Histological Observation Related to the Use of Laser and Ultrasound on Bone Fracture Healing

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ABSTRACT

Objective: To study the effect of both laser and ultrasound radiation on bone fracture healing process. **Materials and Methods:** Nd:YAG laser (1064 nm wavelength, 135 mW power, 16 joules energy) and ultrasound (1 MHz frequency, 50 mW/cm^2 power intensity) were used in this work. Fifteen mature, male, albino rats, were divided into three groups and subjected to a partial fracture on the lateral aspect of femur by a sharp blade. The first group of these animals served as control group. The second group was illuminated by the Nd:YAG laser for two minutes; the first dose was given immediately after surgical fracture induction; the other doses were given on days two, three, six and then one dose weekly for the next three weeks while the third group were treated by the addition of the CW ultrasound perpendicular to the laser treatment in the second group. **Results:** The present study showed that ultrasound increases the penetration of laser power through the tissue. The histological assessments at day 28 after the fracture of first group showed incomplete healing of the bone with disfiguration and disarrangement of Haversian system and the periosteum was not yet well developed. Treatment with laser showed irregularity and lack of Haversian system formation in bone healing of the second group. The laser and ultrasound treated group (third group) expressed a complete healing at the site of fracture with a complete layer of periosteum and a well arranged Haversian system. **Conclusion:** Combination of laser and ultrasound in therapy can enhance healing process of a fractured bone more than laser therapy alone, as ultrasound increases the depth of laser penetration in tissue.

KEYWORDS: Bone, Fracture, Laser, Ultrasound.

INTRODUCTION

Tissue healing is a complex process that involves local and systemic responses.1 Wound healing is a dynamic process of restoring cellular structures and tissue layers. The process of wound healing involves several types of cells; enzymes; growth factors and other substances. Bone healing process is slower than that of soft tissues and differs from it because of its morphology and composition.[1] Healing of tendons and other dense connective tissues take as long as 6 - 8 weeks.[2]

Low-level laser therapy (LLLT) is a treatment refers to the use of red-beam or near-infrared lasers with a wavelength between 600 and 1000nm power from 5–500 milliwatts to stimulate the repair or reduction of pain of a wide variety of body parts. These types of lasers have been advocated for use in a wide range of medical conditions encompassing musculoskeletal conditions such as chronic low back pain,[3] wound healing[4] and acute and chronic Achilles tendinitis.[5] LLLT has stimulatory effects on bone cell proliferation[6] and gene expression[7] and can be used to enhance bone repair at cellular and tissue levels.[8] Laser effects on tissues may be attributed to thermal and non thermal effects; the non thermal effects of laser beams on tissue include photodynamic therapy and photo-biostimulation therapy. The beneficial biological effects of laser light therapy on tissues can be summarized as to accelerate tissue repair and cell growth, improve vascular activity and increase metabolic activity.[9]

Nd:YAG (neodymium-doped yttrium aluminum garnet; Nd:Y3Al5O12) is a crystal that is used as a lasing medium for solid-state lasers. Nd:YAG lasers are optically pumped using laser diodes and typically emit light with a wavelength of 1064 nm, in the infrared. Nd:YAG lasers are one of the most common types of laser, and are used as LLLT for many different applications.[10] Nd:YAG lasers have been used to treat chronic periodontitis[11] and to speed healing of infections around dental implants.[12]

Currently, therapeutic ultrasound is also used primarily in physical medicine. It is a method of

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stimulating tissue beneath the skin's surface using sound waves, of a very high frequency, typically between 800,000 Hz and 2,000,000 Hz. The application of ultrasound to living tissues results in the transmission of mechanical energy in the form of acoustic pressure waves which are transmitted by propagation through the tissue, due to absorption, dispersion or scattering of the wave.[13] The waves produce forces in tissues equal to micromechanical stresses and strains. It has been established that the micromechanical strains produced by these pressure waves in biological tissues led to biochemical events that regulate fracture healing.[14,15] Like LLLT, the biological effects of ultrasound can also be categorized as thermal and non-thermal effects.[16]

The purpose of the present investigation is to study the effect of administration of Nd:YAG laser and ultrasound radiation on the histological changes possibly occurring during bone fracture healing process and to investigate whether ultrasound radiation increases or decreases the beneficial effect of Nd:YAG laser.

MATERIALS AND METHODS

Pilot study

A cross section through the thigh of 5 male Albino rats (Rattus norvegicus), weighing 250-350 gm and aged 6 months were selected to measure the amount of laser power that pass the skin and other tissues to hit the bone surface. The hairs were shaved, then section blocks containing skin, muscle and bone layers were taken from the thigh and their thickness was measured by vernier. These blocks of tissue were subjected to Nd:YAG (1064 nm wavelength, 135 mW power)(The laser-Edmund optics worldwide Inc.) from one side of tissue section. Laser power output was measured using power meter that was placed on the opposite side of the section. The measurements were repeated by adding the effect of a CW ultrasound source (50 mW cm2 intensity, 1 MHz freq.). The measured output laser power P for each section of thickness Z is used; the mean attenuation coefficient in a certain thickness and wavelength for skin, muscle and bone using the input power P^0 for Nd:YAG 135 mW was calculated using the Beer-Lambert law equation:

$$p=p_0 *\exp(-az)$$

Experimental Fracture

Fifteen mature (Rattus norvegicus) rats weighing 250-350 gm were housed in plastic cages, had free access to laboratory chow and water and kept under suitable environmental conditions such as a room temperature that was maintained at about (24±2)0C and exposed to 12 hour/day light program. Each animal was anesthetized with single intraperitoneal dose of 0.2 ml of Ketamine (Hyprochloride) (50 mg/ml) (OUBARI PHARMA-Helepo-Syria) and 0.05 ml Xylazine (Rompun 2%) (23.32 mg/ml) (OUBARI PHARMA-Helepo-Syria). The left leg was shaved, the skin was cut with sharp razor, the femoral muscle was displaced with forceps, then a partial fracture was made in the lateral aspect of the upper third of the femur using surgical blade, after that the muscle was replaced and the skin was sutured. A back slap using POP was placed at the site of fracture for all groups to minimize mobilization.The animals were divided into three groups of five rats each. Group 1, was subjected to partial femur fracture and left to be healed without any interference. Group 2, was treated with CW Nd:YAG laser of 1064 nm wavelength, power of 135 mW and energy of 16 joules for duration of 2 minutes; the first dose was given immediately after surgical fracture induction; the subsequent doses were given on days 2, 3, 6, and then one dose weekly for the next 3 weeks. Group 3, was treated by the addition of the CW ultrasound perpendicular to the laser treatment in group 2, the used ultrasound is of 1 MHz frequency and 50 mW/cm2 intensity per session, for 2 minutes on every occasion of laser treatment (The ultrasonic therapy unit-digital Helosonic delux India). Healing site was assessed weekly by X-ray. X-ray showed healing of the site at the 28th day. By the end of 4th week, the rats were sacrificed and the removed femoral bone samples were fixed in fisher fixative for 12 hours, dehydrated through ascending graded alcohols (50%, 70%, 90% 100% ethanol), cleared in two changes of xylene and embedded in paraplast tissue embedding media of 57 oC melting point (Sigma). Serial sections of 5-6µm thickness were stained by Harris's hematoxylin and eosin.

RESULTS

Table 1 showed the penetrated laser radiation through various tissue thicknesses to reach bone surface. It is clear that the power output of the Nd: YAG laser was increased when ultrasound was added.

Table 1. Power output after passing through a multi-layers tissue consisting of skin, muscle, and one, with and without ultrasound effect.

Tissue thickness (mm) (skin + muscle + bone)	Laser output power (mW)	Laser + Ultrasound output power (mW)
5.5	29	41
6.1	27	38
7.5	25	34
9.5	23	30

Histological Results

Group 1 (Control group). The present histological observation showed that bone healing was incomplete; disfiguration and disarrangement of Haversian system is observed (Figure 1 A). Separation of the bone, and discontinuity was a marked feature in most of the sections (Figure 1 B). New bone matrix lying was observed as bone spicules scattered along the site of injury. Some sections showed the presence of bone spicules at the site of injury with incomplete formation of periosteal layer (Figure 1 C).

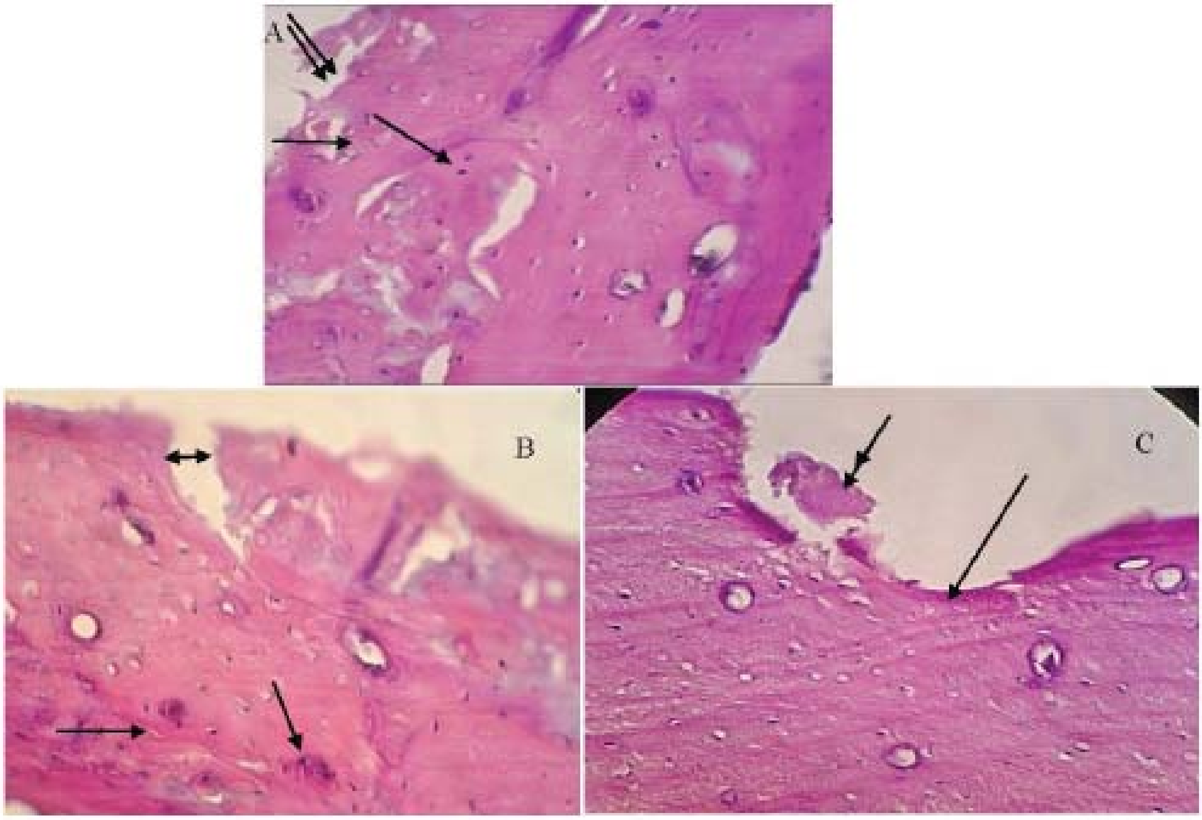

Figure 1A. Sample photomicrographs from bones of control group showing disarrangement of Haversian systems at the site of injury with incomplete bony alignment “ → ” and the periosteum is not yet developed at the site of fracture “Double arrows”, Figure 1B. Discontinuity “↔” of the bone with disarrangement of Haversian systems and bone matrix “→”, Figure 1C. Clear defect in the surface of the bone and the periosteum”→” with small bone fragments at the site of fractures “→→”. (H&E stain, original magnification, X 40).

Group 2 (Laser group). The site of healing of the fractured bone showed a complete layer of bone matrix (Figure 2 A).

The observed healing area was lacking Haversian system formation, representing Woven bone; there was an irregular cell orientation in the matrix of the healed area (Figure 2B).

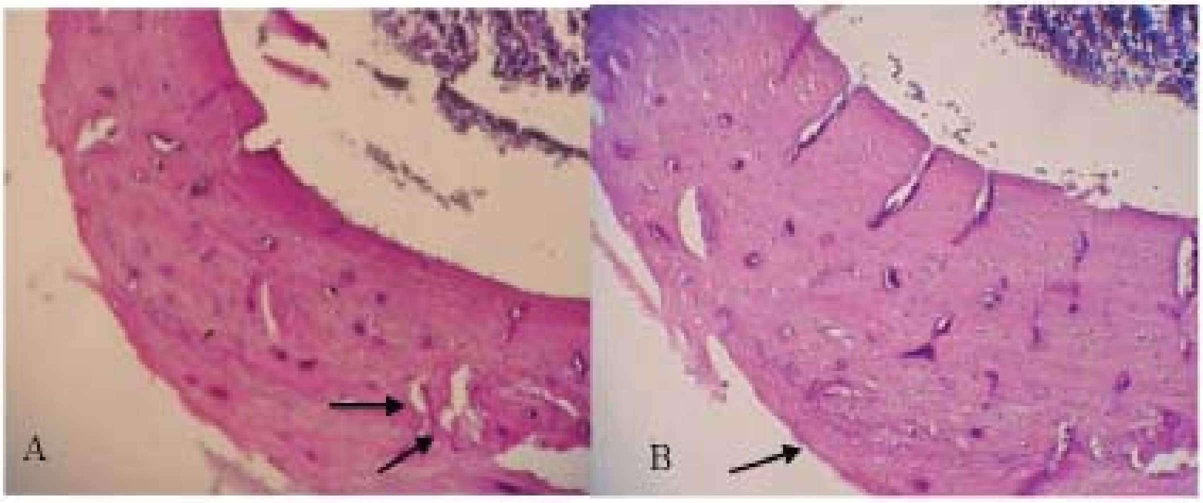

Figure 2. Sample photomicrographs from bones of laser treated group showing the layer of bony material and matrix at the site of injury, but Haversian system is not well developed yet “→” (A), the site of injury “→” with matrix filling the site and small newly formed Haversian system is not yet developed (B), (H&E stain, original magnification, X25).

Group 3 (Laser and Ultra*Group 3 (Laser and Ultrasound group)*. The site of the fractured bone showed a complete healing area at the site of injury. A cross section of the femur showed a regular continuity of the bone surface with regular array of bone marrow inside the bone and a complete layer of periosteum (Figure 3 A). The healed area of fracture appeared lightly stained than the adjacent tissue (figure 3 B). This lightly stained area showed a well developed complete periosteum layer of well arranged parallel fibers and cellularity (Figure 3 C & D). A well arranged Haversian systems with well developed lamellae arranged concentrically around a vascular Haversian canal and Volkmann canals were observed (Figures 3 D & E).

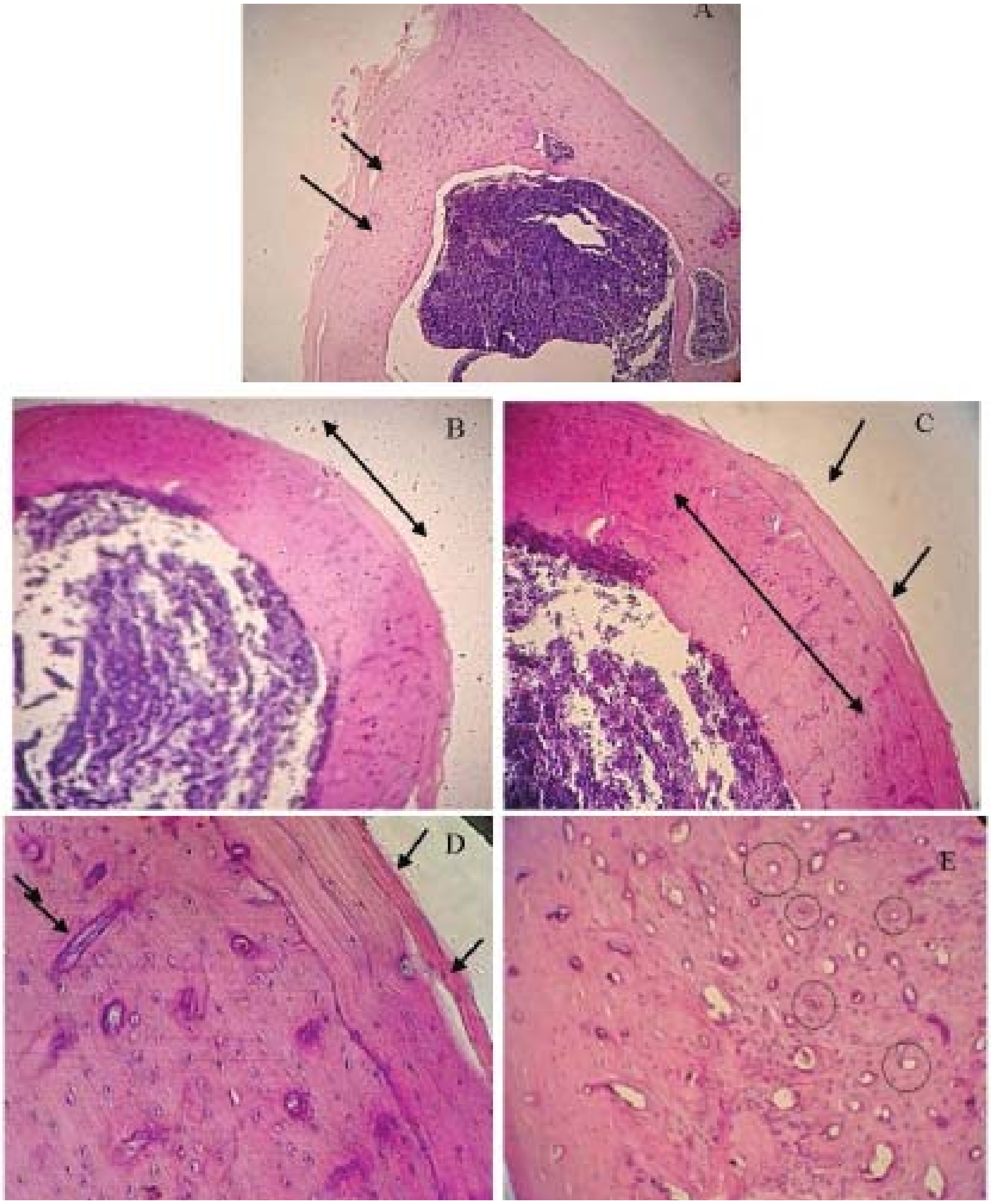

Figure 3. Sample photomicrographs from bones of laser and ultrasound treated group showing a complete bone matrix and a complete layer of periosteum “⟶” (A), completely healed fractured femur, lighter stained area represents the site of injury and healing “⟷” (B), a higher magnification of the area in (B) showing lighter stained matrix at the site of fracture “◄──►“ with a complete layer of periosteum “⟶” (C), a well arranged Haversian system, rich vascularity and Volkmann canals “─►─►”, a well developed complete periosteal layer “⟶” (D), well arranged complete formation of Haversian system at the healed area (E). (H&E stain, original magnification, “A X10”, “B & C X25”, “D & E X40”).

DISCUSSION

The use of LLLT in health care has been documented in the literature for more than two decades and has been used in the treatment of a variety of diseases and conditions. The LLLT positive effects on the repair of soft tissues and bone involve metabolic events which may reduce total treatment time and ensure greater patient comfort17 and has a positive effect on the percentage of newly formed bone.[18]

The use of lasers for therapy depends on the interaction of laser beams with tissue; several types of interaction may take place depending on the wavelength of the laser, temporal nature of the beam "continuous wave or pulsed", energy delivered, and special nature of the beam "focused or unfocused".[19] The interaction of ultrasound with tissues can be also described by reflection that occurs at the tissue surface, absorption by tissues which is often due to coloring agents such as melanin, water and hemoglobin; the absorbed light is generally converted to heat and scattering that causes the light to spread in different directions. All these factors are highly dependent on the wavelength of the incident light.[20]

The present study has shown that the penetration of Nd:YAG laser in the tissue sections "skin, muscle and bone" has increased when the therapeutic ultrasound was added to the applied Nd:YAG. This is due to the effect of the perpendicularly ultrasound application. Ogulu[21] postulated two mechanisms through which ultrasound modulate the optical field in tissues. First, as light travels the tortuous path of the ultrasonic field, accumulates a phase shift due to multiple reflections from the moving particles of the tissue. Secondly, the index of refraction of the tissue is altered by the pressure field due to the piezo-optic effect of the ultrasound, leading to an additional phase change.

Because of its deeper penetration, the Nd:YAG laser was chosen in the present study to illuminate externally an experimentally made partial fracture. The timing of illumination doses was set depending on the fact that the revascularization and the removal of bone debris usually occurs on day 1, 2, and 3 after fracture, while the activation of osteoblast formation occurred on day 6 and 7.[16]

The influence of daily energy doses of He-Ne laser irradiation on the repair of surgically produced tibia damage was investigated in Wistar rats;22 laser treatment was initiated 24 hours after the trauma and continued daily for 7 or 14 days in two groups of nine rats; the course of healing was monitored using morphometrical analysis of the trabecular area; there was a significant increase in the area of new formed trabeculae in tibiae after 7days of irradiation. Trelles and Mayayo[23] performed histological measurements to determine bone repair with low-power He-Ne laser irradiation in mice; they observed an important increase in vascularization and faster formation of osseous tissue with a dense trabecular net compared to the control group, and concluded that the laser effect might modulate the function of osteocytes, promoting faster metabolism and reaction of bone callus.

In the present study, healing of fractured bones responded to combination of Nd:YAG laser and ultrasound treatment faster than using laser alone. This combination accelerated fracture healing process when complete bone healing was identified in four weeks where the site of healing of the fractured bone showed a regular continuity of bone with regular array bone marrow inside the bone and a complete layer of periosteum, the lamellae of osteons were well arranged around Haversian canals. These observations are in accordance with Trelles and Mayayo[23] and may be related to the ability of laser irradiation to increase the bone cells activity around the site of the healing area.[8] Junior et al,[24] concluded that laser therapy accelerates proliferation of osteoprogenitor cells, enhances osteoblastic calcification and promote bone regeneration. Lirani Galvao et al,[25] compared the consequences of LLLT and low-intensity pulsed ultrasound (LIPUS) on bone repair; they found that bone histomorphometry revealed a significant increase in osteoblast number and surface, and osteoid volume in the LLLT group, and a significant increase in eroded and osteoclast surfaces in the LIPUS group; they suggested that LIPUS enhanced bone repair by promoting bone resorption in the osteotomy area, while LLLT accelerated this process through bone formation .

Many researchers have discussed the role of using continuous type of laser and its superiority upon pulsed type. The effect of pulse frequency of LLLT on bone nodule formation in rat was studied by Ueda and ShimizuIn;[26] they isolated Osteoblast-like cells from fetal rat calvariae and irradiate them once with a low-energy Ga-Al-As laser in different irradiation modes "continuous and pulsed irradiation". They observed that laser irradiation in all groups significantly stimulated cellular proliferation, bone nodule formation, Alkaline Phosphatase "ALP" activity, and ALP gene expression, as compared with none irradiation group and concluded that the pulse frequency of LLLT constitutes an important factor affecting biological responses in bone formation. In a computerized morphometry; Silva Junior et al. [27] applied Ga-Al-As laser irradiation on surgical wounds created in the femur of rats and found a significant increase in the mineralization of bone repair at early bone healing in the group subjected to LLLT.

Previous studies reported variable duration of bone healing in fractured bones exposed to various laser's radiation. David et al.[28] studied the effects of He-Ne laser on fracture healing in the tibia of rats and found a gradual increase in healing after 6 weeks of treatment. Chen et al.[29] studied the effect of a CO2 laser on bone repair; experimental bilateral mandibular osteotomies were performed in the ramus of 24 rabbits, the calcium and Phosphorus content in the irradiated calluses was found to be significantly higher than in the control calluses on the 14th, 21st and 28th post-operation days. Morrone et al.[29] studied the effect of a GaALAS laser on the osteochondral lesion repair of the knee in rabbits, and found more hyaline-like cells mixed with fibrocartilage in the matrix after 6 and 12 weeks than in the untreated specimens. Enwemeka and Reddy[30] tested the effect of ultrasound on 632.8 nm He-Ne laser, and 904 nm Ga-As laser on healing of experimentally severed and repaired rabbit Achilles tendons as evidenced by biochemical, biomechanical, and morphological indices of healing and found that combinations of the laser with ultrasound stimulation further promote collagen synthesis on through the 14 day period of the study. These variations may be related to species differences and/or to the type of bone used, and type, modes, wave length and episode of laser irradiation. The present investigation has demonstrated that continuous Nd:YAG laser has a healing outcome; and addition of ultrasound to Nd:YAG laser showed a well developed layer of periosteum. Although structural and cellular healing have occurred after four weeks, it seems that mineralization is still in process and needs further time to complete healing. Further biochemical and ultrastructural studies are needed to study in detail bone healing under the effect of combination of Nd: YAG laser and ultrasound.

CONCLUSION

NA:YAG Laser therapy has been proved practically to speed up the healing process of bone fractures and the combination of laser and ultrasound in therapy can enhance the healing process of a fractured bone more than laser therapy alone, as the ultrasound increase the depth of laser penetration in tissue.

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