



Research Article

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Study of the topical use of *Hyptis pectinata* extract (sambacaita) in wound healing in rats

Talita Lúcio Chaves Vasconcelos¹, Fabiani Tenório Xavier Povoas¹, Thais Honório Lins Bernardo¹, Patrícia de Albuquerque Sarmiento¹, Regina Célia Sales Santos Veríssimo¹, Ricardo Luiz Simões Houly², Círia Vieira Barbosa¹, Eliane Aparecida Campesatto³ and Maria Lysete de Assis Bastos¹

¹Escola de Enfermagem e Farmácia, Universidade Federal de Alagoas. Av. Lourival Melo Mota, s/n, Tabuleiro dos Martins, Maceió/Alagoas, Brasil

²Faculdade de Medicina, Universidade Federal de Alagoas. Av. Lourival Melo Mota, s/n, Tabuleiro dos Martins, Maceió/Alagoas, Brasil

³Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas. Av. Lourival Melo Mota, s/n, Tabuleiro dos Martins, Maceió/Alagoas, Brasil

ABSTRACT

This study aimed to evaluate the healing action of the ethanol extract of Hyptis pectinata leaves in induced clean wounds in rats. It is a preclinical experimental study approved by the Ethics Committee on the use of animals at the Federal University of Alagoas, under No. 058/2014. After dried, the H. pectinata leaves have undergone the process of extraction by maceration in ethanol to obtain the ethanol extract. We used 27 Wistar rats divided into three groups: experimental group (sambacaita ointment 5%), positive control (dexpanthenol ointment 5%) and negative control (nonionic ointment base), all subject to excisional lesions in the middle of the back area. The rats were treated daily for 14 days and the statistical analysis was performed using the Graph Pad Prism System® 6. During the experiments, the animals were observed for the clinical parameters weight, temperature and macroscopic aspects of lesions and their diameter. The mean weight at the postoperative day (POD) was 198.9 ± 1.4 g, reaching 175.2 ± 4.06 g on the 14th POD. It was possible to see an increase in the lesion area on the 3rd POD, followed by a decrease in subsequent days in all groups studied. The value of the body temperature of the animals ranged between 35.2 and 37.5° C. The macroscopic evaluation identified the presence of signs of inflammation, as perilesional blush and exudate in all groups on the 3rd POD and only in PC and NC groups in the 7th POD, not being observed in the following days. During this period, histological specimens were collected for evaluation of the healing process. The ethanol extract did not show better results in reducing the diameter of the wound when compared to the positive control group. There were no significant changes between the groups in histological findings of the wounds. The ethanol extract of H. pectinata showed no healing activity when compared to the positive control group.

Keywords: Medicinal plants; Healing; Nursing.

INTRODUCTION

The use of medicinal plants for the treatment of wounds is mentioned since prehistoric times, when plants and plants and extracts were used as poultices, for the purpose of stopping bleeding and promote healing [1].

Currently, studies have been developed in the pursuit of discovering active compounds isolated from plants, that have effective role in the healing process, and there are products available on the market derived from plants for medicinal purposes in wounds [2,3].

The species *H. pectinata*, popularly known as sambacaita, is widely used in traditional medicine by the local population for the treatment of conditions such as rhinopharyngitis, nasal congestion, skin disorders, gastric disorders, fever and counter infections caused by bacteria and fungi [4]. It is one of the most commercialized plants for medicinal purposes in a county in the state of Alagoas[5]. Its leaves and husks are used in infusion for the treatment of skin inflammations and bacterial infections [6].

As for its healing action, there are no studies to prove it. However, a study of the species *Hyptis suaveolens*, belonging to the same genus and family, showed that such species promoted a greater re-epithelialization of wounds produced in rats [7].

Despite the technological advances, the failure in preventing injuries and their treatment is still an interdisciplinary challenge that should not be underestimated [8]. Since its inception as a profession, wound care is part of nursing care. There is a large number of published studies on this subject, which shows the important role and responsibility of nurses in this process [9,10].

In wound care, it is essential for nurses to seek new ways to care, based on individual and subjective reality of each carrier, and to be attentive to the innovations of care in this area, aiming at improving the quality of care [11]. In this context, the use of medicinal plants in wounds is a tool for nursing in the discovery of new technologies for the care of wounds.

In view of this, the present study aims to evaluate the healing action of the ethanol extract of *H. pectinata* leaves on induced clean wounds in rats.

EXPERIMENTAL SESSION

Plant and preparation of extracts

Hyptis pectinata leaves were collected at the Arboretum of the Federal University of Alagoas - UFAL (Geographic coordinates S9° 33' 11,9"W35°46'11,81"), on 09.25.2014, at 9:30 am. A sample of the material collected was sent to the Herbarium of the Environment Institute of Alagoas (IMA-AL), in which the exsiccate is deposited under the MAC No. 23601.

The leaves were placed for drying in the shade with circulating air for 30 days until it was possible to identify that they were dry. Later, they were crushed and subjected to maceration with Ethyl Ethanol (C₂H₅OH) 97%. Filtering was conducted every 72 hours until the exhaustion of the extraction. In each filtration, the extract solution was concentrated on a rotary evaporator at 40 ° C and kept in a desiccator with silica gel for evaporation of the residual solvent and to obtain the ethanol extract of the *H. pectinata* leaves.

In vivo healing assay

The design of this study was submitted to the Ethics Committee on Use of Animals of the Federal University of Alagoas and approved under No. 058/2014.

We used 27 adult rats (*Ratusnor vegicusalbinus* - Wistar), females, weighing between 160 and 210 grams. The animals were under observation for 21 days before the bioassay to verify the clinical conditions and identify variables that could influence the results of the experiment. Later, they were weighed and separated by the probabilistic method of random choices into 3 groups (n = 9) and identified from the therapy: Positive Control (PC); Negative Control (NC); Experimental T (ET). The animals were kept individually in plastic cages covered with sawdust in photoperiod of 12 hours of light and dark, minimum noise and room temperature 21 ± 1° C, maintained by air conditioning. They were fed with commercial food (Nuvilab®) and water "*ad libitum*".

Each animal underwent examination of body weight for anesthesia calculation. The anesthesia was performed by intraperitoneal injection with 50 mg / kg ketamine 10% and 10 mg / kg xylazine 2%, by administering 0.1 ml per 100 g of body weight of the animal.

Then, we proceeded to check the rectal temperature, the back hair removal and skin antisepsis with chlorhexidine degerming 2%. In the depilated area, it was held an excise lesion with a punch No. 12 (12 mm diameter) in the animal's back from the dorsal midline to the level of the aponeurotic tissue. Then, the injuries were cleaned with saline solution 0.9% and covered with sterile gauze and bandages, waiting up to 24 hours to initiate the therapy. After 24 hours, it was initiated the therapy in groups daily until the 14th POD.

Ointments used in this experiment were derived from a non-ionic base without preservatives. For the PC, it was added dexpanthenol 5% to that base; for the ET, 5% of ethanol extract of *H. pectinata*. The NC was treated only with the non-ionic base. All wounds were treated daily during 14 days of experiment, cleaned with saline solution 0.9%, followed by the application of specific treatment of each group and covered with sterile dry gauze and crepe bandage.

In the 3rd, 7th, 11th and 14th POD, it was performed a macroscopic evaluation of the wound in all the animals. The clinical aspects, macroscopic examination of the wounds regarding the presence or absence of granulation tissue, inflammation, local bleeding, fibrin, exudate, crustal extension or necrotic tissue (total, partial or absent) and measurement of lesion size were observed. On the 3rd, 7th and 14th POD, three animals from each group (a total of 9 animals per day) were killed to remove the wound.

All material removed for microscopic examination (Table 1) was fixed with formalin solution at 10% and then subjected to histological procedure by staining with hematoxylin-eosin (HE).

Table 1 - Scores used to assess the histopathology

Variables	Absent	Present	Discreet	Moderate	Intense	Total factor
Crust	+1	+2	-	-	-	-1
Acuteinflammation	-	-	+3	+4	+5	-4
Collagenization	-	-	+3	+4	+5	+5
Fibroblastproliferation	-	-	+3	+4	+5	+5
Neovascularization	-	-	+3	+4	+5	+5
Reepithelization	-	-	+3	+4	+5	+5
Granulationtissue	+1	+2	-	-	-	+10
Collagenfibers	+1	+2	-	-	-	+10

The sum of these products corresponded to the total score for each animal, which was subsequently added to the scores of other animals in the group and the average was obtained. The higher the animal's score, the better is the healing process [12].

The intensity of the variables (+1 to +5) was multiplied by positive or negative factors (constituents of the seventh column of the table) based on their importance to healing. For the quantitative analysis of the results of histological evaluation, the scores were elected based on the literature.

Statistical tests

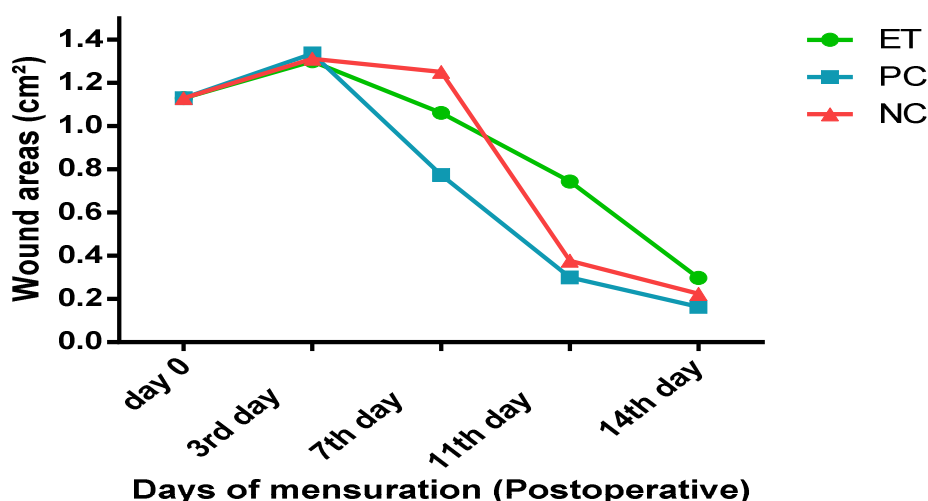
Statistical analysis was performed using the GraphPad PRISM ®6 system. Numerical variables were evaluated by analysis of variance (ANOVA) with a factor of interaction with each other, with Dunnett's post-test for biochemical testing of animals and Tukey's to test for potential healing effect for analysis between groups. The significance level was 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Macroscopic evaluation of lesions

During the experiments, the animals were observed for the clinical parameters weight and temperature. The diameter of wounds and the macroscopic aspects of the lesions were also observed. The mean weight at the POD was 198.9 ± 1.4 g, reaching 175.2 ± 4.06 g on the 14th POD. The value of the body temperature of the animals ranged between 35.2 and 37.5° C, and it was observed a slight increase in temperature of the animals in groups ET and NC at the 3rd POD. There was no statistically significant difference between the means of weight and temperature of the treated groups.

Measuring the size of the wounds was held on days 0, 3, 7, 11 and 14 after operation. The measurement was made with the aid of a caliper to calculate the area of lesions and observing the contraction of the edges. The average area of the wounds on their days of measurement is shown in Figure 1.

Figure 1. Average of the areas of animals' wounds in the *in vivo* healing test

Note: The data represent the mean \pm S.E.M. of the areas of the wounds of the groups ET, PC and NC by ANOVA (One-way ANOVA) followed by Tukey's test.

It was possible to see an increase in the lesion area on the 3rd POD, followed by its decrease in the following days. It is observed that the control groups PC and NC showed decrease in the sharper area when compared to the experimental group ET, with a statistically significant difference between the means of ET and PC groups on the 11th POD ($p = 0.0365$).

The initial increase in the wound area, observed on the 3rd POD, is an expected phenomenon and occurs as a result of the centrifugal retraction of the surrounding skin, for soon after the removal of a skin fragment there is a retraction of the wound margins resulting from the centrifugal action of the elastic skin fibers, which results in an immediate expansion of the injured area [13].

The wound contraction began around the 4th POD, in which the fibroblasts are differentiated into myofibroblasts, which contain an increased amount of actin and myosin filaments and have the ability to contract and expand, moving throughout the wound bed. This contractile ability of fibroblasts is responsible for closing wounds after injury. Thus, the contraction of wounds, which started around the 4th POD, occurs by the movement of existing tissue at the wound edge, and not due to tissue formation [14,15].

Study using *Hyptis suaveolens* cream, which is a plant belonging to the same genus of *H. pectinata*, in the treatment of cutaneous lesions in mice, corroborates the findings in this study. In that study, the wounds treated with *H. suaveolens* cream showed a larger area when compared to the positive control group, observing the late closing of the experimental group injuries [7].

The macroscopic evaluation identified the presence of signs of inflammation, as perilesional blush and exudate in all groups in the 3rd POD and only in PC and NC groups in the 7th POD, not being observed in the following days. Granulation tissue was present at the PC group, in the 3rd PDO, and 100% in the other groups from the 7th POD and persisted until the 14th POD. As for the presence of non-viable tissue, such as fibrin and crust, it was observed fibrin in all groups in the 3rd POD and in the 7th POD and only in ET and NG groups in the 11th POD and in the 14th POD. Crust was partially present in the wound bed only in the ET group, in the 3rd, 7th and 11th POD. This crust, around the 9th DPO, during the course of the healing, began to spontaneously detach from the wound bed.

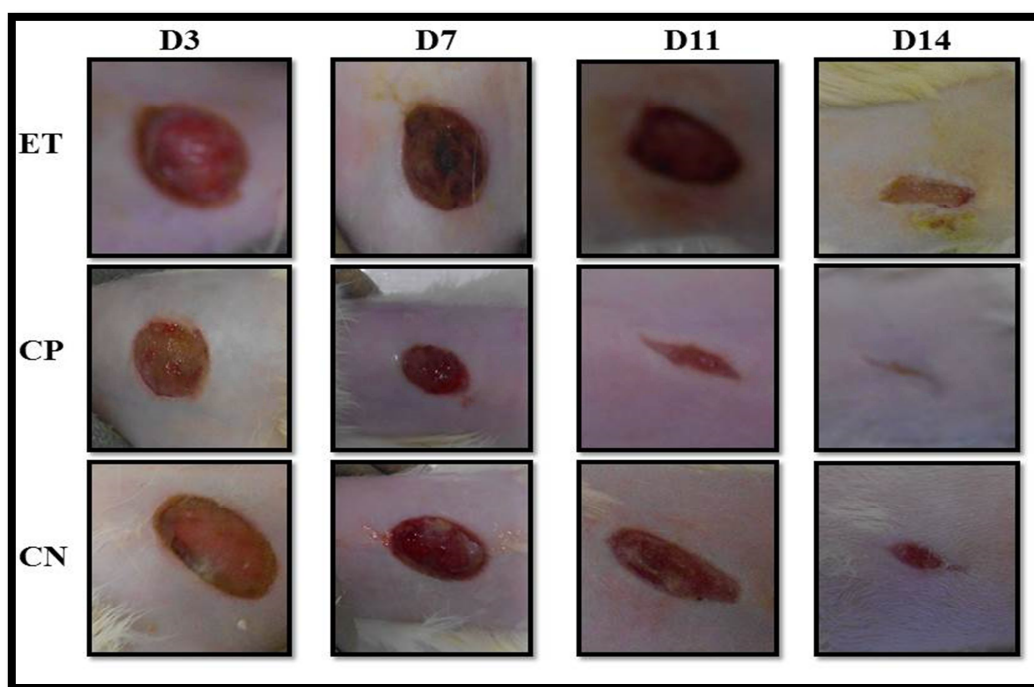
As seen in most groups, the inflammatory phase can last from 48 to 72 hours. At this stage, classical signals can be observed mediated by chemicals released by platelets and mast cells, causing vasodilation and increasing vessel permeability, which favors the migration of neutrophils and macrophages, which, in turn, act phagocytosing foreign bodies in the wound bed. These vascular changes are responsible for signs of inflammation: pain, redness, heat and swelling [16].

The observation of perilesional flushing and exudate in all rats in the 3rd POD is indicative of this expected inflammatory process, as described, for this stage of healing. The exudate is an inflammatory liquid with high protein concentration and large amount of cellular debris, and is expected in the inflammatory phase [17].

However, researchers did not expect to find perilesional flushing, exudation and inflammation in the 7th POD, since, according to the literature, this phenomenon is not present in the second phase of healing, called proliferative. The extension of fibroblasts inhibits inflammation and, consequently, the production of collagen, preventing epithelialization [18]. Thus, these findings in 16.7% of the PC group and 50% of perilesional flushing the NC group in the 7th DPO represent signals that do not match the healing period.

Figure 2 shows the progression of wound healing in the different treatment groups.

Figure2.The progression of healing of the lesions in accordance with the treatment used



Source: AUTHOR, 2015.

Each horizontal sequence of photos corresponds to the treatment used in the respective day of macroscopic evaluation (3rd, 7th, 11th and 14th POD). It is noteworthy the presence of fibrin and progressive reduction of the lesion in all groups, with better results of sharp edges contraction in the PC group.

Histopathological evaluation of lesions

The granulation tissue, as expected according to the literature, was observed in the groups ET, NC and PC since the 7th POD. This tissue is the result of growth of new vessels from the proliferation of preexisting vessels, adjacent to the wound edge. Also, it consists of macrophages and fibroblasts. Its formation is part of the last stage of the proliferative phase. Slowly, this tissue is enriched with collagen fibers, which begins to give the injured area the appearance of scar resulting from fibrous mass accumulation [19].

The fibrous tissue present in the ET and NC groups up to the 14thPOD and in the PC until the 7th POD is a devitalized yellowish tissue, with thin consistency, soft and that may or may not be firmly attached to the wound bed and edges, presented as cords or scabs. It consists of bacteria, fibrin, elastin, collagen, intact leukocytes, cell fragments, exudate and large quantities of DNA [17]. Its presence in the wound bed interferes with the healing process, and there is need to remove until the total exposure of healthy tissue so that the tissue repair occurs in the expected period [20].

About the crust observed only in the ET group, studies conducted to evaluate the plant healing activity also showed prevalence of crust in the group of animals treated with the plant extract formulations when compared to control groups. It is not known for sure whether the formation of this crust is bound to substances present in these plants [21,22].

Table 2 describes the results of the histological scores of the wounds, showing that the higher the animal's score, the better the healing process. One can see that the mean scores in the three days of biopsy and evaluation remained

similar between the groups, and, at histological level, there was not a statistically significant difference in the healing process between the groups ET, PC and NC.

Table 2. Histological study of wounds

POD	Scores			p <0.05*
	ET Group	PC Group	NC Group	
3rd	46	41	44.67	0.8458
7th	64	91	90	0.2939
14th	115.7	115.7	119	0.4921
Mean	75.23	82.57	84.56	0.8398

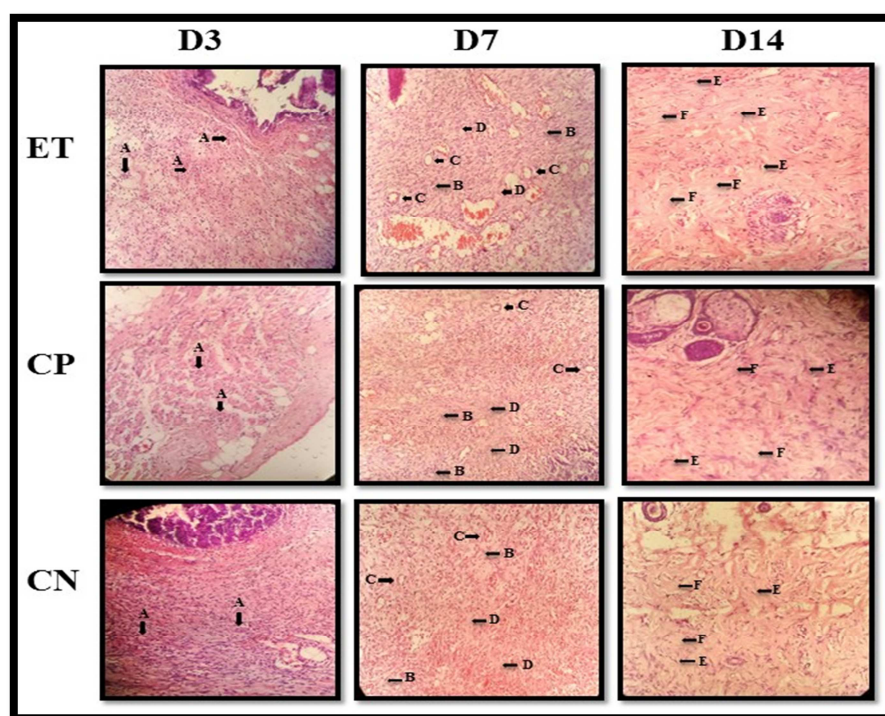
Source: AUTHOR, 2015

The histological analysis of wounds in the 3rd POD aimed at evaluating the first phase of the healing process, the inflammatory phase, identified the presence of moderate vascular proliferation in 66.7% of the animals ET group and discreet in 100% of animals of the groups PC and NC. Regarding the presence of mononuclear cells (monocytes) and polymorphonuclear cells (neutrophils), both were present discretely in 100% of the animals of the three groups.

The analysis of the proliferative phase in the 7th POD identified moderate presence of fibroblast proliferation in 66.7% of animals in the ET group and severe in 33.3% of PC and NC groups. It was also possible to observe moderate neovascularization in 33.3% of animals in the ET group, 100% of the PC group and 66.7% of the NC group. Granulation tissue was observed in 100% of the animals of the three groups. Collagen fibers were observed discretely in 33.3% of animals in the ET group and in 66.7% of the PC and NC groups. The evaluation of the remodeling phase at the 14th POD was able to identify the total re-epithelialization and the presence of collagen fibers in 100% of animals of the groups ET, PC and NC. Figure 3 depicts the histological progress of the wounds in all three groups in the respective evaluation days.

Figura 3. Histological progression of wound healing in accordance with the treatment used

D3: A- Inflammatory infiltrate, D7: Granulation tissue formed by B-fibroblasts and C – Newly formed vessels; D- Collagen fibers, D14: E- Fibroblasts; F – Collagen fibers. Hematoxylineosinstaining. 40x magnification.



Source: AUTHOR, 2015.

The findings of the microscopic analysis of animals' wounds of the groups ET, PC and NC corresponded to the expectations in every time and phase of wound healing, except for the presence of crust / necrosis in the 3rd and 7th POD in the animals of the ET group, with no significant difference between the groups. Study evaluating the healing potential of lectin obtained from seeds of plant species *Parkia pendula* also showed the crust formation in the wound bed, in the experimental group, until the 11th POD. This crust formation corroborates the present study, in which the

crust was also present in the ET group, only. The *P. pendula* contains tannins in its phytochemical constitution [22], similar to the plants of Lamiaceae family, from which *H. pectinata* is part [23].

Another study evaluating wound healing describes the high healing power of the plant *Biophytum sensitivum* and the absence of crusting, differing from the results found in this study [24].

These data indicate the ability of some plants to form crusts or otherwise, which are possibly formed by the presence of some secondary metabolites, mainly by the tannins, that have the capacity to develop hydrogen bonds and long lasting hydrophobic bonds with proteins, polysaccharides or both. The formation of the complex tannin-protein or tannin-polysaccharide is insoluble in water, thus forming a protective layer, called crust, on the wound. Below this layer, the healing process occurs naturally. This precipitation ability of the protein also favors hemostasis after injury [25].

CONCLUSION

The ethanolic extract of *H. pectinata* leaves was not able to promote contraction of the wounds edges faster when compared to the positive control and the negative control. Macroscopic evaluation of organs of animals showed no significant morphological changes, and there were no statistical differences in the histological evaluation of wounds. However, this negative result does not rule out the biological potential of plants as regards the healing action. It is suggested to carry out studies using other plant parts, such as stems or twigs, and also the exploration of their anti-inflammatory activity.

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