Original Article

Application of Solidago chilensis and laser improved the repair of burns in diabetic rats

Juliana A.R. Moreira a, Israel C. Vasconcelos a, José L. Fachi a, Viviane Theodoro a, Rodrigo A. Dalia a, Andrea A. Aro a, Edson R. Pimentel b, Fernanda O.G. Gaspi a, Thiago A.M. Andrade a, Maria E.C. Amaral a, Marcelo A.M. Esquisatto a, Fernanda A.S. Mendonça a, Gláucia M.T. Santos a, *

a Graduate Program of Biomedical Sciences, University Center of Herminio Ometto Foundation – FHO, SP, Brazil
b Graduate Program of Cell Biology and Structural, University of Campinas, UNICAMP, SP, Brazil

Article info

Article history:
Received 9 March 2018
Accepted 15 May 2020
Available online 26 May 2020

Keywords:
Diabetes
Skin burns
InGaP laser
LLLT
Plant extracts
Solidago chilensis

Abstract

Background: The repair of burns in diabetic patients is a clinical problem. It is relevant to study alternative therapies that can improve the healing process. Our aim was to investigate the effects of Solidago chilensis associated or not with laser on burns in diabetic rats.

Methods: The animals were divided in four groups (n = 30): C- without treatment; S- Solidago chilensis extract; L-laser irradiated; LS- laser and Solidago chilensis. In 7, 14 and 21 days samples were collected after the injury to structural, morphometric and molecular analysis.

Results: Our results demonstrate the association of S. chilensis and laser reduced the inflammatory infiltrate and favored the angiogenesis. In the groups treated only with laser or with the plant extract showed higher levels of VEGF. The low-level laser therapy (LLLT) promoted higher collagen I and reduction of collagen III. It was also observed higher MMP-2 activation and a decreasing of the active isoform of MMP-9 in the S, L and LS groups.

Conclusions: The treatments improved the repair of burns in diabetic rats, since it reduced the inflammatory infiltrate and favored the collagen organization presenting similar effects in the burn repair of the diabetics.

A number of complications are observed among diabetic patients highlighting the diabetic skin ulcer that can promote tissue amputation and mortality [1]. Studies in animal models show disorders during healing process of diabetic lesions. In cases the lesion are caused by burns the prognosis is even worse, because they carry several systemic changes such as increased metabolism, loss of fluid volume, high risk of infection and wound healing disorders, requiring more time for healing [2].

* Corresponding author. University Center of Herminio Ometto Foundation – FHO, Av. Maximiliano Baruto, 500. Jardim Universitario, 13607-339, Araras, Sao Paulo, Brazil.
E-mail address: glauciasantos@fho.edu.br (G.M.T. Santos).
Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2020.05.014
2319-4170/© 2020 Chang Gung University. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The great potential of vegetable extracts to treat lesions and burns has been studied and used in the treatment of diabetes [3,4]. Solidago chilensis is known for its activities in the absorption of edema and wound healing, and it is considered medicinal for showing antiseptic, analgesic and healing properties [5]. The phytochemical analysis of Solidago showed that its flowers and leaves when isolated present monoterpenes, sesquiterpenes, diterpenes, flavonoids (quercitrin, quercetin and rutin), sapo- nins and polyacetylenes, tannins, fatty acids, alkaloids, volatile oils, anthranoids and sesquiterpene lactones [6] considered potent compounds in wound healing with antioxidant and anti-inflammatory properties [7,8].

The low-level laser therapy (LLLT) has also been used to treat skin burns in animals [9,10]. The biostimulation is important in accelerating the repair process of injured tissue by promoting proliferation of fibroblasts, re-epithelialization, collagen deposition, triphosphate (ATP) synthesis and granulation tissue formation, as well as the influx of leukocytes, which increase the phagocytic activity of macrophages, angiogenesis, vasodilation, stimulation of mitochondria, increasing cell metabolism [11]. Among its many effects, LLLT has been shown to cause vasodilation [12] and facilitate the absorption of active compounds in herbal of simultaneous applications.

Considering the importance of the new treatments which improve the wound healing process of burns in diabetic patients, the aim of this study was to investigate the association of Solidago chilensis and laser therapy to benefit the wound healing in diabetic animal model.

Materials and methods

Plant material

The leaves of Solidago chilensis were collected in the campus of the University Center of Herminio Ometto Foundation – FHO (Araras, Sao Paulo, Brazil), in the Medicinal Plants Garden, during the morning in the month of August (winter). In the Herbarium of School of Agriculture Luiz de Queiroz (ESA) USP/ESALQ (Piracicaba/Sao Paulo, Brazil was deposited a voucher specimen (ESA114268).

Preparation of the hydroalcoholic extract and phytochemical analysis

After collection, the fresh leaves (50 g) were selected, cleaned under running water to remove impurities and macerated dynamically with 300 mL of an ethanol aqueous solution (7:3, v/v) for 7 days at room temperature. This procedure was repeated three times with the same powder and the same solvent. After filtration, the solvent was completely evaporated under vacuum at 40 °C in a rotary evaporator, and the hydroalcoholic leaf extract of S. chilensis (S) was obtained after lyophilization. The yield of the lyophilized extract was 10%. The experimental procedures were carried out using dry crude extract dissolved in saline solution [6].

Animals

All surgical and experimental procedures used in this study were conducted according to the experimental requirements and biodiversity rights of the National Institute of Health for the Care and Use of Laboratory Animals (NIH Publication 80–23, reviewed in 1996). Studies have been done in accordance to the rules established by Arouca Law and approved by the Ethics Committee on Animal Use (CEUA) of University Center of Herminio Ometto Foundation – FHO, under number 092/2011.

One hundred and twenty male Wistar rats were obtained from the Center of Animal Experimentation, University Center of Herminio Ometto Foundation – FHO (Araras, Sao Paulo, Brazil) with 120 days and average weight 300 g. Throughout the experimental procedure, that did not promote stress, the animals were housed in individual cages, in ambient with humidity of 55%, temperature 23 ± 2 °C, 12/12-h light/dark cycle, and water and chow ad libidum.

Induction of experimental diabetes

The induction of experimental diabetes consisted of Alloxan solution [(Sigma®, Co., USA) (2,4,5,6 tetraoxohexahydropyr- imidine) in single dose of 32 mg/kg body weight, diluted in citrate buffer pH 4.5 [13]. The characterization of the diabetic animal model was performed by measuring the peripheral blood glucose from tails of rats after seven and 30 days after the of diabetes induction, using reagent strips for readings in portable blood glucose meter (Accu-Chek Active®, AM Roche Diagnostics, EUA). The animals considered to the protocol had blood glucose >200 mg/Dl [13]. Before the euthanasia of rats (7, 14 and 21 days) was performed the measurement of glucose to check the stability of inducing diabetes.

Experimental procedures

The animals were anesthetized with peritoneal administration of xylazine hydrochloride (0.2 mg/kg) and ketamine hydrochlo- ride (1 mg/kg) [14] and received analgesics (sodium dipyrone) [15]. The experimental second-degree burns were inflicted on the dorsal skin of all animals produced in the back of animals.
with an aluminum plate measuring 2 cm in diameter connected to a temperature-controlling device that maintained a constant temperature of 120 °C. This plate was applied to the animal’s skin for 20 s for the production of a second-degree burn. To ensure the same pattern of burns in all animals, we used a graduated support rod for sustaining the aluminum plate with the same pressure on the dorsal skin of the animals [16].

The four experimental groups randomly formed by 30 animals were: C, untreated; S, treated with the S. chilensis hydroalcoholic extract; L, irradiated with an LLLT; and LS, irradiated with an LLLT after treated with the S. chilensis hydroalcoholic extract. Lesion samples were collected from ten animals/group in each experimental period (7, 14, and 21 days after the burning procedure) for morphometric analysis (n = 5 animals) and for protein expression analysis by Western blotting, quantitative analysis of glycosaminoglycans, hydroxyproline, and zymography for metalloproteinases (n = 5 animals). The method of euthanasia of the animals was by an overdose of anesthesia and cervical dislocation.

LLLT was performed daily with a Physiolux Dual Bioset® - InGaP (Indium Gallium Phosphide) diode emitting a wavelength of 670 nm (visible red) with an output power of 30 mW, energy density of 4.93 J/cm², and total energy dose of 0.36 J, with the beam covering an area of 0.073 cm². Non-contact laser irradiation was performed punctually, in the continuous mode, at a distance of ±2 mm and an angle of 90° in relation to the wound surface. The time of laser application (12 s) was adjusted on the equipment in four points applied on the wound edges aimed to stimulate the healthy tissue from borders [6,14]. The calibration of the instrument was made by the manufacturer.

The dosimetry for application of S. chilensis hydroalcoholic extract was applied with a Pasteur pipette, dripping 1 mL over whole wound surface [6,15].

**Morphometric analysis**

Standardized samples (25 mm in diameter/10 mm in deep) were collected from the burned skin. The samples were fixed for structural and morphometric analysis [6,15]. The samples were cut into 6-μm longitudinal sections and stained with Dominici (detection of intracellular granules of inflammatory infiltrate), Mallory Trichrome (structural analysis of the epidermis and dermis) and with toluidine blue in McIlvaine buffer pH 4.0 (measuring the number of fibroblasts and blood vessels). From each sample were captured the first 16 cuts, distributed in eight slide blades. The number of inflammatory infiltrate, newly formed blood vessels, fibroblasts (n/10⁵ μm²) in the repaired area were determined in longitudinal sections stained. Five samples of 10³μm² were made for each section from the center of the specimen of each animal per group and for each method. Using a Leica DM2000 photomicroscope the images of sections were captured and digitized in bright field. For the morphometric analysis, samples were examined by one evaluator using the virtual Leica Image Measure® grid and the Sigma Scan Pro 6.0® program [16].

**Western blotting and biochemical analysis**

The densitometry values of TGF-β1, VEGF, Collagen type I and type III signals were developed according to a protocol developed by Ni et al. [17] and expressed relative to proteins stained with β-tubulin, which were taken as 100%.

**Quantitative analysis of glycosaminoglycans**

The glycosaminoglycans extraction of tissue fragments was made according to the DMBB method [18]. It was used one light visible spectrophotometer (526 nm) for the reading.

**Quantification of hydroxyproline**

Fragments of tissue were immersed in acetone (48 h) and then in chloroform: ethanol (2:1) for 48 h. The samples were hydrolyzed (HCl 6 N – 1 mL for each 10 mg of tissue, 16 h, 110 °C) and neutralized (NaOH 6 N). The quantification of hydroxyproline was performed according to the method of Stegmann, & Stalder [19] with some modifications. Hydroxyproline concentrations between 0.2 and 6 μg/mL were used for the standard curve.

**Zymography to metalloproteinases**

The supernatant from each sample (50 μg protein) was used according to the protocol of Aro et al. [20] for the analysis of MMP-2 and MMP-9 activity. The intensity of the bands of different isoforms, for each group was determined by densitometry using Alpha 4.0.3.2 software (Scion Corporation, USA).

**Statistical analysis**

The results of the morphometric analysis, Western Blotting and quantitative of Hydroxyproline and GAGs were reported by mean and standard deviation (X ± SD) and the values were compared by ANOVA and Tukey’s post-test (p < 0.05) using the GraphPadPrism® 3.0 software.

### Table 1 Morphometric parameters evaluated in the wound healing process of second-degree burns in rats diabetics in different treatment groups and experimental periods.

| Parameters | Groups | Experimental periods |
|------------|--------|----------------------|
|            |        | 7 d  | 14 d  | 21 d  |
| N of fibroblasts (n/10⁵ μm²) | C | 13.2 ± 3.4 | 22.8 ± 3.8 | 27.4 ± 3.9 |
| | S | 12.1 ± 3.9 | 20.8 ± 4.2 | 27.9 ± 3.4 |
| | L | 23.2 ± 4.1 | 31.1 ± 3.2 | 37.2 ± 4.3 |
| | LS | 24.2 ± 3.5 | 31.2 ± 4.1 | 37.4 ± 3.5 |
| N of inflammatory infiltrate (n/10⁵ μm²) | C | 20.6 ± 2.8 | 17.1 ± 2.1 | 7.7 ± 1.2 |
| | S | 17.2 ± 1.7 | 13.7 ± 2.2 | 5.2 ± 1.3 |
| | L | 17.1 ± 1.3 | 12.7 ± 1.8 | 5.1 ± 1.6 |
| | LS | 16.2 ± 1.5 | 11.9 ± 2.2 | 5.2 ± 1.5 |
| N of vessels (n/10⁵ μm²) | C | 1.4 ± 0.6 | 1.7 ± 0.6 | 1.8 ± 0.4 |
| | S | 1.7 ± 0.6 | 1.9 ± 0.8 | 2.0 ± 0.5 |
| | L | 1.8 ± 0.3 | 2.7 ± 0.3 | 2.8 ± 0.5 |
| | LS | 1.9 ± 0.3 | 2.8 ± 0.4 | 2.9 ± 0.4 |

Values are the mean and standard deviation of each group and were compared by ANOVA with Tukey’s post-test (p < 0.05).

* Significant difference.
After 7 days of diabetes induction by intravenous Alloxan, animals presented an average glucose of 347.48 mg dL-1 that was maintained during the 30 experimental days.

The morphometric analysis showed that the total number of fibroblasts increased in the L and LS groups in relation to the others. The amount of inflammatory infiltrate decreased in the treated groups compared to the group C, on the 21st day after experimental protocol. It was observed that the number of blood vessels increased in the L and LS groups on the 14th and 21st day [Table 1].

Collagen I analysis showed higher amount during the experimental period with a difference on the 14th day in L and LS groups compared to the other groups, and on the 21st day in the S and L groups. On the other hand, Collagen III reduced during the experimental period, with difference on the 14th day
in the L and LS groups, and on the 21st day in all treated groups, especially in the LS group [Fig. 1]. The results showed no differences for the TGF-β1 level between the experimental groups. The VEGF level was increased at day 14th in the groups treated with S and L. The quantification of bFGF had no differences throughout the study period in all experimental groups [Fig. 1].

Regarding to GAGs, there was a gradual increase between groups during the experimental period, particularly in the L and SL groups which had higher values in relation to the others [Table 2]. Considering the collagenesis, by hydroxyproline quantification, which infers the total collagen content in the tissue, no difference between groups was observed in all experimental periods [Table 2].

The active MMP-9 form was detected in all groups and periods, with the exception of S, L and LS, on the 21st day, where there was a marked decrease in MMP-9 activity in relation the control [Fig. 2, Table 3]. In the zymography analysis for MMP-2, the intermediate isoform was detected in all groups and experimental periods, except for the C group on the 21st day. Higher values were observed for catalytic activity of this isoform in the S group 14th and 21st days [Fig. 2, Table 3].

### Table 2 Biochemical parameters evaluated in the wound healing process of second-degree burns in rats diabetics in different treatment groups and experimental periods.

| Experimental periods | Groups | 7 d     | 14 d    | 21 d    |
|----------------------|--------|---------|---------|---------|
| Parameters           |        |         |         |         |
| Glycosaminoglycans (µg/mg of dry tissue) | C      | 1.26 ± 0.14 | 1.39 ± 0.17 | 1.67 ± 0.14 |
|                      | S      | 1.29 ± 0.16 | 1.45 ± 0.16 | 1.65 ± 0.15 |
|                      | L      | 1.79 ± 0.13a| 1.93 ± 0.13a| 1.96 ± 0.13a|
|                      | LS     | 1.78 ± 0.16 | 1.92 ± 0.14 | 1.93 ± 0.14  |
| Hydroxyproline (µg/mg of dry tissue)    | C      | 108.9 ± 14.1 | 93.1 ± 17.2 | 89.8 ± 14.2 |
|                      | S      | 117.2 ± 16.2 | 96.4 ± 17.1 | 89.6 ± 15.4 |
|                      | L      | 116.4 ± 17.2 | 91.6 ± 13.4 | 89.4 ± 18.3 |
|                      | LS     | 112.9 ± 16.4 | 92.7 ± 14.6 | 92.2 ± 17.1 |

Values are the mean and standard deviation of each group and were compared by ANOVA with Tukey post-test (p < 0.05).

**Discussion**

Study of experimental phytotherapy is important to verify its efficacy to the healing of diabetic lesions, since the use of different extracts in the treatment of skin lesions is a common practice [21,22]. The orientation for use of the species *S. chilensis* and its products is only externally [23]. The plant has no toxicity for external use, which was used in this research and was included in the list of medicinal plants of SUS (Systema Único de Saúde - Brazil) interest for use by the population. In phychotherapy analysis of the constituents of the extract of *S. chilensis* leaves was identified the following classes of chemical compounds: flavonoids, saponins, tannins, fatty acids, alkaloids, volatile oils and anthranoids [6]. Especially flavonoids, tannins and alkaloids, considered potent compounds in wound healing, can influence the migration of fibroblasts, collagen stabilization and in the release of VEGF [24].

The LLLT has also been widely used to solve or minimize burn lesions [25]. The choice of the InGaP 670 nm laser occurred due to its performance in the healing of second-degree burns as was found in previous studies of our group and other reports in the literature [5,9,10,14,15,26,27]. Disorders in tissue repair are a feature of Diabetes mellitus where the synthesis and deposition of collagen fibers, fibroplasia, epithelialization, angiogenesis and inflammatory phase are compromised [28].

The analysis of inflammatory infiltrate indicates that the extract and laser, isolated or in association, have been effective in reducing inflammatory phase during the studied period improved the tissue repair. It is known that the persistence of inflammation is detrimental to tissue repair in both burned and diabetic patients [6,28]. According to Tamura et al. [29] the anti-inflammatory activity of this extract was attributed mainly to the flavonoids and tannins present in the leaves and flowers of this species. The anti-inflammatory property is characteristic of arnicas [30] and this effect has also been shown in other studies [5,31]. Besides that, it is known that the use of LLLT in skin burned treatment of healthy and diabetic rats has anti-inflammatory action [32,33]. Data of our group showed that the use of laser on burned lesion in non-diabetic rats favorably modulated the initial inflammatory process [6]. It is important to mention that both treatments used in our study, isolated or in association, reduced the inflammatory infiltrate.

The analysis of VEGF demonstrated that the treatments favored the angiogenesis, an important process for the repair of tissue, because it provides oxygen and energy substrate. It is also known that VEGF and bFGF are growth factors that have pro-angiogenic properties and the activities of these are essential for an appropriated tissue repair [34]. bFGF is a potent angiogenic agent that acts as a chemoattractant in

![Fig. 2 Zymogram for analyzing the isoforms latent (92 kDa) and active (83 kDa) of MMP-9; and the intermediate isoforms (68 kDa) and active (62 kDa) of MMP-2 in C, S, L and LS groups 7 (7 d), 14 (14 d) and 21 (21 d) days after lesions.](image-url)
different cell types and influence the proliferation and differentiation of endothelial cells and fibroblasts [35].

Increased numbers of fibroblasts was observed in the groups treated with laser in all periods. The LLLT stimulates the fibroblast proliferation in diabetic lesions [36]. Catarino et al. [6] found similar results in non-diabetic rats using the same experimental model used in this study.

The hydroxyproline is an indicator of the collagen concentration in the tissue, which is organized in fibers bundles [37]. No difference in the hydroxyproline concentration was observed between the groups in all periods, but considering the collagen I and III, some differences were detected. The laser isolated or in association with the extract, stimulated the collagen I synthesis, and contributed to decreasing of collagen III, some differences were detected. The collagen I and the decrease of collagen III, might indicate an increase in enzyme activity, protein synthesis, cell proliferation, adenosine ATP production, and collagen organization [12,43–46], thus contributing to tissue repair in association with the phytophoretic.

Conclusions

Our results show that the treatment with LLLT (670 nm InGaP laser) and with the extract of S. chilensis improved the tissue repair in burns of diabetic rats with decrease in the inflammatory infiltrate and increase in the collagen I, MMP-2 activity and amount of GAGs. Therefore, they can be used as an alternative therapy in the treatment of this type of lesion.

Conflicts of interest

We certify that there is no actual or potential conflicts of interest related to this article exist.

Acknowledgments

This research was supported by the Hermínio Ometotto Foundation.

REFERENCES

[1] Motley TA, Gilligan AM, Lange DL, Waycaster CR, Dickerson JE Jr. Cost-effectiveness of clostridial collagenase
ointment on wound closure in patients with diabetic foot ulcers: economic analysis of results from a multicenter, randomized, open-label trial. J Foot Ankle Res 2015;8:7.

[2] Zhao J, Li YG, Deng KQ, Yun P, Gong T. Therapeutic effects of static magnetic field on wound healing in diabetic rats. J Diabetes Res 2017;2017:630570.

[3] Sandhya S, Sai Kumar P, Vinod KR, Banji D, Kumar K. Plants as potent anti-diabetic and wound healing agents – a review. Hygeia J Drugs Med 2013;3:11–9.

[4] Kaya H, Gokdemir MT, Sogut O, Demir T, Kocarslan S. Effects of folk medicinal plant extract ankaferd blood stopper on burn wound healing. Acta Med Mediterr 2013;29:497–502.

[5] Goulart S, Moritz MI, Lang KL, Liz R, Schenkel EP, Frode TS. Anti-inflammatory evaluation of Solidago chilensis Meyen in a murine model of pleurisy. J Ethnopharmacol 2007;113:346–53.

[6] Catarino HR, de Godoy NP, Scharlack NK, Neves LM, de Gaspi FO, Esquisatto MA, et al. InGaP 670-nm laser therapy combined with a hydroalcoholic extract of Solidago chilensis Meyen in burn injuries. Lasers Med Sci 2015;30:1069–79.

[7] de G de Gaspi FO, Foglio MA, de Carvalho JE, Santos GM, Testa M, Passarini JR Jr, et al. Effects of the topical application of hydroalcoholic leaf extract of Orzicum flexuosum Sims (Orchidaceae) and microcurrent on the healing of wounds surgically induced in Wistar rats. Evid Based Complement Alternat Med 2011;2011:950347.

[8] Migliato KF, Chiosini MA, Mendonca FA, Esquisatto MA, Salgado HR, Santos GM. Effect of glycolic extract of Dillenia indica L. combined with microcurrent stimulation on experimental lesions in Wistar rats. Wounds 2011;23:111–20.

[9] Núñez SC, França CM, Silva DF, Nogueira GE, Prates RA, Ribeiro MS. The influence of red laser irradiation timeline on burn healing in rats. Lasers Med Sci 2013;28:633–41.

[10] Belli M, Fernandes CR, Neves LM, Mourão V, Barbieri R, Esquisatto M, et al. Application of 670 nm InGaP laser and microcurrent favors the healing of second-degree burns in rats. J Cosmet Laser Ther 2011;13:237–42.

[11] Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng 2012;40:516–33.

[12] Lenzen S. The mechanisms of alloxan- and streptozocin-induced diabetes. Diabetologia 2008;51:216–26.

[13] Chiotto GB, Neves LM, Esquisatto MAM, dos Santos GM, Chiosini MA, et al. Activity of Porphyrum ruderale leaf extract and 670-nm InGaP laser during burns repair in rats. BMC Compl Altern Med 2015;15:274.

[14] Miller RG. Simultaneous statistical inference. New York: Berlin Heidelberg: Springer-Verlag; 2000.

[15] Ni D, Xu P, Gallagher S. Immunoediting and immunodepression. Curr Protoc Protein Sci 2017;8:10.10.1–37.

[16] Farddale RW, Buttie DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylimethylene blue. Biochim Biophys Acta 1986;883:173–7.

[17] Stiegemann H, Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267–73.

[18] Aro AA, Nishan U, Perez MO, Rodrigues RA, Foglio MA, Carvalho JE, et al. Structural and biochemical alterations during the healing process of tendons treated with Aloe vera. Life Sci 2012;91:885–93.

[19] Pirbalouti AG, Azziz S, Koohpayeh A, Hamedi B. Wound healing activity of Malva sylvestris and Punica granatum in alloxan-induced diabetic rats. Acta Pol Pharm 2010;67:511–6.

[20] Mekala S, Kumar N, Das L, Shetty N, Amuthan A, Vulli V, et al. Evaluation of wound healing activity of ethanolic extract of Lantana camara in streptozotocin induced diabetic rats. Int J Pharm Pharmacuet Sci 2014;6:631–3.

[21] Valverde SS, de Oliveira TB, de Souza SP. Solidago chilensis meyen (asteraceae). Rev. Fitos 2012;7:131–6.

[22] Tsala DE, Amadou D, Habtetamariam S. Natural wound healing and bioactive natural products. Phytopharmcol 2013;4:532–60.

[23] Lorette EH, Pascoal VL, Nogueira BV, Silva IV, Pedrosa DF. Use of laser therapy in the healing process: a literature review. Photomed Laser Surg 2015;33:104–16.

[24] Schlager A, Oehler K, Huebner KU, Schmuth M, Spoettl E. Healing of burns after treatment with 670-nanometer low-power laser light. Plast Reconstr Surg 2000;105:1635–9.

[25] Al-Watban FA, Delgado GD. Burn healing with a diode laser: 670 nm at different doses as compared to a placebo group. Photomed Laser Surg 2005;23:245–50.

[26] Falanga V. Wound healing and its impairment in the diabetic foot. Lancet 2005;366:1736–43.

[27] Tamura EK, Jimenez RS, Waismam K, Gobbo-Neto L, Lopes NP, Malpezzi-Marinho EA, et al. Inhibitory effects of Solidago chilensis Meyen hydroalcoholic extract on acute inflammation. J Ethnopharmacol 2009;122:478–85.

[28] Bergonzini MC, Bilia AR, Casirighi A, Cilurzo F, Minghetti P, Montanari L, et al. Evaluation of skin permeability of sesquiterpenes of an innovative supercritical carbon dioxide arnica extract by HPLC/DAD/MS. Pharmazie 2005;60:36–8.

[29] Castro FC, Magre A, Cherpinis R, Zelante PM, Neves LM, Esquisatto MA, et al. Effects of microcurrent application alone or in combination with topical Hypericum perforatum L. and Arnica montana L. on surgically induced wound healing in Wistar rats. Homeopathy 2012;101:147–53.

[30] de Moraes JM, Eterno de Oliveira Mendonça D, Moura VB, Oliveira MA, Afonso CL, Vinaud MC, et al. Anti-inflammatory effect of low-intensity laser on the healing of third-degree burn wounds in rats. Lasers Med Sci 2013;28:1169–76.

[31] Ma H, Li YX, Chen HL, Kang ML, Liu TYC. Effects of low-intensity laser irradiation on wound healing in diabetic rats. Int J Photoenergy 2012;2012:7.

[32] Wong VW, Crawford JD. Vasculogenic cytokines in wound healing. BioMed Res Int 2013;2013:190486.

[33] Feito MJ, Lozano RM, Alcade M, Ramirez-Santillan C, Arcos D, Vallet-Regl M, et al. Immobilization and bioactivity evaluation of FG-1 and FG-2 on powdered silicon-doped hydroxyapatite and their scaffolds for bone tissue engineering. J Mater Sci Mater Med 2011;22:405–16.

[34] Al-Watban FA, Zhang XY, Andres BL. Low-level laser therapy enhances wound healing in diabetic rats: a comparison of different lasers. Photomed Laser Surg 2007;25:72–7.

[35] Nayak SB, Pinto Pereira L, Maharaj D. Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. Indian J Exp Biol 2007;45:739–43.

[36] Novais RD, Goncalves RV, Cupertino MC, Araujo BM, Rezende RM, Santos EC, et al. The energy density of laser light differentially modulates the skin morphological reorganization in a murine model of healing by secondary intention. Int J Exp Pathol 2014;95:138–46.
[39] Oshiro W, Lou J, Xing X, Tu Y, Manske PR. Flexor tendon healing in the rat: a histologic and gene expression study. J Hand Surg Am 2003;28:814–23.

[40] De Aro AA, Guerra Fda R, Esquisatto MA, Nakagaki WR, Gomes L, Pimentel ER. Biochemical and morphological alterations in the Achilles tendon of mdx mice. Microsc Res Tech 2015;78:85–93.

[41] Guerra Fda R, Vieira CP, Almeida MS, Oliveira LP, de Aro AA, Pimentel ER. LLLT improves tendon healing through increase of MMP activity and collagen synthesis. Lasers Med Sci 2013;28:1281–8.

[42] Trowbridge JM, Gallo RL. Dermatan sulfate: new functions from an old glycosaminoglycan. Glycobiology 2002;12:117R-25R.

[43] Longo L. Nonsurgical laser and light in the treatment of chronic diseases: a review based on personal experiences. Laser Phys Lett 2010;7:771–86.

[44] Karu TI, Kolyakov SF. Exact action spectra for cellular responses relevant to phototherapy. Photomed Laser Surg 2005;23:355–61.

[45] Karu TI, Pyatibrat LV, Kalendo GS. Photobiological modulation of cell attachment via cytochrome c oxidase. Photochem Photobiol Sci 2004;3:211–6.

[46] Karu TI, Pyatibrat LV, Afanasyeva NI. A novel mitochondrial signaling pathway activated by visible-to-near infrared radiation. Photochem Photobiol 2004;80:366–72.