Manual Therapy, Posturology & Rehabilitation Journal
formerly REVISTA TERAPIA MANUAL

RESEARCH ARTICLE

https://doi.org/10.17784/mtprehabjournal.2019.17.727

Photobiomodulation with Led 627nm associated to resveratron in cutaneous injuries of rats: a double-blind controlled clinical study.

Patricia Pacheco Tyski Suckow[1], Ana Carolina Dorigoni Bini[1], Paulo Renato de Oliveira[1], Larissa Sakis Bernardi[1], Danillo Barbosa[2], Ivo Ilvan Kerppers[2], Alice Pansera[3], Gabriela de França Cury[2], Jaqueline Emiliano[2], Andressa Panegalli Hosni[2], Andressa Leticia Miri[4]

ABSTRACT

Introduction: Drugs formulated as Resveratrol, associated with LED 627nm photobiomodulation, can increase the efficacy of active release, increased local circulation, cell proliferation and collagen synthesis, accelerating the healing process. **Objective:** To analyze the effects of 627nm LED photobiomodulation associated with Resveratrol on the tissue repair of induced wounds in Wistar rats. **Methodology:** We used 18 animals corresponding to the control groups, group LED 627nm with association to Resveratrol cream (GLed + Resv) 3 and 7 days. **Results:** Treatment with the use of LED associated with Resveratrol cream provided an efficient healing. In the statistical test, the significance level was observed between the groups of P <0.0156. In the multiple comparison between the pairs the Tukey's test showed significance between the groups CG vs GLed627nm + Resv 7Day. **Conclusion:** The GLed + Resv group showed efficient inflammatory phase of healing, promoting a greater activation of fibroblasts and remodeling of the collagen fiber when compared to the control group.

KEYWORDS: Punch; Healing; Photobiomodulation; Resveratrol.

INTRODUCTION

The skin is the largest organ of the human body. It is also called the integumentary system, with protection functions, water barrier, body temperature regulation, defense against microorganisms and salt excretion and vitamin D synthesis. It consists of three layers firmly adhered some to the others, which are termed the epidermis, dermis and hypodermis[1,2]. The dermis is the supporting layer of the epidermis, the most abundant type of tissue in the body. It has a function of support and connection with other types of tissues, besides providing resistance, support, nutrition and defense for the other tissues[3-6]. It is formed of a fibrous component, containing collagen and elastin, together with the so-called fundamental substance, which is divided into two layers: the papillary dermis, the more superficial and the deeper reticular dermis[7]. Tissue regeneration, which is common to all tissue types of the body, can be divided into three phases, characterized by inflammation, proliferation and remodeling. The exact duration of each phase is impaired due to the overlapping phases[8,9]. The tissue inflammatory process is the immediate response to an injury, whose external cardinal signs are flushing, tumor, heat, pain and loss of function. The acute or early phase of inflammation lasts for 24 to 48 hours, followed by a subacute or late phase, which lasts for another 10 to 14 hours. The purpose of the inflammatory phase is to rid the area of dead tissue and secretions elicited by tissue injury[10,11]. The proliferative phase occurs around the third or fourth day, persisting for two to three weeks and forms the beginning of granulation tissue formation, which precedes the development of mature cicatricial tissue, consisting of a neovascularized neomatrix with macrophages and fibroblasts. As soon as they reach the wound, the fibroblasts begin to synthesize hyaluronic acid, fibronectin and collagen types I and III that form the initial extracellular matrix[9,12].

The application of photobiomodulation in tissue repair[13] is being widely described in the scientific literature[14-16]. Photobiomodulation with light emitting diode (LED) is a diode, based on p-n (p-positive; n-negative) junctions, which when emitted emits light. The process of light emission by the application of an electric source of energy is called electroluminescence[17,18]. Photobiomodulation (LED) causes increased local circulation, cell proliferation and collagen synthesis, improved mitochondrial oxidative metabolism and energy production leading to a tissue repair stimulus, decreased pain and an aesthetically more satisfactory scar

Corresponding Author: Patricia Pachego Tyski Suckow. Adress: Department of Physical Therapy (Defisio) Cedeteg Campus of the Central -West State University – UNICENTRO. Rua Simeão Varela de Sá, 03 - Vila Carli, Guarapuava - PR, 85040-080. Phone number: (55-42 99977-4033). Email: patysuko@gmail.com

[1] Post-Graduate Program Phamaceutical Sciences - Universidade Estadual do Centro Oeste do Paraná – UniCentro, Santa Cruz, Guarapuava – PR, Brasil.

Full list of author information is available at the end of the article.

Financial support: nothing to declare

Submission date 27 March 2019; Acceptance date 08 May 2019; Publication date 13 December 2019

production[19,20]. Resveratrol (3,4,5-trihydroxystilbene) is a phytoalexin present in a wide variety of plant species, including blackberries and grapes, so it is a constituent of the human diet. This compound, like other members of the stilbene family, is produced in plants in response to pathogen attack, ultraviolet radiation and exposure to ozone. Vitis vinifera, or grape, synthesizes Resveratrol as a means of protecting fungal infections and is therefore found in high concentrations in wine[21]. Recent studies have revealed that Resveratrol is a potential candidate for multi-spectrum drug because it has been shown to be a therapeutic application in the treatment of neurodegenerative diseases, diabetes, tumors and heart disease, with anti-inflammatory and anticancer properties [22]. Its topical application of treatment is the most commonly used in skin cancer models and has been used as an active ingredient in skin care products when associated with Vitamin C, calico, polyphenols, methylsulfonylmethane or proanthocyanidins[23]. Therefore, the present study proposes to analyze whether the isolated LED, or associated with resveratrol can influence the healing of lesions in the skin of rats, in different experimental times.

METHOD

Type of study and ethics

This is an induced, descriptive and observational experimental study, which followed all the guidelines of Federal Law No. 6638 and the recommendations of the Brazilian College of Animal Experimentation, with approval and registration in the approval of the Animal Ethics Committee by the protocol 013/2018.

Sample

Eighteen Rattus Norvegicus Wistar rats were used in the central laboratory of the State University of Londrina, weighing between 200 and 250G. They were allocated 3 in 3 in acrylic cages, unbreakable, self-draining, in the measures of 41x34x16cm, with carbon steel arrame cover and watering bottle, made of polypropylene, with lateral graduation of 100 to 700ml, total capacity of 700ml and curved beak. They received rations (Nuvital® Nuvilab CR-1) and water ad libitum, a light/dark cycle of 12 hours and a room temperature of 23± 1 ° C, following the guidelines of Federal Law No. 6638 and the recommendations of the Brazilian College of Animal Experimentation.

Experimental groups

The animals were divided into 3 groups with 6 rats in each group:

I. Control group (CG): composed of 6 animals with lesions in the dorsal region, without treatment and with material collection and euthanasia on the eighth day;

II. Group treated with LED627nm + Resveratrol (GLed + Resv): composed of 6 animals with lesion in the dorsal region and treated with Resveratrol application, for 3 days associated with LED and with material collection and euthanasia on the fourth day;

III. Group treated with LED627nm + Resveratrol cream (GLed + Resv): composed of 6 animals with lesions in the dorsal region and treated with Resveratrol application for 7 days, cream formulation, associated with LED and with material collection and euthanasia on the eighth day.

Surgical procedures

The animals were previously anesthetized with 80 mg / kg of ketamine hydrochloride and 15 mg / kg of xylazine hydrochloride, after verification of the anesthetic status of the animals by the manual compression test of the lower third of the tail, a tricotomy of the dorsal region, between the fourth vertebra and twelfth vertebra, approximately 2cm wide and 5cm long. Shortly thereafter, a punch of 7 mm was used to perform the lesion, on the epidermis to the hypodermis, observing the muscular fascia limit for depth parameter. The animals corresponding to the treatment group (GLED + Resv) received daily irradiation of LED627nm during 60s and topical application of Resveratrol cream, since the animals of the control group (CG) did not receive any type of treatment.

Photobiomodulation application protocol Led 627nm

The LED's used in the study were from the Superbrightled® models RL5-R12008 and RL5-IR27, for the emission lengths of 627nm. The output powers of the devices, measured using an optical wattmeter brand Thorn Labs model PM100D, were 70 mW to 627nm. The application method was punctual, with spacing between the equipment and the tissue 1 cm and perpendicular to the skin surface, with irradiation times of 60 s for LED 627 nm, corresponding to a dose of 4.2 J/cm 2. The irradiations were performed for 3 and 7 consecutive days.

Protocol for formulation of Resveratrol

Resveratrol and non-ionic moisturizing cream were purchased in a manipulation pharmacy and handled by the Laboratory of Technology and Biopharmaceutical Analysis of the Pharmacy Department of Unicentro-Pr. Resveratrol at 10% was solubilized in Propylene Glycol and incorporated into the nonionic moisturizing cream.

Euthanasia procedures

The animals were previously anesthetized, and then received a lethal dose of intraperitoneal sodium thiopental (250mg / kg). This is the recommended method of euthanasia for rodents and other small mammals contained in Resolution 714 of the Federal Council of Veterinary Medicine of June 20, 2002.

Procedures for histological analysis and histomorphometry

The pieces were included in 10% formalin for 48 hours to maintain the morphological characteristics. Subsequently the pieces were sent to the laboratory of the Center of Pathology and Cytopathology Ltda, without identification, only with markers provided by the researcher (ex: X1, X2). The pieces were re-cut, divided into two parts, and embedded in paraffin for histological sections 2 µm thick with a microtome, from the medial region to the borders. Three sections were stained and stained with hematoxylin-Eosin (HE). histomorphological analysis of tissue sections. Histology was performed using Olympus CX31 ® microscope, with UIS (Universal Corrected Infinity) optical system with 5 megapixel DP25 digital color camera, being photographed at 1280 X 960 resolution, and saved in JPEG. Macroscopic (in vivo) analysis of the lesions was performed by the ImageJ Launcher program, after quantification of lesion size the data were arranged and analyzed statistically.

Statistical analysis

Data were analyzed by GraphPad Prism software. For the statistical analysis, the Tukey's Multiple Comparasion and ANOVA test were used, both with a statistical significance level of $p < 0.05$.

RESULTS

In Figure 1, macroscopic lesions of the skin are depicted every day (day 1 to 8 day) after injury in the experimental groups. It is observed a change in the shape of the edges of the lesions as well as in the diameter. At the end of the treatment period, the edges were approximated, granulation tissue, presence of crust and reduction of lesion depth.

Figure 2. (1A and 1B and 2A and 2B) show images of histological sections of the dorsal region of Control Group 3 and 7 days, with the microscopic photos of HE staining 4x (referring to image 1A) and 10x (referring to image 1B), respectively. In frames 1A and 1B of both days, there is presence of developing hypodermic and dermal tissue, underlying cellular tissue (adipose) and discrete layer of keratin. In frames 2A and 2B of both days, there is presence of underlying tissue suggestive of adipose tissue, epidermal layer and developing dermis, and discrete presence of fibroblasts and fibrocytes in the upper left corner.

In the Figure 3, frames 3A and 3B show images of histological sections of the GLed + Resv Group dorsal region 3 and 7 days, with the microscopic photos of HE staining 4x (referring to image 1A) and 10x (referring to image 1B) respectively. In frame 4A of both days, we observed the presence of a large marginalized area of underlying cellular tissue (adipose). Dense layer of epidermis, thin dermal layer and presence of a thin layer of keratin. Frame 4B shows the presence of adipose, adjacent epidermal and dermal layers, and a large marginal area of keratin. There are large numbers of fibroblasts and fibrocytes distributed in the margin of the tissue characterizing a localized regenerative process.

Figure 4 shows the mean values obtained in the ANOVA and Tukey tests of the Control Group and GLed + Resv Group 3 and 7 days respectively. In the statistical test, a significance level was observed between the groups of $P < 0.0156$. In the multiple comparison between the pairs the Tukey's test showed significance between the groups CG vs GLed627nm + Resv 7Day

Was compared the effects of low blue level (470 nm) and red (629 nm) light emitting diodes (LEDs) on wound healing in vivo in a circular wound excision in rats. They observed that the irradiation caused by the blue LED had positive influences on the healing and the epithelization process, significantly reducing the wound size when compared to the control group. In addition, they also noticed positive effects on keratin, which plays an important role in the healing of normotrophic wounds[1]. Studies involving photobiomodualization with specific wavelengths provides a reinforcement in the rate of healing, as well as in the proliferation of specific cells and their mechanisms of action. However, it reinforces that further studies are still needed to determine the wavelengths and combinations that are most effective in healing wound rapid and complete healing[17]. When we compare the biomodulatory effects of LED and ultrasound combined with semipermeable dressings in the repair of cutaneous lesions in rats

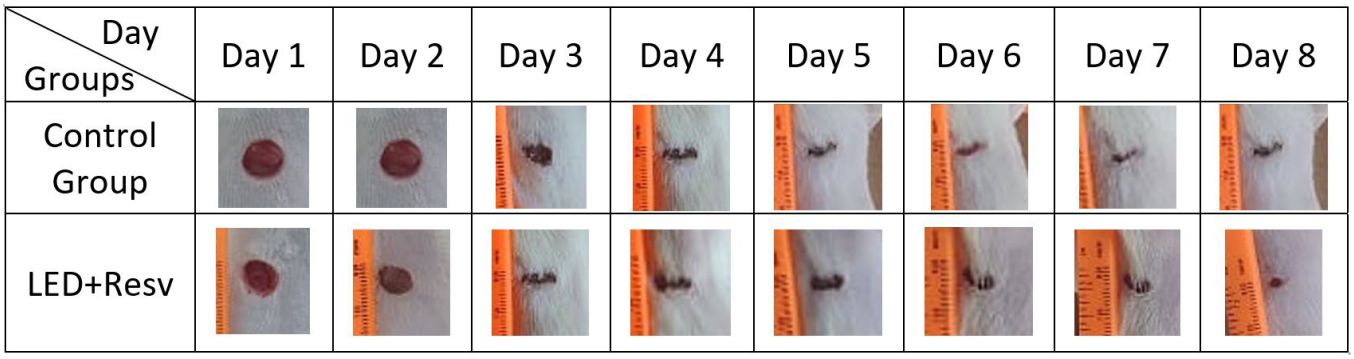

Figure 1. Macroscopic representation of animal skin

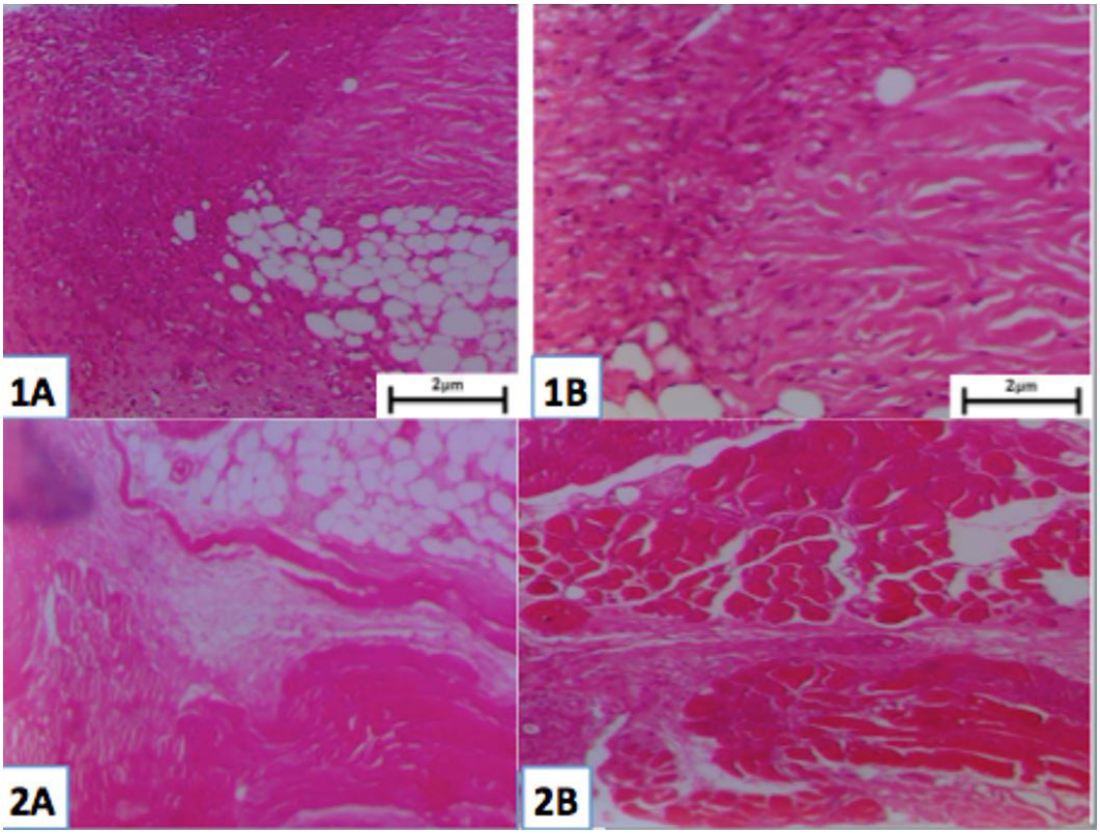

Figure 2. Histological sections of animal skin of the Control Group 3 days. **Note.** HE staining 4x (referring to image 1A) and 10x.

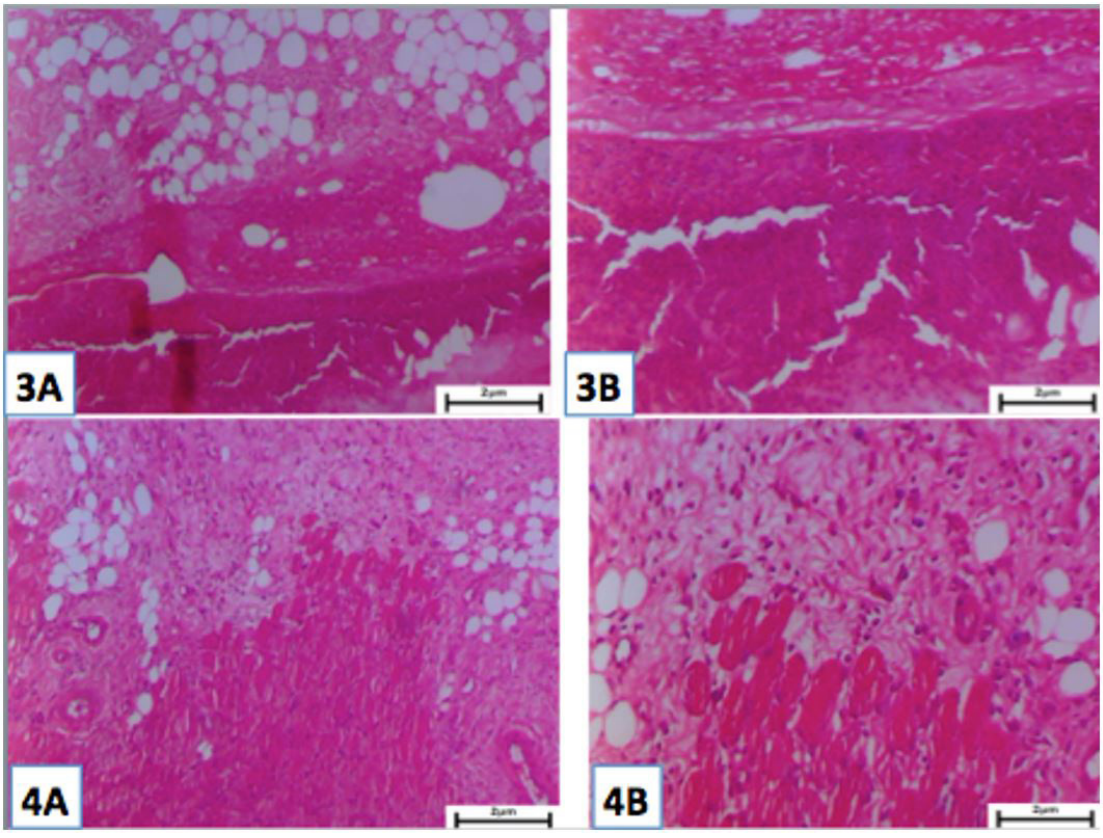

Figure 3. Histological sections of animal skin of the Control Group 7 days. **Note:** HE staining 4x (referring to image 1A) and 10x.

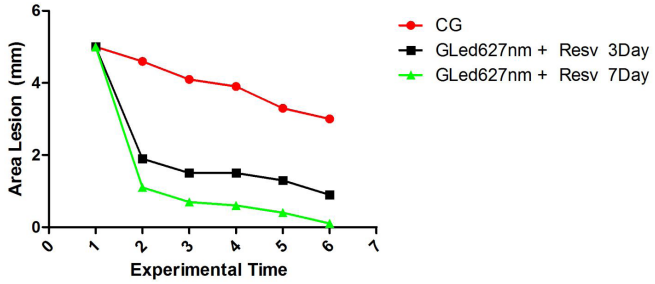

Figure 4. Comparative data about area of lesion.

Note: One-way analysis of variance test, P value 0.0156. Tukey's Multiple Comparison Test, CG vs. GLed627nm + Resv 7Day was significant.

and observed in the LED group intense proliferation of fibroblasts and greater production and organization of collagen fibers in the long term according to histological and histological macroscopic qualitative analysis. The study also noted a more satisfactory cosmetic aspect of healing[21]. Was investigated the effects of resveratrol in the healing process of total uterine thickness lesion in rats and concluded that the use of resveratrol significantly increased the healing of uterine wall thickness by its antioxidant effects[24]. In the present research the gel containing the extract of Resveratrol the treatment was also carried out in an animal model and anti-inflammatory activity was observed, suggesting that the components contained in the extract are able to stimulate the cicatrization process. Was analyzed the effects of Resveratrol [3] on angiogenesis in healing a complete incision of the thickness of the dermis on the skin of the dorsum of rats, obtaining a positive result for the use of Resveratrol, which significantly increased vascular endothelial growth factor resulting in a greater granulation of the tissue, as well as a more accelerated healing of the wounds.

CONCLUSION

The treatment of the present study was effective during the cicatricial phase in both the experimental weights of 3 and 7 days, and the drug Resveratrol associated with the LED627nm optimized wound healing, promoting a greater fibroblast activation and remodeling of the collagen fiber when compared to the control group.

AUTHORS' CONTRIBUTIONS

PPTS: Final design and data collection; ACDB: Data collection and data processing; PRO: Guidance, design and article correction; LSB: Guidance, design and article correction; DB: Guidance, design and article correction; IIK: Guidance, design and article correction; AP: Guidance, design and article correction.

CONFLICT OF INTEREST

nothing to declare.

AUTHORS DETAILS

[2,3] Physiotherapy Department - Universidade Estadual do Centro Oeste do Paraná – UniCentro, Santa Cruz, Guarapuava – PR, Brasil. [4] Rehabilitation Sciences Program – Universidade Estadual de Londrina, Londrina – PR, Brasil.

REFERENCE

1. Adamskaya, N. et.al. Light therapy by blue LED improves wound healing in an excision model in rats. Injury, Int. J. Carre Injured, 2011;42(9):917-921.
2. Afaq F, Adhami VM, Ahmad, N. Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. Toxicology and Applied Pharmacology, 2003;186(1):28-37.
3. Afshar M, Hassanzadeh-Taheri MM, Zardast M, Moghaddam A. The Angiogenetic Effect of Resveratrol on Dermal Wound Healing in Balb/C Mice. Modern Care Journal, 2017;14(4).
4. Alerico GC, Beckenkamp A, Vignoli-Silva M, Buffon A, Von Poser, GL. Proliferative effect of plants used for wound healing in Rio Grande do Sul state, Brazil. Journal of ethnopharmacology, 2015;176:305-310.
5. Allen LV, Popovich NG, Ansel HC. Formas farmacêuticas e sistemas de liberação de fármacos. Porto Alegre: Artmed, 9° ed, 2013.
6. Boateng J, Catanzano O. Advanced therapeutic dressing for effective wound healing – a review. Journal of Pharmaceutical Sciences, 2015;104(11):3653-3680.
7. Catão MH, Costa RO, Nonaka CF, Junior RLA, Costa IR. Green LED light has anti-inflammatory effects on burns in rats. Burns, 2016;42(2):392-396.
8. Ferreira CLR, Nicolau RA, de Oliveira MA, Ribeiro D, Costa ACGPJ. Efeito da terapia LED (945nm ± 20nm) de baixa intensidade sobre tecido epitelial de ratos diabéticos em processo de reparo. Revista Brasileira de Engenharia Biomédica, 2013; 29(4), 404-441.
9. Gainza G, Chu WS, Guy RH, Pedraz JL, Hernandez RM, Delgado-Charro B, Igartua, M. Development and in vitro evaluation of lipid nanoparticle-based dressings for topical treatment of chronic wounds. International journal of pharmaceutic, 2015;490(1-2):404-411.
10. Guirro e Guirro. Fisioterapia Dermato-Funcional. 3ª ed. Barueri:Manole, 2002. p:584.
11. Guyton AC, Hall JE. Tratado de Fisiologia Médica. 13ª ed. Elsevier, p:1176-2017.
12. Higgins PE, Hews K, Windon III L, Chasse P. Light-emitting diode versus sham in the treatment of plantar fasciitis: a randomized trial. Journal of Chiropratic Medicine, 2015;14(1):10-14.
13. Junqueira LCU, Carneiro J. Histologia Básica. 11ª Ed. Rio de Janeiro: Guanabara Koogan, 2008. p:524.
14. Junqueira LCU, Carneiro J. Biologia Celular e Molecular. 8ª Ed. Rio de Janeiro: Guanabara Koogan, 2005. p:352.
15. Kierszenbaum AL. Histologia e Biologia Celular: Uma Introdução à Patologia. Ed. Elsevier, 2004. p:654.
16. Kuffler DP. Photobiomodulation in promoting wound healing:a review. Regenerative medicine, 2016;11(1):107-122.
17. Ishida Y, Gao JL, Murphy PM. Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. The Journal of Immunology, 2008;180(1):569-579.
18. Mandelbaum SH, Di Santis ÉP, Mandelbaum MHSA. Cicatrização: conceitos atuais e recursos auxiliares-Parte1. An. Bras. Dermatol, 2003;78(4).
19. Nemen D, Lemos-Senna E. Preparation and characterization of resveratrol-loaded lipid-based nanocarriers for cutaneous administration. Química Nova, 2011;34(3):408-413.
20. Nogueira VC, Coelho NPMDF, Barros TLD, Silva SMMDS, Martins M, Arisawa EAL. Biomodulation effects of LED and therapeutic ultrasound combined with semipermeable dressing in the repair process of cutaneous lesions in rats. Acta cirurgica brasileira, 2014;29(9):588-595.
21. Oshima Y, Coutts RD, Badlani NM, Healey RM, Kubo T, Amiel D. Effect of light-emitting diode (LED) therapy on the development of osteoarthritis (OA) in a rabit model. Biomedicine & Pharmacotherapy, 2011;65(3):224-229.
22. Pangeni R, Sahni JK, Ali J, Sharma S, Baboota S. Resveratrol: review on therapeutic potential and recent advances in drug delivery. Expert opinion on drug delivery, 2014;11(8):1285-1298.
23. Sayin O, Micili SC, Goker A, Kamaci G, Ergur BU, Yilmaz O, Akdogan GG. The role of resveratrol on full–Thickness uterine wound healing in rats. Taiwanese Journal of Obstetrics and Gynecology, 2017;56(5):657-663.