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Evaluating the Use of an Ointment Based on *Prosopis juliflora* Leaves in the Topical Therapy of Wounds

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Authors' contributions

This work was carried out in collaboration between both authors. Author GMC designed the study, provided the scope and structure, and alignment to national and institutional discourse, and read, reviewed and edited the first draft. Author ACFC managed and conducted the literature review, co-wrote first draft, and reviewed and edited the first draft and provided valuable assistance with the research process. Both authors read and approved the final manuscript.

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ABSTRACT

Wound healing is characterized by how a dynamic physiological process must maintain the integrity of the lesion. This study aimed to evaluate morphometrically and histologically, through *in vivo* assays, the healing of skin wounds with topical use of an ointment formulated by a powder base obtained from the leaves of *Prosopis juliflora*. Twenty-seven adult male Wistar rats were divided into three groups and received a daily application of saline 0.9% NaCl (GI), a commercial ointment based on silver sulfadiazine 1% (GII), and an ointment-based powder of the leaves of *P. juliflora* (GIII) for periods of 7, 14 and 21 days. Morphometric evaluation of the wound was done daily and its evolution was followed by observing the appearance of the scar retraction with a digital calliper. The histological assessment was made through the production of blades for the observation of epithelialization processes and re-epithelialization. The animals treated with the ointment base of *P. juliflora* leaves showed a satisfactory healing process from day 7, with the appearance of

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granulation tissue and wound contraction. Topical application of the ointment powder base from the leaves of *P. juliflora* allowed a gradual healing and the stable behaviour of wounds *in vivo*. Further investigation will allow application of this compound to heal human wounds and pharmaceutical use.

Keywords: Prosopis juliflora; Wound Healing; Re-Epithelialization.

1. INTRODUCTION

The wound healing process is intended to restore the integrity and function of damaged tissue by means of a complex physiological process involving cellular, molecular and biochemical events that begin after tissue damage [1]. According to Dinh et al. [2], it is a dynamic process that is characterized by the occurrence of three independent histopathological stages: inflammation, cell proliferation, and repair and tissue remodelling.

During the process of wound healing, the cells release growth factors that influence all stages, so that care for the lesion involves using antibiotics and anti-inflammatory agents, which do not always avoid unwanted side effects and toxicity [3]. This becomes a problem, especially for diabetic patients whose wounds heal slowly.

In this context, plants show a great potential for use in the treatment of wounds, since a large number of species are used empirically as bases as described by Tsala et al. [4]. The therapeutic potential assigned to plants resides in the phytochemical constituents, which have the ability to produce definite physiological actions on living organisms [5].

The phytochemical constituents include various chemical families, such as alkaloids, flavonoids, tannins, terpenoids, saponins and phenolic compounds; besides the essential oils whose pharmacological effects include tissue repair from stimuli that act on contractile events of wound healing, angiogenesis stimulation, re-epithelialization, granulation tissue formation and remodelling of the extracellular matrix from the action on dermal fibroblasts and keratinocytes, among others [6,1]. Particularly for the species *P. juliflora*, Sing [7] reported the presence of tannin, phenolics, flavonoid, alkaloid, terpenes, steroids and saponin in the leaf, pod, flower, stem and root.

According to Tsala et al. [4], the healing potential of plants has been studied extensively in recent years, with the aim of finding substances capable of accelerating the healing process and reducing the complications that occur during this process. Studies also seek to develop alternative treatment options against synthetic therapeutic tools available for wound healing.

Continuing the investigation of the wound-healing activity of *Prosopis juliflora* Sw. DC [8], the aim of this study was to evaluate the wound-healing potential of an ointment formulated to a powder base obtained from the leaves of *P. juliflora* through histological and morphometric analysis of skin wounds and using the model for *in vivo* testing.

2. MATERIALS AND METHODS

This animal research experimental study was conducted in the Biotery and the Multidisciplinary Laboratory of Morphology and Pathology, Faculty of Biological and Health Sciences (FCBS) of Cesmac University Center (CESMAC), Maceió, Alagoas, Brazil after approval by the Ethics Committee that researched CESMAC under No. 067B/09-11 protocol. The study was in compliance with the ethical principles of animal testing recommendations of the Brazilian College of Animal Experimentation (COBEA).

The collection of leaves was performed on individual trees in urban areas in the city of Maceió, Alagoas, Brazil (9° 39′ 59″ S and 35° 44′ 6″ W). The plant species was confirmed by professionals in the Herbarium of the Institute of the Environment of Alagoas (IMA/AL) and a voucher specimen of the deposit was provided to record the species with the herbarium under No. IMAL-9638.

2.1 Preparation of the Ointment Formulation

The *P. juliflora* leaves were dehydrated in a forced-ventilation oven at 45°C, where they remained for three days. They were then promptly ground in an electric grinder and the resulting powder was placed in glass jars.

To prepare the ointment base, 30 g of leaf powder was mixed with 100 g of glycerine as described by Vitorino-Filho and collaborators [9].

2.2 Animals

Twenty-seven male Wistar rats (*Rattus norvegicus*), 90 days old and with an approximate weight of 250 g, were obtained from the Biotery of FCBS/CESMAC for use in this study. During the experiment, the animals were kept in cages under controlled temperature conditions with a well-defined night/day cycle and access to food and water *ad libitum*.

2.3 Experimental Study

The animals were divided into three groups. Group I (control, received a topical application of saline 0.9 %), Group II (control pattern, received a topical application of trade-based silver sulfadiazine 1% ointment) and Group III (experimental group, received topical application of the prosopis ointment). The animals were redistributed into subgroups: GI^{7}, GII^{7} and $GIII^{7}$ (animals treated during the seven-day interval, with three animals in each group); GI^{14}, GII^{14} and $GIII^{14}$ (animals treated during the 14-day interval, with three animals in each group) and GI^{21}, GII^{21} and $GIII^{21}$ (animals treated during the 21-day interval, with three animals in each group).

After the acclimation period of the animals, experimental wounds were made by a surgical method with anaesthesia by intramuscular injection, mixing xylazine (50 mg/kg) and ketamine (15 mg/kg) in accordance with the recommended dosage. After anaesthesia, the target areas were shaved and disinfected with povidone-iodine (PVPI). The demarcation area of the skin consisted of a sterile fenestrated field of 1 cm^2 and the excision of the skin flap around the area was marked with the aid of scissors and anatomical forceps. Subsequent to surgery, skin flaws were treated daily by the treatments described in the corresponding periods.

During the postoperative (PO) periods, daily microscopic evaluations of the wounds were made with the occurrence of hyperaemia, exudation and crusting. The morphometric analysis of the wounds was made with the aid of a calliper, using the equation $\mathbf{A=\pi.R.r}$ (where A is the area value calculated in mm^2, R corresponds to half the largest diameter of the wound and r is half of the smaller diameter of the wound) for the size of the area of each wound to monitor the healing process.

After the seventh, 14^{th} and 21^{st} postoperative day tissue samples in the areas of the lesion and surrounding areas were surgically removed in all the animals. The tissue samples were fixed in 10% formalin for 24 hours and then subjected to standard histological techniques [8] for microscopic analysis using an Olympus® optical microscope. This analysis was performed according to the degree of re-epithelialization for the histological findings proposed by Garros et al. [10], which were grouped according to three qualitative parameters: no change, slight alterations and intense modifications.

2.4 Statistical Analysis

Data were analysed by Bioestat version 5.0. The wound area (mm^2) was assessed by Student's t-test and the comparison of means was assessed by Tukey's test with a null significance level of 5% ($p<0.05$).

3. RESULTS AND DISCUSSION

After the surgery, there was no record of complications among the animals. They showed normal behaviour for all physical activities, daily mobility, and food intake to provide sufficient recovery.

The macroscopic evaluation of the effects of the ointment formulated with the powdered leaves of *P. juliflora* with different administration periods in the topical treatment of skin wounds demonstrated a remarkable improvement on the incision of wounds as compared to other groups (Fig. 1) and observed statistically significant differences in wound contraction between the groups I and III (Table 1). The results are in line with those of previous tests with the same species that were conducted by the research group, which indicates a satisfactory healing potential of the species *P. juliflora* [8].

According to Pereira and Bartolo [11], using healing agents that enhance tissue repair or act as an antiseptic agent is favourable for the healing process once the process of wound exudate is normal. However, when persistent it generates crust disintegration and promotes the growth of microorganisms between the crust and the granulation tissue and indicates problems with healing [12].

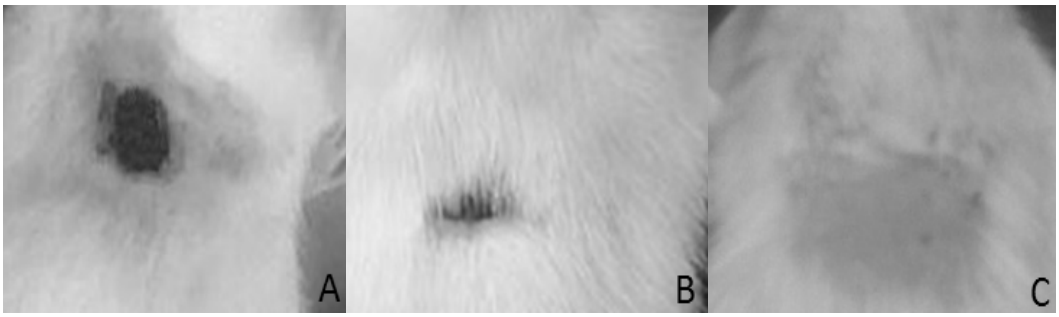

Fig. 1. Macroscopic evaluation of wounds treated with an ointment formulated with powdered leaves of *P. juliflora* in different periods of time. (A) 7th day, (B) 14th day and (C) 21st day

Table 1. Morphometric data of the averages of the wound areas among groups treated with solution saline (GI), treated with Kollagenase ® (GII) and treated with ointment of prosopis (GIII), at 7th, 14th, 21st and days postoperatively (PO)

Groups	7th day	14th day	21st day
GI	0,53±1,01	0,51±0,83	0,39±1,06
GII	0,34±1,78	0,26±0,97*	0,19±0,76*
GIII	0,36±1,06	0,24±1,02*	0,18±1,09*

** Groups tagged with asterisks indicate a statistically significant difference compared with the group treated with saline 0.9 % (p<0.05).*

Natural products have been identified as effective wound healing agents and several studies have shown satisfactory results for crude extracts and isolated compounds derived from plants. Meenakshi and collaborators [13] reported healing activity for the ethanol extracts of *Plagiochasma appendiculatum*. Upadhyay et al. [14] discussed the wound healing potential of the aqueous leaf extract of *Cleome viscous*. Clericuzio et al. [15] tested the wound-healing potential of a galactosyldiacylglycerol compound, a mixture of 1.2-di-*O*-linolenoyl-3-*O*-β-*D*-galactopyranosyl-glycerol, isolated from *Ophioglossum vulgatum* L., and achieved significant results in *in vivo* assays.

Regarding the healing potential of the *Prosopis* genus, the few records available point to the healing potential of aqueous extracts from the bark of the *Prosopis african* stem in animal studies [16] and particularly the species *P. juliflora.* Costa & Cavalcante [8] noted a gradual healing, a satisfactory re-epithelialization and a stable behaviour of skin wounds when treated daily with a topical application of aqueous extracts of the leaves and stem bark.

In this study, we observed a satisfactory evolution of skin wounds, delicate crust exudation and draining being recorded from the second postoperative day. By traversing the different periods of PO, it was possible to observe the epithelialization from the wound margins in the seventh postoperative day and re-epithelialization on the 14th postoperative day. According to Pereira and Bartolo [11], this process is important for wound closure.

When we compared the level of epithelialization among the groups, we observed the superiority of GIII as compared to GI. GI epithelialization was absent on the seventh postoperative day, while in GIII this epithelialization was already performing with discrete characteristics, considering the assessment level proposed by Garros et al. [10] that indicated that by the seventh postoperative day the healing process in GIII animals was accelerated compared to GI (Fig. 2). On the 21st postoperative day, GIII showed a marked degree of epithelialization, which characterized the terminal stage of the healing process with the connective tissue covering the tissue injury, as stated by Thakur et al. [6].

Importantly, the use of saline 0.9% did not positively influence the healing process, unlike the ointment based on silver sulfadiazine 1%, standard control, and leaves of the ointment-based *P. juliflora*.

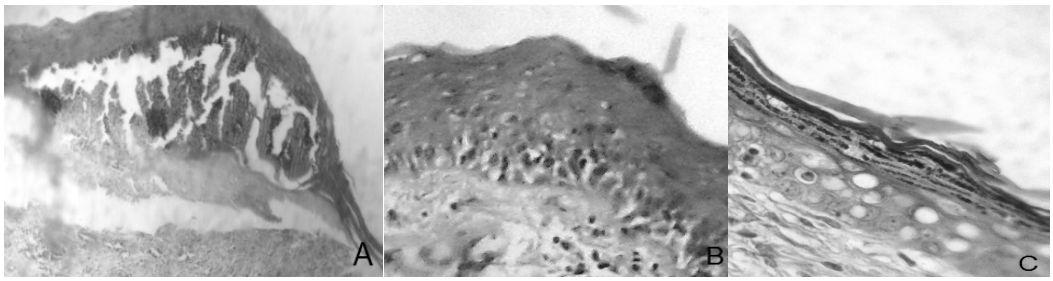

Fig. 2. Histopathological evaluation of wound healing and epidermal/dermal remodelling in the group treated with ointment formulated from the powdered leaves of *P. juliflora* at different periods. (A) 7th day, (B) 14th day and (C) 21st day

The phytochemical analysis of the extracts of *P. juliflora* reported by Sukirtha and Growther [17] showed the presence of alkaloids, flavonoids, saponin steroids, glycosides, phenolics and tannins.

The antimicrobial activity presented by *P. juliflora* is related to the presence of the alkaloids juliprosinene and juliflorinine isolated from the leaves [18], as well as the antifungal activity of these compounds in the alkaloidal fraction of the ethanolic extract of the leaves [19] and wound healing process in diabetic patients [20]. As such, the wound healing process reported in this study may be attributed to the presence of these same compounds.

4. CONCLUSION

The topical application of ointment-based, *P. juliflora*-leaves powder enabled a gradual healing and stable behaviour of wounds in the study conducted with animals, with a satisfactory level of repeating the study.

It is necessary to conduct subsequent investigation in order to determine sensitive bacteria in the ointment form of *P. juliflora.*

It is necessary to expand experimental trials with the products derived from this species on animals to assess their applicability to humans.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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