ORIGINAL ARTICLE

Evaluation of topical administration of mucoadhesive gel containing triamcinolone and extracts of *Aloe vera* and propolis for surgical wound tissue repair in the tongue of rats

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ABSTRACT

Triamcinolone acetonide (TA) is a drug used to relieve signs and symptoms of oral ulcers, which can trigger adverse effects when administered for a long period. Thus, Aloe Vera (AV) and propolis appear as therapeutic alternatives for the management of oral wounds, due to their healing properties and few side effects. This study aimed to evaluate the influence of AV and propolis on the repair of surgical wounds in the tongue of rats, and compare it with TA, through clinical and histopathological analyses. A lesion was made on the back of the tongue of 48 *Wistar* rats. The animals were divided into 4 groups: NCG (0.9% saline solution); PCG (TA 1 mg/g); AVG (0.5% AV); PG (5% propolis). Drugs were administered for 12/12 h. Euthanasia occurred on the 3rd and 7th days of the experiment. On day 7, AVG and PG had intermediate sized ulcers, not differing from PCG and NCG. Histological analysis showed that the reepithelialization score was significantly higher in NCG and AVG than in PCG and PG. Thus, it was demonstrated that the administration of 0.5% AV mucoadhesive gel of 12/12 h was not able to optimize the healing process of oral ulcers.

Keywords: *Aloe*, Propolis, Oral ulcer, Wound healing.

INTRODUCTION

Oral ulcers are lesions of the oral mucosa characterized by a loss of continuity of the epithelial tissue and exposure of nerve endings in the lamina propria, which usually results in pain (Schemel-Suárez et al. 2015). Their etiopathogenesis varies but may include trauma, chemical stimulation, autoimmune and infectious processes, and immunologically mediated dermatoses (Lehman and Rogers 2016; Fitzpatrick et al. 2019; Dudding et al. 2019).

Although common, oral ulcers have no gold standard treatment, though several medications have been proposed to reduce pain and accelerate tissue repair (Lehman and Rogers 2016; Nagieb et al. 2021). Triamcinolone acetonide (TA), a topical corticosteroid, is commonly used to relieve the symptoms of various oral inflammatory conditions (Nagieb et al. 2021). However, chronic use can trigger local adverse effects, such as allergic dermatitis, tissue atrophy (Fani et al. 2012), and secondary infections (Suter et al. 2017; Fani et al. 2012) as well as systemic adverse effects, such as adrenal suppression, modification of glucose and protein metabolism, and peptic ulcers. Furthermore, TA is contraindicated in patients with fungal, viral, and bacterial infections because of its immunosuppressive effects, which can aggravate infectious conditions (Martorelli et al. 2012).

Although synthetic substances are widely used in healthcare, natural therapeutic agents have gained traction in recent years because of their low cost, ease of access and handling, and minimal adverse effects (Faleiro et al. 2009; Kumar et al. 2022). In addition, the literature reports that the active substances found in natural agents can exert immunomodulatory activity and control inflammatory responses, altering the processes of coagulation, inflammation, re-epithelialization, collagenization, and wound contraction (Liang 2020). However, much

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of the information available on natural products lacks a scientific basis, thus limiting their potential for widespread use (Teplicki et al. 2018; Kumar et al. 2022). In this context, *Aloe vera* (L.) Burm.f. (AV) and propolis are two such therapeutic alternatives for the treatment of various conditions (Bolouri et al. 2015; Cuba et al. 2016; Ernawati et al. 2018; Shi et al. 2020).

AV is a plant belonging to the Liliaceae family, and because of its wide range of therapeutic properties, it is used for the treatment of various conditions. This plant has 400 identified species, with *Aloe barbadensis* Mill. being preferred for medicinal use due to its curative actions. The mucilaginous gel found inside the leaf is rich in nutrients (Mercês et al. 2017; Teplicki et al. 2018). Its pharmacological properties help in the modulation of the tissue repair process, and its effects on healing are related to its ability to keep wounds moist, increase the migration of epithelial cells, stimulate the proliferation of fibroblasts, accelerate collagen maturation, reduce the inflammatory process, and exert antimicrobial action in the presence of anthraquinone (Gupta and Malhotra 2012; Liang 2020).

Propolis is a natural product originating from bees of the species *Apis mellifera* and is formed by the combination of various resinous, gummy, and balsamic substances collected by bees and mixed with their salivary enzymes (Almeida et al. 2016; Abbasi et al. 2018). Propolis is widely used because of its therapeutic properties due to the presence of phenolic compounds and pharmacologically active substances. These bioactive agents can act on a variety of physiological processes including wound healing and immunomodulation. Previous studies have also confirmed the antimicrobial, anti-inflammatory, and antioxidant properties of propolis (Almeida et al. 2016; Oryan et al. 2018; Jongjitaree et al. 2022).

In the literature, the use of these natural agents has been increasingly described for the management of oral ulcers. The beneficial effects of AV and propolis have been demonstrated in the treatment of oral mucositis (OM) (Noronha et al. 2014; Bolouri et al. 2015; Cuba et al. 2016), recurrent aphthous ulcerations (RAU) (Lotufo et al. 2005; Pensin et al. 2009; Babaee et al. 2012; Mansour et al. 2014; Shi et al. 2020), and traumatic ulcers (Grégio et al. 2005; Ernawati et al. 2018; El- Batal and Ahmed 2018).

Mucoadhesive systems have been developed to improve drug retention in the oral mucosa. These systems demonstrate greater adhesion to the mucosa, allowing the drug to remain at the site of action for a longer period of time than conventional delivery systems, such as solutions or suspensions (Roque et al. 2018). In addition, they are more stable than oral gels (Alaei and Omidian 2021).

The pharmacological properties and benefits of AV and propolis in the healing process are well established in the literature. In addition, their therapeutic dosages and possible toxicities have been widely studied to ensure the safe use of these natural agents. The present study aimed to evaluate the influence of AV and propolis on the repair of oral surgical wounds in the tongue of rats and to compare the findings with those of AT through clinical and histopathological analysis of the wound area.

METHODS

Ethics committee

The present study was carried out in the Bioterium of Faculdade Adventista da Bahia (FADBA), in Cachoeira, Bahia, Brazil. The methodology used was carried out in accordance with Resolution 196/96 of the National Health Council of Brazil, under approval of the Ethics Committee in the Use of Animals (CEUA) of FADBA under registration number 55/2018. The experimental protocol followed the ethical principles for the use of animals as prepared by the Brazilian College of Animal Experimentation, affiliated with the International Council of Laboratory Animal Science, which establishes the conduct that must be carried out in animal experimentation based on the principles of sensitivity, good sense, and good science.

Animals

Experiments were performed on 48 male *Wistar* rats (*Rattus norvegicus albinus*) provided by the Bioterium of FADBA. The rats were two to three weeks old, weighed 250-360 g, and had no genetic modifications.

The animals were housed in shared cages, with three animals per cage, in a temperature-controlled environment (20-22 ºC) with well-defined 12-h day/night cycles. The animals were provided with Nuvilab feed (Quimtia, Colombo, Paraná), which is suitable for the species, and filtered water *ad libitum* throughout the experiment.

The animals were randomly divided into four groups of 12 animals each, according to the formulation used: negative control group (NCG), saline 0.9%; positive control group (PCG), TA 1 mg/g; *A. vera* group (AVG), 0.5% AV mucoadhesive gel; and propolis group (PG), 5% mucoadhesive propolis gel.

Formulations used

AV mucoadhesive gel 0.5%, as recommended for topical use (Syed et al. 1996;

Khorasani et al. 2009; Eshghi et al. 2010; Mansour et al. 2014.), was obtained by adding concentrated dry AV extract of certified quality (Fagron) to the vehicle (a mucoadhesive gel). The concentrated dry AV extract consisted of a fine, hygroscopic, white-to-yellowish powder with a characteristic odor, and was soluble in water. It had a pH of 4.52, apparent density of 0.3930 mg/ml, loss of desiccation of 3.6%, heavy metals <10 ppm, acid-insoluble ash of 0.14%, and total ash of 0.37%. The results of the microbiological tests showed the absence of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, and bile-tolerant Enterobacteriaceae, with <10 CFU/g of total bacteria, fungi, and yeasts. The components used in the preparation of the vehicle were 10.0% carboxymethyl cellulose (CMC), 5.0% gelatin, 10.0% pectin, 10.0% polyethylene grains, 0.1% nipagin, and petroleum jelly (L) q.s.p. The mucoadhesive gel was prepared by mixing the polyethylene grain and nipagin in vaseline, applying heat until the powders were completely dissolved, and finally cooling and homogenizing the mixture. After cooling, the base became more consistent in texture, and CMC, gelatin, and pectin were added.

The mucoadhesive propolis gel was obtained by combining 5% (v/w) of the glycolic propolis extract (Pensin et al. 2009) (produced from 80% propolis and 20% hydroglycolic solvent) with 95% of the vehicle (mucoadhesive gel) (Pensin et al. 2009). This vehicle was the same used one used in the preparation of the AV formulation. The drugs were formulated by a qualified pharmacist at the company A Formula, located in Feira de Santana, Brazil.

TA (Omcilon-A), which is widely prescribed in dentistry for the treatment of oral ulcers, was obtained in its commercial form from a private pharmacy and was used as a positive control (Martorelli et al. 2011).

Experimental procedures

Generating the oral wound

Under aseptic conditions in the FADBA laboratory, general anesthesia was induced in the animals with an intraperitoneal (IP) injection containing a mixture of ketamine (90 mg/kg; Vetbrands, Brazil) and xilazine (5 mg/kg; Syntec, Brazil) at 6 am. Using a circular scalpel (Biopsy punch, Kolplast, Brazil), a single calibrated operator created a uniform, well-defined, and standardized wound, 6 mm in diameter and 1 mm in depth, in the center of the dorsum of the tongues of the animals. The lesion was characterized as a wound limited to the mucosa without muscle involvement. After the procedure, post-anesthesia monitoring of the rats was performed until the level of consciousness was completely restored.

Administration of formulations

Each formulation was first applied immediately after the surgical procedure once adequate hemostasis was achieved in the area through topical application with sterilized flexible cotton swabs (Swabs, Johnson & Johnson, Brazil). Thereafter, the formulations were administered daily at 12-h intervals throughout the experiment.

Euthanasia

On days 3 and 7 of the experiment, six animals from each experimental group were euthanized with an injection of 75 mg/kg ketamine and 10 mg/kg xylazine administered via IP injections. Thereafter, the iatrogenic lesion was excised through an incision at the base of the tongue with a conventional scalpel, and the tissue was fixed in buffered formalin for a minimum period of 48 h. The specimen was then processed histologically, and 4-µm thick tissue sections were obtained and stained with hematoxylin-eosin and Sirius red.

Macroscopic analysis

The evolution of tissue repair in the ulcers was clinically analyzed on all days of the experiment. Tissue was classified as either repaired or unrepaired. To assess the surgical wound contraction process, the ulcer was measured using a universal analog caliper (Monaliza Import, China) at the time of euthanasia (days 3 and 7 of the experiment). After measuring the diameter of the wound, the area was calculated ($A = \pi \times R^2$). Assessments were performed by a single-blinded, calibrated operator to avoid bias prior to the analyses.

Microscopic analysis

Images of stained tissue sections were captured using Motic Images Advanced software (version 3.0; Motic China Group). A standard area (13107,200000 pixels) was established for the analysis of all cases. Ten standard images corresponding to each case were captured with the established dimensions, and the percentages of polymorphic and monomorphonuclear inflammatory cells, vascular density, and collagen-containing areas were measured. Each area was captured at 400× magnification, and the images were saved in JPEG format.

All analyses were performed by the same examiner, blinded and calibrated prior to the analysis. The degree of tissue inflammation was measured according to the method described by Sampaio et al. (2018). A semi-quantitative study of the sections was performed by analyzing the variables of the inflammatory process, adopting the following grading criteria: absent (0), mild (+), moderate (++), and intense (+++). The following criteria were used:

when alterations were present in a percentage equal to or greater than 50% in the analyzed histological section, the grade was considered intense; for 25 to 50% of the tissue, moderate; and less than or equal to 25%, discrete.

Statistical analysis

A database was created in Excel 2016 using R software (version 3.3.0). Descriptive and exploratory analyses (mean, median, and standard deviation) were performed on the ulcer size data and histological slides. As the data did not meet the presuppositions of the parametric analysis, the nonparametric Mann–Whitney test was used for comparisons between groups on different days of analysis, the paired Wilcoxon test for comparisons between different periods of analysis, and the Kruskal–Wallis and Dunn tests for comparisons between treatment types. All analyses were performed using R software with a significance level of $p<0.05$.

RESULTS

Clinical analysis

All experimental groups (NCG,12 animals; PCG,12 animals; AVG,12 animals; PG,12 animals) showed a significant decrease in ulcer size after treatment ($p<0.05$) on days 3 and 7. However, when the groups were compared on day 3, there were no significant differences between the four groups ($p > 0.05$) even though AVG demonstrated a greater numerical clinical reduction in wound area, followed by PG, PCG, and NCG. On day 7, the clinical reduction in the size of the ulcer was greater in NCG, followed by AVG, PG, and PCG; however, statistical significance was observed only when comparing NCG and PCG, wherein the ulcer sizes were smaller in the former group ($p<0.05$). The animals in the groups that received treatment with AV and propolis exhibited ulcers that were intermediate in size and not significantly different from those in PCG or NCG ($p>0.05$). It was also noted that, with the exception of PCG, all groups had smaller ulcer sizes on day 7 when compared to day 3 of treatment ($p<0.05$) (Table 1). With regard to clinical ulcer healing, all animals in the experiment exhibited unrepaired ulcers on days 3.

Histopathological analysis

In each group, the inflammatory infiltrate score was significantly lower on day than on day ($p<0.05$). On day, there was no significant difference between the groups ($p>0.05$). On day, the inflammatory infiltrate score was significantly higher in PCG and PG when compared to AVG ($p<0.05$).

Table 1. Mean (standard deviation), median (minimum and maximum value) of the ulcer area (mm) as a function of the group, day of assessment and time.

Time	Group	Assessment day				*p*-value
		Third		Seventh		
		Mean (standard deviation)	Median (minimum and maximum value)	Mean (standard deviation)	Median (minimum and maximum value)	
Initial	Positive control	28.26 (0.00)	28.26 (28.26 – 28.26)	28.26 (0.00)	28.26 (28.26 – 28.26)	-
	Negative control	28.26 (0.00)	28.26 (28.26 -28.26)	28.26 (0.00)	28.26 (28.26 -28.26)	-
	Propolis	28.26 (0.00)	28.26 (28.26 -28.26)	28.26 (0.00)	28.26 (28.26 -28.26)	-
	A. vera	28.26 (0.00)	28.26 (28.26 -28.26)	28.26 (0.00)	28.26 (28.26 -28.26)	-
p-value		-	-	-	-	
Final	Positive control	3.77 (2.35)	*3.46 (0.78-8.03) Aa	4.95 (2.07)	*4.43 (3.14-7.54) Aa	0.8102
	Negative control	9.55 (7.70)	*7.06 (3.14-23.74) Aa	0.13 (0.20)	*0.01 (0.007-0.5) Bb	0.0039
	Propolis	3.09 (1.32)	*3.14 (0.78-4.9) Aa	1.11 (0.62)	*1.13 (0.12-1.76) Bab	0.0306
	A. vera	3.02 (2.11)	*2.45 (1.53-7.06) Aa	0.97 (0.91)	*0.64 (0.007-2.54) Bab	0.0250
p-value			0.0524		0.0006	

*Significant decrease in ulcer size in relation to initial time ($p\leq0.05$). Different letters (upper case comparing horizontally between days of assessment and lowercase comparing vertically between groups) indicate statistically significant differences ($p\leq0.05$).

For the polymorphonuclear cell score, lower scores were observed on day 7 than on day 3 for PCG, NCG, and AVG (p<0.05). PG had a p-value close to the threshold (p=0.0516). On day 3, there was no significant difference between the groups, and on day 7, the score was significantly higher in PCG when compared to AVG (p<0.05). For mononuclear cells, no significant differences were observed between the groups or evaluation times (p > 0.05). The edema score was significantly lower on day 7 in all the groups (p<0.05). There was no significant difference between the groups on day 3, but the p-value was close to the threshold (p=0.0534). On day 7, the edema score was significantly higher in PG when compared to AVG (p<0.05).

The degree of vascular density was higher on day 7 in all groups (p<0.05). On day 3, there were no significant differences between the groups (p>0.05), and on day 7, the scores were significantly higher in AVG and NCG when compared to PCG and PG (p<0.05). The re-epithelialization score was higher on day 7 for PCG, NCG, and AVG (p<0.05). On day 3, the re-epithelialization score was significantly higher in NCG and AVG when compared to PCG (p<0.05). On day 7, the scores in NCG and AVG were significantly higher than those in the PCG and PG (p<0.05). The collagen score was significantly higher on day 7 for NCG, PG, and AVG (p<0.05). On day 3, the score was significantly higher in PCG when compared to the other groups (p < 0.05). On day 7, the score was significantly higher in NCG when compared to PCG (p<0.05) (Table 2).

DISCUSSION

To avoid the undesirable effects of TA, which have been reported in the scientific literature (Martorelli et al. 2011; Fani et al. 2012), the use of propolis and AV has been proposed as a therapeutic alternative for the treatment of oral ulcers; in addition to presenting minimal adverse effects, they are inexpensive and easy to access and manipulate (Lotufo et al. 2005; Martins et al. 2009; Nimma et al. 2017). In order to increase the permanence time of propolis and AV in wounds, we decided to use them in the form of a mucoadhesive gel. Mucoadhesive materials are known to have a great affinity for and adherence to mucosal surfaces; therefore, they persist at the applied site for longer periods of time, which increases the absorption of the medication and confers prolonged contact with the mucosal area of interest (Roque et al. 2018; Komati et al. 2019; Desai et al. 2020; Alaei and Omidian 2021).

There were no statistically significant differences between AVG and the other groups on the third day of the experiment, despite a more pronounced wound reduction in this group. On day 7, the reduction of the size of the ulcer was even smaller than that in NCG. Similarly, Coelho et al. (2015) evaluated the effect of 0.5% topical AV gel on oral wound healing in rats for 14 days and found that the group treated with AV showed the greatest decrease in wound area from days one to five; however, this effect was not observed from days five to 10, which mirrors the results of the present study. The authors also found no significant difference between the AV group and other experimental groups (p>0.05). In contrast, Mansour et al. (2014) evaluated a mucoadhesive gel containing 0.5% AV as an active ingredient in the treatment of recurrent aphthous stomatitis and found that on days four and six of the experiment, the mean ulcer sizes were significantly different between the groups (p<0.05), and the AV group demonstrated the greatest reduction in size in the two days of clinical evaluation.

Possible explanations for these varied results may be related to differences in the methodologies of the studies. Mansour et al. (2014) used a mucoadhesive gel as a vehicle, unlike Coelho et al. (2015), who used a standard gel without bioadhesive properties. In addition, the study by Mansour et al. (2014) involved human participants with four daily applications, whereas the experiment conducted by Coelho et al. (2015) and the current study were performed in rats with two daily applications. Therefore, the use of human participants, who were aware of their injuries and were receptive to guidance aimed at improving clinical symptoms, and the higher number of daily AV applications may have led to a significant reduction in the wound area in the study by Mansour et al. (2014). Another possible explanation is related to the amount of polysaccharides found in AV, which are important constituents in the healing process. Polysaccharide levels can be altered by seasonal changes, modifications in plant cultivation, extraction, and processing, and geographic location (Freitas et al. 2014). Therefore, it is possible that the polysaccharide levels differed across the studies, thus affecting the clinical outcomes.

The histopathological findings did not suggest beneficial effects associated with the use of AV when compared with NCG. In the present study, the inflammatory infiltrate score was significantly higher on day 7 in PCG and PG when compared to AVG (p<0.05), and the polymorphonuclear cell score on day 7, was significantly higher in PCG when compared to AVG (p<0.05). On day 7, the edema score was significantly higher in PG than in AVG (p<0.05). On day 7, the re-epithelialization score was significantly higher in NCG and AVG when compared to PCG and PG (p<0.05). This is in contrast to the findings by Coelho et al. (2015), possibly because this study used a gel without bioadhesive properties,

Table 2. Median (minimum and maximum value) of the histopathological analysis scores as a function of the group and the evaluation time.

Variable	Group	Assessment day		p-value
		Third	Seventh	
Inflammatory infiltrate	Positive control	3.0 (3.0; 3.0) Aa	2.0 (1.0; 3.0) Ba	0.0081
	Negative control	3.0 (2.0; 3.0) Aa	1.0 (1.0; 2.0) Bab	0.0049
	Propolis	3.0 (2.0; 3.0) Aa	2.0 (1.0; 3.0) Ba	0.0325
	A. vera	3.0 (2.0; 3.0) Aa	1.0 (0.0; 1.0) Bb	0.0030
p-value		0.5125	0.0033	
Polymorphonuclear cells	Positive control	3.0 (3.0; 3.0) Aa	2.0 (1.0; 2.0) Ba	0.0022
	Negative control	2.5 (2.0; 3.0) Aa	1.0 (0.0; 1.0) Bab	0.0038
	Propolis	3.0 (1.0; 3.0) Aa	1.5 (1.0; 2.0) Aab	0.0516
	A. vera	3.0 (2.0; 3.0) Aa	0.5 (0.0; 1.0) Bb	0.0037
p-value		0.3235	0.0070	
Mononuclear cells	Positive control	0.0 (0.0; 0.0) Aa	0.0 (0.0; 1.0) Aa	0.1740
	Negative control	0.0 (0.0; 1.0) Aa	0.3 (0.0; 1.0) Aa	0.3360
	Propolis	0.0 (0.0; 1.0) Aa	0.5 (0.0; 1.0) Aa	0.6400
	A. vera	0.0 (0.0; 1.0) Aa	0.0 (0.0; 0.0) Aa	0.4050
p-value		0.5125	0.2443	
Neovascularization	Positive control	1.0 (1.0; 1.0) Ba	2.0 (1.0; 2.0) Ab	0.0067
	Negative control	1.0 (1.0; 1.0) Ba	3.0 (2.0; 3.0) Aa	0.0018
	Propolis	1.0 (0.0; 2.0) Ba	2.0 (2.0; 2.0) Ab	0.0088
	A. vera	1.0 (1.0; 1.5) Ba	3.0 (3.0; 3.0) Aa	0.0018
p-value		0.6271	0.0002	
Edema	Positive control	2.5 (2.0; 3.0) Aa	1.0 (0.0; 2.0) Bab	0.0080
	Negative control	1.5 (1.0; 2.0) Aa	0.0 (0.0; 1.0) Bab	0.0063
	Propolis	2.0 (1.0; 3.0) Aa	1.0 (0.0; 1.0) Ba	0.0096
	A. vera	2.0 (1.0; 2.0) Aa	0.0 (0.0; 0.0) Bb	0.0018
p-value		0.0534	0.0126	
Re-epithelialization	Positive control	0.0 (0.0; 0.0) Bb	1.0 (1.0; 2.0) Ab	0.0018
	Negative control	1.0 (1.0; 1.0) Ba	2.0 (2.0; 3.0) Aa	0.0022
	Propolis	1.0 (0.0; 1.0) Aab	1.0 (0.0; 2.0) Ab	0.3860
	A. vera	1.0 (1.0; 1.0) Ba	3.0 (2.0; 3.0) Aa	0.0022
p-value		0.0006	0.0008	
Collagen	Positive control	2.0 (1.0; 2.0) Aa	2.0 (2.0; 3.0) Ab	0.2180
	Negative control	1.0 (1.0; 1.0) Bb	3.0 (3.0; 3.0) Aa	0.0013
	Propolis	1.0 (1.0; 2.0) Bb	2.5 (2.0; 3.0) Aab	0.0063
	A. vera	1.0 (1.0; 1.0) Bb	3.0 (2.0; 3.0) Aab	0.0018
p-value		0.0023	0.0181	

*Distinct letters (lowercase vertically and uppercase horizontally) indicate statistically significant differences ($p \leq 0.05$). Scores: absent (0); discrete (1); moderate (2); sharp (3).

thus minimizing the adhesion of the formulation to the mucosa and consequently decreasing the bioavailability of the medication in the area of the wound.

Although previous studies have shown that propolis aids in the healing process of oral wounds by increasing vascular endothelial growth factor (VEGF) (Ernawati and Sari 2018), elevating fibroblast growth factor-2 expression (Puspasari et al. 2018), re-epithelializing the lesion (Grégio et al. 2005), and significantly reducing the healing time (Pensin et al. 2009), the present study did not find

similar results. On day 3, the clinical behavior of the PG wounds was inferior to that of the AVG wounds, and on day 7, it was inferior to that of the AVG and NCG wounds. Pensin et al. (2009) evaluated the effects of propolis in orabase 5% in patients with a history of RAU and found that participants who used propolis for three months, three times a day, had a reduction in healing time between 2 and 5 days. Regarding the histopathological evaluation in the present study, the PG wound on the seventh day had inflammatory infiltrate, polymorphonuclear, and edema scores higher than those of the AVG wound, and lower neovascularization than the AVG and NCG wounds. Furthermore, PG was the only group that did not show significant re-epithelialization on day 7. These histological results also support the clinical findings. In contrast, Grégio et al. (2005) evaluated the effect of pure propolis extract diluted in ethyl alcohol 30% PA on induced wounds on the dorsum of rat tonguesand observed complete re-epithelialization on day seven in ulcerated lesions treated with propolis on day 7, with only two animals exhibiting a mild inflammatory infiltrate.

Possible explanations for the varying results with the use of propolis may be related to methodological differences, including the use of mucoadhesive propolis gel, which was administered twice instead of three times a day, as shown in the study by Pensin et al. (2009), as well as the use of different application vehicles, as shown in the studies by Díaz et al. (1997) and Grégio et al. (2005).

The topical use of TA is the treatment of choice for the management of ulcerations of the oral cavity (Oliveira et al. 2016). However, an important finding of the present study was that PCG, which corresponded to the group treated with TA, had worse outcomes than NCG, which was the only group that did not show a statistically significant reduction in the wound area between days 3 and 7 (p>0.05) and had a lower re-epithelialization score than NCG and AVG on day of the experiment. Previous studies have corroborated these findings. Oliveira et al. (2016) found that only the group treated with oral-based TA did not show a decrease in the size of the ulcer from day five to 10. In addition, histological analysis on day 10 of the experiment showed that the TA group had a median score of four, meaning that the group was characterized by the presence of ulcers and intense acute inflammatory processes. Wahyuni et al. (2022) observed that topical application of 0.5%, 1%, 2%, and 4% ethanolic extract of *Kaempferia galanga* L. rhizoma (EEKG) was superior in macroscopic ulcer area reduction than TA 0.1% orabase on days 3 and 6 of the experiment. The study further noted that treatment with 0.5%, 1%, and 4% EEKG was better than TA at reducing the inflammation score. Martorelli et al. (2011) compared the healing effect of 30% Aroeira in orabase with TA, 5% dexpanthenol in orabase, and one orabase vehicle (negative control) for 14 days and observed that TA had the worst clinical healing performance compared to the other groups, including the negative control. None of the animals in this group presented with complete healing on day 14 of the experiment. Furthermore, Martins et al. (2009) demonstrated that topical corticosteroids prolonged tissue repair in secondary wound infections. These results suggest that the commercial use of this drug is overestimated by the population and by the dental professionals responsible for its wide prescription, since studies have shown adverse effects and few significant results regarding its ability to accelerate wound healing.

It is important to emphasize that tissue repair in the oral cavity of humans is more complex when compared to the repair of oral lesions in animals. Given this limitation, further in vivo studies of the topical use of AV and propolis are strongly recommended.

In conclusion, the results obtained in this study showed that the administration of 0.5% AV mucoadhesive gel and 5% propolis at 12-h intervals was not able to optimize the healing process of oral wounds. Thus, new randomized controlled clinical trials are needed to standardize effective therapeutic parameters for the clinical use of AV and propolis in the treatment of oral ulcers.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by J Azevedo, E Julião, FL Sousa, and A Medrado. The first draft of the manuscript was written by J Azevedo, E Julião, J Borges, and J Néri, and all authors commented on the previous versions of the manuscript. All authors have read and approved the final manuscript.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

REFERENCES

Abbasi AJ, Mohammadi F, Bayat M, Gema SM, Ghadirian H, Seifi H, Bayat H, Bahrami N (2018) Applications of Propolis in Dentistry: A Review. Ethiop J Health Sci 28(4):505-512. https://www.doi.org/10.4314/ejhs.v28i4.16.

Alaei S, Omidian H (2021) Mucoadhesion and Mechanical Assessment of Oral Films. Eur J Pharm Sci 159:1-17. https://doi.org/10.1016/j.ejps.2021.105727.

Almeida DC, Alves DCB, Jardim Júnior IJ, Mendonça SMS (2016) Própolis na Odontologia: Uma Abordagem de suas Diversas Aplicabilidades Clínicas. Rev Flum Odontol 2(46):1-12.

Babaee N, Zabihi E, Mohseni S, Moghadamnia AA (2012) Evaluation of the therapeutic effects of Aloe vera gel on minor recurrent aphthous stomatitis. Dent Res J. (Isfahan) 9(4):381–85. https://www.doi.org/10.4103/1735-3327.102758

Bolouri AJ, Pakfetrat A, Tonkaboni A, Aledavood SA, Najaf MF, Delavarian Z, Shakeri MT, Mohtashami A (2015) Preventing and Therapeutic Effect of Propolis in Radiotherapy Induced Mucositis of Head and Neck Cancers: A Triple-Blind, Randomized, Placebo-Controlled Trial. Iran J Cancer Preven 8(5):1-6. https://www.doi.org/10.17795/ijcp-4019

Coelho FH, Salvadori G, Rados PV, Magnusson A, Danilevicz CK, Meurer L, Martins MD (2015) Topical Aloe Vera (Aloe barbadensis Miller) Extract Does Not Accelerate the Oral Wound Healing in Rats. Phytother Res 29(7):1102-5. https://www.doi.org/10.1002/ptr.5352

Cuba LF, Braga Filho A, Cherubini K, Salum FG, Figueiredo MAS (2016) Topical application of Aloe vera and vitamin E on induced ulcers on the tongue of rats subjected to radiation: clinical and histological evaluation. Support Care Cancer 24(6):2557-64. https://www.doi.org/10.1007/s00520-015-3048-3

Desai VC, Shirsand SB, Malpani A, Hiremath S (2020) Avaliação do gel mucoadesivo de dexametasona fosfato sódico no tratamento da fibrose submucosa oral induzida por arecolina em ratos albinos wister: um estudo transversal. Indian J Dent Res 31(5):685-693. https://doi.org/10.4103/ijdr.IJDR_685_19.

Díaz JCQ, Rodrígues AO, Velázques MD, Milián ML (1997) Empleo de la tintura de propóleo al 5 en la cura de herida **sépticas** faciales. Rev Cuba Estomatol 34(1):347-51. https://www.doi.org/

Dudding T, Haworth S, Lind PA, Sathirapongsasuti JF, Tung JY, Mitchell R et al (2019). Genome wide analysis for mouth ulcers identifies associations at immune regulatory loci. Nat Commun 10(1052):1-12. doi: 10.1038/s41467-019-08923-6

El-Batal AI, Ahmed SF (2018) Therapeutic effect of Aloe vera and silver nanoparticles on acid-induced oral ulcer in gamma-irradiated mice. Braz Oral Res 32:1-9. https://doi.org/10.1590/1807-3107bor-2018.vol32.0004

Ernawati DS, Puspasari A (2018) Expression of vascular endothelial growth factor and matrix metalloproteinase-9 in Apis mellifera Lawang propolis extract gel-treated traumatic ulcers in diabetic rats. Vet World 11(3):304-309. https://doi.org/10.14202/vetworld.2018.304-309

Eshghi F, Hosseinimehr SJ, Rahmani N, Khademloo M, Norozi MS, Hojati O (2010) Effects of Aloe vera cream on posthemorrhoidectomy pain and wound healing: results of a randomized, blind, placebo-control study. J Altern Complement. Med 16(6):647-50. https://www.doi.org/ 10.1089/acm.2009.0428

Faleiro CC, Elias STH, Cavalcanti LC, Cavalcanti ASS (2009) O extrato das folhas de babosa, Aloe vera na cicatrização de feridas experimentais em pele de ratos, num ensaio controlado por placebo. Natureza Online 7(2):56- 60.

Fani MM, Ebrahimi H, Pourshahidi S, Aflaki E, Shafiee Sarvestani S (2012) Comparing the Effect of Phenytoin Syrup and Triamcinolone Acetonide Ointment on Aphthous Ulcers in Patients with Behcet's Syndrome. Iran Red Crescent Med J 14(2):75-8.

Fitzpatrick SG, Cohen DM, Clark AN (2019). Ulcerated Lesions of the Oral Mucosa: Clinical and Histologic Review. Head Neck Pathol 13(1):91–102. doi: 10.1007/s12105-018-0981-8

Freitas VS, Rodrigues RAF, Gaspi FOG (2014) Propriedades farmacológicas da Aloe vera (L.) Burm. f. Rev Bras Plantas Med 16(2):299-307. https://www.doi.org/10.1590/S1516-05722014000200020

Grégio AMT, Lima AAS, Ribas MO, Barbosa APM, Pereira ACP, Koike F, Repeke CEP (2005) Efeito da propolis mellifera sobre o processo de reparo de lesões ulceradas na mucosa bucal de ratos. Estud Biolog 27(58):43-47. https://www.doi.org/10.7213/reb.v27i58.21912

Gupta VK, Malhotra S (2012) Pharmacological attribute of aloe vera: Revalidation through experimental and clinical studies. Rev AYU 33(2):193-6. https://www.doi.org/10.4103/0974-8520.105237

Jongjitaree S, Koontongkaew S, Niyomtham N, Yingyongnarongkul BE, Utispan K (2022) The Oral Wound Healing Potential of Thai Propolis Based on Its Antioxidant Activity and Stimulation of Oral Fibroblast Migration and Proliferation. Evid Based Complementary Altern Med. https://doi.org/10.1155/2022/3503164

Khorasani G, Hosseinimehr SJ, Azadbakht M, Zamani A, Mahdavi MR (2009) Aloe versus silversulfadiazine creams for second-degreeburns: a randomized controlled study. Surg Today 39(7):587-91. https://www.doi.org/10.1007/s00595-008-3944-y

Komati S, Swain S, Rao MEB, Jena BR, Dasi V (2019) Mucoadhesive Multiparticulate Drug Delivery Systems: An Extensive Review of Patents. Adv Pharm Bull 9(4):521-538. https://www.doi.org/10.15171/apb.2019.062.

Kumar R, Mirza MA, Naseef PP, Kuruniyan MS, Zakir F, Aggarwal G (2022) Exploring the Potential of Natural Product-Based Nanomedicine for Maintaining Oral Health. Molecules 27(5):1-21. https://www.doi.org/10.3390/moléculas27051725.

Lehman JS, Rogers RS (2016) Acute Oral Ulcers. Clin Dermatol 34(4):470-74. https://www.doi.org/10.1016/j.clindermatol.2016.02.019

Liang J, Cui L, Li J, Guan S, Zhang K, Li J (2021) Aloe vera: A medicinal plant used in skin wound healing. Tissue Eng Parte B Ver 27(5):455-474. https://www.doi.org/10.1089/dez.TEB.2020.0236.

Lotufo MA, Lemos Júnior CA, Shimizu MT, Cabral R, Birman EG (2005) Clinical evaluation of the topical use of propolis in recurrent minor aphthous ulceration. Ciênc Odontol Bras 8(3):6-9. https://www.doi.org/10.14295/bds.2005.v8i3.327

Mansour G, Ouda S, Shaker A, Abdallah HM (2014) Clinical efficacy of new aloe vera- and myrrh-based oral mucoadhesive gels in the management of minor recurrent aphthous stomatitis: a randomized, double-blind, vehicle-controlled study. J Oral Pathol Med 43(6):405–09. https://www.doi.org/10.1111/jop.12130

Martins MD, Marques MM, Bussadori SK, Martins MAT, Pavesi VCS, Mesquita-Ferrari RA, Fernandes KPS

(2009) Comparative Analysis between Chamomilla recutita and Corticosteroids on Wound Healing. An In Vitro and In Vivo Study. Phytother Res 23:274–278. https://www.doi.org/10.1002/ptr.2612

Martorelli SBF, Pinheiro ALB, Souza IA, Higino JS, Bravo F (2011) Efeito aintiinflamatório e cicatrizante do extrato hirdoalcoólico de *Schinusterebinthifolius* Raddi (aroeira) a 30% em orobase – estudo "*in vivo*". Int J Dent 10(2):80-90.

Mercês PL, Araújo LA, Araújo ACV, Santos MHAS, Lemes SR., Melo-Reis PR (2017) Avaliação da Atividade Cicatricial do Aloe vera em Feridas em Dorso de Ratos. Estima (online) 15(1):35-42. https://www.doi.org/10.5327/Z1806-3144201700010006

Nagieb CS, Harhash TAEH, Fayed HL, Ali S (2022) Evaluation of diode laser versus topical corticosteroid in management of Behcet's diseaseassociated oral ulcers: a randomized clinical trial. Clin Oral Investig 26(1):697-704. https://www.doi.org/10.1007/s00784-021-04047-8.

Nimma VL, Talla HV, Bairi JK, Gopaldas M, Bathula H, Vangdoth S (2017) Holistic Healing Through Herbs: Effectiveness of Aloe Vera on Post Extraction Socket Healing. J Clin Diagn Res 11(3): 83-6. https://www.doi.org/10.7860/JCDR/2017/21331.9627

Noronha VRAS, Araujo GS, Gomes RT, Iwanaga SH, Barbosa MC, Abdo EN, Ferreira EF, Campos ACV, Souza AA, Abreu SR, Santos VR (2014) Mucoadhesive Propolis Gel for Prevention of Radiation-Induced Oral Mucositis. **Current Clinical Pharmacology (online).** 9(4):359-64. https://www.doi.org/10.2174/1574884709666140205210051

Oliveira BV, Barros Silva PG, Nojosa JS, Brizeno LAC, Ferreira JM, Sousa FB, Mota MRL, Alves APNN (2016) TNF-alpha expression, evaluation of collagen, and TUNEL of *Matricaria recutita L.* extract and triamcinolone on oral ulcer in diabetic rats. J Appl Oral Sci 24(3):278-90. https://doi.org/10.1590/1678-775720150481.

Oryan A, Alemzadehb E, Moshiri A (2018) Potential role of propolis in wound healing: Biological properties and therapeutic activities. Biomed. Pharmacother 98(1):469-483. https://doi.org/10.1016/j.biopha.2017.12.069.

Pensin NR, Pensin C, Miura CSN, Boleta-Ceranto, DC (2009) Efeito de Pomada de Própolis em Orabase para Tratamento de Ulcerações Aftosas Recorrentes: Um Estudo Piloto. Arq Ciênc Saúde UNIPAR 13(3):199-204.

Puspasari A, Harijanti K, Soebadi B, Hendarti HT, Radithia D, Ernawati DS (2018) Effects of topical application of propolis extract on fibroblast growth factor2 and fibroblast expression in the traumatic ulcers of diabetic Rattus norvegicus. J Oral Maxillofac Pathol 22(1):54-8. https://doi.org/10.4103/jomfp.JOMFP_82_17.

Roque L, Alopaeus J, Reis C, Rijo P, Molpeceres J, Hagesaether E, Tho I, Reis C (2018) Mucoadhesive assessment of different antifungal nanoformulations. Bioinspir Biomim 13(5):1-31. https://www.doi.org/10.1088/1748-3190/aad488.

Sampaio LAS, Costa JS, Freire TFC, Reis SRA, Deiró TCBJ, Medrado ARAP (2018) Influence of protein malnutrition on cutaneous wound healing in rats. Rev Nutr. (online) 31(5):433-442. https://www.doi.org/10.1590/1678-98652018000500001

Schemel-Suárez M, López-López J, Chimenos-Küstner E (2015) Úlceras orales: diagnóstico diferencial y tratamento. Med Clin (Barc) 145(11):1-5. https://www.doi.org/10.1016/j.medcli.2015.04.017.

Shi Y, Wei K, Lu J, Wei J, Hu X, Chen T (2020) A Clinic Trial Evaluating the Effects of Aloe Vera Fermentation Gel on Recurrent Aphthous Stomatitis. Can J Infect Dis Med Microbiol 2020:1-9. https://doi.org/10.1155/2020/8867548.

Suter VGA, Sjölund S, Bornstein MM (2017) Effect of laser on pain relief and wound healing of recurrent aphthous stomatitis: a systematic review. Lasers Med Sci 32(4):953-963. https://www.doi.org/10.1007/s10103-017-2184-z

Syed TA, Ahmad SA, Holt AH, Ahmad SA, Ahmad SH, Afzal M (1996) Management ofpsoriasiswith Aloe vera extract in a hydrophilic cream: a placebo-controlled, double-blindstudy. Trop Med Int Health 1(4):505-9. https://www.doi.org/10.1046/j.1365-3156.1996.d01-91.x.

Teplick E, Ma O, Castillo DE, Zarei M, Hustad AP, Chen J, Li J (2018) Os efeitos do Aloe Vera na cicatrização de feridas na proliferação, migração e viabilidade celular. 30(9):263-268.

Wahyuni IS, Sufiawati I, Nittayananta W, Levita J (2022) Anti-Inflammatory Activity and Wound Healing Effect of *Kaempferia galanga L*. Rhizome on the Chemical-Induced Oral Mucosal Ulcer in Wistar Rats. J Inflamm Res 15:2281-2294. https://www.doi.org/10.2147/JIR.S359042. eCollection 2022.