Lasers in Medical Science (2019) 34:721–728
https://doi.org/10.1007/s10103-018-2647-x

ORIGINAL ARTICLE

Effect of blue LED on the healing process of third-degree skin burns: clinical and histological evaluation

José de Alencar Fernandes Neto[1] · Cassiano Francisco Weege Nonaka[1] · Maria Helena Chaves de Vasconcelos Catão[1]

Received: 13 March 2018 / Accepted: 21 September 2018 / Published online: 1 October 2018

Abstract

The aim of this study was to evaluate the effects of blue light-emitting diode (LED) on the healing process of third-degree skin burns in rats through clinical and histological parameters. Forty male Wistar rats were divided into two groups: control (CTR) ($n = 20$) and blue LED (BLUE) ($n = 20$), with subgroups ($n = 5$) for each time of euthanasia (7, 14, 21, and 28 days). LED (470 nm, 1 W, 12.5 J/cm^2 per point, 28 s) was applied at four points of the wound (total, 50 J/cm^2). Feed intake was measured every other day. It was observed that there were no statistically significant differences in the Wound Retention Index (WRI) of the BLUE group in relation to CTR group ($p > 0.05$) at the evaluation times. After 14, 21, and 28 days, it was observed that the animals in the BLUE group consumed more feed than animals in the CTR group ($p < 0.05$). At 7 days, there was a statistically significant increase in the angiogenic index (AI) in BLUE (median: 6.2) when compared to CTR (median: 2.4) ($p = 0.01$) and all animals in BLUE had already started re-epithelialization. This study suggests that blue LED, at the dosimetry used, positively contributed in important and initial stages of the healing process of third-degree skin burns.

Keywords Burns · Healing · Low-intensity light therapy · Phototherapy

Introduction

Burns are considered a serious public health problem, causing approximately 265,000 deaths per year worldwide. They are one of the main causes of morbidity, prolonged hospitalization, disfiguration, and incapacity, often causing great physical and psychological impact on the victims [1].

Therapies that accelerate the repair process increase the quality of life of patients and reduce treatment costs have been the focus of several scientific researches. Photobiomodulation using LED (light-emitting diode) is among the therapeutic methods currently tested [2–4].

The complete mechanism of action of photobiomodulation is not entirely clear, but it is known that light at low intensity can interact with cells, leading to changes in molecular, cellular, and tissue levels. Each tissue, however, responds to this interaction differently [5], since light is absorbed or not by target tissue molecules that are termed chromophores or photoreceptors.

Studies have shown that the effects of light on tissues occur independently of the light coherence, since this property is lost in the first skin extract before light is absorbed by chromophores and is therefore not determinant for the treatment result [6, 7].

The biological effects promoted by this therapy in the repair process found in literature are related to the attenuation of the inflammatory response [4, 8], increased proliferation of fibroblasts [9], stimulation of angiogenesis [7, 10], increased synthesis of collagen [3, 11], stimulus to re-epithelialization [12], and analgesic effects [13, 14].

Although blue LED (400–470 nm) has tissue penetration of approximately 1 mm, targeting the deep epidermis [15], research has evaluated the in vivo effects of this light on the healing process of third-degree skin burns [16, 17].

Therefore, the aim of this study was to evaluate the effects of blue LED photobiomodulation on the healing process of third-degree skin burns in rats through clinical and histological parameters.

✉ Maria Helena Chaves de Vasconcelos Catão
mhcvcatao@gmail.com

[1] Postgraduate Program in Dentistry, State University of Paraíba, R. Baraúnas, 351, Bodocongó, Campina Grande, PB 58429-500, Brazil

Materials and methods

Animals

Forty healthy Wistar rats aged 60–90 days, weighing between 200 and 250 g, from the animal facility of the Faculty of Medical Sciences (FCM), Campina Grande, Paraíba, Brazil, were selected. Animals received water ad libitum, standardized feed, and the temperature in the animal facility was 12 h of light/dark cycle. This research was approved by the Committee of Ethics on Animal Use (CEUA) of the Center for Higher Education and Development (CESED) (protocol number 6809092016) and respected standards issued by the National Council for the Control of Animal Experimentation—CONCEA. Animals were divided by simple randomization into two experimental groups: control group (CTR) ($n = 20$) and LED group (BLUE) ($n = 20$), with subgroups of five rats for each time of euthanasia (7, 14, 21, and 28 days after burn).

Anesthesia/trichotomy/burn

All animals were anesthetized with an anesthetic association of 100 mg/kg of 10% ketamine (Cetamin®, Syntec, Santana de Parnaíba, SP, Brazil) and 5 mg/kg of 2% xylazine (Dopaser®, Hertape, Juatuba, MG, Brazil), applied in the intraperitoneal region with insulin syringe. Once the deep anesthetic plane was established, the animal was placed in the ventral decubitus position and tricotomy was performed in the dorsal region, using a razor blade, needle holder, and 4% chlorhexidine digliconate solution (Riohex® 4%, Rioquímica, São José do Rio Preto, SP, Brazil). Burn was performed by a single calibrated operator with a flat active tip metal instrument measuring 1 cm^3. The instrument was heated by direct contact with the blue flame of a torch for 40 s and then touched against the skin of the animal's back for 20 s, according to methodology of Meyer and Silva [18], modified by Catão et al. [3]. Third-degree skin burns were confirmed by complete destruction of epidermis and dermis in tissue sections stained with hematoxylin and eosin (HE), as previously reported by Catão et al. [3].

LED application

The Easy Bleach® equipment (Clean Line Indústria e Comércio de Produtos Odontológicos LTDA, Taubaté, São Paulo, Brazil) was used. LED was continuously applied (470 nm, 1 W, 12.5 J/cm^2 per point, 28 s) in contact with four points coincident with the wound angles (total, 50 J/cm^2), daily except for days of animal's euthanasia. The CTR group received no treatment.

Feed intake

Feed intake was calculated by the difference of its weight between two consecutive days, through an analytical scale. Every 2 days, during the evaluated times, the box with the animals received an amount of 200 g ± 5 g of feed.

Euthanasia/wound retraction index/histological processing

After the experimental period of each subgroup, animals were euthanized, housed in a hermetically sealed box containing cotton soaked with halothane (Tanohalo® 1 mL/mL, Cristália, Itapira, SP, Brazil). After euthanasia, lesions were photographed with a digital camera at a standard distance of 15 cm, with the help of a tripod, and the images were analyzed in the Image J software version 1.50i (National Institutes of Health, Bethesda, MD, USA). The wound was carefully circumvented by a single evaluator, and the WRI was calculated by the following equation: WRI (%) = initial area − area on the day of euthanasia ÷ initial area × 100. The specimen was removed with a scalpel with a safety margin of at least 0.5 cm. After fixation in 10% formalin, the sample was included in paraffin and submitted to cuts of 3 μm thickness. Subsequently, tissue sections were extended on glass slides and submitted to routine HE-staining methods.

Histological evaluation

Two previously calibrated and blinded examiners evaluated the degree of wound re-epithelialization according to criteria adopted by Meireles et al. [19] (Table 1). The parameters used for histological evaluation of the inflammatory response intensity in the wound areas were based on the study of Souza et al. [20] (Table 2). The angiogenic index (AI) was measured by counting vessels in five fields (× 400 magnification) of greater vascularity per specimen [7]. Each of these areas was photomicrographed (ICC 50HD, Leica Microsystem Vertrieb GmbH, Wetzlar, DE) and the images obtained were counted with the help of the Image J software version 1.50i (National Institutes of Health, Bethesda, MD, USA). Subsequently, the mean number of vessels was established for each animal. The granulation tissue formation and collagen deposition of each subgroup were descriptively analyzed.

Statistical analysis

The distribution of feed intake, WRI, and AI data were evaluated by the Shapiro-Wilk test, which indicated the absence of a normal distribution. Thus, the non-parametric Mann-Whitney U test was used to compare the median number of feed intake, WRI, and AI, considering a level of significance of 5% ($p < 0.05$). Re-epithelialization and inflammatory

Table 1 Criteria used for the microscopic analysis of the wound re-epithelialization area, according to Meireles et al. [19]

		Scores
Re-epithelialization	Absent (0)	Present, covering < 50% of the wound (1)
		Present, covering > 50% of the wound (2)
		Present, covering 100% of the wound, with irregular thickness (3)
		Present, covering 100% of the wound, with regular thickness

response data were descriptively analyzed. The IBM® SPSS Statistics software (version 20.0; IBM Corp., Armonk, NY, USA) was used for analyses.

Results

Wound retraction index (WRI)

There were no statistically significant differences in WRI between BLUE and CTR groups at 7 ($p = 0.28$), 14 ($p = 0.53$), 21 ($p = 0.83$), and 28 days ($p = 0.57$) (Table 3). The clinical aspects of burn retraction of both groups at the times evaluated can be seen in Fig. 1.

Feed intake

It was observed that animals from the BLUE group consumed more feed than those from the CTR group, with a statistically significant difference at 14 ($p < 0.01$), 21 ($p = 0.01$), and 28 days ($p < 0.01$) (Table 4).

Re-epithelialization

Seven days after burn, wound re-epithelialization (score 1) was observed in 100% ($n = 5$) of animals from the BLUE group, whereas in CTR, in 60% ($n = 3$) of cases, this process had not started (score 0). At 14 days, score 1 was observed in 100% ($n = 5$) in the CTR group and in 80% ($n = 4$) of animals in the treated group, a more advanced re-epithelialization stage was observed (score 2). In 21 days, it was observed that score 2 was predominant in 60% ($n = 3$) and 80% ($n = 4$) of cases, in CTR and BLUE groups, respectively. In the last evaluation period, 28 days, while 80% ($n = 4$) of wounds were completely re-epithelialized in the CTR group (score 4), in the BLUE group, all cases ($n = 5$) remained in score 2 (Fig. 2a). In wounds with epithelial discontinuity, presence of serohematic crust of variable thickness covering the region was frequently observed.

Granulation tissue/inflammatory infiltrate

At 7 days, both groups presented predominance of acute (score 5) and subacute inflammatory response (score 4) (Fig. 2b), with inflammatory infiltrate present throughout the wound region. Dilatation of arterioles and venules could be observed, as well as degeneration of muscle fibers (Fig. 3a, b).

Fourteen days after the burn, there was predominance of subacute inflammatory response (score 4) in both groups, with presence of neutrophils mainly in superficial areas of the lesion. In BLUE, 40% ($n = 2$) of animals presented moderate chronic inflammatory response (score 2) (Fig. 2b). Exuberant granulation tissue was observed in both groups, with young fibroblasts and newly formed blood vessels amid an extracellular matrix composed of thin and elongated collagen bundles.

At 21 days, moderate inflammatory infiltrate was observed in both groups (Fig. 2b and 3c, d). Compared to 14 days, collagen fibers were thicker and more densely organized.

In the last evaluation period, 28 days, both groups presented predominance of chronic inflammatory infiltrate of mild intensity (score 1) (Fig. 2b and 3e, f). In 60% ($n = 3$) of BLUE cases, the connective tissue had much resemblance to the tissue found at 21 days, with predominance of thinner collagen fibers and cells more randomly arranged in the extracellular matrix.

Table 2 Description of parameters used to evaluate the intensity of the inflammatory response in wound areas, according to Souza et al. [20]

Score of the inflammatory response	Semiquantification of the inflammatory response	Classification of the inflammatory response
0	Absent	Absent
1	Mild	Chronic (predominance of lymphocytes and histiocytes)
2	Moderate	
3	Intense	
4	Intense	Subacute (balance of neutrophils, lymphocytes, and histiocytes)
5	Intense	Acute (predominance of neutrophils)

Table 3 Wound Retraction Index (WRI) values of both groups at different evaluation times

Group	Median	Minimum	Maximum	95% Confidence interval		p*
				Minimum	Maximum	
CTR 7	− 40.00	− 60.00	− 33.00	− 54.49	− 37.50	0.28
BLUE 7	− 53.00	− 122.00	− 24.00	− 86.49	− 36.30	
CTR 14	− 17.00	− 84.00	27.00	− 72.82	27.62	0.53
BLUE 14	− 36.00	− 70.00	− 6.00	− 70.34	− 4.05	
CTR 21	77.00	61.00	92.00	62.02	94.37	0.83
BLUE 21	81.00	− 34.00	94.00	− 36.69	121.09	
CTR 28	96.00	83.00	100.00	85.40	102.99	0.57
BLUE 28	100.00	82.00	100.00	86.22	105.77	

*$p < 0.05$ for statistically significant differences

Angiogenic index (AI)

At 7 days, there was a statistically significant increase of AI in the BLUE group (median: 6.2, range: 4.6–9.8) when compared to CTR group (median 2.4, range 0.6–8.6) ($p = 0.01$) (Fig. 3a, b). At the other evaluation times, there were no statistically significant differences between groups ($p > 0.05$) (Fig. 4). At 14 days, the AI of the BLUE group (median 4.80, range 0.6–7.8) was higher than that of the CTR group (median 4.2, range 3.0–5.4) ($p = 0.83$). After 21 days, the AI of both groups assumed equal values: BLUE (median 5.2, range 2.8–9.4) and CTR (median 5.2, range 2.4–9.8) ($p = 1.00$). At 28 days, the BLUE group presented lower AI (median 3.2, range 2.4–8.8) when compared to the CTR group (median 3.8, range 1.8–7.2) ($p = 0.83$).

Discussion

The introduction of LED phototherapy has reduced some concerns previously associated with lasers, simplifying, for example, photobiomodulation in large skin areas [21] and reducing acquisition and treatment costs, since LEDs are generated in cheaper devices [22].

In this study, it was observed that blue LED stimulated the proliferation of blood vessels in the period of 7 days. Other studies that evaluated the effects of LED on angiogenesis during the repair process of skin burns have not been found in literature; however, it has been reported that photobiomodulation influences endothelial cell proliferation [23]. Dungel et al. [24] observed that low-intensity light therapy with LED of different wavelengths (blue and red) induces angiogenesis and improves the healing of ischemic wounds.

When analyzing the wound 7 and 14 days after the burn, negative WRI was found in both groups. This is possibly due to the interstitial edema generated, which increases the macroscopic dimensions of wounds, as pointed out by Catão et al. [3]. At 21 and 28 days, wounds were observed to regress, with no statistically significant difference between groups, possibly due to the transformation of fibroblasts into myofibroblasts, in addition to reduction of edema and vascularization.

The key phenomenon of wound contraction is the phenotypic differentiation of preexisting fibroblasts in myofibroblasts characterized by the expression of smooth muscle α-actin (α-SMA), which gives these cells the property of contracting and, consequently, contracting the tissue. It usually occurs from the second week of healing, where fibroblasts become the most numerous populations of cells in the granulation tissue [25, 26].

Fekrazad et al. [17] studied the effects of blue LED (405 nm, 1.5 J/cm^2, 21 days) on the healing of third-degree burns in rats and found no statistically significant differences in wound retraction between treated and control groups.

Sousa et al. [9] histologically evaluated fibroblastic proliferation in dorsal cutaneous wounds of rats daily treated with LED of three wavelength (red, green, and blue) for 7 days and observed that blue LED (460 ± 20 nm, 22 mW, 10 J/cm^2)

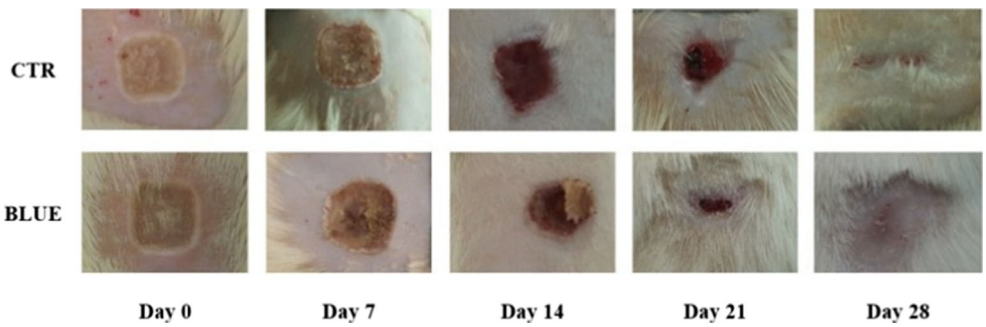

Fig. 1 Clinical regression of wounds up to 28 days after burn

Table 4 Comparison of feed intake (grams) per animal between experimental groups at different evaluation times

Group	Median	Minimum	Maximum	95% Confidence interval		p*
				Minimum	Maximum	
CTR 7	33.24	30.36	33.75	30.73	34.16	0.27
BLUE 7	37.79	31.64	40.38	32.38	40.81	
CTR 14	31.63	29.22	34.32	29.73	33.45	< 0.01
BLUE 14	40.05	40.02	40.39	39.96	40.31	
CTR 21	36.56	31.05	40.00	33.94	38.17	0.01
BLUE 21	39.99	32.60	40.60	37.00	40.90	
CTR 28	32.51	28.03	35.66	30.85	33.37	< 0.01
BLUE 28	40.10	40.00	40.06	40.06	40.33	

*$p < 0.05$ for statistically significant differences

showed no significant increase in fibroblast proliferation when compared to control, unlike the other two wavelengths.

In vitro studies [27–29] have pointed out that blue LED can dose dependently inhibit the proliferation and the migration rate of dermal fibroblasts. However, further in vitro and in vivo studies are needed to elucidate the subject.

Adamskaya et al. [12] investigated the in vivo effects of blue LED (470 nm, 1 W, 50 mW/cm^2) on cutaneous wounds of rats and found that light significantly reduced wound size within 7 days and strongly stimulated re-epithelialization. The findings in the present study suggest that re-epithelialization in treated animals was stimulated by LED in the first days, since re-epithelialization began in all wounds in animals from the BLUE group in 7 days.

It is important to note that the contrasting phototherapy results found in literature can be justified by the lack of standardization of protocols, since studies use different equipment, doses, wavelengths, irradiation times, and potencies, which makes it difficult to compare results. The biological effects are dependent on the application parameters, especially the light wavelength and applied dose, thus highlighting the importance of determining an appropriate protocol before any treatment [30].

The higher feed intake in treated groups observed in this study may suggest possible analgesic and anti-inflammatory effects of LED, since all animals (CTR and BLUE) were standardized for sex, age, weight, and kept under the same housing conditions. Similar reports were pointed out by Catão et al. [3], when evaluating the effects of Lasers and green LED on the healing of third-degree skin burns. Studies have shown that phototherapy can inhibit the expression of the cyclooxygenase 2 enzyme (COX-2), thus preventing the release of

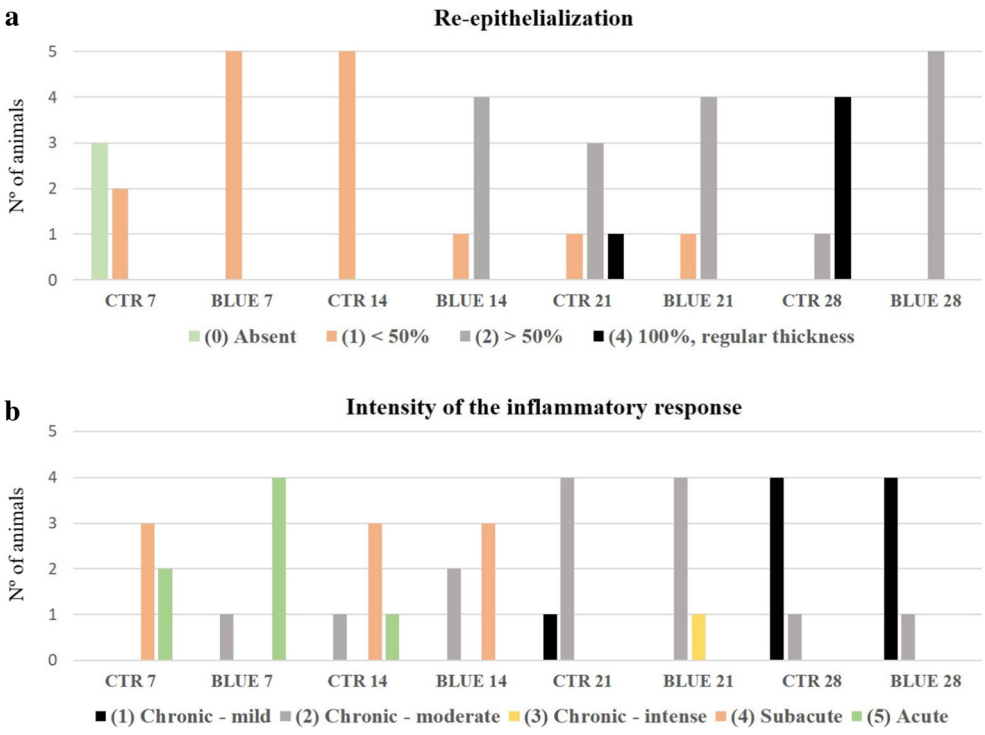

Fig. 2 Re-epithelialization and intensity of the inflammatory response in burns of both experimental groups at different euthanasia times (7, 14, 21, and 28 days). **a** Absolute distribution of cases regarding the re-epithelialization scores of wounds. **b** Absolute distribution of cases regarding the intensity of the inflammatory response in burns

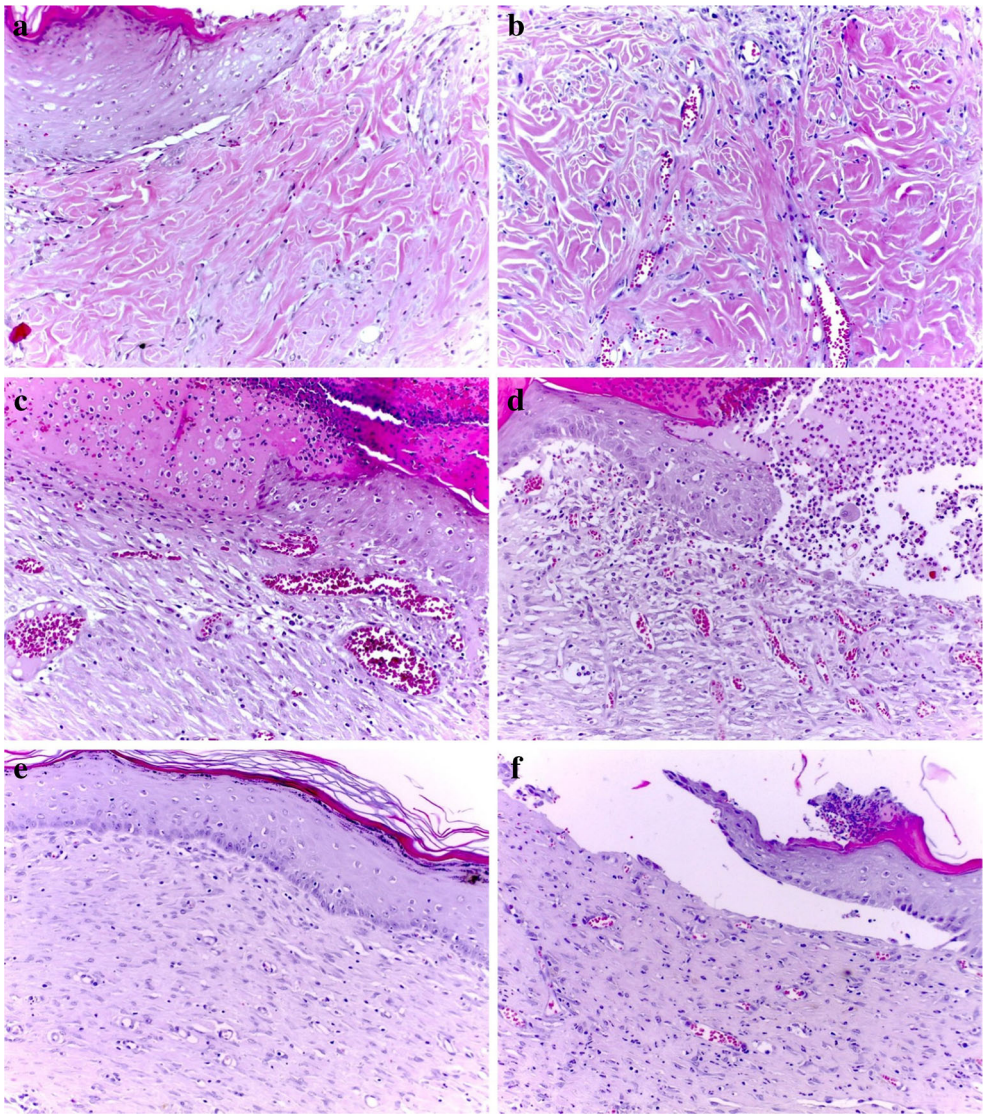

Fig. 3 Histological evaluation of wounds in different periods. **a** Wound of CTR group in 7 days with the presence of inflammatory infiltrates (neutrophils, lymphocytes, and histiocytes) and onset of re-epithelialization (HE, × 200) **b** BLUE group in 7 days, showing a greater number of blood vessels and presence of inflammatory infiltrate (neutrophils, lymphocytes, and histiocytes) (HE, × 200). **c** Wound in re-epithelialization process of CTR group after 21 days with the presence of serohematic crust, blood vessels, and inflammatory infiltrate (HE × 200). **d** BLUE wound appearance in 21 days with blood vessels arranged in connective tissue and chronic inflammatory infiltrate (HE × 200). **e** Complete wound re-epithelialization in CTR after 28 days of injury with small blood vessels and small numbers of inflammatory cells in the connective tissue (HE × 200). **f** Absence of complete re-epithelialization in BLUE in 28 days, and blood vessels of larger diameters in the connective tissue (HE × 200)

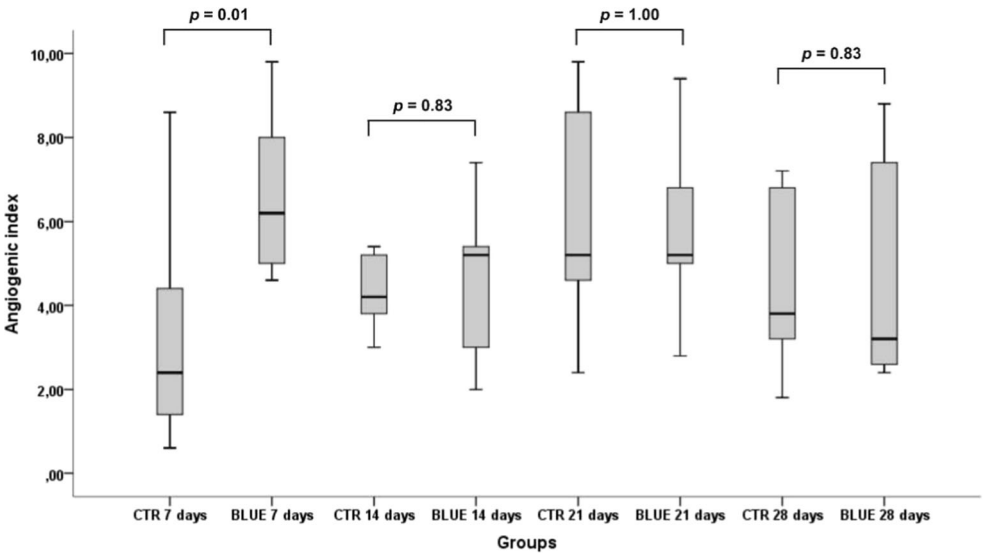

Fig. 4 Box-plot graph of the angiogenic index in third-degree skin burns of both groups at different evaluation times

prostaglandins, generating relief from symptoms of inflammation and pain [31, 32]. Despite these findings, since calorie consumption is directly related to weight and can also contribute to the healing process, it would be important to assess the weight of each animal during the entire experimental period to calculate the exact feed intake. This is a limitation of the present study that should be addressed.

Considering the limitations of the histochemical techniques used in this study, future studies should be carried out in order to evaluate in more detail the photobiomodulatory effects of blue LED on the repair process of third-degree skin burns.

Conclusion

Based on the findings of this study, it is suggested that blue LED has stimulated important steps in the healing of third-degree skin burns, such as re-epithelialization and angiogenesis in the initial periods, accompanied by possible analgesic effects. However, future studies are needed to more clearly understand the photobiomodulatory effects of blue LED in the repair process of these lesions.

Acknowledgments To UniFacisa (Campina Grande/PB) for having authorized the development of part of this research in its animal facilities and the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting a postgraduate scholarship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research was approved by the Committee of Ethics on Animal Use (CEUA) of the Center for Higher Education and Development (CESED) (protocol number 6809092016) and respected standards issued by the National Council for the Control of Animal Experimentation—CONCEA.

References

1. World Health Organization WHO (2016) Burns. http://www.who.int/mediacentre/factsheets/fs365/en/. Accessed 30 March 2017
2. Fushimi T, Inui S, Nakajima T, Ogasawara M, Hosokawa K, Itami S (2012) Green light emitting diodes accelerate wound healing: Characterization of the effect and its molecular basis in vitro and in vivo. Wound Repair Regen 20:226–235
3. Catão MHCV, Nonaka CFW, Albuquerque RLC Jr, Bento PM, Oliveira RC (2015) Effects of red laser, infrared, photodynamic therapy, and green LED on the healing process of third-degree burns: clinical and histological study in rats. Lasers Med Sci 30: 421–428
4. Catão MHCV, Costa RO, Nonaka CFW, Albuquerque Junior RLC, Costa IRRS (2016) Green LED light has anti-inflammatory effects on burns in rats. Burns 42:392–396
5. Freitas LF, Hamblin MR (2016) Proposed mechanisms of photobiomodulation or low-level light therapy. IEEE J Sel Top Quantum Electron. https://doi.org/10.1109/JSTQE.2016.2561201
6. Agnol MAD, Nicolau RA, Lima CJ, Munin E (2009) Comparative analysis of coherent light action (laser) versus non-coherent light (light-emitting diode) for tissue repair in diabetic rats. Lasers Med Sci 24:909–916
7. Sousa AP, Paraguassú GM, Silveira NT, Souza J, Cangussú MC, Santos JN et al (2013) Laser and LED phototherapies on angiogenesis. Lasers Med Sci 28:981–987
8. Gupta A, Keshri GK, Yadav A, Gola S, Chauhan S, Salhan AK et al (2015) Superpulsed (Ga-As, 904 nm) low-level laser therapy (LLLT) attenuates inflammatory response and enhances healing of burn wounds. J Biophotonics 8:489–501
9. Sousa AP, Santos JN, Reis JRJA, Ramos TA, Souza J, Cangussú MC et al (2010) Effect of led phototherapy of three distinct wavelengths on fibroblasts on wound healing: a histological study in a rodent model. Photomed Laser Surg 28:547–552
10. Medeiros ML, Araújo-Filho I, Silva EMN, Queiroz WSS, Soares CD, Carvalho MGF et al (2016) Effect of low-level laser therapy on angiogenesis and matrix metalloproteinase-2 immunoexpression in wound repair. Lasers Med Sci 32:35–43
11. Fiório FB, Albertini R, Leal-Junior EC, Carvalho PT (2014) Effect of low-level laser therapy on types I and III collagen and inflammatory cells in rats with induced third-degree burns. Lasers Med Sci 29:313–319
12. Adamskaya N, Dungel P, Mittermayr R, Hartinger J, Feichtinger G, Wassermann K (2011) Light therapy by blue LED improves wound healing in an excision model in rats. Injury 42:917–921
13. Lins RD, Dantas EM, Lucena KC, Catão MHCV, Granville-Garcia AF, Carvalho Neto LG (2010) Biostimulation effects of low-power laser in the repair process. An Bras Dermatol 85:849–855
14. Fabre HSC, Navarro RL, Oltramari-Navarro PVP, Oliveira RF, Pires-Oliveira DAA, Andraus RAC et al (2015) Anti-inflammatory and analgesic effects of low-level laser therapy on the postoperative healing process. J Phys Ther Sci 27:1645–1648
15. Opel DR, Hagstrom E, Pace AK, Sisto K, Hirano-Ali SA, Desai S et al (2015) Light-emitting diodes: a brief review and clinical experience. J Clin Aesthet Dermatol 8:36–44
16. Fekrazad R, Nikkerdar A, Joharchi K, Kalhori KAM, Abbas FM (2014) Effect of laser photostimulation on the healing of third-degree burn wounds in rats. J Arch Mil Med. https://doi.org/10.5812/jamm.22315
17. Fekrazad R, Nikkerdar A, Joharchi K, Kalhori KA, Abbas FM, Vahid FS (2017) Evaluation of therapeutic laser influences on the healing of third-degree burns in rats according to different wavelengths. J Cosmet Laser Ther 19:232–236
18. Meyer TN, Silva AL (1999) A standard burn model using rats. Acta Cir Bras. https://doi.org/10.1590/S0102-86501999000400009
19. Meireles GC, Santos JN, Chagas PO, Moura AP, Pinheiro AL (2008) Effectiveness of laser photobiomodulation at 660 or 780 nanometers on the repair of third-degree burns in diabetic rats. Photomed Laser Surg 26:47–54
20. Souza ICL, Nascimento MF, Souza Neta RG, Santos JC, Costa LP, Cardoso JC et al (2013) Effect of the maltodextrin-induced chemical reticulation on the physical properties and healing potential of collagenbased membranes containing Brazilian red propolis extract. Int J Med Sci 5:514–524
21. Kim WS, Calderhead RG (2011) Is light-emitting diode phototherapy (LED-LLLT) really effective? Laser Ther 20:205–215
22. Avci P, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N et al (2013) Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg 32:41–52
23. Szymanska J, Goralczyk K, Klawe JJ, Lukowicz M, Michalska M, Goralczyk B et al (2013) Phototherapy with low-level laser influences the proliferation of endotelial cells and vascular endotelial

growth factor and transforming growth factor-beta secretion. J Physiol Pharmacol 64:387–391
24. Dungel P, Hartinger J, Chaudary S, Slezak P, Hofmann A, Hausner T et al (2014) Low level light therapy by LED of different wavelength induces angiogenesis and improves ischemic wound healing. Lasers Surg Med 46:773–780
25. Darby IA, Laverdet B, Bonté F, Desmoulière A (2014) Fibroblasts and myofibroblasts in wound healing. Clin Cosmet Investig Dermatol 7:301–311
26. Olczyk P, Mencner L, Komosinska-Vassev K (2014) The role of the extracellular matrix components in cutaneous wound healing. Biomed Res Int. https://doi.org/10.1155/2014/747584
27. Mamalis A, Garcha M, Jagdeo J (2015) Light emitting diode-generated blue light modulates fibrosis characteristics: fibroblast proliferation, migration speed, and reactive oxygen species generation. Lasers Surg Med 47:210–215
28. Masson-Meyers DS, Bumah VV, Enwemeka CS (2016) A comparison of four methods for determining viability in human dermal fibroblasts irradiated with blue light. J Pharmacol Toxicol Methods 79:15–22
29. Opländer C, Hidding S, Werners FB, Born M, Pallua N, Suschek CV (2011) Effects of blue light irradiation on human dermal fibroblastos. J Photochem Photobiol B 103:118–125
30. Chaves MEA, Araújo AR, Piancastelli ACC, Pinotti M (2014) Effects of low-power light therapy on wound healing: LASER x LED. An Bras Dermatol 89:616–623
31. Lim W, Lee S, Kim I, Chung M, Kim M, Lim H (2007) The anti-inflammatory mechanism of 635 nm lightemitting-diode irradiation compared with existing COX inhibitors. Lasers Surg Med 39:614–621
32. Rocha CLJV, Rocha Júnior AM, Aarestrup BJV, Aarestrup FM (2012) Inhibition of cyclooxygenase 2 expression in NOD mice cutaneous wound by low-level laser therapy. J Vasc Bras 11:175–181