Assessment of the healing activity of jucá pods \textit{[Libidibia ferrea (Mart. ex Tul.) L. P. Queiroz]} in cutaneous lesions of rats

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ABSTRACT. The study aimed to evaluate the healing activity of the powdered pod of \textit{L. ferrea} in cutaneous wound healing in preclinical test in rats. Eighteen rats were divided into two groups: the positive control group (PCG) treated with Kollagenase® and the experimental group (EG) treated with an ointment prepared with the powder of \textit{Libidibia ferrea}. The lesions were clinically evaluated on 0 - 21st days, when histopathological analysis was also performed. In this study, the clinical analysis showed that although the rate of contraction of the lesions in EG was lower than in PCG, there was significant reduction in the wound of the group treated with ointment obtained from the powder of \textit{L. ferrea}. Furthermore, the morphometric data showed that from the 3rd to 21st day after operation, the EG presented significant reduction in the rate of contraction of the skin lesions. Histological analysis revealed that the clinical and histological parameters of EG were similar to PCG. Although the biological activity of the powder remains unclarified, our results clearly showed the wound healing with the use of the powder of the pod of \textit{Libidibia ferrea} in skin lesions. These finds provide subsidies for a similar research.

Keywords: medicinal plants, wound healing, healing activity.

Avaliação da atividade cicatrizante de vagem do jucá (\textit{Libidibia ferrea} (Mart. ex Tul.) L.P.Queiroz) em lesões cutâneas de ratos

RESUMO. O objetivo do estudo foi avaliar a atividade cicatrizante da pomada obtida com o pó da vagem da \textit{Libidibia ferrea} em lesões cutâneas em testes pré-clínicos em ratos. Dezoito ratos foram divididos em dois grupos; grupo controle positivo (GCP), tratado com Kollagenase® e grupo experimental (GE), tratado com o pó de \textit{Libidibia ferrea}. As lesões foram clinicamente avaliadas em 0 - 21º dias, quando a análise histopatológica também foi realizada. Neste estudo, a análise clínica mostrou que, embora a taxa de contração das lesões em GE foi menor do que no GCP, houve uma diminuição significativa na ferida do grupo tratado com a pomada do pó de \textit{L. ferrea}. Além disso, os dados morfométricos mostraram que nos dias 3 - 21º após a operação, o GE apresentou uma redução significativa na taxa de contração das lesões na pele. A análise histológica revelou também que os parâmetros clínicos e histológicos para GE foram semelhantes aos do GCP. Embora a atividade biológica do pó da vagem utilizado para a cicatrização de feridas permaneça sem esclarecimento, os nossos resultados mostram claramente a cicatrização em lesões cutâneas com o uso do pó da vagem de \textit{Libidibia ferrea}. Estes achados fornecem subsídios para outra pesquisa similar.

Palavras-chaves: plantas medicinais, cicatrização de feridas, atividade cicatrizante.

Introduction

Wound healing is a dynamic process involving a well-coordinated cascade of cellular and molecular events mediated by biochemical and physiological reactions that lead to repair and reconstitution of damaged tissue (Mandelbaum & Di Santis, 2003). Healing is part of the response to lesions and represents an attempt to maintain structure and function of normal cells following the inflammatory process (Rubin & Faber, 2002).

As far back as 3000 BC, man has learned to use various parts of plants such as willow, pine, cypress, and poppy mint, among others, to facilitate healing process (Gomes & Carvalho 2002). The therapeutic potential of medicinal plants and some of their constituents, such as flavonoids, alkaloids, triterpenes, sesquiterpenes, tannins, lignans, among others, has been the subject of continuous studies, highlighting their various pharmacological actions through preclinical tests on animals (Cechinel Filho & Yunes, 1998). Indeed, a good number of these
substances have been explored and successfully used as medicinal agents in addressing diverse health problems. Although many synthetic healing agents are commercially available in the market, a good number of them may be inaccessible to the public (Vitorino Filho et al., 2007).

Libidibia ferrea, popularly known in Brazil as 'jucá' or “pau-ferro”, belongs to the Leguminosa-Caesalpinoidae family and it is easily recognized because of the presence of pale spots on its trunk. Morphologically, it is characterized by small leaves, bearing yellow flowers (Lima, Almeida, Dantas, Silva, & Moraes, 2006). Its bark is widely used in folk medicine. Fruits, pods and roots of L. ferrea have been used for years, as infusions, to treat various inflammatory disorders, such as bruises, rheumatism, healing, lung hemoptysis (Braga, 1976; Pereira et al., 2012), fever (Correa, 1984), enterocolitis (Balbach, 1972) and diabetes (Ueda, Tachibana, Moriyasu, Kawanishi, & Alves, 2001). Furthermore, some research in the plant has shown that it has antibacterial and antifungal properties (Sampaio et al., 2009), antiulcer (Bacchi & Sertié, 1994; Bacchi, Sertié, Villa, & Katz, 1995). It has also been shown to possess anti-inflammatory as well as analgesic properties (Carvalho et al., 1996).

Although the L. ferrea powder has been reported to be widely used by Brazilian folk medicine for the treatment of skin wounds, there are few scientific reports in the literature to investigate the claim. The few studies regarding its healing property have been restricted to stem bark. Here, we report on the evaluation of the healing activity of the powdered pod of L. ferrea in cutaneous wound healing in preclinical test in rats.

Methodology

Clinical analysis

Eighteen (18) female albino Wistar rats (Rattus norvegicus), aged 90 days, with average weight of 162.35 ± 10.58 g were divided into two groups as follows: Positive Control Group (PCG), which was treated with ointment Kollagenase®, a commercially available healing balm, and the other was the Experimental Group (EG), which was treated with ointment prepared from the powder of the pods of L. ferrea and sterile petroleum jelly (Vaseline).

To obtain the powder, pods were dried in an oven at 50°C for two days, and then grinded with mortar and pestle. Test was conducted using a 1:1 w/w mixture of powder of the pod L. ferrea and sterile petroleum jelly (Vaseline).

The pods of the plant used in the study were collected from Industrial Avenue Gil Martins, in the city of Teresina - Piauí State; latitude 5° 6' 55.17" and longitude 42° 47' 42.60" and cataloged under Nº 28216 in the Herbarium Graziela Barroso of Universidade Federal do Piauí (UFPI) in Teresina – Piauí State, Brazil. They were stored in sterile plastic bags and transported to the Faculdade de Saúde, Ciências Humanas e Tecnológicas do Piauí - Novafapi.

All wounds were postoperatively evaluated for daily clinical condition. The lesions were photographed at a fixed height using a tripod stand and major and minor diameters were measured using caliper. To calculate the area of lesions, we used the equation 1:

\[ A = \pi.\text{Rr} \]  

where:

A represents the area expressed in cm²;
R, the larger radius and;
\( \text{r} \) the smaller radius of the lesion (Prata et al., 1988).

The degree of contraction expressed as a percentage was measured by the equation proposed by Ramsey et al. (1995) which is given by equation 2:

\[ 100 \times (W_0 - W_i) / W_0 = M \pm SD \]  

where:

\( W_0 \) = initial area of the wound;
\( W_i \) = area of the wound on days 3rd, 7th, 14th and 21st;
M = medium and
SD = standard deviation. The data obtained from the measurement of the diameter of the lesion was subjected to statistical analysis of variance (ANOVA) at a level of significance of p < 0.05.

**Histopathological analysis**

Water was placed on the slide and that on a hot plate (50°C). After cooling, the excess water was drained, when necessary a paper filter was used. With the aid of a brush, the cut was positioned on the blade and taken to oven drying (40°C) for 15 to 30 min. The HE staining method (Hematoxylin and Eosin) was used on the study. Blades were stained for 3 to 5 min with hematoxylin. Following, the slides were washed for one minute in running water and stained for 15 min in eosin. After dehydration they were mounted which is the final stage of histological technique consisting of gluing the coverslip on the cut with Canada Balsam, a resin which is soluble in xylene and insoluble in water. The coverslip prevents hydration on the cut by the ambient air humidity, thus allowing these blades to remain stable indefinitely.

The drop of oil was placed on the material and then it was covered, avoiding forming air bubbles. They were then taken to the greenhouse horizontally, where they remained for at least 48 hours (36°C) to dry the balm. Thus, the slides were ready for labeling and reading.

Slides were prepared and stained with hematoxylin-eosin (HE). Parameters considered during observation were fibroblast proliferation; tissue granulation, vascular proliferation; presence of mononuclear and polymorphonuclear cells; collagen deposition; re-epithelialization and formation of crust. The changes noticed were diagnosed, classified and quantified in numerical values and grouped according to the severity of the injury. Results were expressed in percentages.

The procedures followed the Ethical Principles in Animal Experimentation, recommended by the National Council (Conceia) and the Brazilian Law of Experimental Animals, Law No. 11.794, of October 8, 2008. The research project was approved by the Ethics Committee (CEP) of the Faculdade de Saúde, Ciências Humanas e Tecnológicas do Piauí - Novafapi under protocol number 0040/11.

**Results**

**Clinical analysis**

The mean and standard deviation of the injured area showed healing in all animals. However, it was observed that PCG presented greater reduction in the wound area (cm²) than EG. There was a statistically significant difference in lesion size amongst the groups. The EG presented less reduction on the lesions, which were 20 times greater than the PCG at day 21st (Table 1). However, there was a significant reduction in the wound in the group treated with ointment prepared with the powdered pod of *Libidibia ferrea*, which appeared to be favorable to the healing process leading to a reduction of the lesion area.

**Table 1. Mean and standard deviation of the lesion area per group and day.**

<table>
<thead>
<tr>
<th>Day</th>
<th>Lesion area (cm²)</th>
<th>Rate of contraction of the lesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group (PCG)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1,221</td>
<td>1,221, 0,108, 0</td>
</tr>
<tr>
<td>3rd*</td>
<td>0,81</td>
<td>0,155, 17,399, 4,418</td>
</tr>
<tr>
<td>7th*</td>
<td>0,585</td>
<td>0,183, 29,167, 8,228</td>
</tr>
<tr>
<td>14th*</td>
<td>0,046</td>
<td>0,072, 77,976, 11,471</td>
</tr>
<tr>
<td>21st*</td>
<td>0,001</td>
<td>0,002, 99,81, 0,467</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group (EG)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1,287</td>
<td>1,287, 0,08, 0</td>
</tr>
<tr>
<td>3rd*</td>
<td>0,975</td>
<td>0,131, 11,996, 5,746</td>
</tr>
<tr>
<td>7th*</td>
<td>0,833</td>
<td>0,098, 17,995, 5,526</td>
</tr>
<tr>
<td>14th*</td>
<td>0,141</td>
<td>0,037, 61,447, 3,5</td>
</tr>
<tr>
<td>21st*</td>
<td>0,02</td>
<td>0,008, 83,745, 2,889</td>
</tr>
</tbody>
</table>

Statistical analysis showed significant difference in the degree of contraction of injury amongst groups (Table 1), where PCG showed higher wound healing than the EG. Wound contraction is a process by which the edges of a wound tend to shrink as a result of centripetal movement of intact skin adjacent to the center of the lesion. This phenomenon is important in wound healing and leads to reduction in size of wound area (Coelho, Rezende, Nunes, & Simões, 1999).

In our morphometric data showed that on the 3rd, 7th, 14th and 21st day after operation, EG presented significant reduction in the rate of contraction of the lesions on the skin. This is in spite of the fact that there was lower retraction of the lesion compared to the PCG as presented in the Figure 1, which makes it favorable to process healing (Figure 1 and Table 1).
Figure 1. Evolution of healing activity. Development of healing activity using Positive Control Group, treated with ointment Kollagenase® and Experimental Group treated with an ointment prepared from the powder of the pods of *Libidibia ferrea* (Mart. Ex Tul.) L. P. Queiroz with ointment formulation (50 g pod powder *L. ferrea* + 50 g sterile Vaseline).

**Histological analysis**

Just PCG group showed a uniformity regarding histological parameters. The EG showed several experimental histological parameters (Table 2).

Table 2. Percentage of histological findings by group at 21st day.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Positive Control Group (%)</th>
<th>Experimental Group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast</td>
<td>Abs</td>
<td>Pres</td>
</tr>
<tr>
<td>Proliferation</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Granulation Tissue</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Vascular Proliferation</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Mononuclear Cells</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Collagenization</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Reepithelialization</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Crust</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend: Legend: Abs = absent; Pres = present; Lig = light; Mod = Moderate; Int = Intense.

In the microscopic analysis, we observed that EG presented more intense inflammatory response than PCG, as evidenced by increased number of mononuclear and polymorphonuclear cells. On the other hand, the parameters that characterize the healing such as epithelialization, fibroblast proliferation and collagen appeared in smaller degree in EG, as can be seen in Figure 2.

**Figure 2.** Histological analysis of injured tissues at 21st day. A: Positive Control Group; B: Experimental Group.

**Discussion**

Plants have been used by man for thousands years and still have their value not only in traditional communities but are also objects of interdisciplinary studies in the search for new drugs. Although there is large number of products available for the treatment of wounds, the need to expand available therapeutic arsenals of topical substances requires further search for alternatives (Macedo, Carvalho, & Nogueira, 2002; Ferreira, Tuma Junior, Carvalho, & Kamamoto, 2006).

In this work, the wound healing activity of ointment prepared from the powder of the pod of *Libidibia ferrea* on cutaneous lesions of rats was evaluated. The results showed that the treatment of PCG with Kollagenase® was efficient, because the group presented a greater reduction in wound area. However, we also observed that treatment with ointment prepared from the powdered pod of *Jucá* presented beneficial effects in wound healing of the rats. Furthermore, there was a significant injury reduction in the group treated with the plant, which favored the healing process and led to reduction in lesion area as shown in Table 1, 2 and Figure 1. Although the injury reduction with the powdered pod of Jucá was less than PCG, we suggested that EG reveal a significant reduction in the wound, in
which appeared to be favorable to process healing leading to reduction of lesion area.

In this regard, *Libidibia ferrea* presents one of such alternatives for being a plant widely used in folk medicine with numerous therapeutic properties. Notably, its stem bark has been used in the treatment of wounds, bruises, asthma, chronic cough (Braga, 1976). It is still used as a decongestant, for the treatment of rheumatism and against diabetes (Gomes & Carvalho, 2002), showing yet possible benefits in the cardiovascular system of the users.

We found in studies using the pod *L. ferrea* as anti-bacterial activity (Sampaio et al., 2009), (Sudhakar et al., 2006), anti-inflammatory action (Lima et al., 2006), (Pereira et al., 2012), (Carvalho et al., 1996) and anticancer activities were reported (Nakamura et al., 2002). Other studies using goats, which showed the efficiency of ointment with powder stem of *L. ferrea* in cutaneous wound repair (Sudhakar et al., 2006).

Macroscopic evaluation was performed on the tissues of lesion to verify wound contraction regarding measurements performed on the day of surgery. This was necessary, since the contraction is important for the centripetal movement of the edges around the central region of the wound, which forms part of the mechanism of healing (Mandelbaum & Di Santis, 2003). The macroscopic observation revealed that the wounds of animals treated with the powder of the pod *L. ferrea* (Figure 1) showed less uniform results when compared with the group treated with Kollagenase® (PCG). However, the EG exhibited advances in the wound treatment, since the treatment of 0 day up 21 days almost covered the entire lesion area as shown in Figure 1 and in Table 1 and 2. This suggests that ointment from the powder of the *L. ferrea* was useful in healing the studied tissues.

Following wound, healing involves a complex sequence of physiological events which results in the release of local and systemic factors, among which we can mention nutrition (especially vitamin C), tissue necrosis, deficiency of irrigation, mineral deficiency, anti-inflammatory agents, diabetes, arteriosclerosis, ischemia, lymphopathy, etc. These events involve migration, proliferation and differentiation of cells and collagen formation to aid the process of tissue regeneration (Muscara et al., 2000).

In microscopic analysis we observed that on day 3 following operation, wounds in EG had dried up, presenting no exudates and bearing regular and thick crust containing many mononuclear red blood cells and fibrin, in the form of blood clot around the edges of the wound. According Mandelbaum and Di Santis (2003) these crusts hinder the healing process. After the 14th day, there was distinct thickening of the crust. The crusting on microscopic evaluation, in turn, showed a difference between groups, where animals in EG had higher number (66.6%).

With respect to collagen, we observed intense deposition of the molecule in PCG. According to Mendonça and Coutinho-Netto (2009) this phase of healing process presents cellular attempt of recovery of the structure and is characterized by normal tissue maturation and changes in extracellular matrices of proteoglycan and collagen. In our results, there was evidence of granular tissue formation, proliferation of vascular tissues and mononuclear cells in all groups. However, in the PCG, these changes appeared more uniform.

In microscopic analysis, we observed that animals in EG presented a more intense inflammatory process than PCG as evidenced by the greater number of mononuclear cells. In the EG, higher number of polymorphonuclear cells was observed, which was absent in positive control group. According Biasi et al. (2003) and Arnhold (2004), polymorphonuclear leukocytes are the first cells that are activated in host immune defense against infection. These cells migrate and infiltrate into the inflammatory site by chemotactic gradients, which together with macrophages, phagocytize and destroy the causative agent of inflammation. Furthermore, there was a complete reepithelization in positive control group (100%), which was lower in the experimental group (66.6%).

In all slides observed, hemosiderin was present but appeared to be more intense in the experimental group. According Halliwell and Gutteridge (1990) lesions associated with bleeding tissue may also release hemoglobin (Hb) and iron, favoring reactions of reduction when iron is released from Hb. The hemoglobin resulting from digestion of erythrocytes unfolds to release heme and globin. The globin fraction is broken down into its constituent amino acids which are released into the bloodstream while molecules of iron, heme fraction, within macrophages, are grouped together to form the so-called hemosiderin pigment (Alencar, Kohayagawa, & Campos, 2002).

Research on the use of stem of *L. ferrea* in skin lesions using stem bark extract showed the acceleration of healing process. However, there was no sufficient evidence to prove the healing activity of the ointment from the powder of the pod *Libidibia ferrea* in skin lesions since the lesions in the EG had a lower rate of contraction compared with PCG.
However, there was significant retraction in the lesion caused on the experimental animals, which favored the healing process and reduction of lesion area. Thus, the findings on this study indicate an important healing activity of the ointment from the powder of the pod *Libidibia ferrea* when applied in rats showing the acceleration of healing process. Therefore, further research is required to identify and study its other active components with possible medicinal values, such as chemical profiling and mechanisms of actions with the biological activity that remain unclarified.

**Conclusion**

Although we could not find enough evidence to prove the efficiency of healing ointment from the powder of the pod of *Libidibia ferrea* in skin lesions, the lesions GE presented clear lower rate of contraction when compared with the GCP.

In spite of the fact that *Libidibia ferrea* revealed wound healing activity, showing a significant retraction of injury in the lesion caused on the experimental animals, thus favoring the healing process, further research is required to identify and study their bioactive components in the plant.

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**References**


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