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RESEARCH ARTICLE

EFFECT OF 790-805NM DIODE LASER THERAPY ON MAST CELL IN CUTANEOUS WOUND HEALING IN MICE.

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Key words:-

Biostimulation, low level laser therapy (LLLT).

Abstract

Background and objective: -The use of low level laser therapy (LLLT) has been increased now a day to accelerate healing of soft tissue injuries because of some biostimulatory effects. The goal of this study was to investigate the effect of 790-805nm diode laser on the inflammatory effect of mast cells during wound healing in rodents.

Materials and Methods: -A cut wound (1.5cm) was made on the cheek of 40 albino mice. 20 of them exposed to LLLT (360 J/cm²) at 790-805 nm immediately post wounding procedure. The animals were sacrificed and the wound area was prepared and stained by toluidine blue.

Results: - Mean mast cell count of 10.2 in the first day of control group while in laser group 8.4. The control and laser group showed gradual inclination in the mean value to be return to increase at the day 14 of experiment. There was significant difference (P< 0.05) in control group at the first day. While significant difference (P≤0.05) was in the day 7. Pearson correlation showed significant correlation (P≤0.01) between the control group at the day 1 and the laser group at the day 7. While there was significant correlation (P≤0.05) between the control group at the day 14 and laser group at the day 1.

Conclusions:- LLLT may induce an anti-inflammatory effect on wound healing process by its inhibitory action on mast cells; while it may have a biostimulatory effect on the proliferation of mast cells at proliferative phase of wound healing which indirectly affects fibrous tissue regeneration in subcutaneous area.

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Introduction:-

Mast cells can be activated by trauma or immune mediated mechanisms^[1]; and they are present in mucosa of respiratory and digestive system in addition to the skin.^[2] They are arising in the bone marrow from a multipotent CD34+ precursor and distribute throughout the body by circulation to act locally and systemically by releasing inflammatory mediators through degranulation^[3].

Mast cells have been improved to play a role in wound healing by releasing these mediators^[4] that are released by mast cells are neuropeptides and cytokines and pro-inflammatory mediators selectively without degranulation particularly IL-1 induces selective release of IL-6 while corticotropin-releasing hormone secreted under stress induced the release of vasculoendothelial growth factor^[5]. The anti-inflammatory effect of LLLT in wound healing had

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been studied by many researches. This effect vary from accelerating the inflammatory phase by activation of different cells involved in wound healing to deactivation of other types of cells that may be delay the healing process.^{[6][7][8][9]}

One of these cells that had been studied for their relation to the application of LLLT was mast cell. The results vary also from activation to inhibition of anti-inflammatory effect ^{[10] [11][12][13]}. The mechanism by which LLLT act on mast cells is still under research. In this study we tried to explain how this mechanism could be occurring.

Materials and methods:-

40 albino mice were used in our study weighing 100-150 g. They were housed in plastic cages which nurtured properly and kept in a temperature- and humidity- controlled environment at 23 °C in a 12/12-h day/night light cycle.

Wound procedure:-

The animals were anesthetized with diethyl ether and 1.5cm² standardized cut wound was performed on the face , then they were arbitrarily divided into two chief groups; control and laser (20 each), then subdivided into four groups(5 each) as fellow: day 1, 3, 7 and 14group.

Laser procedure:-

Animals inlaser groups were exposed toArGaAl laser beam of 790-805-nm wavelength gotten from a laser device (K-LASER-ITALY) immediately following surgical procedure. The laser beam characters consisted of 780-905 nm for 90 s, 4W (output power), and an energy density of 360 J/cm² and focal spot was 8mm. Laser beam was directly positioned over wound without contact(0.5 cm)away from the edge of the wound with one spot to cover the wound area.

Histological procedure:-

The animals were sacrificed by overdose of diethyl ether in closed jar then; the histological specimens were taken from wound and adjacent areas. The specimens were fixed in buffered 10% formalin, and then embeddedin paraffin. Many sections of 5 µm thickness were taken from each block and put on microscopic slides.

Toluidine blue stain (1% toluidine blue in 1% sodium chloride) was used as a special stain for enumeration of mast cells. Both intact and degranulated mast cells were recorded under high magnification of light microscope (oil lens x1000) for counting. Five fields from high power view for each specimen's slide were used to obtain the data results.

Ethical considerations:-

The study was led in full agreement with the World Medical Association Declaration of Helsinki. Before starting the study; astudy proposal was submitted, and approved bythe InstitutionalEthical Committeein Baghdad University- College of Dentistry.

Statistical analysis:-

Statistical Package for Social Sciences, Windows 7, software version 14 (SPSS)was used to process the data gained. Descriptive, ANOVA, Persons correlation were used to obtain the results.

Results:-

Microscopical viewing of the histological slides is seen in figures (1-4) for both control and laser group. The observations of the sections showed no obvious variance in the distribution of mast cells between the two groups.

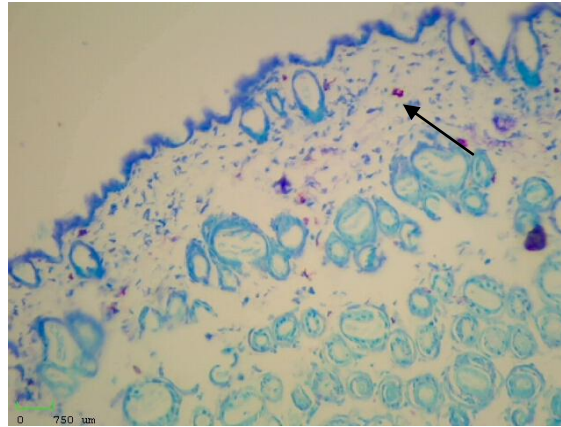


Figure-1:- Site of the wound in control group at day 1 showed proliferation of mast cells (pointed) x20

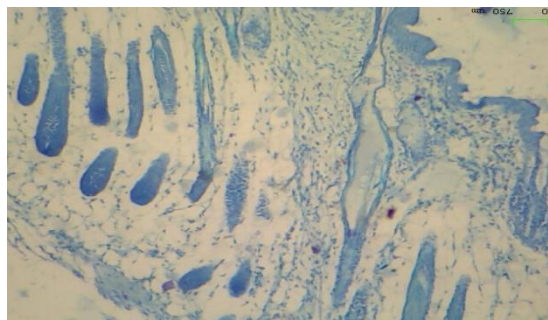


Figure 2:- Wound healing area at day 14 showed marked reduction in mast cells count x10

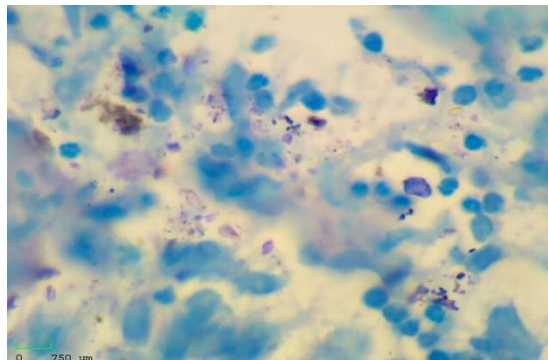


Figure 3:- Wound healing area in laser group at day 3 in sub-epithelial area x100.

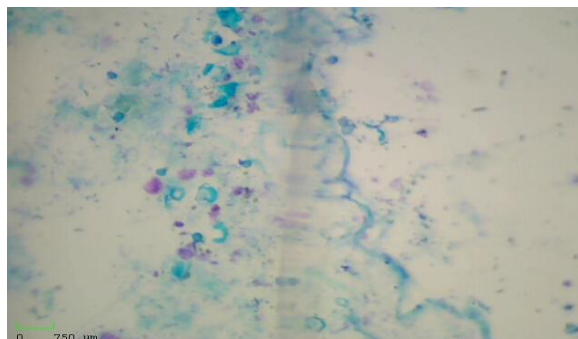


Figure 4:- Wound healing area in laser group at day 14 X10

(Table 1) showed mean value of mast cell count of (10.2) in the day 1 of control group while in laser group (8.4). Both the control and laser group showed gradual inclination in the mean value at day 3 to be return to increase at the day 7 of experiment. The laser group showed high mean value of mast cells (9) at day 14 than control group.

Table 1:-mean value, standard error and standard deviation of total mast cell number.

| group | Mean | | Standard deviation |
|------------|-----------|----------------|--------------------|
| | statistic | Standard error | |
| Control 1 | 10.2 | 5.774 | 12.911 |
| Laser 1 | 8.4 | 4.19 | 9.37 |
| Control 3 | 7.2 | 2.244 | 5.019 |
| Laser 3 | 5 | .836 | 1.87 |
| Control 7 | 8.6 | 1.224 | 2.733 |
| Laser 7 | 5 | 2.4 | 5.366 |
| Control 14 | 7.2 | 1.496 | 3.346 |
| Laser 14 | 9 | 2.07 | 4.636 |

(Table 2) showed statistically significant difference ($P \leq 0.05$) in control group at the day 1.

Table 2:-ANOVA test between groups.

| group | Sum of square | Mean square | F value | Sig. |
|------------|---------------|-------------|---------|-------|
| Control1 | 666.3 | 222.1 | 444.2 | .035* |
| Laser 1 | 109.2 | 36.4 | .15 | .918 |
| Control 3 | 68.8 | 22.933 | .717 | .677 |
| Laser 3 | 14 | 4.667 | - | - |
| Control 7 | 29.5 | 9.833 | 19.667 | .167 |
| Laser 7 | 114.7 | 38.233 | 76.46 | .084 |
| Control 14 | 42.8 | 14.267 | 7.133 | .267 |
| Laser 14 | 45.5 | 15.167 | .374 | .799 |

Pearson correlation showed significant difference ($P \leq 0.01$) between the control group at the day 1 and the laser group at the day 7.

While there was significant difference ($P \leq 0.05$) between the control group at the day 14 and laser group at the day 1. (Table 3).

Table 3:-Pearson correlation between control and laser group.

| Pearson correlation | Laser7 | Laser1 |
|---------------------|--------|--------|
| Control 1 | .997** | |
| Control14 | | .953* |

** At level 0.01

At level 0.05

Discussion:-

Wound healing represents one of the most common fields that use LLLT in various researches and clinical studies in humans.^[14-17] Additional studies in animals has also reinforced the role of LLLT in accelerating wound healing^[18-19] In contrast, many investigators proved that there was no benefit in healing by using LLLT^[20-23] The concept of light absorption is specific to cell and tissue type and the variations between tissues in the same organ could be seen^[24] decreasing the ability to generalize the outcomes of animal data results on human wounds. The molecular level of clinical trials and researches could be more informative about the specific cells and structures that affected by laser therapy.

The results of our study in day 1 showed increased in mast cells' count in control group in comparison to laser group. This count made a greater difference in mean value (8.6) at day 7 in control group.

On day 14, the laser group exhibited means 9 of mast cells higher than control group. This result disagree with that of Bayat et al, and Vasheghani^[10-11] who found that LLLT effects significantly on the increase of total number of mast cells during the inflammatory phase of wound healing in the experimental third degree burns.

This difference may be due the type of laser and type of wound. In contrast there was major increase in the overall number of mast cells in day 7 up to the day 14 in laser group which represents the phase of proliferation and remodeling in wound healing process.

This disagreed with the results of DeCastro et al^[25] who approved that mast cells affect the proliferation of fibroblast and there was a correlation between their number and myofibroblasts in control wound repair but in the excisional wound treated with laser; there was increased in collagen deposition in relation to reduction in mast cells 'count.

Though both groups showed complete healed wounds without complications because there was no sign of infection, clinically by day 14; but on cellular level; LLLT appeared to accelerate the remodeling phase of healing, in which the granulation tissue would be converted to more strong fibrous connective tissue.

The effect of use of LLLT on mast cells in cutaneous wound and its effect on wound healing was performed by many clinicians and investigators to investigate the most beneficial use of specific therapeutic modality. These studies have been done on different types of wounds and correlate the results with different types of laser, laser devices and frequencies concluded that many errors in the methods among studies made using LLLT did not allow sufficient conclusions to be tired relative to the value of the modality.^[27-31]

These studies didn't explain and quantify the actual differences between laser and non-laser-treated wounds and many of them often employ a variety of approaches in the same study; which makes it difficult to evaluate the effects of a single parameter. The cellular level of investigation for several intrinsic and extrinsic factors related to the inflammatory response and healing make well-designed studies more reliable.

The precise mechanism by which LLLT accelerates wound healing is still unknown. But some theories could explain how laser energy stimulates cells in wound healing. In some of these studies that were experimental; have shown an increase in fibroblast count after irradiation^[32] suggesting that LLLT may accelerate fibroblasts proliferation during the remodeling phase of wound healing which support our results in this study. We found that LLLT has a significant difference at day 7, when wounds start to be in the remodeling phase of cutaneous healing. However; other studies found no in vitro changes in fibroblast count after LLLT^[33-35]. This could be due to difference in laser device settings, such as wavelength, duration, power density, and intensity^[24]

Accelerated wound contraction could be explained by the work of Pourreau-Schneider et al^[36] who showed that LLLT transforms fibroblasts into myofibroblasts which are mainly involved in granulation tissue contraction, so increased their count could accelerate wound contraction. The role of mast cells seems to affect the proliferation of fibroblast and there was a correlation between their number and myofibroblasts in control wound repair.^[3]

The mechanism by which the two edges of wound become close and causing contraction of granulation tissue is by the action of cytoplasmic fibrils of actomyosin of myofibroblasts and then reducing the size of the wound during the remodeling phase of healing process.^[37] Our data providing support to the fact of that LLLT enhanced wound healing by its stimulate the proliferation of mast cells hence fibroblasts proliferation but not necessarily enhance other variables associated with wound healing

This study showed that LLLT may have an indirect effect on wound's surrounding tissues. Both the laser exposed and unexposed wounds showed enhancement of healing. These data are agreed with that of Braverman et al,^[38] who found that both control and laser group showed the same results of wound healing. This may be due to release of growth factors into circulation, then affect surrounding tissues or other parts of the body. Mast cells may modify inflammatory response by playing a role in neoangiogenesis and tissue remodeling^[39]. This is an indirect effect on healing that could be a very valuable effect of this modality in treating tissue injury of large size, multiple sites, and deep wounds that could be affected by laser therapy.

The uniform wound model in this study was restricted to acute, full skin thickness incisional wounds that extend into the dermis that may show the same healing process to that in other types of tissues in the body, but there was no obvious results that similar effects of LLLT would be seen in wounds of other sites or in chronic wounds although mast cells both acute and chronic inflammation due to different types of inflammatory mediators released from them. Among these mediators are histamine, heparin, hyaluronic acid, proteoglycans, proteases growth factors, and cytokines^[3].

The results in this study were related to the specific device settings. These settings reflect common clinical practice and the manufacturer's recommendations and guidelines.^[15] 790-805nm diodes represents a common clinical practice diode lasers^[24] there may be a gap between the head of the probe and the wound and some assumed divergence of the laser light and reduction of irradiation intensity to the tissue. The variations were seen from other studies could be due to be the result of individual differences in tissue elasticity and compliance among different animals' model.

Conclusions:-

Mast cells proliferation and degranulation could be affected by LLLT in wound if applied directly after the trauma or wound process. This stimulation may be less than normal wound healing in the inflammatory phase but would be more in the proliferation phase.

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