Effects of Fitoscar™ (dry extract of Stryphnodendron adstringens 50%) on the healing of acute wounds in Wistar rats

Efeitos do Fitoscar™ (extrato seco de Stryphnodendron adstringens 50%) na cicatrização de feridas agudas em ratos Wistar

Efectos de Fitoscar™ (extracto seco de Stryphnodendron adstringens 50%) sobre la cicatrización de heridas agudas en ratas Wistar

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ABSTRACT
Several studies show the potential of Stryphnodendron adstringens (Mart.) Coville bark preparations in wound healing. A medicine called Fitoscar™ is based on this herbal medicine, which is commercially available and facilitates its acquisition by the patient. However, studies with Fitoscar™ are necessary to determine its effects during the healing processes. The aim of this study was to evaluate the effects of Fitoscar™ on different wound healing events induced in Wistar rats. Excisional wounds were experimentally induced in Wistar rats, divided into two groups: control: wound cleaning with warm 0.9% saline solution (39° to 40°C) and treated with Fitoscar™, subdivided into 04 subgroups, corresponding to 3, 7, 14 and 21 days of experimental period. Morphometric, macroscopic and microscopic analyzes were performed. Fitoscar™ influenced fibroblast migration and stimulated angiogenesis at days 3 and 7 respectively. While there was greater intensity of miofibroblasts at day 14. At 21 days, Fitoscar™ induced more crust and hypergranulation. While Fitoscar™ group presented lower percentage of wound closure on days 7 and 14, however all wounds healed completely in a similar time, 21 days. Fitoscar™ may favor wound healing, leading to fibroblast migration and angiogenesis during the inflammatory and proliferative phases in acute experimental wounds.

Keywords: healing, herbal medicines, nursing wounds, wound closure techniques.

RESUMO
Vários estudos mostram o potencial das preparações de casca de Stryphnodendron adstringens (Mart.) Coville na cicatrização de feridas. É baseado neste medicamento fitoterápico um medicamento chamado Fitoscar™, que está disponível comercialmente e facilita sua aquisição pelo paciente. Porém, estudos com Fitoscar™ são necessários para determinar seus efeitos durante os processos de cicatrização. O objetivo deste estudo foi avaliar os efeitos do Fitoscar™ em diferentes eventos de cicatrização de feridas induzidos em ratos Wistar. As feridas excisionais foram induzidas experimentalmente em ratos Wistar, divididos em dois grupos: controle: limpeza das feridas com solução salina 0,9% morna (39° a 40°C) e tratadas com Fitoscar™, subdivididos em 04 subgrupos, correspondentes a 3, 7, 14 e 21 dias de período experimental. Foram realizadas análises morfométricas, macroscópicas e microscópicas. Fitoscar™ influenciou a migração de fibroblastos e estimulou a angiogênese nos dias 3 e 7, respectivamente. Enquanto houve maior intensidade de miofibroblastos no dia 14. Aos 21 dias, Fitoscar™ induziu mais crosta e
hipergranulação. En quanto o grupo Fitoscar™ apresentou menor percentual de fechamento da ferida nos dias 7 e 14, porém todas as feridas cicatrizaram completamente em tempo semelhante, 21 dias. Fitoscar™ pode favorecer a cicatrização de feridas, levando à migração de fibroblastos e angiogênese durante as fases inflamatória e proliferativa em feridas experimentais agudas.

Palavras-chave: cicatrização, fitoterápicos, tratamento de feridas, técnicas de fechamento de feridas.

RESUMEN

Varios estudios muestran el potencial de las preparaciones de corteza de Stryphnodendron adstringens (Mart.) Coville en la cicatrización de heridas. A base de esta medicina herbaria se encuentra un medicamento llamado Fitoscar™, el cual está disponible comercialmente y facilita su adquisición por parte del paciente. Sin embargo, son necesarios estudios con Fitoscar™ para determinar sus efectos durante los procesos de cicatrización. El objetivo de este estudio fue evaluar los efectos de Fitoscar™ en diferentes eventos de cicatrización de heridas inducidos en ratas Wistar. Se indujeron experimentalmente heridas escisionales en ratas Wistar, divididas en dos grupos: control: limpieza de la herida con solución salina tibia al 0,9% (39° a 40°C) y tratadas con Fitoscar™, subdivididas en 04 subgrupos, correspondientes a 3, 7, 14, y 21 días de período experimental. Se realizaron análisis morfométricos, macroscópicos y microscópicos. Fitoscar™ influyó en la migración de fibroblastos y estimuló la angiogénesis en los días 3 y 7 respectivamente. Mientras que hubo mayor intensidad de miofibroblastos el día 14. A los 21 días, Fitoscar™ indujo más costra e hipergranulación. Mientras que el grupo Fitoscar™ presentó menor porcentaje de cierre de heridas los días 7 y 14, sin embargo todas las heridas sanaron completamente en un tiempo similar, 21 días. Fitoscar™ puede favorecer la cicatrización de heridas, lo que lleva a la migración de fibroblastos y a la angiogénesis durante las fases inflamatorias y proliferativas en heridas experimentales agudas.

Palabras clave: cicatrización, medicinas herbarias, enfermería de heridas, técnicas de cierre de heridas.

1 INTRODUCTION

Wound healing is a physiological reaction of injured tissues and aims to recover skin integrity (Eming et al. 2014; Sorg et al. 2017). The wound healing process is divided into three phases: inflammatory, proliferative and tissue remodeling (Majewska; Gendaszewska-Darmach, 2011). This whole process can be optimized with proper wound care such as with the aid of adequate dressings, a complex procedure that involves cleaning and applying a product or dressing to accelerate the physiological healing process (Skórkowska-Telichowska et al. 2013).

Phytotherapy is one of the predominant integrative and complementary practices in health (PICS) used in the care of patients with wounds, being encouraged by the Brazilian Unified
Health System (SUS) and by the National Program of Medicinal and Phytotherapeutic Plants that support the use of natural resources as a way to obtain active compounds for the treatment of diseases, and in the context of wounds as an intervention with a curative purpose (Lemos et al. 2018). A medicinal plant that has gained prominence is *Stryphnodendron adstringens* (Mart.) Coville; a plant species native to Brazil, commonly known as barbatimão, belonging to the *Fabaceae* family (Farmacopeia brasileira, 2010).

Traditionally *Stryphnodendron adstringens* bark has been used as astringent and has been reported to present healing properties. Its use has been documented since the Brazilian colonization due to its use by indigenous and was intensified in the XX century. Its healing properties have been recognized by August Saint-Hilaire, a French naturalist (Albuquerque et al. 2007; Brandao et al. 2012; Ricardo et al. 2018). Other species from the same genus, *S. rotundifolium* Mart., Fabaceae, has been used to treat allergic reactions which is an effect clearly related to the anti-inflammatory properties of these plants (Oliveira et al. 2014). The Brazilian Pharmacopoeia states that the drug derived from *S. adstringens* has at least 8% of total tannins (Farmacopeia brasileira 2010; Pellenz et al. 2019). It is also part of the National List of Medicinal Plants of Interest to SUS (Renisus) (Brazil, 2013). The wound healing properties of *S. adstringens* are probably related to its chemical matrix which is rich in bioactive molecules such as tannins, mucilage, flavonoids and saponins (Pellenz et al. 2019).

This medicinal plant has been used as a healing agent in different types of lesions, in topical treatment and to alleviate dermatological problems, it has angiogenic activity and has the property of stimulating the proliferation of keratinocytes facilitating the re-epithelialization of the wound (Pinto et al. 2015; Chaves et al. 2016; Rodrigues et al. 2019; Alves et al. 2022). Anti-inflammatory and vasoconstrictor action has also been demonstrated in the microenvironment of wounds in Wistar rats followed during a 10 days experimental period (Hernandes et al. 2010).

Other studies also show as *S. adstringens* effects antibacterial and antifungal action, and stimulus in the production of collagen fibers during the wound healing which are beneficial effects throughout the repair process (Audi et al. 2004; Ishida et al. 2009; Pinto et al. 2015).

This herbal medicine produces several secondary metabolites to which its therapeutic effects and success in topical therapies aimed at wound healing are attributed, including flavonoids, terpenes, steroids, alkaloids, saponins and the main one is the tannins that attribute therapeutic value to this plant (Fiori et al. 2013; Nascimento et al. 2013).
A herbal medicine produced with 50% of the dry extract of *S. adstringens* bark, in which there is 60 mg of the extract corresponding to 30 mg of total phenols and 27 mg of total tannins is commercially available as Fitoscar™ (*Stryphnodendron adstringens*, 5% dry extract, produced by Apsen Pharmaceuticals and approved by Anvisa (National Health Surveillance Agency) (Apsen, 2017). This medicine is presented in the form of an ointment and according to the manufacturer the product has a healing, anti-inflammatory and antimicrobial action (Apsen, 2017). Despite several studies carried out showing the potential of this herbal medicine in healing, there are still important gaps to be clarified about its ethnotherapeutic potential (Chaves et al. 2016; Hernandes et al. 2010; Ishida et al. 2009; Pinto et al. 2015; Rodrigues et al. 2019).

Fitoscar™ has already been evaluated in other studies, but with methodological weaknesses that limit the complete analysis of its effects, such as monitoring the wounds only up to the 10th day after the injury induction, or the absence of a control group, without an active ingredient like saline solution (Ballaben et al. 2013). Vieira et al. (2015) tested Fitoscar™ on wounds on the back of rabbits, demonstrating the course of healing, however, only up to 14 days were evaluated, and daily cleaning and occlusion were not mentioned, in addition collagen and myofibroblasts were not quantified (Vieira et al. 2015). Also, in another study by the same group, they followed the course of wound healing in rabbits only up to 14 days without evaluating the outcome of wound closure (complete healing) (Vieira et al. 2019). These studies were also carried out without occluding the wounds, a practice considered the gold standard in the treatment of secondary intention wounds and also recommended by the product manufacturer (Apsen 2017; Atkin et al. 2019).

The use of this herbal medicine in wound healing is a plausible alternative to be adopted by health professionals. Despite recognizing the importance of its use more scientific research should be conducted in order to provide more information regarding its effects and to help determine the efficiency and correct indication during the treatment, aiming to provide adequate assistance to patients with skin wounds, care takers and health professionals (Marmitt et al. 2018; Ribeiro Neto et al. 2020).

Therefore, the present study will contribute in the evaluation of the effects of Fitoscar™ in several aspects of the healing cascade, aiming to provide consistent evidence that can support its indication for wound healing, clarifying in which processes it acts and, consequently, in which phase of healing its effects can be further exploited to enhance the physiological healing process.
Thus, the objective of this study was to evaluate the effects of Fitoscar™ on the different events on the healing of acute excisional wounds induced in Wistar rats.

2 MATERIAL AND METHODS

2.1 ETHICAL ASPECTS

This is an experimental study approved by the Ethical Committee in Animal Use from the Federal University of Goias, protocol number 136/17.

2.2 EXPERIMENTAL PROCEDURES

The experiments were performed using male Wistar rats, weighing 250 to 300 grams, supplied by the Animal Facility of the Federal University of Goias (UFG). The animals were housed in 40x40cm cages suitable for rats. The brightness, noise intensity and relative humidity of the air were those of the general environment, the temperature was monitored, with an average of 25.8°C in the room where the dressings were applied, and 25.9°C in the room where the animals were housed. Three animals were placed per cage, receiving water and food ad libitum. Beds were changed twice a week, with replacement of water, ration and wood shavings on alternate days.

The animals were divided into 02 paired experimental groups as follows: G1 – control group: the wounds were cleaned with warm saline solution (39°C to 40°C); and G2 - Fitoscar™ (Apsen Farmacêutica S.A.) treated group (Apsen, 2017). Each group presented 6 animals which were euthanized in the following experimental days 03, 07, 14 and 21. Therefore there were 4 control groups and 4 test groups, totaling 48 animals.

The animals received of anesthesia using 10% ketamine and 2% xylazine intraperitoneally, at a dose of 0.01mL/g of weight followed by trichotomy of the dorsal region, degermation using chlorhexidine digluconate - solution with surfactants (Riohex™ 2 % - Riochemistry) and antisepsis with chlorhexidine digluconate - Alcoholic solution (Riohex™ 0.5% - Riochemistry).
The wounds were surgically induced in the upper region of the animal's back using a 4 cm² acrylic mold as a parameter, which was used to demarcate the area to be excised. The wounds were surgically induced by incising the right lateral portion of the marked square with a scalpel and following the dissection of the skin segment with scissors in the areolar anatomical plane, at the suprafascial level. The other edges were sectioned with scissors, always on the inner margin of the demarcation. From the cut area, the skin was detached from the *Panicullus carnosus*, exposing the dorsal muscle aponeurosis (Figure 01) (Meza-Valle *et al.* 2021).

Figure 1. Excisional wound induction procedures. A) Acrylic mold with internal measurement of 2x2cm. B) Beginning of the tissue removal procedure after demarcation on the trichotomized back. C) Researcher performing the procedure. D) Excisional wound on the back of the animal.

The dressing was performed immediately after surgery, and according to each experimental group. In G1 – control group: the wounds were cleaned with 9 ml of warm 0.9%
saline solution (39 to 40ºC) daily; in G2 – Fitoscar™ treated group: the wounds were cleaned with 9 ml of warmed 0.9% saline and subsequently received a sufficient layer of ointment to cover the entire wound. In both groups, cleaning was standardized with 3 irrigations of 3 ml each, followed by cleaning with sterile gauze. After the treatments, the wounds were covered with sterile cotton cloth (primary coverage) and a cotton cloth garment to stabilize the dressing (secondary coverage), ensuring that the wound remained occluded and maintaining free movement of the animal.

During the postoperative period, the animals were sent to the maintenance room and were followed up for about 03 hours. The animals received analgesia with Tramadol (40 mg/kg), diluted in the drinking water for 07 days after the injury induction.

2.3 MORPHOMETRIC ANALYSIS

To assess the percentage of wound closure over the experimental days, the wounds were photographed on the day of induction, day 0, and at 03, 07, 14 and 21 days after injury induction (DAI). All photos were taken with a digital camera at a distance of 20 cm from the back of the animal which was positioned in ventral decubitus.

The obtained images were submitted to analysis using the Image J software version 1.3.1 (NHI, United States). To determine the percentage of wound closure, the following formula adapted from Moraes et al. (2013) and Bernardes et al. (2022) was used:

\[
\text{Percentage of wound closure (\%) = \left[ \frac{\text{Area}_{T_0} - \text{Area}_{\text{euthanasia day}}}{\text{Area}_{T_0}} \right] \times 100,}
\]

in which \(T_0 = \) day of injury induction and \(T_{\text{euthanasia day}} = \) days after the injury induction, which could be 3, 7, 14 or 21.

2.4 MACROSCOPIC ANALYSIS

The macroscopic evaluation of the lesions was performed with the analysis of the presence of crust. This alteration was classified semi-quantitatively, according to the following criteria: absent (score = 0); mild with involvement of up to 25% of the area (score = 1); moderate, from 26 to 50% of area involved (score = 2) and severe, above 50% of committed area (score = 3) (Bernardes et al. 2022; Moraes et al. 2013).
2.5 MICROSCOPIC ANALYSIS

On days 3, 7, 14 and 21 after the induction of the lesion, the 06 animals of each group were euthanized, the injured areas were photographed and skin fragments from the injured region were removed for evaluation. The animals were euthanized according to Regulation 37 of the National Council for the Control of Animal Experimentation – CONCEA using an CO₂ (Carbon Dioxide) inhalation chamber. The injured tissue was removed through biopsy, processed for paraffin embedding and the fragments were cut into 5 µm thick and stained using the hematoxylin and eosin (HE) technique.

The general pathological processes analyzed in the lesions were necrosis, fibrin, mononuclear inflammatory infiltration, polymorphonuclear inflammatory infiltration, fibroblast, angiogenesis, re-epithelialization and granulation tissue. These alterations were classified in a semi-quantitative way, according to the following criteria: absent (score = 0); mild with involvement of up to 25% of the area (score = 1); moderate, from 26 to 50% of committed area (score = 2) and severe, above 50% of committed area (score = 3) (Bernardes et al. 2022; Moraes et al. 2013).

The collagen quantification was performed on histological sections stained with picrosirius, observed under a binocular microscope (Zeiss Axiostar Plus) and photographed with a digital camera (Sony Alpha Nex-3) throughout the experimental days. The fibers were examined under polarized light and the entire length of the slides was photographed. The photographs were digitalized and analyzed through the ImageJ 1.3.1 software.

Myofibroblasts were quantified through immunofluorescence, for which tissue sections were submitted to immunofluorescence for the detection of alpha-smooth muscle actin (α-SMA) through incubation with mouse monoclonal antibody (1A4, sc-32251, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Histological sections were mounted using equal parts of phosphate-buffered saline (PBS) and glycerin. Slides were digitized using a confocal microscope (TCS SP8 DMI8, Germany) and subsequently analyzed using Image J 1.3.1 software. As this marking also marks the vessel wall, which is one of the origins of myofibroblasts, this region referring to the vascular wall was disregarded for analysis and only the area related to myofibroblasts in the wound bed was quantified (Manso et al. 2021).
2.6 STATISTICAL ANALYSIS

Statistical analysis was performed using the Graph Pad Prism 8.2.1 software. All variables were tested for normal distribution and homogeneous variance. For wound contraction and collagen quantification, the T Test was used, for analysis of the crust and microscopy and myofibroblasts quantification, the Mann-Whitney test was used. The observed differences were considered significant when (p<0.05).

3 RESULTS AND DISCUSSION

All wounds were completely closed at 21 days after the injury induction (DAI). However, at 07 and 14 DAI (proliferative and maturation phases, respectively) there was a significant difference between the groups treated with Fitoscar™ and the control one, showing less percentage of wound contraction in the treated groups (p<0.05) (Table 1, Figure 2).

Table 1: Macroscopic analysis of the percentage of wound closure in excisional wound experimentally induced in Wistar rats after the use of Fitoscar™ compared to the control group.

<table>
<thead>
<tr>
<th>DAI</th>
<th>G1-Control</th>
<th>G2-Fitoscar</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± SD</td>
<td>M ± SD</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15.25 ± 3.80</td>
<td>31.93 ± 14.12</td>
<td>0.088</td>
</tr>
<tr>
<td>7</td>
<td>57.78 ± 4.54</td>
<td>46.40 ± 5.29</td>
<td><strong>0.0015</strong>*</td>
</tr>
<tr>
<td>14</td>
<td>99.45 ± 1.03</td>
<td>94.37 ± 1.80</td>
<td><strong>0.0003</strong>*</td>
</tr>
<tr>
<td>21</td>
<td>100.0 ± 0.00</td>
<td>100.0 ± 0.00</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Legend: DAI: Days after injury induction; G1: Control (heated saline solution group) and G2: group treated with Fitoscar™. M: mean; SD: standard deviation. T test. Statistically significant result when p<0.05. n=06. Source: The authors.
Figure 2: Macroscopic analysis of the lesions in the experimental groups at 03, 07, 14 and 21 DAI (days after lesion induction). G1- Control (heated saline solution) and G2- Group treated with Fitoscar™.

G1- Control group    G2- Fitoscar treated group

Source: Author’s personal archives

The macroscopic analysis showed that on day 14 the Fitoscar™ treated group presented more crust intensity than the control group; on days 3, 7 and 21, there was no difference in crust formation between the analyzed groups (Table 2, Figure 2).
Table 2. Macrophscopic analysis of crust formation in excisional wounds experimentally induced in Wistar rats after Fitoscar™ treatment in comparison to the control group.

<table>
<thead>
<tr>
<th>DAI</th>
<th>G1-Control Median (max–mín)</th>
<th>G2-Fitoscar Median (max–mín)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.0 (1.0-3.0)</td>
<td>2.0 (1.0-2.0)</td>
<td>0.6364</td>
</tr>
<tr>
<td>7</td>
<td>1.5 (1.0-3.0)</td>
<td>2.0 (2.0-3.0)</td>
<td>0.1667</td>
</tr>
<tr>
<td>14</td>
<td>0.0 (0.0-1.0)</td>
<td>2.0 (0.0-2.0)</td>
<td>0.0281*</td>
</tr>
<tr>
<td>21</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>&gt;0.9999</td>
</tr>
</tbody>
</table>

Legend: DAI: Days after injury induction; G1: Control (heated saline solution group) and G2: group treated with Fitoscar™. The statistical analysis was performed in a semi-quantitative form as follows: absent (score = 0); mild with involvement of up to 25% of the area (score = 1); moderate, from 26 to 50% of committed area (score = 2) and severe, above 50% of committed area (score = 3). The results are presented in median (maximum-minimum) and the statistic test used was Mann Whitney and difference were considered when p<0.05, n=6.

Source: The authors.

Considering the recognized traditionality of *Stryphnodendron adstringens* in the treatment of wounds, it was expected that the wounds in our model would close more quickly, however the percentage of closure was lower on days 7 and 14, despite this, at point final evaluation at 21 days the wounds of both groups were completely healed (Ricardo et al. 2018). We believe that intense inflammation and the presence of necrosis/crust may have contributed to the delay in closing speed at these assessment points. Also, because it is an acute wound and rapidly healing, it was not possible to precisely detect the impact of the effects of Fitoscar on this outcome, unlike studies with chronic wounds, as in the case of diabetic patients with chronic wounds on the feet (diabetic foot), treated with ointment containing 5% *S. adstringens* extract, which showed improvement in healing with reduction of wound area (Aguiar et al. 2021; Hernandes et al. 2010; Ribeiro Neto et al. 2020).

The formation of necrosis/crust was evaluated microscopically and macroscopically, and we observed that at 3 and 7 days this aspect was more intense in the Fitoscar™ treated group in the microscopic evaluation, and macroscopically the crust was evidenced on the 14th day; despite being a harmful factor for healing (Vieira et al. 2015 and 2019). According to the manufacturer, the crust is predicted and attributed to the impregnation of the tissues by the materials that are part of the composition of the product, especially the tannins that can bind to proteins and polysaccharides; leading to the formation of a protective film in the region of the lesion, which can culminate in a thick, dry and irregular crust (Apsen, 2017). Although crust formation is predicted due to the high astringent power of this plant, which can lead to decreased hydration and dryness of the wound, as was also demonstrated in healing studies on dog skin and horses; this process tends to impair healing, prolonging the inflammatory phase and altering cell migration, re-epithelialization and formation of granulation tissue, in addition to favoring

The microscopic analysis showed that in the inflammatory phase of the healing process (3 and 7 DAI) the treatment with Fitoscar™ induced a significant increase in necrosis, mononuclear and polymorphonuclear inflammatory infiltration, fibroblasts and granulation tissue (p<0.05). The significant increase in angiogenesis was observed only at the initial days of this phase (p<0.05) (Table 3, Figure3).

Table 3: General pathological processes observed on excisional wounds experimentally induced in Wistar rats after treatment with Fitoscar™.

<table>
<thead>
<tr>
<th>Pathological Processes</th>
<th>DAI</th>
<th>G1-Control Median (mín-max)</th>
<th>G2-Fitoscar Median (mín-max)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>1.0 (1.0-1.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>0.0079*</td>
</tr>
<tr>
<td>Necrosis</td>
<td>7</td>
<td>1.0 (0.0-3.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>0.0476*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-2.0)</td>
<td>0.4545</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>MN inflammatory infiltration</td>
<td>3</td>
<td>1.0 (1.0-1.0)</td>
<td>2.0 (2.0-2.0)</td>
<td>0.0079*</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.0 (1.0-2.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>0.0079*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.0 (1.0-2.0)</td>
<td>1.0 (1.0-2.0)</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.0 (1.0-1.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0476*</td>
</tr>
<tr>
<td>PMN inflammatory infiltration</td>
<td>3</td>
<td>2.0 (1.0-2.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>0.0079*</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.0 (0.0-3.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>0.0476*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-2.0)</td>
<td>0.4545</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>3</td>
<td>1.0 (1.0-2.0)</td>
<td>2.0 (1.0-2.0)</td>
<td>0.0476*</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.0 (1.0-2.0)</td>
<td>3.0 (3.0-3.0)</td>
<td>0.0079*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.0 (2.0-3.0)</td>
<td>2.0 (2.0-3.0)</td>
<td>0.2424</td>
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<td>3.0 (3.0-3.0)</td>
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<td>2.0 (2.0-3.0)</td>
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<td>1.0 (1.0-1.0)</td>
<td>0.0476*</td>
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<td></td>
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<td>1.0 (1.0-2.0)</td>
<td>0.0 (0.0-1.0)</td>
<td>0.0108*</td>
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<td></td>
<td>21</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Legend: DAI: Days after injury induction; G1: Control (heated saline solution group) and G2: group treated with Fitoscar™; min: minimum; max: maximum; MN: mononuclear cells; PMN: polymorphonuclear cells. For statistical analysis, a semi-quantitative analysis was performed as follows: 0 – absent; 1 – discreet; 2 – moderate; 3 – accentuated; and subjected to the Mann Whitney test. Statistically significant result when p<0.05. n=06.

Source: The authors.
Figure 3: Photomicrographs of histological sections at 3, 7, 14 and 21 DAI (days after lesion induction). G1-Control (heated saline solution) and G2-group treated with Fitoscar™. Hematoxylin and Eosin staining. Scale 100µm, Insert 20µm. n=06. Dotted area: necrosis, inflammatory infiltrate: asterisk, white arrow: fibroblasts, red arrow: angiogenesis and double asterisk: granulation tissue.

While in the proliferative phase (14 DAI) the Fitoscar™ treatment induced a significant decrease in fibroblasts and granulation tissue and an increase in angiogenesis (p<0.05) (Table 3,
Figure 3). In the remodeling phase (21 DAI) the Fitoscar™ treatment induced a decrease in the mononuclear cells’ inflammatory infiltration and an increase in angiogenesis (p<0.05) (Table 3, Figure 3).

The fibrin intensity and re-epithelialization did not present significant difference between the analyzed groups during the experimental period (data not shown).

The treatment with Fitoscar™ in the present study intensified several physiological processes during the healing phases. For example, in the inflammatory phase represented in our model by the 3rd experimental day, we observed in the Fitoscar™ treated group intense inflammatory activity with a predominance of polymorphonuclear cells followed by mononuclear cells, fibroblasts and angiogenesis. In the first days, inflammation is expected, since soon after the injury, in response to vascular and biochemical changes, there are stimuli for the arrival of neutrophils in the injured area to prevent the invasion and proliferation of microorganisms (Eming et al. 2014; Majewska, Gendaszewska-Darmach 2011; Skórkowska-Telichowska et al. 2013; Sorg et al. 2017). This reaction was more intense with the use of Fitoscar™, demonstrating its ability to enhance inflammation, which is important to trigger the other phases of wound healing, and with the greater intensity of angiogenesis and fibroblasts, resulted in the formation of granulation tissue that was also more intense in the Fitoscar™ treated group in the proliferative phase of the healing process (14 DAI).

Among the general pathological processes analyzed in this study it was possible to observe that Fitoscar™ induced a rapid polymorphonuclear inflammatory response that remained intense during the inflammatory and proliferative phases, ceasing after 14 days, evidencing a prolonged and vigorous stimulation of cell chemotaxis, with late control of angiogenesis. Mononuclear cells were also in greater numbers in the Fitoscar™ treated group. It is known that in the early stages of inflammation in a murine model, profiles of classically activated macrophages (M1) predominate with intense pro-inflammatory and phagocytic activity, coinciding with the findings of greater infiltrate on days 3 and 7 in the Fitoscar™ treated group (Daley et al. 2010). For the control of the inflammatory response and progression to the other phases of the healing process, the emergence of alternatively activated macrophages (M2) with the function of modulating inflammation and stimulating angiogenesis and fibroplasia is essential (Daley et al. 2010; Das et al. 2015; Hassanshahi et al. 2022). In this study, we did not evaluate the macrophage profiles, but due to the healing events and their products, the two profiles were
accentuated in the group treated with Fitoscar™ in different phases of the analyzed experimental period.

In addition, it was possible to observe that Fitoscar™ was an inducer of greater intensity of fibroblasts, collagen and angiogenesis, consistent with the activation/differentiation of macrophages of the M2 profile. The angiogenic activity of *Stryphnodendron adstringens* was also observed in the study by Chaves *et al.* (2016), where the treatment with an aqueous solution of *S. adstringens* bark on the chorioallantoic membrane of embryonated chicken egg, culminated in the increase of the vascular networks formed in relation to the control and inhibitor groups, proving its capability to induce neovascularization. Angiogenesis is crucial for the formation of granulation tissue in order to provide oxygen to the forming tissue and supplying it with nutrients, promoting improved healing (Moreira, Marques 2022).

In the remodeling phase (21 DAI), it is expected that angiogenesis is ceasing, however, this did not occur in the group treated with Fitoscar™, suggesting that it is capable of inducing neovascularization even in a physiological situation in which this process should not be observed, as shown by its persistence in the proliferative and remodeling phases, at 14 and 21 DAI, respectively. That, added to the induction of myofibroblasts and fibroplasia, favors the indication of the use of this product in chronic wounds, in which the processes of the proliferative and maturation phase are impaired. These effects can be well directed with a careful evaluation of the wound by the health care professional and the indication of Fitoscar™ predominantly in wounds in the proliferative phase.

The collagen quantification showed significant increase in the group treated with Fitoscar™ at 21 DAI (p<0.05) (Table 4, Figure 4).

<table>
<thead>
<tr>
<th>DAI</th>
<th>G1-Control M ± SD</th>
<th>G2-Fitoscar M ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>4.90 ± 5.64</td>
<td>3.68 ± 2.40</td>
<td>0.0873</td>
</tr>
<tr>
<td>21</td>
<td>5.31 ± 2.52</td>
<td>11.67 ± 4.47</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

Legend: DAI: Days after injury induction; G1: Control (heated saline solution group) and G2: group treated with Fitoscar™. M: mean; SD: standard deviation. T test. Statistically significant result when p<0.05. n=06. 
Source: The authors.
The quantification of myofibroblasts by immunofluorescence showed that on day 14 these cells were more intensely present in the G2-Fitoscar when compared to G1-Control, there was no significant difference on day 21 (Table 5, Figure 5).

Table 5: Quantification of total myofibroblasts in excisional wound experimentally induced in Wistar rats after the use of Fitoscar™ compared to the control group.

<table>
<thead>
<tr>
<th>DAI</th>
<th>G1 - Control M ± SD</th>
<th>G2 - Fitoscar M ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>3.7 (0.1 - 8.5)</td>
<td>5.2 (2.3 - 10.8)</td>
<td>0.0178*</td>
</tr>
<tr>
<td>21</td>
<td>2.0 (0.3 - 4.5)</td>
<td>2.6 (0.6 - 9.4)</td>
<td>0.1873</td>
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</table>

Legend: DAI: Days after injury induction; G1: Control (heated saline solution group) and G2: group treated with Fitoscar™. M: mean; SD: standard deviation. T test. Statistically significant result when p<0.05. n=03.

Source: The authors.
The increased number of fibroblasts in the initial phases (3 and 7 days) ensured greater collagen production than the control group at 21 days. The results of the treatment with Fitoscar™, in the present study, led to findings similar to those observed by Lima et al. who demonstrated that the cream containing 5% *S. adstringens* bark extract promoted the activation of fibroblasts and the deposition of collagen in skin wounds in rabbits (Lima et al. 2016).

During the healing process, fibroblasts differentiate into myofibroblasts, starting to express α-SMA, a molecule by which these cells were identified in this study and confer contractile capacity to myofibroblasts, favoring wound closure and homeostasis of the extracellular matrix (Monika et al. 2021).

It was not possible to identify in the literature studies that evaluated the effect of *S. adstringens* extract on the differentiation of myofibroblasts. The differentiation of this cell is
mainly due to the action of transforming growth factor beta 1 (TGF-b1), which, when it activates its receptor in the fibroblast, triggers a cascade of events that regulates the expression of α-SMA and other components that confer contractile capacity to this cell (Hinz, 2010). It has been shown that *S. adstringens* has the potential to suppress the M1 profile and polarize the M2 profile, which produces TGF-b1, which is consistent with the increased presence of myofibroblasts in the group treated with Fitoscar™ (Carvalho *et al.* 2020; Oishi *et al.* 2016).

In short, we observed that Fitoscar™ has the ability to accelerate processes, inducing early responses and maintaining proliferative phase events for a longer time, which in our model did not accelerate or delay healing compared to what was observed in the control groups. Therefore, the processes observed here can be considered valuable findings when we think about the clinic aspects of patients with complex wounds who need fast and intense responses so that they can progress through a precise and timely repair sequence.

Under experimental conditions of venous hypertension in rats, which is a condition known to delay the progress of the wound healing process, it was found that the use of an ointment containing 10% aqueous extract of the bark of *S. adstringens* in venous ulcers induced an intensification of inflammation, neovascularization, presence of fibroblasts and collagen in comparison to the control group, treated with 0.9% saline solution (Coelho *et al.* 2010). These findings are similar to the ones found in our study, after the Fitoscar™ treatment.

Fitoscar® also acted as a collagen inducer, as observed on the 21st day of treatment. This result is similar to the ones described in another study that demonstrated the effectiveness of a gel containing 1% crude extract of *S. adstringens*, in stimulating collagen deposition in wounds of diabetic rats, a condition known to impair healing (Pinto *et al.* 2015). We demonstrate, for the first time, the potential of a herbal medicine containing *S. adstringens*, in inducing myofibroblast differentiation, as it strengthens the recommendation of this product in wound healing.

4 CONCLUSION

Fitoscar™ is an herbal medicine capable of intensifying cell migration and angiogenesis during the inflammatory phase, migration of fibroblasts and inflammatory infiltrate during the proliferative phase, and during the proliferative and remodeling phases leading to greater intensity of myofibroblasts and collagen deposition. Although crust formation occurred, all
wounds in the present study closed completely within 21 days, therefore, we consider that complex chronic wounds can be remarkably benefited by the processes induced by treatment with Fitoscar™.

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