Clinical Assessment of Antimicrobial Effects of Brazilian Green Propolis on Chronic Wounds

Pascalle Sousa Rocha1,3, Fernando Luiz Affonso Fonseca2,7, David Feder2, Luiz Eldio Gregório6, Joserlan Nonato Moreira3, Luis Rafael Leite Sampaio8, Glaciu Luciano da Veiga7, Fulvio Alexandre Scorza5,6, Beatriz da Costa Aguiar Alves7, Thais Gascón7, Carla Alessandra Scorza5,6, Fabio Ferreira Perazzo1,2

INTRODUCTION

Infections are responsible for 75% of deaths after injuries.[11] The prevalence of bacteria in patients with non-specific chronic wounds challenges health professionals and the Public Health Network to identify these microorganisms and develop a plan to curb or prevent possible infections and complications, such as amputations.

These lesions usually contain some bacteria that hinder the healing process, becoming chronic, infected, or even complicated due to polymicrobial infestations in 60 to 80% of cases. Gram-positive bacteria, Staphylococcus aureus, and Streptococcus sp. have a high recurrence in moderate lower limb infections. In addition to these, Gram-negative (glucose-fermenting bacilli from the Enterobacteriaceae family and non-fermenting glucose, such as Pseudomonas spp. and Acinetobacter spp.) and anaerobic (mainly bacteroid) are also frequent.[1]

Brazilian green propolis, produced by bees, has as the primary source the bush Baccharis dracunculifolia (Asteraceae) found in southeastern Brazil. This propolis has compounds with important biological activities, such as flavonoids, coumaric acid, and ferulic acid that have antitumor and antimicrobial action.[12] Flavonoids are a kind of marker so that the quantification of total flavonoids tests the quality of propolis. Its antioxidant pharmacological action occurs mainly due to the structural characteristics, a tricyclic compound, and the presence of radicals attached to the rings.[13] Observations in beehives suggest that propolis has antimicrobial properties. Carcasses of heavy intruders that cannot be discarded are covered by propolis and undergo an embalming process, which protects the hive from a generalized bacterial infection.[14] Thus, this study aims to answer the following questions: Is a green propolis ointment capable of inhibiting the proliferation of bacteria in chronic wounds in humans? What clinical evidence justifies that green propolis ointment has bactericidal action?

ABSTRACT

Objectives: Chronic wounds in patients of several diseases, especially diabetics, comprise a public health problem in Brazil. The aim of this study was evaluate the effects of topical application of 5% green propolis ointment on nonspecific chronic wounds of patients from a health unit in Cajazeiras, Paraiba. Materials and Methods: The patients were divided into two groups: 20 for the intervention group, treated with 5% green propolis ointment, and 20 participants for the control group, treated with an essential fatty acid, plus vitamins A and E, which has standard application in the Brazilian Public Service, both during 30 days. The following clinical variables were observed: complications (necrosis, crust), aspects of the skin around the lesion, appearance and amount of exudate, signs of infection, presence of pain during treatment, appearance of any complication and odor. Forty participants were surveyed from May 2017 to October 2018. Results: Most of them were women (52.5%) aging from 76 to 95 years (55%), and illiterate (77.5%). The prevalent bacteria in the wounds were Pseudomonas aeruginosa, and most of the sensitive antibiotics were aminoglycoside. In the control group, two of eight participants evaluated on the first day remained infected, whereas, in the group treated with green propolis ointment, all nine patients had infection cleared, with reduction of pain, 81.8% decrease of lesions with purulent exudate, and 73.4% debridement of devitalized tissue. Conclusion: These results confirm the potential of the green propolis ointment as a debridement, bactericide, and the ability to inhibit the growth of microorganisms, and adsorb odors.

Keywords: Chronic wound, Propolis, Wound infection, Clinical trial, Treatment.
We chose Brazilian green propolis in 5% extract due to its composition comprising terpenic compounds, steroids, flavonoids, vitamins, and minerals, aiming to evaluate the bactericidal or bacteriostatic capacity in chronic wounds of different origins.

**MATERIALS AND METHODS**

**Research Type and Location**

We conducted a controlled randomized clinical trial, without blinding, using a descriptive exploratory approach of a qualitative character. The study occurred at the School Clinic of Santa Maria Faculty, Cajazeiras (06°53′24″ S; 38°33′43″ W), Paraíba, Brazil.

In the clinical trial, the investigator applied a treatment, called intervention, and observed its effects on the outcome, to answer questions regarding the effectiveness of new drugs or treatments.

**Sampling and Ethical Aspects**

The trial participants comprised 40 patients who attended the consultation space with the vascular doctor at the school clinic. The selection followed the pre-established criteria, which comprised patients with chronic nonspecific wounds, over 18 years old, of both sexes, registered at the college school clinic. The selection occurred from March to October 2018, after approval in the Research Ethics Committee (CAAE 64526217.9.0000.5180; Opinion number 2.016.083) to guarantee the confidentiality of personal information. Registered on the Rebec Platform with Identifier RBR-294d68.[3]

The patients were divided into two groups: 20 for the intervention group, treated with 5% green propolis ointment, and 20 for the control group, treated with an essential fatty acid, plus vitamins A and E, which has standard application in the Brazilian Public Service. The division into groups occurred at random; the patients chose a form containing group A or B and were referred to the respective treatment. A single observer guided the respective treatments of each patient and followed them for 30 days.

This study was submitted to the appreciation of the Research Ethics Committee, complying with the formal requirements of the National Health Council/Ministry of Health, which provides for research involving human beings according to resolution 466/2012.

**Data Collection**

We carried out the previous evaluation and registered the lesions on the first, seventh, fourteenth, and thirtieth days of experiment. On the first day, samples were collected from the wound using cotton swabs to perform the microorganism culture. Then, both groups received daily dressing, cleaning with 0.9% saline, and application of green propolis ointment in the intervention group and essential fatty acids in the control group, ending with coverage with gauze.

The collected material followed hygiene recommendations. The samples were stored and transported in a thermal box to a laboratory for analysis and identification of bacteria. Aerobic organisms were grown in Brain Heart Infusion Broth and Brain Heart Broth for 24 hr, followed by inoculation in Blood Agar, Chocolate Agar, and MacConkey Agar.

The following clinical variables were recorded: etiology; location; complications (necrosis, infection, crust); aspects of the skin around the lesion (intact, marked erythema, or ulceration); aspect and amount of exudate (high, medium, low); signs of infection (edema, heat, redness, pain, purulent discharge, changes in the lesion bed); presence of pain during treatment; appearance of some complication (pain, bleeding, worsening of signs of infection and increased area of necrosis); and odor.

**Green Propolis Extract and Ointment Production**

Green propolis was harvested directly from the hives by scraping. The raw propolis was washed with distilled water and sanitized in a bath of 150 ppm solution of active chlorine for 15 min to reduce the microbial load. The samples were dried in a dry heat oven at 45°C for one hour. The temperature range covered the melting point of most propolis (60 to 70°C).[6]

Propolis was broken into small pieces to remove possible debris using sterile tweezers or gloves. The solvent (cereal alcohol) was added to the propolis in the proportion of 1:10 in a sterilized glass container with a fabric cover for a minimum period of 10 days. Then, the samples were filtered on sterile filter paper and submitted to microbiological analyzes. Once the product had no contamination, it was ready for ointment preparation.

After the extraction process described above, the green propolis ointment was produced, consisting of lanovaslin (sine ointment), with Lanolin 30%, BHT 0.02%, solid Vaseline q.s. 100%, and subsequently added the green propolis extract at 5%. The components were weighed, the BHT was solubilized in liquid Vaseline q.s., and then all mixed with the propolis extract with a mortar and pestle. The ointment samples were deposited in sterile containers to tests on chronic wounds. Physical, chemical, and microbiological tests were carried out, checking the appearance at 25°C, color, density, water content (KF), total bacteria, molds, and yeasts (as certified). The results of these tests are found in the supplementary materials.

**Chemical Composition of Green Propolis**

Brazilian green propolis is rich in phenylpropanoids, including cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid, and its derivatives. Among these substances, pre-methylated cinnamic acids have an inbreeding influence on the antimicrobial activity of green propolis.

In recent years, researchers have identified several phenylpropanoid derivatives in Brazilian propolis, suggesting that the source of stilbenes is not limited to a few plants. Lignans, as the main chemical compounds in tropical propolis, have attracted worldwide interest in research. Over the past 12 years, researchers have identified three lignans in propolis in Brazil.[7]

**Data Analysis**

Data were organized according to CONSORT recommendations and analyzed using SPSS statistical software (VERSION 24, 2018). In addition to descriptive statistics of absolute and relative frequency, we applied Pearson’s chi-square test or Fisher’s exact test. The p < 0.05 was used for statistical significance.

**RESULTS**

Table 1 shows the socio-demographic data. The control group comprised 13 (65%) women, 14 (70%) elderly 65 and 95 years old, 10 (50%) married, 17 (85%) without education, 15 (75%) retired, 18 (90%) had their residence, and 19 (95%) had sanitation in their homes. The intervention group contained 12 (60%) men, 13 (65%) elderly 65 and 95 years old, 10 (50%) married, 14 (70%) without education, 12 (60%) retired, 20 (100%) had their residence, and 19 (95%) had sanitation in their homes.

Chronic wounds of the lower limb were the most frequent type of injuries in the control group, 9 (45%), whereas pressure wounds, 8 (40%), and chronic wounds of the lower limb, 8 (40%) were the most frequent in the intervention group (Table 2).

Wounds both in control and in the intervention group occurred more frequently in the lower third of the leg, 6 (30%) and 5 (25%), respectively (Table 3).
The most common bacterium in the control group was *Pseudomonas aeruginosa*, and aminoglycosides were the class of antimicrobial with higher sensitivity to this microorganism. The most common bacteria in the intervention group were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, or *Pseudomonas* sp., and aminoglycosides were the antimicrobial class with the highest sensitivity to all these microorganisms (Table 4).

Table 5 shows the characteristics of wounds. Although 20 patients were recruited for both groups, we evaluated 21 wounds, both in control and intervention groups, making up 42 injuries. We assessed the Kind of Tissue, Type of Bed Exudate, Boundary Characteristics, Microbial Characteristics, and Pain. These parameters were measured in the program Image J.

The tissue evaluation, in the control group, revealed devitalized tissue, in eight (38.1%) patients evaluated on the first day, and in the intervention group 13 (61.9%). On the thirtieth day, six (28.6%) remained with the presence of necrotic tissue in control, and only two (9.5%) in the intervention.

The viable tissue showed granulation in eleven (52.4%) patients at the beginning of the control treatment, and in eight (38.1%) at the end. On the first day of the intervention group, eight (38.1%) wounds had granulated tissue, rising to ten (47.6%) patients on the thirtieth day.

The wounds in both groups had significant epithelialization. On the first day, no lesions had epithelialization. On the thirtieth day, seven (33.3%) lesions epithelized in the control group (p-value = 0.021), and nine (42.9%) in the intervention group (p-value < 0.001).

Regarding the exudate evaluation, purulent exudate was the most common type of exudate in wounds. At the beginning of treatments, the control group had 12 (57.1%) patients with purulent exudate, and the intervention group had ten (47.6%). On the thirtieth day, the control group maintained four (19.0%) purulent lesions while the intervention reduced to one (9.1%).

On the first day, all wounds had exudates in both groups. However, on the thirtieth day, both the control and intervention group showed the absence of exudate in six (28.6%) wounds (p-value = 0.011 in the control group, and P-value = 0.002 in the intervention group).

There was a predominance of lesions with irregular edges. The control group started with 12 (57.1%) injuries in this condition, and the intervention group with 14 (66.7%). At the end of treatment, the control had eight (38.1%) lesions with irregular edges (p-value = 0.885), while the intervention group increased the value to 18 (85.7%; p-value = 0.002).
Regarding characteristics, wounds in the clean-contaminated category prevailed on the first day of treatment, with ten (47.6%) in the control group and eight (38.1%) in the intervention. On the thirtieth day, the control increased the number of clean-contaminated wounds to 16 (76.2%) and the intervention to 19 (90.5%).

On the first day, the control group had eight (38.1%) infected wounds, and the intervention had nine (42.9%). On the thirtieth day, the control group had two (9.5%) infected wounds (p-value = 0.012), while the intervention group did not have infected wounds (p-value < 0.001). The presence or absence of pain was assessed through the participant’s report. The control group started with 14 (66.7%) of patients reporting pain, and the intervention with 13 (61.9%). On the thirtieth day, the control increased the number of clean-contaminated wounds to 16 (76.2%) and the intervention to 19 (90.5%).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>0 (0.0%)</td>
<td>3 (14.3%)</td>
</tr>
<tr>
<td>Clean-contaminated</td>
<td>10 (47.6%)</td>
<td>16 (76.2%)</td>
</tr>
<tr>
<td>Infected</td>
<td>2 (9.5%)</td>
<td>4 (19.0%)</td>
</tr>
<tr>
<td>Critical colonization</td>
<td>3 (14.3%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Some research explains the synergistic effect between propolis and other antibiotics. From this perspective, propolis can enhance the antibacterial effect of antibiotics sensitive to each type of injury. In the present study, the antibiotic with the highest sensitivity was from the group of aminoglycosides. Probably, propolis can cause partial bacterial lysis and inhibit protein synthesis. When used in combination with most antibiotics, propolis can increase the antibacterial effect and reduce the recovery period.[6]

Many studies confirm the synergism between antibiotics and propolis. The synergistic effect of Brazilian propolis inhibits RNA polymerase in protein synthesis. Thus, the use of propolis can improve antibiotic therapy.[6]

Brazilian propolis decreases the resistance of bacterial wall to antibiotics (amoxicillin, ampicillin, and cephalaxin) and has synergistic effects
CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BHT: Butylated hydroxytoluene; KF: Karl Fisher method; LP: Pressure injury; RNA: ribonucleic acid; DNA: deoxyribonucleic acid; MMII: Treated group.

SUMMARY

Green propolis has a bactericidal action, both against gram-positive and gram-negative bacteria, with the removal of the surface layer of bacteria, the biofilm.

REFERENCES

5. ReBEC. Registro brasileiro de ensaios clínicos. Interfacerebec. Rio de Janeiro, RJ: Instituto de Informação Científica e Tecnológica em Saúde (Brazil); 2010: Identifier RBR-294d68.