Different Wavelengths of LEDs on Cutaneous Wound Healing in Wistar Rats

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Abstract: This study evaluates different wavelengths of LED therapy in *Wistar* rats skin injuries. LEDs (Light Emitting Diodes) are phototherapeutic resource nowadays, since it is considered a good alternative to Low Level Laser Therapy in injury healing because of the lower cost. Twenty-five male *Wistar* rats were divided in five groups: Control, Red LED (630-780 nm), Green LED (490-565 nm), Blue LED (440-490 nm) and Yellow LED (590-630 nm). It´s a experimental research that it was performed during 4 weeks. Twenty-four hours after surgical injury (1cm²) was applied LED therapy for 6 minutes during five days. Red LED presented best anti-edematous effects in comparison to the other wavebands. The perimeters were reduced in all groups, but in Green and Red LED groups were significantly diminished ($p<0,05$) when compared to control and Blue LED groups. The best result of area was with Green LED and worst results with Blue LED. The use of non collimated phototherapy with Red, Green, Yellow and Blue LEDs improves the wounds healing process, mainly with Red wavelength. The non collimated phototherapy with LEDs could be included in Physiotherapy practice for wound healing improvement because of the lower cost in comparison with Low Level Laser Therapy and the benefits of irradiate a bigger area.

Keywords: Phototherapy, wound healing, LED, Physiotherapy, Wistar rat.

INTRODUCTION

This study investigates the effects of different wavelengths of the LED in the healing process. It is important to professional health including the Physiotherapy to demonstrate that the LED with your specific wave length of Red (630-780 nm), Green (490-565 nm), Blue (440-490 nm) or Yellow (590-630 nm) is more effective in wound healing.

The idea of using LED in the healing process was based on the literature that confirms their antibacterial, anti-inflammatory and healing effects. According to some authors [1], it is a new, painless, fast results and using extremely simple therapeutic devices. It does not cause tissue damage and the action is through direct intercellular stimulation, acting specifically in mitochondria, rearranging cells, inhibiting the action of some cell groups and encouraging others. There are different biological and physiological effects according to each wavelength of Blue LED (440-490nm) bactericidal, Green LED (490-565nm) stimulates fibroblast on wound healing, Yellow LED (590-630) stimulates collagen on healing mature and the Red LED (630-780nm) is anti-inflammatory.

Over the years the healing process has deserved extra attention from researchers, especially regarding the factors that delay or hinder this process. This complex physiological process is highly organized and begins with an inflammatory response characterized by increased blood flow and capillary permeability and leukocyte migration into the injured area. There are factors that hinder repair as contaminated environment in which infection occurs. The deficient nutritional status, especially hypoproteinemia, associated systemic diseases like Diabetes, chemoradiation, chemotherapy, and use of immunosuppressive drugs hinder too. Solutions to improve the quality of life of patients who are affected by disabilities in the tissue repair process have been investigated [2-4].

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Physiotherapy may play an important role in facilitating the repair process, since it uses therapeutic resources to modulate this process. Among these resources highlights the use of laser therapy, ultrasound therapy, sonic waves, microcurrent and light therapy. Today, one phototherapy option is the LEDs. Those are semiconductor diodes subjected to an electric current that emits light and the wavelength ranging from 405nm (blue) to 940nm (infrared). The photo-stimulation caused that light acts on the cell and in the permeability, then stimulating on mitochondria, the synthesis of ATP, the proteins collagen and elastin. This light also acts as an antimicrobial and anti-inflammatory and it had been indicated for many inflammatory disorders [1]. Laser sources and LEDs are similar from the point of view of the light emitted, or both produce a relatively narrow wavelength band, while the LED has somewhat broader spectrum. The important difference of these sources is the fact that the emerging LED light is neither collimated nor coherent. So, the LED is being justified as a good alternative to low-intensity laser to promote wound healing [5].

The energy dose is a question for many researchers and clinicians. Regarding the energy threshold biomodulation, there are several controversies. The lasers and LEDs with different energy doses produce certain effects on the blood vessels of the skin. Several studies following phototherapy based on the basic curve Arndt-Schultz as ideal to stimulate endothelial cell and subsequently the angiogenesis, besides the production of fibroblasts and collagen [6, 7]. On the other hand, some authors justify the use of high fluencies photo modular for wound healing [8]. Whom is correct?

MATERIALS AND METHODS

This is experimental, controlled and randomized study in which it was analyzed the healing process of skin wounds produced in rats.

The population studied was 25 males Wistar rats (*Rattos norvegicos Albinus*), albino variation and randomly selected. All animals were originated from the Animal Facility and Laboratory Animal Experimentation of *Universidade Potiguar, Rio Grande do Norte, Natal / RN*. The study sample was composed of 5 groups of 5 animals weighing between 250g and 300g. They were divided into Control Group, Green LED Group, Red LED Group, Yellow LED Group and Blue LED Group.

It´s an experimental research that it was performed during 4 weeks. After that, the animals groups were to an experimental lesion procedure. The animals were previously sedated by the anesthetic (Zoletil 50) intramuscularly in the right quadriceps. The calculated dose was according to the animal weight (50 to 75 mg / kg). After ten minutes, the shaving and disinfecting of the dorsum of each animal with chlorhexidine 2% was taken, followed by surgical incision of 1 cm^2 in the dorsal region of 25 rats. Twenty-four hours after the surgical incision, the therapy was initiated with LED equipment (Figure **1**).

LED application was performed at 90° from the treatment area according to the cosine law Lambert without skin contact. The distance from the application zone is the area under the lesion so that the light emitted by the LED covering the entire wound. The application was daily, five applications followed, lasting 6 minutes and 3 watts of power in each application, with the appliance manufacturer's BioLight Demox Eletromédica. Then, surgical incision of the skin lesions, daily if accompanied her area and perimeter of

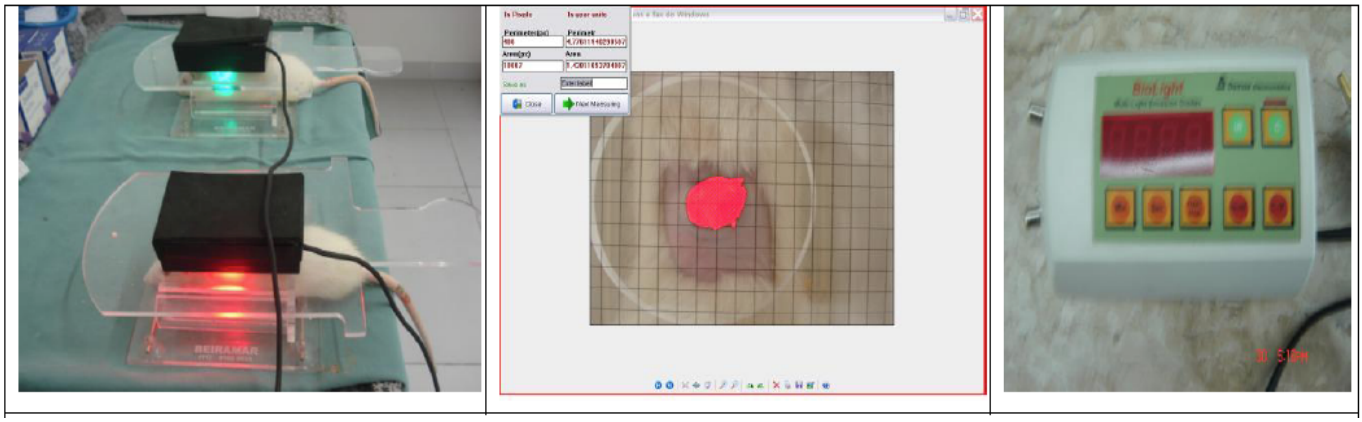


Figure 1: Aplication of the LED, lesion zone perimeter and utilized device.

the lesion. A total of 5 applications were performed with sacrifice after 24 hours, so on the 6th day.

Lesions were photographed daily with a transparent film brought grid (Figure **1**), enabling the calculation of area and perimeter, with the Sony Cyber Shot digital camera, 6.0 mega pixels. The perimeter and area calculation was made with the Universal Desktop Ruler 9.2.1124 version (AVPSpft) software.

Moreover, histological analysis, in which it was, researched the inflammatory reaction and the repair processes with the following items were taken:

Inflammation: type of inflammatory reaction (acute, chronic); Presence of crust; Presence of giant cells; Edema (moderate and intense light).

Repair Procedure: epithelialization or regeneration (Complete, Incomplete), granulation tissue - early repair process (vascular paucity, moderate vascular, plurivascular, And paucity cellular, cellular and multicellular moderate) and Healing (collagen Type: Young or Ripe). Data were collected by trained professionals in a blinded fashion.

Animal Investigations

The project was submitted to the Ethics Committee (CEP) and approved in accordance with paragraph 214/2009 protocol, as the standards of animal research

Statistics

Data were passed to Excel spreadsheets where the percentage difference was calculated for each group using the formula: Percentage difference = (initial measure - end measure) x 100 / (initial + final measure).

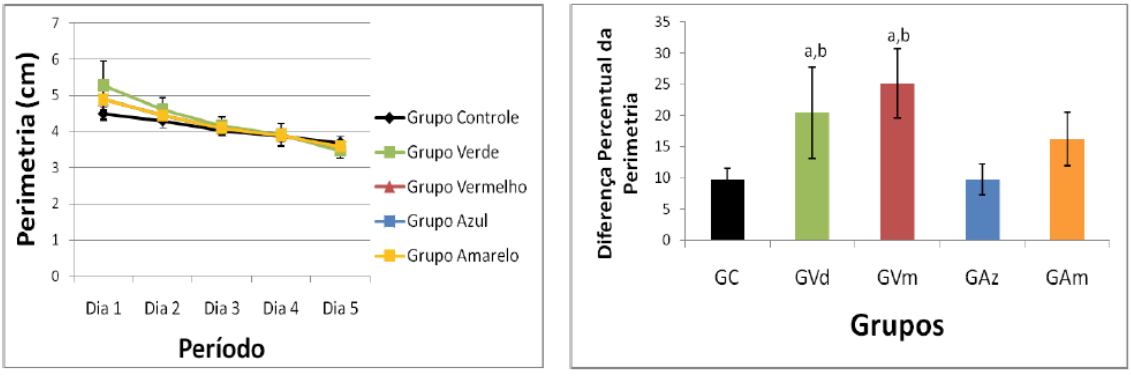
Variations in the magnitude of this difference indicate changes of area and perimeter. Subsequently, the Kolmogorov-Smirnov test was performed to assess the normality of distribution. Then the ANOVA followed by Turkey's test were used. The programs used in this evaluation were in the Sigma Stat version 3.5 (SYSTAT software, Erkrath, Germany) and in the Prism version 5.0 (Graph Pad Software Inc., La Jolla, CA, USA). Results whose descriptive levels (p values) were less than 0.05 which were considered statistically significant.

RESULTS

Perimeter

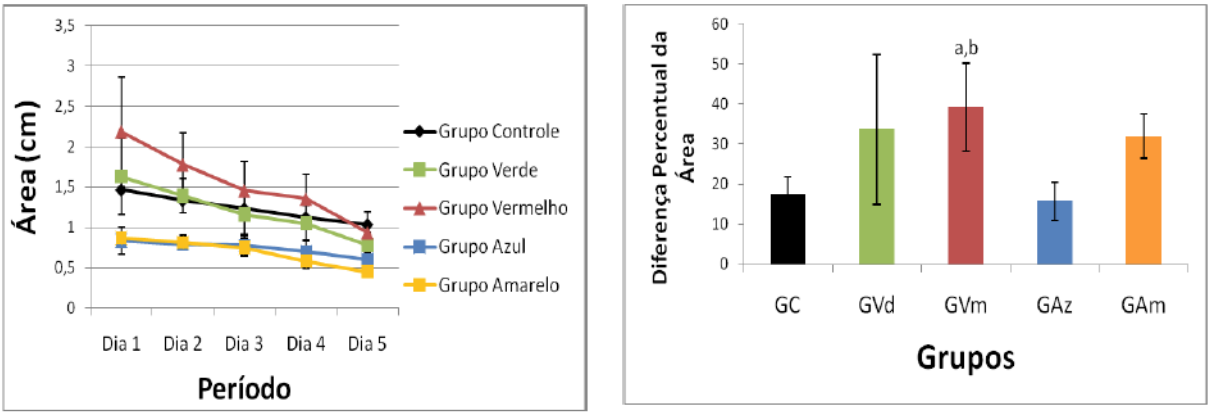
Figure **2A** shows the evolution of the perimeter of all groups. It was observed that all groups had reduced perimeter during the 1st to the 5th day of treatment. The online Red LED and Blue LED group are overlapped by the other lines.

Figure **2B** shows the percentage difference perimeter in studied groups. It was observed that the Red LED group was significant compared to the Control group ($p < 0.001$) and Blue LED group ($p = 0.032$). The Green LED group was also significant compared to the Control group ($p = 0.014$) and Blue LED group ($p = 0.014$). The Red LED Group obtained the best results with regard to the reduction of perimeter being followed by the Green LED and Yellow LED group and the group with the worst result was the Blue LED group.



A **B**

Figure 2: A: Evolution of the studies groups perimetry during the 5 days of treatment. The results are expressed in media ± standard deviation. GC: Control group; GVd: Green group; GVm: Red group; GAz: Blue group; GAm: Yellow group. **B:** Perimetry percentage difference in relation to the studies groups. The results are expressed in media ± standard deviation. a – Significative in relation to the GC; b – Significative in relation to the GAz. GC: Control group; GVd: Green group; GVm: Red group; GAz: Blue group; GAm: Yellow group.



A **B**

Figure 3: A: Evolution of the area to the studies groups during the 5 days of treatment. The results are expressed in media ± standard deviation. GC: Control group; GVd: Green group; GVm: Red group; GAz: Blue group; GAm: Yellow group. **B:** Percentage difference of the area in relation to the studies groups. The results are expressed in media ± standard deviation. a – Significative in relation to the GC; b – Significative in relatio to the GAz. GC: Control group; GVd: Green group; GVm: Red group; GAz: Blue group; GAm: Yellow group.

Figure **3A** shows the evolution of the area of all groups. It was observed that all groups had reduction in the area during the 1st to the 5th day of treatment. Figure **3B** shows the percentage difference of the area in relation to the studied groups. It was observed that the Red LED group was significant compared to the Control group ($p = 0.026$) and Blue LED group ($p = 0.014$). The Red LED Group achieved the best results in relation to the reduction of the wound area being followed by the Green and Yellow LED group and the group with the worst result the Blue LED group. The Control group had a slight improvement over the Blue LED group.

In Figure **4A** and **B** it can observe the findings of the Control group, which revealed acute inflammation with edema and mild presence of fibrin in two samples. Chronic Inflammation was moderate. The crust was presented in two of the samples and discrete epithelialization in the margins and in one per fifth of the lesion. Granulation tissue not completely filled and the wound area was constituted by young connective tissue and loose collagen high deposition young (80%). Two samples showed giant cells.

Figure **4C** and **D** shows the results of the group treated with Red LED, it is possible to see that the acute inflammation was moderate (100%) in the presence of fibrin mass. The swelling was 100% of the discrete samples. Chronic inflammation presented predominantly moderate. The crust was presented in all animals, but epithelialization on the banks reached a larger area, compared to the control group. Granulation tissue appeared subdued and filled 100% of the sample, this group was more developed, with less edema and increased deposition of collagen young (compact) occurred when compared to the control group. One sample showed giant cells.

In Figure **4D** and **E** the results shows the applying of green LED, it exhibits acute inflammation moderated. The edema had predominantly moderate (60% - 3 samples) with worse outcome in relation to the Red LED group with mild edema. Chronic inflammation was moderate; however, in the group treated with Green LED crust was also present in all animals with fibrin deposition. Epithelial regeneration was slightly higher than in the control group, but lower than the Red LED group. The scar showed high deposition of young collagen (100%).

Figure **5A** and **B** shows the effects of the Yellow LED group, it was found that showed mild acute inflammation with better response compared to the Red LED and the Green LED group. Chronic inflammation showed moderate in all samples. The edema had lower results compared with control groups, red LED and green LED, and categorized as moderate. Epithelial regeneration was incomplete in sample 100%, 20% and 80% marginal covering 1/5 of the wound. The scar showed moderately young collagen in all samples and showed a slight presence of mature collagen in all samples, which was not observed in control groups, Red and Green LED.

Figure **5C** and **D** shows the effects of the Blue LED. It can observe the acute inflammation with the presence of discrete light fibrin in all samples. Chronic

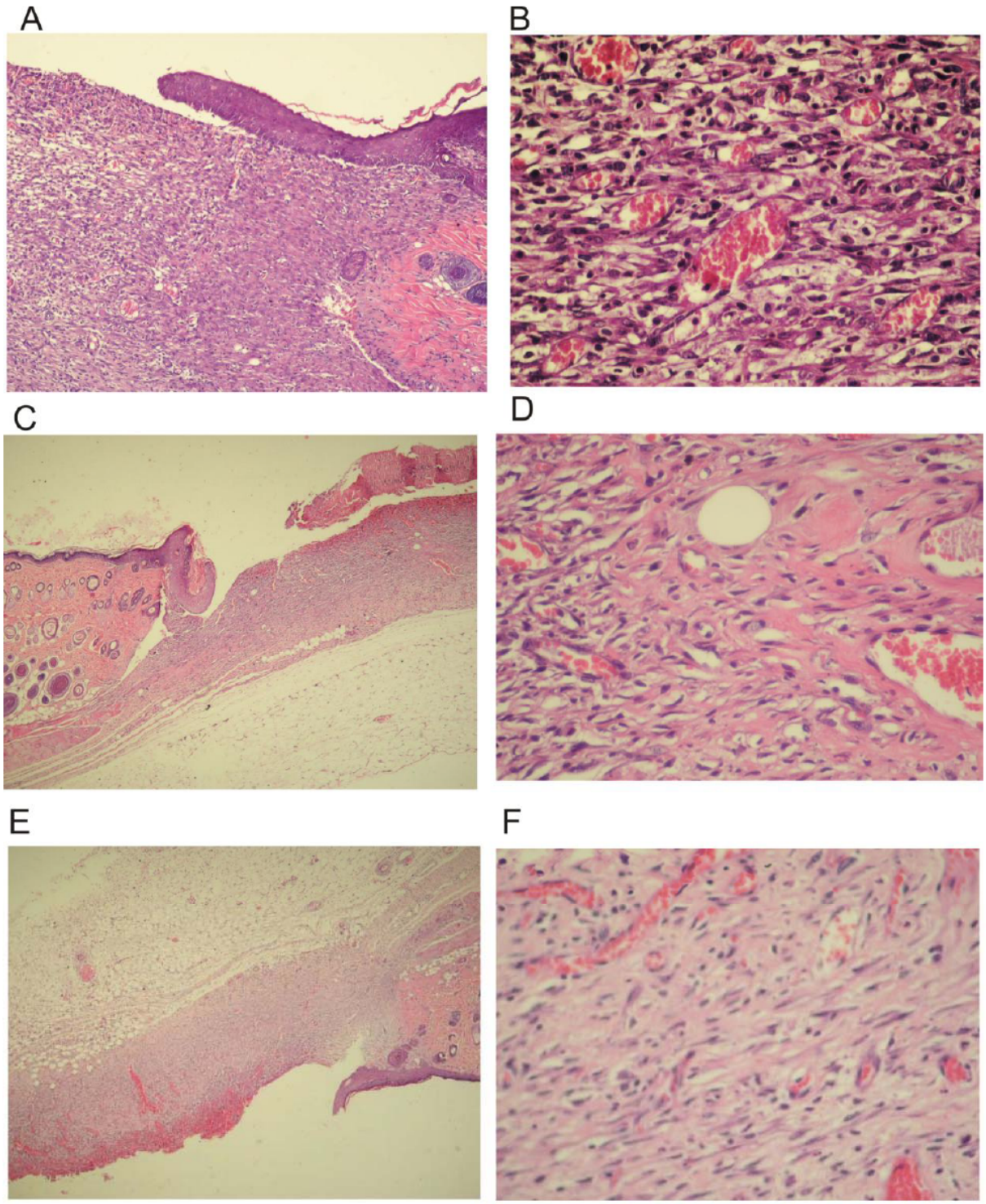


Figure 4: A: Control group showing epithelia and granulation tissue. HE 100x. **B:** Control group showing young collagen and mast cells. Gomori Trichrome 400 X. **C:** Red LED group with discrete epithelialization, less edema. HE 40X. **D:** Red LED group demonstrating more mature e dense collagen. HE 400X. **E:** Green LED group – Presented less epithelial tissue, granulation tissue, and fibrin presence. HE 40X. **F:** Green LED presents accumulation of granulation tissue. HE 100X.

inflammation showed moderate in all samples. Mast cell in the presence of high amount was observed in both samples. Edema presented moderate, however the result was lower compared with the Control, Red LED, Green LED and Yellow LED groups. Epithelial regeneration was incomplete in 100% of the samples and had the worst result for the wound healing among all groups. Granulation tissue was observed in the group considered too young appear very high neovascularization. The scar showed high young

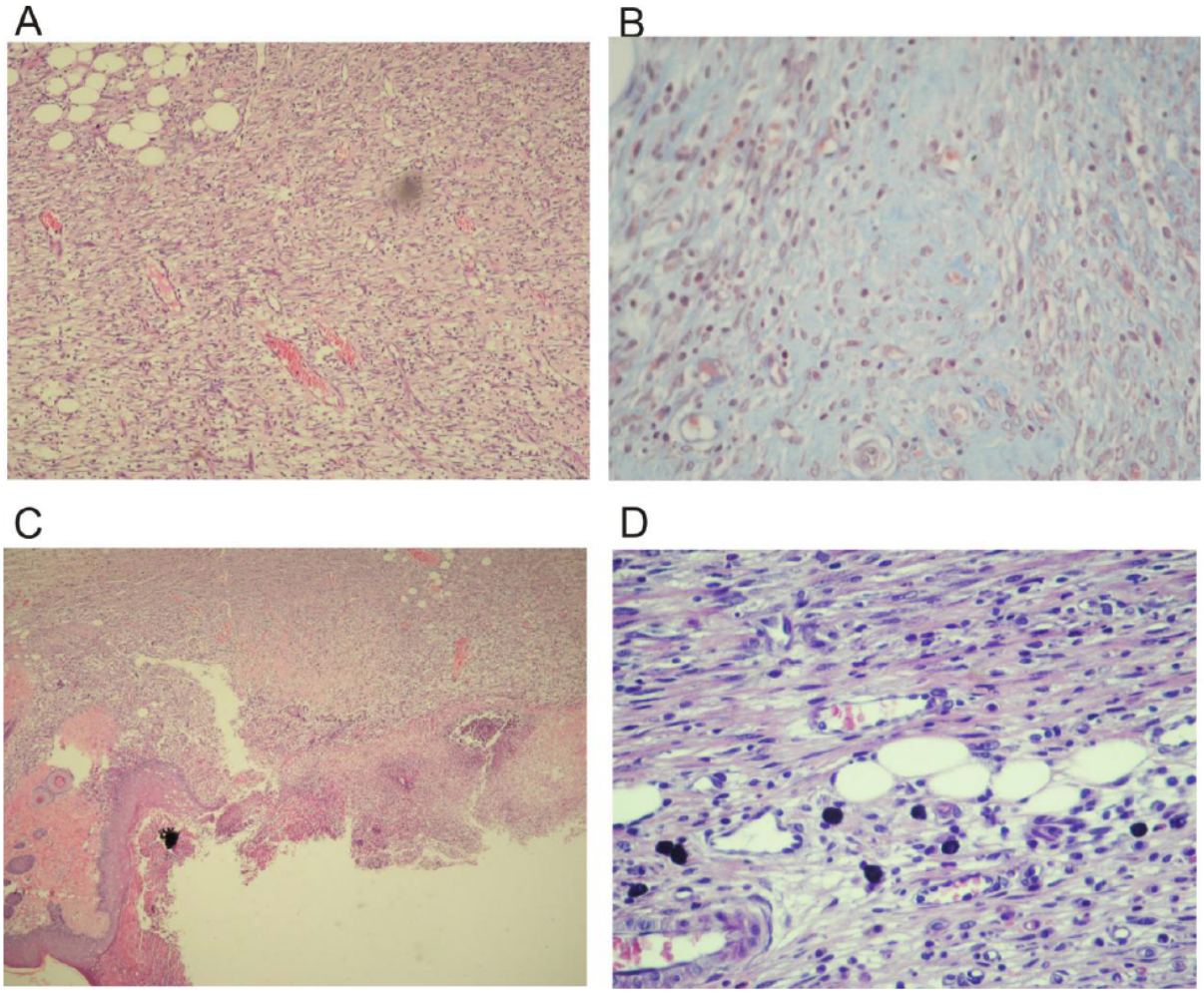


Figure 5: A: Yellow LED presenting less quantity of mast cells, acute inflammation signals, marked edema. HE 400X. **B:** Yellow LED presenting dense collagen, more mature. Gomore trichrome 400X. **C:** Blue LED showing the crusts presence, fibrin, slow epithelialization. HE 40X. **D:** Blue LED presenting considerable increase of mast cells. HE 400X.

collagen in all samples. Giant cells were observed in three samples.

Table **1** summarizes the results found in different groups:

Table **1** shows the comparison of the groups, divided by the features presented by histological examination. Acute inflammation had better outcome in the control group, however Blue LED group with fibrin in high quantity which is an important component to healing. The worst outcome in relation to acute inflammation was found in the Red and Green LED groups that had the same result. In chronic inflammation the result was significant at the best and worst Red LED group on the Blue LED group with loads of mast cells during this phase. Regarding epithelial regeneration, all groups were incomplete. The Red LED group had the best results followed by the Yellow LED group. The worst result was the Control group. Acting scar the most efficient result was obtained by the Yellow LED group having stimulated mature collagen in 100% of samples and their worst result where only the Blue LED stimulation of young poor quality collagen was observed. In the presence of giant cells the group that got the best result was the Red LED, which showed 20% of them and the worst result was observed in the Green LED group, as has been seen 100% of giant cells. Regarding the presence of edema the best result was observed in the group because red LED display discreet presence, already the worst result was the Blue LED group had moderate edema in all its samples.

DISCUSSION

As expected after a skin lesion is an inflammatory response triggered by a specific immunologic mechanism initially occurring concentration of leukocytes, predominantly neutrophils, having the

Table 1: Analysis of the Results of the Different Groups Studied

	Control	Red LED	Green LED	*Yellow LED*	Blue LED
Acute inflammation	60% of acute inflammation / 40% absence of acute inflammation	Moderate acute inflammation – presence of fibrin	Moderate acute inflammation 100% presence of fibrin	80% discrete acute inflammation / 20% moderate presence of fibrin 100%	100% discrete acute inflammation/ presence of fibrin 100%
Chronic Inflammation	Normal chronic Inflammation	80% of Moderate chronic Inflammation/ 20% discrete chronic inflammation	Normal chronic Inflammation	Normal chronic Inflammation	Normal chronic Inflammation 40% of mast cells presence
Epithelial Regeneration	Incomplete 100% / 40% margin/ 60% covering 1/5 of the wound	20% epithelial margin 80% covering 1/6 of the wound	40% epithelial margin 60% covering 1/6 of the wound	Incomplete 100% / 20%margin and 80% covering 1/5 of the wound	Incomplete 100% / 60% covering the margin 1/ 6 of the wound 40% covering 1/5 of the wound
Granulation tissue	Vascular moderate Cellular moderate	100% vascular moderate 100% Cellular moderate	Moderate 95% vascular / 5% cellular 5% multicellular	100% vascular moderate 90% cellular moderate	20% vascular moderate 80% cellular moderate 80% multivascular
Scar Young collagen/ Mature	80% young collagen 20% mature collagen 20% discrete	100% young collagen 100% denser strong	100% young collagen 100% marked 0% mature collagen	100% moderate young collagen 100% discrete mature collagen	Young collagen 100% marked 0% mature collagen
Giant cell	40% giant cells	20% giant cells	100% giant cells	60% giant cells 40% absence	60% giant cells
Edema	100% discrete edema	100% discrete edema	60% moderate edema 40% discrete edema	80% discrete edema 20% moderate edema	100% moderate edema

Source: research.

function of dead cells phagocytize and digest and microorganisms. They release enzymes which attract other inflammatory cells and initiate the formation of granulation tissue. The longer cell accumulation signals an extension of the inflammatory response, slowing the repair phase and remodelagem [9].

The Red LED decreases the time resolution of side effects such as erythema, edema and bruising in half the time a third, for their effective action anti inflammatory [10]. The statement confirms that the results shown in the histological analysis of this study, which viewed the reduction of edema and inflammatory response. Also observed, collagen deposition as early as possible in the lesion area, a result which agrees with other authors [11] who claims that the Red LED significantly increases the amount of collagen and elastin by stimulating fibroblasts.

The effects of Green LED promoted a reduction of edema and acute inflammation and sloughing of greater healing than the control group, however to a lesser extent than the Red LED. These results demonstrate the persistence of chronic inflammation, an accumulation of fibroblasts, formation of new blood vessels and capillaries occurs, forming an infiltrated tecido [12].

The results obtained with the application of Yellow LED showed better effects on the acute phase of inflammation more effectively than red and Green LED, but with persistent edema. The results agree with the findings of other studies [13] in which significant results were demonstrated in relation to the stimulation of fibroblasts in cell culture at this wavelength.

The application of Blue LED was demonstrated inferior results compared to other types of LEDs

studied. The Blue LED has a wavelength very close to ultraviolet light, thus showing similar effects as bactericidal action and is today a substitute in the ultraviolet treatment where it is seeking to decrease the contamination, which may explain their lower share in healing [14].

The application of LED therapy with the colors Red (630-780nm), Green (490-565nm), Yellow (590-630) and Blue (440-490nm) with a duration of 6 minutes was able to produce qualitative results in scar formation second intention. Epithelialization the banks and scarring occurred with best quality Yellow LED followed by Red LED. The Yellow LED got a greater presence of collagen best quality (mature collagen) in relation to the Red LED. Phototherapy not collimated of 630-780 nm (red) and 490-565 (green) produced better effects in relation to the circumference and area average significantly declining. The Yellow LED and the Blue LED did not obtain good results in relation to the circumference and area average.

The edema had good results with phototherapy not collimated 630-780nm (Red) and 590-630nm Yellow followed by Green LED. The worst result obtained in relation to edema with phototherapy was not collimated Blue 440-490nm.

Given these responses with phototherapy not collimated Red, Green, Yellow and Blue, it may conclude that it is possible to use with benefits, predominantly Red LED.

This study has limitations concerning the parameters used in respect of power, since it cannot be modulated. It is suggested to use LED therapy combined with different wavelengths using other parameters to check the effects of the different phases of the healing process.

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