrin at the injured area, but no thickening in the epitenon. Fibroblasts and collagen deposition were absent, while there was light infiltration of neutrophils (Figure 3A). In group A, there were minor hemorrhages and thickening of the epitenon (10-12 cells), along with fibroblast infiltration, but no new blood vessel generation. Loose collagen areas were also observed (Figure 3B). In group B, there were minor hemorrhages and thickening in the epitenon (12-15 cells). Fibroblasts and neutrophils infiltrated the area and new blood vessels were present. Collagen fibers were deposited but they were irregular and incoherent. In group D (Figure 3C), there was no hemorrhage and the epitenon thickness increased (12-15 cells). There was severe infiltration of fibroblasts and neutrophils and many blood vessels in different growth periods. Irregular collagen fibers were clearly deposited. In the group E, there was hemorrhage, increased thickness in the epitenon layer (13-15 cells), infiltration of fibroblasts and inflammatory cells, and loose deposition of collagen fibers with new angiogenesis.

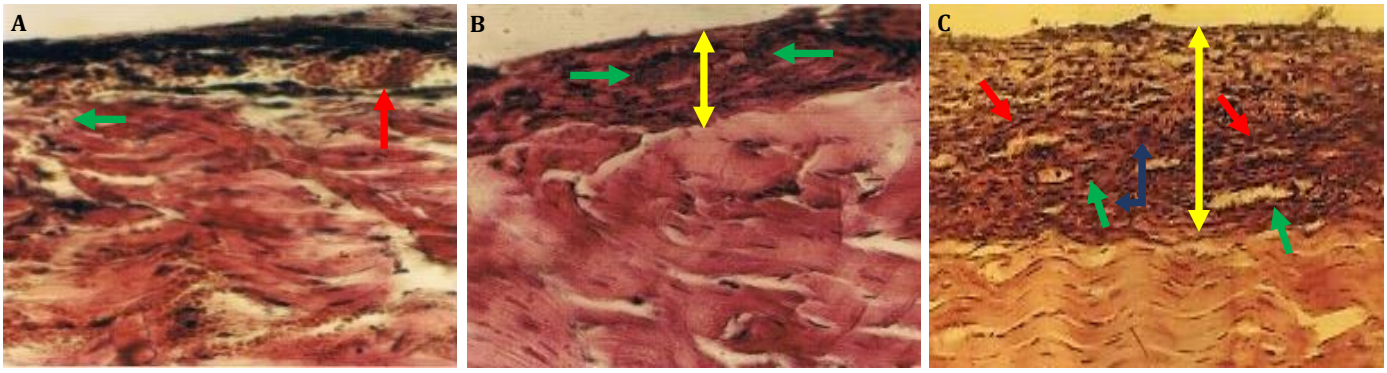

Figure 3. Representative sections of injured semimembranosus muscle tendon of local rabbit on 5th postoperative day in **(A)** control group showing hemorrhages (red arrow), neutrophils (green arrow), H&E, 40×; **(B)** Group A, irradiated with diode impulse laser at 1800 rad for 15 min showing thick epitenon (yellow double-arrow), neutrophils (green arrows), H&E, 40×; **(C)** Group D, irradiated with diode impulse laser at 4200 rad for 35 min showing thicker epitenon (yellow double-arrow), severe infiltration of inflammatory cells (green arrows), fibroblasts (red arrows), and collagen fibers precipitation (blue left-up arrow), H&E, 20×

Eighth postoperative day

In the control group, there was hemorrhage, no thickening in the epitenon, light infiltration of fibroblasts and inflammatory cells, few immature blood vessels, and soft collagen fibers (Figure 4A). In group A (Figure 4B), there was a minor hemorrhage, an increase in the epitenon layer (15-18 cellular layers), severe infiltration of fibroblasts, neutrophils and lymphocytes, growth of blood vessels and more coherent but less regular collagen fibers. In group B, there was no hemorrhage, the epitenon increased to 18-20 cells, severe infiltration of fibroblasts and inflammatory cells, clearer blood vessels in different sizes and clear coherent irregular collagen (Figure 4C). In group D, there was no hemorrhage, clear thickening of the epitenon, mild infiltration of fibroblasts and inflammatory cells (neutrophils and lymphocytes) and incoherent regular collagen fibers toward the longitudinal axis (Figure 4D). In group E, there were areas of hemorrhage, a noticeable increase in the epitenon (more than 20 cellular layers), a clear increase in fibroblasts and inflammatory cells.

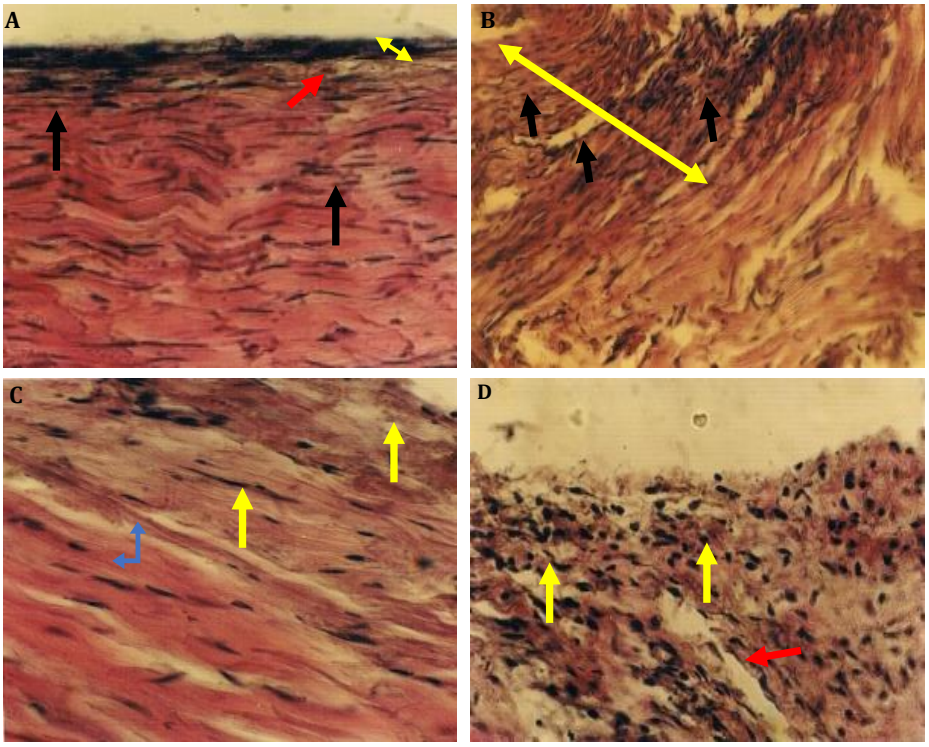

Figure 4. Representative sections of injured semimembranosus muscle tendon of local rabbit on 8th postoperative day in **(A) Control group** showing thin epitenon (yellow double-arrow), hemorrhage (red arrows), light infiltration of inflammatory and fibroblast cells (black arrows), H&E, 40×; **(B) Group A**, irradiated with diode impulse laser at 1800 rad for 15 min showing thick epitenon (yellow double-arrow), severe infiltration of inflammatory and fibroblast cells (black arrows), H&E, 20×; **(C) Group B**, irradiated with diode impulse laser at 3000 rad for 25 min showing severe infiltration of inflammatory and fibroblast cells (yellow arrows) and new blood vessels (red arrow), H&E, 40×; **(D) Group D**, irradiated with diode impulse laser at 4200 rad for 35 min showing mild infiltration of inflammatory cells (yellow arrows), regular collagen fibers (blue left-up arrow), H&E, 40×

Fifteenth postoperative day

In the control group, there was diffuse hemorrhage, slight thickening in the epitenon compared with those rabbits that were euthanized on the 8th postoperative day, randomly scattered increase in fibroblasts, infiltration of neutrophils, small and few blood vessels and there were no collagen fibers. In group A, there was minor hemorrhage, thickening of the epitenon (18-20 cellular layers), severe infiltration of fibroblasts which appeared mature (fibrocytes) with elongated nuclei (fusiform shape), mild inflammatory cell infiltration, and collagen fibers were more coherent and uniform toward the longitudinal axis with clear wave-shape (Figure 5A). In group B, there was no hemorrhage, no thickening in the epitenon compared with the eighth day in the same group, mild numbers of fibroblasts which were more mature (fibrocytes), little infiltration of lymphocytes, an abundance of blood vessels, and despite soft areas of collagen fibers in the bridging area, they were more coherent and uniform toward the longitudinal axis (Figure 5B). In Group D, there were hemorrhages in many areas, the epitenon was still thick, severe infiltration of fibroblasts, mild infiltration of inflammatory cells, many blood vessels and regular collagen fiber deposition (Figure 5C). In group E, the epitenon layer was very thick with the presence of fibroblast and inflammatory cell infiltration. There were diffuse hemorrhagic areas and loose irregular collagen fiber deposition (Figure 5D).

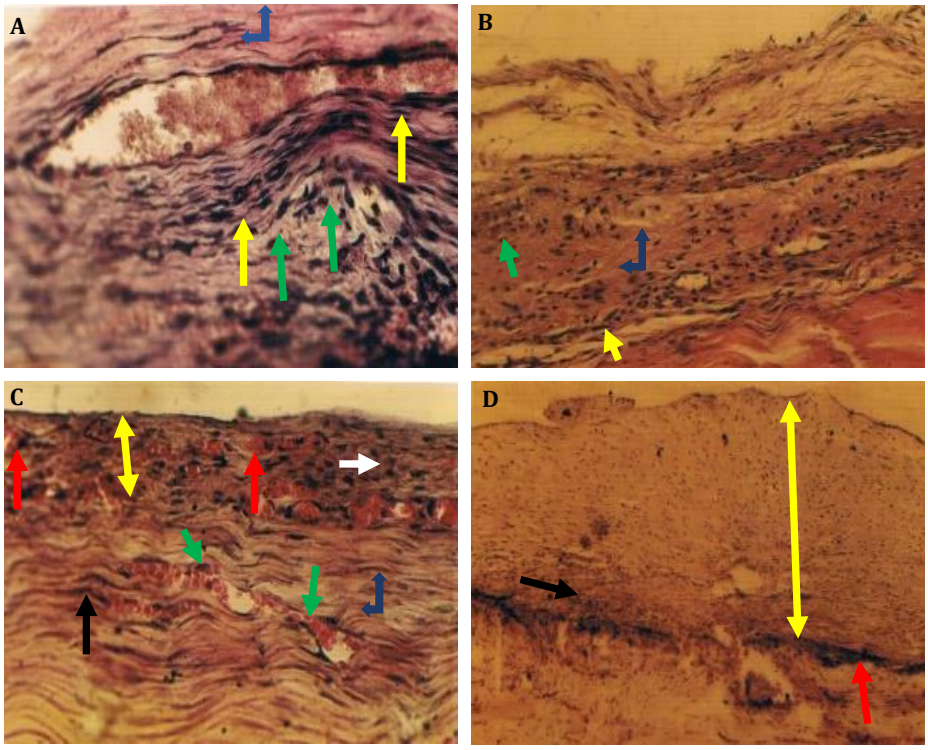

Figure 5. Representative sections of injured semimembranosus muscle tendon of local rabbit on 15th postoperative day in **(A)** Group A, irradiated with diode impulse laser at 1800 rad for 15 min showing severe infiltration of fibroblasts, presence of fibrocytes with elongated nuclei (yellow arrows), mild inflammatory cells (green arrows), and coherent collagen fibers (regular) (blue left-up arrow), H&E, 40×; **(B)** Group B, irradiated with diode impulse laser at 3000 rad for 25 min showing presence of fibrocytes with elongated nuclei (yellow arrow), little inflammatory cells infiltration (green arrow), and coherent regular collagen fibers (blue left-up arrow), H&E, 20×; **(C)** Group D, irradiated with diode impulse laser at 4200 rad for 35 min showing hemorrhages (red arrows), thick epitenon (yellow double-arrow), severe infiltration of fibroblasts (black arrow), mild inflammatory cells infiltration (white arrow), blood vessels (green arrows), and regular collagen deposition (blue left-up arrow), H&E, 40×;; **(D)** Group E, showing very thick epitenon (yellow double-arrow), severe infiltration of inflammatory cells (black arrow) and fibroblasts (red arrow), H&E, 20×

Twenty first postoperative day

In the control group, hemorrhage was still present, there was thickening in the epitenon (20-22 cellular layers), infiltration of fibroblasts, decreased inflammatory cells, and loose incoherent collagen fibers, but they were more pronounced towards the endotenon (Figure 6A). In group A, there was no hemorrhage, thinner epitenon compared to the control group, regular longitudinal fibroblasts towards the axis of the tendon, light infiltration of inflammatory cells with few blood vessels appearing in the growth area, and the collagen fibers were more coherent and regular towards the longitudinal axis of the tendon (Figure 6B). In group B, there was no clear hemorrhage, thin epitenon layer, very few fibroblasts which were fibrocytes, absence of inflammatory cells, very few blood vessels, and more coherent regular fibers with clear wavy-shaped collagen (Figure 6C). In group D, there was hemorrhage, thin epitenon, and dense deposition of coherent regular collagen fibers (Figure 6D). In group E, the hemorrhagic areas were still present, very thick epitenon layer, intense infiltration of fibroblasts and inflammatory cells (mono and multiple nuclei), and dense regular wavy-shaped collagen fibers. Using Masson's Trichrome dye, the collagen fibers appeared green in color and were dense and wavy, showing the effect of laser irradiation on collagen fiber formation (Figure 6E)).

DISCUSSION

Macroscopic findings showed adhesions in the control group until the 5th postoperative day, which disappeared on the 21st postoperative day. There were mild adhesions in the irradiated groups, except in group B on the 8th postoperative day. From gross observations, it can be concluded that the healing in the treated groups did not show significant signs in tendon healing. This fact was indicated by faster and better healing in the treated group compared to the control group, which showed adhesions until the 15th postoperative day. It also revealed the effect of laser radiation in reducing post-tendon injury adhesions. Such findings were consistent with earlier documentation (2). In fact, adhesion and healing processes may be affected by fibronectin, which may be involved in restorative processes through orderly interaction with growth factors or function as a source of adhesion formation, which can determine healing and tensile strength during convalescence after tendon restoration (6).

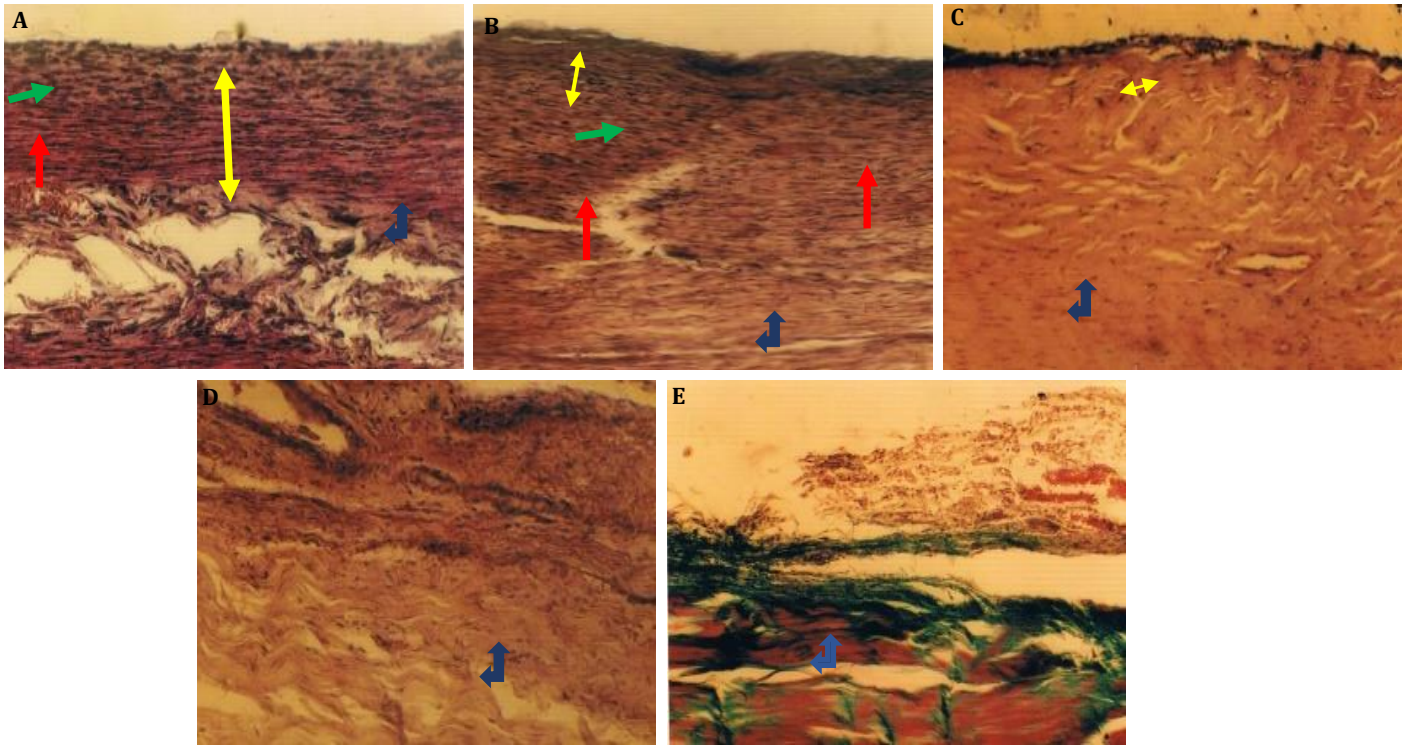

Figure 6. Representative sections of injured semimembranosus muscle tendon of local rabbits on 21st postoperative day in **(A) Control group** showing thick epitenon (yellow double-arrow), inflammatory cells (green arrow) and fibroblasts infiltration (red arrow), and incoherent collagen fibers (blue left-up arrow), H&E, 20×; **(B) Group A**, irradiated with diode impulse laser at 1800 rad for 15 min showing thinner epitenon (yellow double-arrow), longitudinal arranged fibroblasts (red arrows), and regular collagen (blue left-up arrow), H&E, 20×;**(C) Group B**, irradiated with diode impulse laser at 3000 rad for 25 min showing thin peritenon (yellow double-arrow) and wavy shape collagen fibers (blue left-up arrow), H&E, 10×; **(D) Group D**, irradiated with diode impulse laser at 4200 rad for 35 min showing regular dense collagen deposition blue left-up arrow), H&E, 20×; **(E) Group E**, irradiated with continuous diode laser at 5400 rad for 30 min showing dense, regular, and wavy collagen fibers (blue left-up arrow) green in color (Masson's Trichrome)

In the control group, there was hemorrhage in the injured area. The wounded area in group D darkened due to internal bleeding from the tendon wound, but this did not appear in other laser-treated groups because of their faster healing. This is consistent with what was pointed out by (7), that the tendon initially loses its white to yellowish color and appears red, gray, moist, and swollen as a result of inflammatory perfusion. On the lump surface, small points of hyperemia and hemorrhage appear.

Histopathological Findings

On the 5th postoperative day, there were obvious differences in the thicknesses of the epitenon layer, which appeared thick in the treated groups and continued to increase until the 8th postoperative day, reaching its peak on the 15th postoperative day, especially in the group irradiated for 25 minutes. The thickness of the epitenon in the control group did not reach that of the irradiated groups until the 21st postoperative day, and the laser-treated groups revealed a reduction in epitenon layer thickness. These findings were inconsistent with (8), who reported that the increase in epitenon thickness in the deep digital flexor tendon in dogs had increased significantly on the 7-11th postoperative days and that a cellular response was found in the restoration area. However, our findings were consistent with the observations of reference (9), who reported an increase in epitenon thickness six weeks after inducing Achilles tendon inflammation in rabbits, along with an increase in capillary blood vessels and fibroblast cell numbers.

Since the 5th postoperative day, fibroblasts appeared in the treated groups but were not observed in the control group. Their numbers were low until the 8th postoperative day in the control group. This is consistent with (10)'s statement that only migratory fibroblasts multiply, while tendon cells are classified to the stage where they do not have more susceptibility to division. They only work on the production and retention of the main materials of the tendon, and this showed the source of the increase in the numbers of fibroblasts in the healed tendon. This is also suggested by (11), who noticed more numbers of fibroblasts in irradiated tissue, indicating a significant increase in fibroblast precipitation and decreased inflammatory cell infiltration. This concludes that low-level laser therapy (LLLT) expedited the process of tissue repair.

On the 15th postoperative day, the shape of fibroblast nuclei began to change. More elongation was observed after

they were large and rounded, indicating the maturation of fibroblasts according to (12). Fibroblasts have large, rounded nuclei with a clear nucleus, while mature fibroblasts have an elongated nucleus, and the cytoplasm has a spindle shape with cytoplasmic extensions extending into the matrix. referred to as fibrocytes by (13). They were less active than fibroblasts and this clearly showed the maturity of the restored tissue as the function of fibroblasts is the formation of collagen fibers. Maturity meant a decrease in the level of precipitation when it was enough to repair the wound area.

When comparing the experimental groups in the precipitate of collagen fibers, the present findings revealed that it started on the 5th postoperative day in the treated groups and only appeared on the 15th day in the control group. The fibers evolved and appeared coherent and uniform towards the longitudinal axis of the tendon, and their wavy shape appeared on the 15th day in the laser irradiation groups. The formation of collagen fibers depended on the presence and number of fibroblasts. Therefore, the current observations showed an increase in collagen fiber formation that coincided with an increase in the number of fibroblasts in the experimental groups, which may have occurred due to the effect of the laser in increasing the number of fibroblasts and their activity. Low-power laser radiation had an effect on fibroblasts and fiber formation. This was in good agreement with (14) postulation of strengthening collagen fiber contraction in the wound. They suspected an increase with low-energy laser by affecting the activation of the fibroblasts or increasing the growth of fibroblasts, as suspected by (15). The appearance of coherent and regular fibers on the 15th day in the laser-treated groups reflects the improvement in the maturity of the restored tissue. According to (16), the use of GaAS laser affected the tendon repair processes in dogs and led to good maturation of collagen fibers, accelerating healing. They explained that the collagen fibers in the treated groups were of medium and large diameters. In fact, the current observations revealed both small and medium-sized collagen fibers in all groups of the study. Additionally, (17) mentioned that the positioning of collagen fibers, wavy shape, and the degree of periodic repetition showed improvement in tissue maturation. It was reported by (18) that the helium-neon laser caused an increase in collagen construction, and (19) concluded that soft laser irradiation caused the metabolic activity of fibroblasts to increase, resulting in increased production of collagen. It was reported by (20) that the collagen fibers in laser-irradiated groups were denser, thicker, more regular, and communicated with existing fibers compared to untreated animals, which is consistent with our results using diode laser in Rabbit's tendons. The good organization and coherence of the collagen fibers with the visibility of the wavy shape reflected the remodeling stage, which was observed on the 15th day in the treated groups. This corresponds with what (21) stated, that laser irradiation led to collagen accumulation and influenced the shape of granulation tissue.

Tendon injury leads to rupture of its fibers, resulting in bleeding from capillaries within the tendon. This can lead to the formation of fibrin and hypoxia in the injured area, causing congestion, tenocyte necrosis, and accumulation of fluid between the fibers, as mentioned by (22) and (1). The presence of hemorrhage in group D was a result of high-dose irradiation, consistent with what was stated by (23), where the effect of laser exposure depended on the dosage given. Angiogenesis, or the formation of new blood vessels, was observed to start on the 5th postoperative day in the treated groups, but only appeared on the 8th postoperative day in the control group. Blood vessels showed good growth in the treated groups during the treatment course, indicating the potential bio-stimulatory effect of low-level laser, which may impact the release of growth factors, as demonstrated by (24) who showed that tendon-related growth factors play a role in the vascular response during the initial weeks after tendon injury and repair.

The presence of basic fibroblast growth factor (bFGF) protein stimulates angiogenesis and affects differences in the repair process (25), while laser radiation has been found to enhance tissue vascularity, as noted in (26). In addition, (27) observed that LLLT facilitated blood circulation and enhanced microcirculation. Other studies have demonstrated increased angiogenesis and proliferation of new fibroblasts, along with decreased infiltration of inflammatory cells in irradiated treated lesions, as reported in (11). Similarly, it has been reported that a diode laser is effective in inducing angiogenesis and encouraging the healing process (28-30). Vascular laser stimulation was consistent with the results in this study, which observed that blood vessels appeared earlier in treated groups and had better growth than in the control group when using a diode laser.

Inflammatory cells appeared on the 5th day in all groups throughout the experimental period, but were very few on the 21st postoperative day in group A and the control group. The proliferation of immune-competent cells was stimulated when a wound occurred, and the phagocytic cells removed the blood clot and the destructive tissue and foreign matters, as mentioned in (13). The white blood cells can affect healing by defending against secondary infection. White blood cells exposed to argon laser appeared normal but phagocytized germs (31). Hiranuma et al. (32) noticed severe infiltration of white cells (polymorphonuclear cells), phagocytes, and lymphocytes during the first and second weeks in White Leghorn chickens aged 4 months and after their tendons were cut. The laser radiation increased the cellular immune response, as shown in (33). Low-energy laser stimulated phagocytic activity of white blood cells, such as lymphocytes, and stimulated the migration of these cells to the area of injury, as mentioned in (2). From the

above, we noted that it was consistent with our observations about the cellular immune response in the treated groups. As the healing process progressed, an increase in the amount of precipitated collagen fibers was observed, as well as increased regularity and consistency with a decrease in the number of inflammatory cells, reflecting improved restoration of tissue healing in the laser treatment groups. The author concluded that the functional characteristics of the injured tissue had returned to normal, as mentioned in (27). This was reported by (32) who stated that as the inflammatory reaction diminished, fibroblasts predominated in the connective tissue and mature collagen fibers accumulated. It is noteworthy that the clarity of the collagen fibers, as well as their ability to take pigment well and their wavy appearance, indicated that these fibers were of large and medium types (types I, III), which indicated good tendon healing. This is because type V and IV collagen molecules do not form fibers or fibrils that can be distinguished from the surrounding matrix except in immunohistochemistry tests, as mentioned in (13). Therefore, the fibers did not appear in the control group until the 15th postoperative day, where mostly type V and IV collagen molecules were deposited but did not appear in the H&E stain, while the collagen fibers appeared in the treated group since the 5th postoperative day. The regularity of the collagen fibers toward the longitudinal axis of the tendon reflected progress in the maturation of the tissue, as pointed out in (17), which mentioned that the deposition of collagen fibers at the beginning of healing was irregular, but as the wound healed, these fibers became longitudinally aligned along the tension lines. It has been demonstrated that the collagen produced in the wound initially consisted of small-diameter, randomly oriented bundles, and as the fibrous tissue matured, larger diameter bundles became predominant and were positioned along the stressed lines (34, 35). The obvious difference between the control group and the laser-treated groups indicated a clear effect of irradiation on the speed of healing, as evidenced by the early appearance of fibroblasts and the early formation of fibers, followed by their regularity, consistency, and wavy shape, as healing progressed. The fibroblasts matured and decreased, inflammatory cells disappeared, and remodeling occurred in the restored tissue.

The epitenon decreased to reach advanced healing after 21 days in the laser treatment groups. This also reflected the effect of irradiation on the vascular response, which is important in healing as it provides nutrition to the fibroblasts that visibly proliferated in the region to perform their role in collagen fiber formation. Low-energy laser irradiation has been shown to improve microcirculation, as stated in (36). This finding is consistent with (37), which reported increased vascularity in the early stages of tendon scar in rabbits, with the distribution of blood vessels disappearing and becoming focused on the late stage. At a later stage of healing, the degree of vascularity was reduced and a regular distribution of vessels was evident, indicating the importance of blood flow in the healing processes of tendons and ligaments. The increase in fibroblasts in the diode laser treated groups would stimulate their activity in producing more collagen fibers. Furthermore, the increased vascularity compared to the control group suggests an increase in the amount of fibronectin due to the laser effect. If fibronectin levels were increased by the effect of laser irradiation, it would indicate that the laser effect not only increases the number of fibroblasts and stimulates them to produce collagen fibers directly, but also indirectly stimulates them by increasing fibronectin. It was reported by (32) that fibronectin plays a role in the organization of collagen in tendon healing.

The use of low-energy diode lasers has been proven effective in accelerating and improving tendon wound healing. It has been shown to reduce the healing duration compared to the control group, which is consistent with some studies that demonstrated significant stimulation of healing in surgical wounds or burns in rats or mice irradiated with LLLT (38). In addition, there was an observed appearance of coherent and regular collagen fibers of medium and large types, which served the function of withstanding tension resulting from movement and muscle tension. Some collagen fibers appeared pigmented in red when exposed to continuous laser radiation using tri-chrome pigment, which aligns with the findings of (39) that laser-affected collagen fibers took on red pigment. However, in their experiment, a high-energy laser was used, which had a significant impact on collagen fibers, resulting in a pattern change with a more homogeneous appearance in wider areas. The difference in interaction of collagen fibers with Trichrome pigment indicated that continuous irradiation had a greater thermal effect than pulsed-laser irradiation. This finding is in line with the observation of (40) who confirmed that the mechanism of continuous laser work is mainly thermal in nature and dependent on linear energy, whereas the pulsed laser effect comes from micro-explosions that occur in the surrounding tissue. However, a challenge in laser therapy is the lack of a standardized protocol, difficulty in fixing the wavelengths, power, and energy density (3, 4).

In conclusion, the use of low-energy lasers has shown a significant effect on healing of injured tendons, with emphasis on the positive effect of impulse lasers compared to continuous lasers, as continuous lasers tend to cause an exaggerated effect on healing. Based on the above, the use of impulse lasers can help accelerate tendon healing through bio-stimulation of tendon tissue. The dosage used also has an impact on healing, as the dosage of 2×10^{-3} for 25 min showed a better healing effect compared to other dosages in the impulse laser-treated groups.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. McCullagh KG, Goodship AE, Silver IA. Tendon injuries and their treatment in the horse. Vet Rec. 1979; 105(3): 54-57.
2. Pryor B, Millis DL. Therapeutic laser in veterinary medicine. Vet Clin North Am Small Anim Pract. 2015;45(1):45-56.
3. Woodruff LD, Bounkeo JM, Brannon WM, Dawes KS, Barham CD, Waddell DL, et al. The efficacy of laser therapy in wound repair: a meta-analysis of the literature. Photomed. Laser Surg. 2004; 22(3): 241–247.
4. Avci P, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N, et al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg. 2013; 32(1):41-52.
5. Richardson CA, Flecknell PA. Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress? Altern Lab Anim. 2005;33(2):119-27.
6. Brigman BE, Hu P, Yin H, Tsuzaki M, Lawrence WT, Banes AJ. Fibronectin in the tendon-synovial complex: quantitation in vivo and in vitro by ELISA and relative mRNA levels by polymerase chain reaction and northern blot. J Orthop Res. 1994;12(2):253-261.
7. Webbon PM. Preliminary study of tendon biopsy in the Horse. Equine Vet J. 1992;18(5): 383-387.
8. Gelberman RH, Khabie V, Cahill CJ. The revascularization of healing flexor tendons in the digital sheath. A vascular injection study in dogs. J Bone Joint Surg Am. 1991;73(6):868-881.
9. Backman C, Boquist L, Fridén J, Lorentzon R, Toolanen G. Chronic achilles paratenonitis with tendinosis: an experimental model in the rabbit. J Orthop Res. 1990;8(4):541-547.
10. Wilson AM, Goodship AE. Exercise-induced hyperthermia as a possible mechanism for tendon degeneration. J Biomech. 1994;27(7):899-905.
11. Rocha Júnior AM, Oliveira RG, Farias RE, Andrade LCR, Aarestrup FM. Modulation of fibroblast proliferation and inflammatory response by low-intensity laser therapy in tissue repair process. An Bras Dermatol. 2006; 81(2): 150-156.
12. Eurell JA, Frappier BL, editors. Dellmann's textbook of veterinary histology. 6th ed. John Wiley & Sons; 2013. 419 p.
13. Paulsen Douglas E. Basic Histology. Examination and Board Review. New York: Prentic-Hall, International Inc.;1990. 215 p.
14. Bosatra M, Jucci A, Olliaro P, Quacci D, Sacchi S. In vitro fibroblast and dermis fibroblast activation by laser irradiation at low energy. An electron microscopic study. Dermatologica. 1984;168(4):157-62.
15. Gamsari SM, Yamada H, Acorda JA, Unno N. Evaluation of low level laser therapy on open wound healing of the teat in dairy cattle. Laser Ther. 1994; 6(2): 113-118.
16. Wanderer C, Buchi DF, Tassini CM, Raiser AG, Schimitt I. Use of lectins to evaluate the effects of GaAs softlaser on dog tendon. Braz J Med Biol Res. 1994;27(9):2241-51.
17. Watkins JP, Auer JA, Morgan SJ, Gay S. Healing of surgically created defects in the equine superficial digital flexor tendon: effects of pulsing electromagnetic field therapy on collagen-type transformation and tissue morphologic reorganization. Am J Vet Res. 1985;46(10):2097-2103.
18. Abergel RP, Mecker CA, Lam TS, Lesavoy MA, Vitto J. Control of connective tissue metabolism by laser: recent developments and future prospects. J Am Acad Dermatol. 1984;11(6):1142-1150.
19. Chomette G, Auriol M, Zeitoun R, Mousques T. Effect du soft laser sur letissueconjoctif gingival: Et de microscopeieelectronique. J Biol Buccale. 1987;15:45-57.
20. Ghamsari SM, Taguchi K, Abe N, Acorda JA, Sato M, Yamada H. Evaluation of low level laser therapy on primary healing of experimentally induced full thickness teat wounds in dairy cattle. Vet Surg. 1997;26(2):114-120.
21. Wanderer CA. Valiacao Clinica, macro e microcopica dos efeitos das radiacoes Laser sobreprocesso [Thesis]. Santa Maria: UFRG;1991.
22. Webbon PM. Equine tendon stress injury. Equine Vet. J. 1973;5(2):58-64.
23. Wolbarsht ML, Landers MB. Lasers in ophthalmology: the path from theory to application. Appl Opt. 1979;18(10):1518–1526.
24. Duffy Jr FJ, Seiler JG, Gelberman RH, Hergrueter CA. Growth factors and canine flexor tendon healing: initial studies in uninjured and repair models. J Hand Surg. 1995; 20(4):645-649.
25. Folkman J, Klagsbrun M. Angiogenic factors. Science. 1987;235(4787):442-447.
26. Mester E, Mester A. The biomedical effect of Laser application. Laser Surg Med. 1985;5(1):31-39.
27. Ihsan FR. Low-level laser therapy accelerates collateral circulation and enhances microcirculation. Photomed Laser Surg. 2005;23(3):289-294.
28. Mirsky N, Krispel Y, Shoshany Y, Maltz L, Oron U. Promotion of angiogenesis by low energy laser irradiation. Antioxid Redox Signal. 2002;4(5):785-790.
29. Omar, RA, Saleh SI. Study of low power laser effect on the healing of tibial fracture treated by intramedullary pin in rabbits. Iraqi J. Vet. Med. 2003;27(1):99–108.
30. Sinan A, Eesa MJ, Omar RA. Histopathological study of the influence of platelet rich-plasma and low level laser therapy on healing of experimentally fractured proximal sesamoid bone in equine. Iraqi J. Vet. Med. 2017;41(1):160-168.
31. Hode L, Tunér J. Laser Phototherapy: clinical practice and scientific background: a guide for researchers, doctors, dentists, veterinarians and other interested parties within the medical field. Grängesberg, Sweden: Prima Books; 2014. 957 p.
32. Hiranuma K, Suzuki K, Hirata K, Nakamura H, Higashi K, Hirano H. Extracellular matrices in peritendinous connective tissue after surgical injury to the chicken flexor tendon. Arch Orthop Trauma Surg. 1996;115(2):63-67.
33. Mester E, Nagylucskay S, Döklen A, Tisza S. Laser stimulation of wound healing. Acta Chir Acad Sci Hung. 1976;17(1):49-55.
34. Steiner M. Biomechanics of tendon healing. J Biomech. 1982;15(12):951-958.
35. Gay S, Miller EJ. What is collagen, what is not. Ultrastruct Pathol. 1983;4(4):365-377.
36. Buyanov VM, Danilov KJV, Zaranko AI, Karitonv SV. Intravascular irradiation of blood in complex Treatment of acute cholecystitis. The Russian Medical State University, First clinical & scientific conference proceeding. Moscow. 1992; pp: 45- 49.
37. Bray RC, Rangayyan RM, Frank CB. Normal and healing ligament vascularity: a quantitative histological assessment in the adult rabbit medial collateral ligament. J Anat. 1996;188(Pt 1):87-95.
38. Dawood MS, Salman SD. Low level diode laser accelerates wound healing. Lasers Med Sci. 2013; 28(3):941-945.
39. Hayashi K, Thabit G 3rd, Vailas AC, Bogdanske JJ, Cooley AJ, Markel MD. The effect of nonablative laser energy on joint capsular properties. An in vitro histologic and biochemical study using a rabbit model. Am J Sports Med. 1996; 24(5):640-646.
40. 40.Zypen EV, Frank H, Bebie F, Shall HVJ. Changes in ultrastructure of the tendon after irradiation with intense light. Adv Opth. 1979; 39: 59-180.

تقييم تأثير التشعيع بالليزرات واطئة الطاقة على التئام الاذى الجراحي المستحدث للأوتار في الارانب

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الخلاصة

تم إجراء دراسة عيانية ونسجية مرضية على أربعين ارنبا محليا بالغا لمعرفة تأثير الليزر واطئ الطاقة على التئام جروح الأوتار للأرانب. تم جرح وتر العضلة النصف غشائية بقطع جزء من سمك الوتر تحت التخدير العام ومن ثم متابعة التئام الوتر في فترات مختلفة. تم تقسيم الحيوانات الى خمس مجاميع متساوية، احداها كانت مجموعة السيطرة (C) والمجاميع الاخرى تم تعريضها للتشعيع بالليزر؛ ثلاثة منها تعرضت لليزر المتذبذب بطول موجي (٩٠٤ nm) بقوة ((A, B, C ،watt 2x103) والمجموعة الاخيرة (E) تعرضت لليزر المستمر بطول موجي (٩٠٤ nm) وبقوة ((watt ٣٠١x وكما يلي: مجموعة تعرضت للتشعيع لمدة (١٥) دقيقة، ومجموعة تعرضت لمدة (٢٥) دقيقة، ومجموعة تعرضت لمدة (٣٥) دقيقة وحسب التسلسل، ومجموعة الليزر المستمر(E) تعرضت للتشعيع لمدة ٣٥) دقيقة), ومجموعة السيطرة (C) لم تتعرض لأي تشعيع. قتل حيوانان في كل فترة لدراسة التغيرات العيانية والنسجية المرضية في الايام التالية (٥, ٨, ١٥, ٢١) بعد العملية الجراحية. وكانت النتائج كما يلي: ازداد سمك طبقة الابيتينون منذ اليوم الخامس في المجاميع التي تعرضت للتشعيع وهذا ما لم يتم ملاحظته في مجموعة السيطرة الا بعد وقت متأخر. واستمر وجود النزف الدموي حتى اليوم الوحد والعشرين في مجموعة السيطرة، بينما لم نلاحظ وجود النزيف الا في المجموعة التي تعرضت للتشعيع بالليزر النبضي لمدة (٣٥ دقيقة) (D) في اليوم الواحد والعشرين. وكانت الاستجابة الخلوية (الخلايا الالتهابية والخلايا المولدة للألياف) واضحة في المجاميع المعالجة منذ اليوم الخامس بعد العملية الجراحية. وظهرت الالياف الغراوية وبشكل مميز منذ اليوم الخامس في المجاميع المعالجة بينما لم تظهر في مجموعة السيطرة الا في اليوم الواحد والعشرين ولم تكن ناضجة ولا منتظمة. وظهرت أحسن صورة للالتئام في مجموعة (B) في اليوم الخامس عشر بعد العملية الجراحية والذي بدأ فيه إعادة الترميم. وكان لليزر المتذبذب تأثير اكبر على التئام الوتر المجروح مقارنة بتأثير الليزر المستمر والذي أظهر في مجموعته (E) استمرار الاستجابة الخلوية الشديدة وتكوين الياف غراوية غير ناضجة وغير منتظمة حتى اليوم الواحد والعشرين.

الكلمات المفتاحية: ليزر، الليزر واطئ الطاقة، وتر، ارنب