Evaluation of the cicatrizant activity of a semisolid pharmaceutical formulation obtained from *Platonia insignis* Mart.

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The use of natural products for skin treatments has been increased. Mainly, those of topical action incorporated to pharmaceutical forms, since they allow the restoring of skin integrity after possible attacks. In this context, the plant *Platonia insignis* Mart has been widely used for the treatment of various skin diseases and as wound healing medicine in folk medicine. The aim of this study is to evaluate the wound healing activity of a cream formulated containing triglyceride isolated from *P. insignis* Mart called 1,3-distearoyl-2-oleoylglycerol (TG1) in skin lesions induced in Wistar rats by macroscopic and histological analysis of the wounds. The animals were randomly divided into 5 groups according to the treatment to be performed. The negative control received saline solution, the positive control received collagenase and the other groups received cream containing the triglyceride at concentrations of 5, 10 and 15%. The results showed effectiveness in the healing of wounds through reduction of their diameter compared to negative control. Histological analysis demonstrated the inflammatory and proliferative phenomena of healing in wounds of rats. The cream with TG1 in three concentrations demonstrated efficacy in wound healing, as evidenced by macroscopic and microscopic analyses of lesions in Wistar rats. Based on this, a further development of phytomedicines for wound care is suggested.

**Key words:** Cutaneous wounds, healing, *Platonia insignis*.

**INTRODUCTION**

Natural products from plants, animals and microorganisms are a huge source of different chemicals...
that can be used by pharmaceutical industries for production of medicines (Ogbourne and Parsons, 2014; Fonseca et al., 2014). Bioactive substances, such as alkaloids, steroids, glycosides, saponins, tannins and flavonoids present mainly in plants, are potential therapeutic agents that can help in wound healing (Adiele et al., 2014; Kuntal, 2013). The healing process involves migration of inflammatory cells, synthesis of granulation tissue, deposition of collagen and proteoglycans and scar maturation, being associated with intense refurbishment. The search for treatments using medicinal plants as a healthier lifestyle alternative has led to complementary medicine to be used as alternative to treat wounds (Mukherjeea et al., 2013; Furumoto et al., 2014; Santos et al., 2006). In this context, the plant *Platonia insignis* Mart has been widely used in treatments of several dermatoses and as a preparation for healing wounds in folk medicine. This species, popularly known as “bacurizeiro”, is a monotype vegetal belonging to the Clusiaceae family. It is a fruit tree species and timber found in the Northeast region of Brazil, mainly in the states of Piauí and Maranhão. Its seeds are used in the processing of oil or "bacuri lard". Recent studies have shown the effectiveness of the oil from seeds in increasing the healing process of skin wounds in rats (Souza et al., 2013; Moura et al., 2007; Santos et al., 2013; Clerici et al., 2011).

Given the growing use of natural products for dermatological treatments, the topical action incorporated in dosage forms should be highlighted because they allow the restoration of skin integrity after possible attacks (Karodi et al., 2009). Thus, the semi-solid pharmaceutical formulations using a natural and typical product as active principle may be an alternative for caring of local population. In addition, it may contribute to the regional flora valorization showing its economic and biological potential. In this scenery, the aim of this study was to evaluate the wound healing activity of a formulated cream containing TG1 (1,3-distearoyl-2-oleoylglycerol) (Figure 1) in skin lesions of Wistar rats by macroscopic and histological analysis.

**MATERIALS AND METHODS**

**Plant**

The seeds were obtained from fruits in the city of Barras, Piauí, Brazil, in March 2013. A voucher specimen was deposited in the Herbarium Graziella Barroso of Federal University Piauí (UFPI), N° ICN TEPB 27,164.

**Extraction and isolation of TG1**

The *P. insignis* seeds were dried at 55°C and powdered, yielding 848 g, and then extracted with hexane in Soxhlet for 8 h. The extract was concentrated in vacuum rota evaporator (534.24 g, yield 63%) and stored at 8°C. A formation of a white precipitate was observed, consisting of TG1. The white precipitate was fractionated by chromatography over Si gel, using n-hexane-EtOAc (19:1) yielded TG1. Its spectra of NMR 1H NMR (Figure 2), 13C NMR (Figure 3), DEPT 135 (Figure 4), COSY (Figure 5) and HMBC (Figure 6) were obtained for identification of TG1: C 57H108O6. NMR spectra description of 1H is 13C NMR (CDCl3, ppm, 125 MHz): \( \delta _{H} = 5.45-5.35 \) (4H, m, -CH-CH-), 5.30-5.28 (1H, m, -CH2-CH2-), 4.34-4.30 (2H, dd, -CH2(CH(O)-CH2-), 4.19-4.15 (2H, dd, -CH2-CH(CH(O)-CH2-), 2.35-2.31 (6H, t, -C(-O)-CH2-CH2-), 2.04-1.96 (4H, m, =CH2-CH2-), 1.65-1.62 (6H, m, -C(-O)-CH2-CH2-), 1.32-1.29 (nH, m, -CH3). NMR spectra description of 13C is 13C NMR (CDCl3, ppm, 500 MHz), \( \delta _{C} = 173.31 \) (C-1, sn 1, 3); 172.87 (C-1, sn 2); 34.06 (C-2, sn 1, 3); 34.21 (C-2, sn 2); 24.88 (C-3, sn 1, 3); 24.88 (C-3, sn 2); 29.22 (C-4, sn 1, 3); 29.14 (C-4, sn 2); 29.50 (C-5, sn 1, 3); 29.50 (C-5, sn 2); 29.35 (C-6, sn 1, 3); 29.35 (C-6, sn 2); 29.70 (C-7, sn 1, 3); 29.70 (C-7, sn 2); 29.72 (C-8, sn 1, 3); 29.72 (C-8, sn 2); 29.72 (C-9, sn 1, 3); 129.69 (C-9, sn 2); 29.72 (C-10, sn 1, 3); 130.03 (C-10, sn 2); 29.72 (C-11, sn 1, 3); 29.72 (C-11, sn 2); 29.72 (C-12, sn 1, 3); 29.72 (C-12, sn 2); 29.38 (C-13, sn 1, 3); 29.38 (C-13, sn 2); 31.94 (C-14, sn 1, 3); 31.94 (C-14, sn 2); 22.71 (C-15, sn 1, 3); 22.71 (C-15, sn 2); 31.94 (C-16); 22.71 (C-17); 14.13 (C-18); 68.88 (C-2, CHO); 62.10 (C-1 and 3, CH2O).

**Cream preparation formulation**

The emulsions developed were O/W type in the pharmaceutical form of creams. It was chosen a nonionic-base cream that allows the vehicle general cosmetic principles (Brazil, 2011). To prepare the nonionic basic cream, the emulsion components were separately weighed on an analytical scale and classified into two distinct phases: aqueous phase and oil phase. The oil and aqueous phases were heated at 80 and 85°C in two beakers, respectively. Subsequently, the internal phase (oil) was poured under the external phase (aqueous) under stirring, and the product was taken from heating. After cooling, with moderate stirring, the preservatives were added at approximately 40°C to reach the room temperature. The base was then homogenized. The cream-based active TG1 were initially weighed 2.5, 5 and 7.5 g of TG1 in order to acquire the dosage forms should be highlighted because they allow the restoration of skin integrity after possible attacks (Karodi et al., 2009). Thus, the semi-solid pharmaceutical formulations using a natural and typical product as active principle may be an alternative for caring of local population. In addition, it may contribute to the regional flora valorization showing its economic and biological potential. In this scenery, the aim of this study was to evaluate the wound healing activity of a formulated cream containing TG1 (1,3-distearoyl-2-oleoylglycerol) (Figure 1) in skin lesions of Wistar rats by macroscopic and histological analysis.

**Animals**

A total of 50 adult male and female Wistar rats (250 ± 50 g) were used in this study. Animals were kept in well-ventilated cages (Alesco, São Paulo) under standard conditions of light (12 h with alternative day and night cycles) and temperature (24 ± 1°C), and they were housed with access to commercial rodent stock diet (Nutrilabor, São Paulo, Brazil) and water ad libitum. The
investigational protocols were approved by the local Ethical Committee on Animal Research at Federal University of Piauí (078/2012). Animals were randomly divided into 5 groups according to the treatment ($n = 10$ rats/group) (Figure 7).

**Procedure for injury obtaining**

After intraperitoneal anesthesia of the rats with sodium pentobarbital (40 mg/100 g), it was performed trichotomy and epilation in the dorsal region, removing skin circular diameter of 4 mm with the aid of a punch (tool provided with a circular cutting surface) to expose the dorsal muscle fascia. Afterwards, animals were put back in their cages and treated with the samples (Figure 7) observed daily for the 7th, 14th and 21st days.

**Wound care**

The wounds were treated with a bandage, which were composed of gauze (first layer) and crepe bandage (second layer), and evaluated. The treatment was topically administered in the injured area once a day during 14 days. The wounds were cleaned with 0.9% saline every new application (Santos et al., 2002; Rahal et al., 2011). Furthermore, in order to avoid interference with wounds regarding a possible cross contamination, the autoclaving of wood shavings was carried out daily. This procedure improves asepsis and reduces friction of animals inside cages.

**Macroscopic evaluation of skin lesions in rats**

Treated animals were monitored daily and observed for the repair of the lesion. The observed parameters were changes in the presence or absence of edema, exudate and crust, and wound color. Digital photographic of the wound were recorded during treatments. In addition, the lesions were measured with analogical caliper in 7th, 14th and 21st day of treatment.

**Histological evaluation of skin lesions in rats**

A rectangular skin fragment (0.5 cm × 2.0 cm) from the back of each animal was dried. Each segment contained an injured central
Figure 3. $^{13}$C NMR Spectrum of TG1 in CDCl$_3$, 125 MHz

Figure 4. DEPT 135° Spectrum of TG1 in CDCl$_3$, 13C NMR (CDCl$_3$, ppm, 125 MHz) MHz.
Figure 5. HMBC Spectrum of TG1 in CDCl$_3$, 500 MHz.

Figure 6. $^1$H-$^1$H COSY Spectrum of TG1 in CDCl$_3$, 500 MHz.

Figure 7. Distribution of the experimental groups for evaluation of cutaneous wound healing in rats.
Figure 8. Diameter of skin lesions in animals on the 7th, 14th and 21st days of treatment with cream containing TG1 (1,3-distearoyl-2-oleoylglycerol).

Figure 9. Macroscopic analysis of the cutaneous lesions of animals after 1, 7, 14 and 21 days of daily treatment. Legend - A: Treatment with collagenase; B: Treatment with saline; C: Treatment with cream containing 5% TG1; D: Treatment with cream containing 10% TG1, E: Treatment with cream containing 15% TG1.
Figure 10. Histological analysis on the 7th day of treatment of skin wounds in rats. Legend - A: Saline; B: Collagenase; C: Cream with 5% TG1, D: Cream with 10% TG1 and E: Cream with TG115%. Hematoxylin & Eosin staining (H&E, Scale bar, 10 µm). Magnification, 100 ×. One representative experiment with n = 5 is shown.

area and a non-injured peripheral area of skin to serve as controls for 7 days, 14 and 21 days of treatment (Coelho et al., 2010). All skin lesion samples obtained were fixed in formalin and sent for histological preparation at the State University of Ceará (UECE). The analysis was performed under optical microscope at (100× magnification) in order to observe inflammatory and healing processes such as: presence of granulation tissue, vascular proliferation, acute and chronic inflammation, presence of collagen and re-epithelization.

Statistical analysis

Statistical analyses were performed with the Graph Pad Prism software, version 5.0 (San Diego, CA, USA). Results are expressed as mean ± standard error of mean (SEM). Data were analyzed by analysis of variance (ANOVA) followed by Student-Newman-Keuls. Differences were considered statistically significant when p < 0.05.

RESULTS

Macroscopic evaluation of skin lesions, the groups that received collagenase (positive control) and cream in three concentrations showed wounds with about 0.1 to 0.2 mm in diameter, while the group that received saline had lesions with about 0.3 mm in diameter (Figure 8). Thus, it was noted that on the 7th day of treatment with collagenase and cream preparations (5, 10 and 15%), the wounds presented smaller diameters when compared with the group treated with saline only (negative control). The macroscopic lesions of rats treated with saline
(negative control), collagenase and cream in three concentrations allowed realizing evolution of the tissue repair. The groups treated with collagenase and cream in a concentration of 5% did not show edema or hemorrhage in the lesion. The groups treated with saline and cream in concentrations of 10 and 15% showed edema and purulent secretion on the 7th day of treatment. After a week of administration, there was formation of browning crust in the majority of groups. There was no evidence of granulation during the evaluations (Figure 9). Figures 8 and 9 indicate that increasing concentration of cream accelerates the healing process, since decreased wounds compared to the three concentrations were noted. This supports the cream effectiveness on the wound healing. In the concentration of 15%, it was observed the absence of injury on the 14th day of treatment; a similar outcome was obtained with the positive control. The analysis of scar diameter revealed early lesion reduction on the 7th day (Figure 10).

Histological analysis on the 14th day of treatment with TG1 showed better results compared with the 7th day. It was possible to observe healing with formation of dermal papillae and hair follicles and total reorganization of collagen with a keratinized and recovered epithelium in the area where previous injury had been done (Figure 11). On day 21 of treatment with cream TG1 (Figure 12) total histofunctional restructuring with angiogenesis and
absence of inflammatory foci and edema was observed.

DISCUSSION

The assessment of the characteristics that the active ingredient incorporated into the base in semisolid pharmaceutical has fundamental importance in the process of wound healing in rats. It allows the verification of the feasibility of the planned formulation for use in other pharmacological models and preclinical trials. Mice and rats are routinely used as an experimental tool to study novel bioactive medicines and healing remedies (Surey et al., 2014; Fernandes et al., 2014; Sá et al., 2012; Magalhães et al., 2010). The Wistar rats were chosen for this study for being small, easy to purchase and due to the standardization concerning age, weight, sex, food, accommodation, cleaning care and experimental manipulation. They have also good resistance to handling, surgical injury and infections (Santos et al., 2006).

Monitoring of histological attributes, the identification of cellular elements and the content of collagen production are parameters usually used for wound healing studies (Rawat et al., 2012; Akela et al., 2012). Thus, histological analysis of groups treated with the cream of TG1 allowed realizing effective healing of the skin lesions. It is noteworthy that the response to tissue injury, whether traumatic or surgical, is composed of three phases: the inflammatory phase, the proliferative phase and remodeling.
phase, all aiming at the definitive tissue repair. The inflammatory phase may take 24 to 48 h and consists of hemostatic mechanisms. The following steps are related to collagen production and tissue remodeling (Guo and Dipietro, 2010). Thus, it is possible to realize a principle of re-epithelialization on the 7th day of treatment of wounds with cream containing TG1. A study with different extracts of Martynia annua Linn leaves demonstrated wound healing in rats. It showed fibroblast proliferation, collagen maturation and epithelialization in the histological analyses, which corroborate to the results of this study (Santram and Singhai 2011).

Other studies also showed intense healing on day 14 of treatment. The treatment induced diabetic wounds in rats with the flavonoid fraction of leaves of M. annua Linn demonstrated organization of collagen fibers, fibroblasts, and increased angiogenesis. The use of crude extract of Jatropha gossypifolia L. also showed intense fibroblastic proliferation and epithelialization on the 14th day (Santos et al., 2006; Santram and Singhai 2013). It is noteworthy that the last phase of tissue repair process (maturation phase) is characterized by endothelial regression. On the 21 day of treatment, there was continuous process of re-epithelialization of the injured area with keratinization in all groups, promoting the corneum stratum, primarily composed of keratin, a protein responsible for the skin impermeability (Garros et al., 2006; Moura et al., 2014).

The angiogenesis is a process of proliferative phase in which new blood vessels are formed and involves the migration and proliferation of endothelial cells. Topical treatment with substance P (a neuropeptide produced by both neuronal and immune cells) in skin wounds of rats allowed realizing effective wound healing with increased fibroblast proliferation, angiogenesis and collagen deposition (Kant et al., 2013). Therefore the treatment with cream containing TG1 in the three concentrations used demonstrated efficacy in wound healing. It was evidenced by macroscopic and microscopic analyses of lesions and wound retraction. Histological analysis showed healing morphological phenomena (intense fibroblast proliferation, angiogenesis and reepithelialization) with all doses tested. The present study showed a possible healing activity of the cream containing TG1, enabling the advanced development of phytomedicines.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES


