UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

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USO TÓPICO DO EXTRATO DE ALOE VERA (Aloe *barbadensis* Miller) NO REPARO DE ÚLCERAS BUCAIS EM RATOS

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Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Cirurgião-Dentista.

Orientadora: Prof[a]. Dr[a]. Manoela Domingues Martins

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Ao meu pai, Angelo, por sempre me proteger e se preocupar com os mínimos detalhes do meu dia-a-dia, priorizando sempre a minha felicidade acima de qualquer coisa.

À minha mãe, Mônica, por torcer, rezar e acreditar constantemente no meu sucesso, por ser minha inspiração como mãe e como mulher.

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RESUMO

COELHO, Fernanda Hack. **Uso tópico do extrato de Aloe Vera (Aloe Barbadensis Miller) no reparo de úlceras bucais em ratos**. 2013. 28 f. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

O efeito da aplicação tópica do extrato de Aloe Vera (Aloe Barbadensis Miller) foi avaliada no reparo de feridas bucais de ratos em um modelo in vivo utilizando 72 ratos machos da linhagem Wistar, divididos em três grupos (n = 24): controle, placebo e Aloe Vera (0,5% em extrato hidroalcoólico). Úlceras cirurgicamente induzidas foram provocados no dorso da língua usando um instrumento punch de 3 mm de diâmetro. O grupo Aloe Vera recebeu duas aplicações diárias da medicação manipulada. Os animais foram sacrificados depois de 1, 5, 10 e 14 dias. Análise clínica (área da úlcera e percentual de reparo) e análise histopatológica (grau de reepitelização e inflamação) foram realizadas. A comparação das diferenças entre os resultados com base em grupo e período experimental, tanto na análise quantitativa ou semi - quantitativa foi realizada utilizando o teste de Kruskal -Wallis. O nível de significância foi de 5%. No dia 1, todos os grupos mostraram predominantemente infiltrado inflamatório agudo. No dia 5, houve reepitelização parcial e infiltrado inflamatório crônico. Nos dias 10 e 14 foi observado reparo total de úlceras. Não houve diferença significativa entre os grupos no reparo de úlceras da boca. Concluiu-se que o tratamento utilizando Aloe Vera não tem capacidade de acelerar o reparo de feridas em ratos.

Palavras-chave: Reparo de feridas. Úlceras orais. Aloe vera. Rato. Fitoterápico.

ABSTRACT

COELHO, Fernanda Hack**. Topical Aloe Vera (*Aloe Barbadensis Miller*) extract on oral wound healing in rats**. 28 f. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

The effect of topical application of Aloe Vera (*Aloe Barbadensis Miller*) extract was assessed on the healing of rat oral wounds in an *in vivo* model using 72 male Wistar rats divided into 3 groups (n = 24): control, placebo and Aloe Vera (0.5% extract hydroalcoholic). Surgically induced ulcers were caused in the dorsum of the tongue using a 3 mm punch tool. The Aloe Vera group received two daily applications. The animals were sacrificed after 1, 5, 10 and 14 days. Clinical analysis was performed (ulcer area and percentage of repair) and histopathological analysis (degree of re-epithelialization and inflammation). The comparison of the differences between scores based on group and experimental period, both in quantitative and semi-quantitative analysis was performed using the Kruskal-Wallis test. The significance level was 5%. On day 1, all groups showed predominantly acute inflammatory infiltrate. On day 5, there was partial epithelialization and chronic inflammatory infiltrate. On the days 10 and 14 total repair of ulcers was observed. There was no significant difference between groups in the repair of mouth ulcers. It is concluded that treatment using Aloe Vera as a herbal formulation did not accelerate oral wound healing in rats.

Keywords: Wound healing. Oral ulcer. Aloe vera. Rat. Phytotherapy.

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVA

O processo de reparo consiste em uma cascata de eventos celulares e moleculares que interagem para que ocorra a repavimentação e a reconstituição do tecido (MANDELBAUM; SANTIS; MANDELBAUM, 2003). Esse processo fisiológico tem como objetivo restaurar a integridade tecidual, podendo ser dividido em três fases que se interrelacionam dinamicamente: inflamatória, proliferativa e de remodelamento (ARAÚJO et al., 2010). A fase inflamatória compreende a agregação plaquetária e a coagulação sanguínea, assim como quimiotaxia de células inflamatórias para eliminação de componentes teciduais danificados. A fase proliferativa é responsável pelo fechamento da lesão compreendendo reepitelização, fibroplasia e angiogênese. Os dois últimos são responsáveis pela formação do tecido de granulação, o qual é essencial ao processo de reparo. Por fim, a fase de remodelamento é uma maturação dos elementos da matriz extracelular, com depósito de proteoglicanos e colágeno (MENDONÇA; COUTINHO-NETTO, 2009).

Lesões ulceradas na mucosa bucal são comuns na clínica odontológica, porém, seu diagnóstico é por vezes difícil pela grande quantidade de afecções que levam a essa condição. Dentre estas podem ser citadas as úlceras traumáticas, ulceração aftosa recorrente, doenças virais e bacterianas, úlceras idiopáticas ou relacionadas a doenças auto-imunes ou sistêmicas. (LEÃO; GOMES; PORTER, 2007).

O tratamento das úlceras em boca não está estabelecido e vários protocolos utilizando medicamentos de uso tópico ou sistêmico vêm sendo descritos (SCULLY; SHOTTS, 2000; FIELD; ALLAN, 2003; LEÃO; GOMES; PORTER, 2007; JURGE et al., 2005; MIZIARA, 2009). Usualmente, o tratamento visa aliviar a sintomatologia e acelerar o fechamento da ulceração dentre os quais são citados na literatura o uso de antisépticos, analgésicos, corticosteróides, antibióticos, imunomoduladores e antiinflamatórios. Tratamentos locais específicos como remoção cirúrgica, debridamento, terapia com laser, ultrasom de baixa densidade, cauterização química, barreiras físicas como adesivos de cianoacrilato também têm sido citados (SCULLY; SHOTTS, 2000; FIELD; ALLAN, 2003; LEÃO; GOMES; PORTER, 2007; JURGE et al., 2005; MIZIARA, 2009).

O uso de fitoterápicos no reparo de feridas vem sendo muito estudado (O'HARA, 1998; SHUKLA; RASIK; DHAWANl, 1999;MARGARET et al., 1998; SUGUNA et al., 2002;MUKHERJEE et al., 2003; PAIVA et al., 2002; BLAZSÓ et al., 2004; CHAUDHARI; MENGI, 2006; MENSAH et al., 2006; LUSBY;COOMBES; WILKINSON, 2006; SHETTY; UDUPA; UDUPA, 2007; SPERONI et al., 2007;MARTINS et al., 2009; ARAÚJO et al.,

2010.) isto porque, várias plantas tem princípios ativos como alcalóides, triterpenos e biomoléculas que influenciam uma ou mais fases do reparo. Nosso grupo vem trabalhando com diferentes fitoterápicos de uso tópico em modelo experimental de reparo de úlceras bucais dentre eles a camomila, folha de goiaba e rubim (FERNANDES et al., 2010; MARTINS et al., 2009; CERVERA et al., 2008).

O Aloe Vera é uma planta que tem sido usada na medicina tradicional por diversas culturas na tentativa de cura de inúmeras doenças (CHITHRA;SAJITHLAL; CHANDRAKASAN, 1998). Também chamada de Aloe *barbadensis* Miller, pertence à família Liliaceae, a qual tem aproximadamente 360 espécies. Aloe Vera é uma planta que cresce facilmente em climas quentes e secos (VOGLER; ERNEST, 1999). Essa planta possui diversas propriedades importantes, inclusive a de interferir no processo de reparo tecidual (HERLIHY et al., 1998).

A ação do Aloe Vera no reparo tecidual tem sido atribuída ao fato desta planta estimular a função dos macrófagos e a produção de óxido nítrico, assim como, aumentar a produção de colágeno no tecido de granulação, ter função vasodilatadora além de antibacteriana e antifúngica (INAN et al., 2007). Essa propriedade é citada na literatura tanto em seu uso tópico quanto sistêmico. Dentre os estudos que relatam o uso tópico de Aloe Vera, alguns verificaram que o processo de reparo foi acelerado (SYED et al., 1996; HEGGERS et al., 1997; CHITHRA; SAJITHLAL; CHANDRAKASAN, 1998; CHOONHAKARN et al., 2007; KHORASANI et al., 2009; MENDONÇA et al., 2009; ESHGHI et al., 2010) e outros verificaram que o processo de reparo foi retardado devido ao uso do fitoterápico (SCHMIDT; GREENSPOON, 1991; PAULSEN; KORSHOLM; BRANDRUPT, 2005).

Neste estudo pretendeu-se avaliar clínica e histopatologicamete a ação tópica do extrato hidroalcoólico de Aloe Vera a 0,5% no reparo de úlceras bucais em ratos.

2 ARTIGO CIENTIFICO

TOPICAL ALOE VERA (*ALOE BARBADENSIS MILLER*) EXTRACT ON ORAL WOUND HEALING IN RATS

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Abstract

The effect of topical application of Aloe Vera (*Aloe Barbadensis Miller*) extract was assessed on the healing of rat oral wounds in an *in vivo* model using 72 male Wistar rats divided into 3 groups (n = 24): control, placebo and Aloe Vera (0.5% extract hydroalcoholic into cream). Surgically induced ulcers were caused in the dorsum of the tongue using a 3 mm punch tool. The Aloe Vera received two daily applications while the placebo group received application of a neutral extract. Topical applications lasted around thirty seconds. The animals were sacrificed after 1, 5, 10 and 14 days. Clinical analysis was performed (ulcer area and percentage of repair) and histopathological analysis (degree of re-epithelialization and inflammation). The comparison of the differences between scores based on group and experimental period, both in quantitative and semi-quantitative analysis was performed using the Kruskal-Wallis test. The significance level was 5%. On day 1, all groups showed predominantly acute inflammatory infiltrate. On day 5, there was partial epithelialization and chronic inflammatory infiltrate. On the days 10 and 14 total repair of ulcers was observed. There was no significant difference between groups in the repair of mouth ulcers. It is concluded that treatment using Aloe Vera as a herbal formulation did not accelerate oral wound healing in rats.

Keywords: Wound healing. Oral ulcer. Aloe vera. Rat. Phytotherapy.

INTRODUCTION

Oral ulcerative lesions are common in dental practice; however, its diagnosis is sometimes made difficult due to the large number of diseases that lead to this condition. Among them traumatic ulcers, recurrent aphthous stomatitis, viral and bacterial diseases, idiopathic ulcers or related to autoimmune and systemic diseases may be cited (Chavan *et al*., 2012; Felix *et al*., 2012; Leão *et al*., 2007). The treatment of these conditions has not been fully established, and several protocols using topical or systemic drugs have been described (Chavan *et al*., 2012; Felix *et al*., 2012; Leão *et al*., 2007). Usually, the aim is to relieve symptoms and accelerate the repair of ulceration to allow tissue reconstitution (Mandelbaum *et al.*, 2003; Eming *et al*., 2007). This process can be divided into three phases that are interrelated dynamically, namely the inflammatory, proliferative and remodeling phases (Mandelbaum *et al.*, 2003; Eming *et al*., 2007; Mendonça and Coutinho-Neto, 2009). These phases are characterized by clotting, chemotaxis of inflammatory cells, re-epithelialization, fibroplasia, angiogenesis, granulation tissue formation and maturation of the elements of the extracellular matrix with deposition of proteoglycans and collagen (Araújo *et al.,* 2010). The use of herbal medicines in wound healing has been extensively studied, because plants have several active ingredients such as alkaloids, triterpenes and biomolecules that affect one or more repair phases (Fernandes *et al*., 2010; Martins *et al*., 2009; Martins *et al*., 2011; Mogosanu *et al.,* 2013; O'Hara *et al*., 1998). Among these herbal remedies, Aloe Vera is a plant that has been used in traditional medicine by many cultures in an attempt to cure numerous diseases (Chithra et al., 1998). Also called Aloe barbadensis Miller belongs to the family Liliaceae, which is approximately 360 species. Aloe Vera is a plant that grows easily in hot and dry climates (Chithra et al., 1998). This plant has several important properties, and may interfere in the process of tissue repair. The mechanism of Aloe Vera in tissue repair has been attributed to the fact that this plant stimulates macrophage function and nitric oxide production, as apart from increasing collagen production in the granulation tissue, Moreover,

this plant species has vasodilator, antibacterial and antifungal action (Herlihy *et al*., 1998; Vogler and Ernest, 1999). This property is cited in the literature in both topical and systemic applications; however, controversial results have been described. Some studies that investigated the topical use of Aloe Vera showed an acceleration of wound healing (Chithra *et al*., 1998; Choonhakarn *et al*., 2007; Eshghi *et al*., 2010; Heggers *et al.*, 1997; Khorasani *et al.*, 2009; Syed *et al.*, 1996), but in other studies wound healing was delayed (Schmidt and Greenspoon, 1991; Paulsen *et al*., 2005). The aim of this study was to evaluate the clinical and histopathological aspects of topical treatment with 0.5% Aloe Vera hydroalcoholic extract in oral wound healing in rats.

MATERIALS AND METHODS

Preparation of 0.5% extract hydroalcoholic (*Aloe Barbadensis Miller*). Commercially available concentrated Aloe Vera granulated powder of certified quality was used (Viafarma LTDA, freeze dried powder, 200:1 concentration). The oral topical cream was always prepared using powder from the same batch, in the same compounding pharmacy and under strict quality control. The components of the cream are: hydroalcoholic extract (solvent), nipagin (preservative) nipazol (preservative) phenonip (preservative) propylene glycol (vehicle), glycerol (vehicle) and distilled water. The final concentration of Aloe Vera was 0.5% as recommended for topical use (Eshghi *et al.*, 2010; Khorasani *et al*., 2009; Syed et al., 1996). The placebo was prepared using the same components of the gel, without Aloe Vera extract.

Animals. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and received approval from the ethics committee of the Porto Alegre University Hospital (HCPA, Brazil) (12-0134). Seventy-two male rats (*Rattus novegicus albinus, rodentia mammalia* – wistar lineage) weighing 150 to 200g were kept under standard conditions of temperature (20 to 24^{o}C) and light/dark cycle, with solid chow and water *ad*

libitum. The animals were randomly divided into three groups of 24 animals each: Control group (no treatment, only daily handling), Placebo Group (topical treatment with a hydroalcoholic extract without Aloe Vera) and Aloe Vera Group (topical treatment with 0.5% Aloe Vera extract hydroalcoholic).

Wound procedure and treatment protocol. Under aseptic conditions, the three groups were anesthetized with an intraperitoneal administration of ketamine (0.1ml/100g) and xylazine (0.05ml/100g). Traumatic ulcers measuring 3 mm in diameter were inflicted on the dorsum of the tongue following a standard punch biopsy technique. The animals in the Placebo and Aloe Vera groups received two daily applications of the product using a swab so as fully cover the wound at 12-h intervals throughout the experimental period. During application animals were kept under isoflurane inhalant anesthesia and it took a while for about thirty seconds for each animal. After the surgical procedure six rats in each group were euthanized using a CO_2 chamber on days 1, 5, 10 and 14. After, the wound area was measured and photographed.

Clinical analysis. Ulcer area was calculated based on length and width using a digital caliper. These measurements were multiplied by the area in square centimeters. The percentages of wound healing and healing time were recorded as described elsewhere (Khorasani *et al*., 2009). Briefly, percentage of healing was calculated as [(initial area– area at respective evaluation time) / (initial area)] X 100.

Histopathological analysis. After euthanasia, the tongues were removed and fixed in a 10% buffered formalin solution for 48 hours. After washing with water, the specimens were dehydrated and embedded in paraffin. Slices measuring 5µm in thickness were obtained and stained with hematoxylin-eosin. Descriptive analysis of each group/evaluation time was performed, followed by a semi-quantitative analysis. The analysis was done by two pathologists to reach a consensual final score. The degree of reepithelialization was determined a grading system (0 to 4), as described elsewhere (Sinha and Gallaher, 2003): Grade 0= reepithelialization on the margins of the wound; Grade 1= reepithelialization

covering less than half the wound; Grade 2= reepithelialization covering more than half the wound; Grade 3= reepithelialization covering the entire wound with irregular thickness; and Grade 4 = reepithelialization covering the entire wound with normal thickness. Inflammation was also evaluated according to a grading system, as described elsewhere (Kumar *et al.*, 2003; Camacho-Alonso and Lopez-Jornet, 2003): Grade 1 =acute inflammation (pyogenic membrane); Grade 2= prevalence of acute diffuse inflammation; Grade 3= prevalence of chronic inflammation (fibroblast proliferation); and Grade 4= resolution and healing (reduction in or disappearance of chronic inflammation, despite the persistence of some inflammatory cells).

Statistical analysis. The data were expressed as mean and standard deviation values. The SPSS version 18.0 was employed for the statistical analysis. Groups, evaluation times and the interaction between group and evaluation time were compared using the Kruskal-Wallis test. The significance level was set at 5% ($p < 0.05$).

RESULTS

Clinical analysis. The analysis of wound area revealed no differences between groups and no detectable signs of repair on Day 1. A decrease in mean wound area was found in all groups on Day 5, with the smallest area in the Aloe Vera group; however, no statistical difference was observed. On Day 10 and Day 14 all groups exhibited repaired lesions with no statistically significant differences between groups (Table 1 and Fig. 1a).

Table 1. Clinical evaluation of mean and standard error of wound area (in mm^2).

Group	Day 1	Day 5	Day 10	Day 14
Control	5,2 ± 1,19 aA	3,18 ±0,69 aA	0,0 ±0,0 bA	0,0± 0,0 bA
Placebo	2,89 ±0,96 aA	1,31 ± 1,31 aA	0,0 ± 0,0 aA	0,0± 0,0 aA
Aloe Vera	3,02 ±0,8 aA	1,01 ±0,81 aA	0,02 ±0,03 aA	0,0± 0,0 aA

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test).

The percentages of wound healing analyzed on Day 1 revealed no significant differences between groups. An increase in percentage of wound healing was gradually observed on Days 5, 10 and 14. On Day 10 a total closure of wound was observed in all groups, without differences (Table 2 and Fig.1b).

Table 2. Clinical evaluation of mean and standard error of healing percentage.

Group	Day 1	Day 5	Day10	Day14
Control	26,2 ± 16,94 aA	54,9 ±9,82 aA	100 ±0,0 bA	100 ± 0,0 bA
Placebo	58,5 ± 13,3 aA	77,4 ± 18,06 aA	100 ±0,0 aA	100 ± 0,0 aA
Aloe Vera	56,5 ± 11,3 aA	85,5 ± 11,5 aA	99,6 ±0,51 aA	100± 0,0 aA

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test).

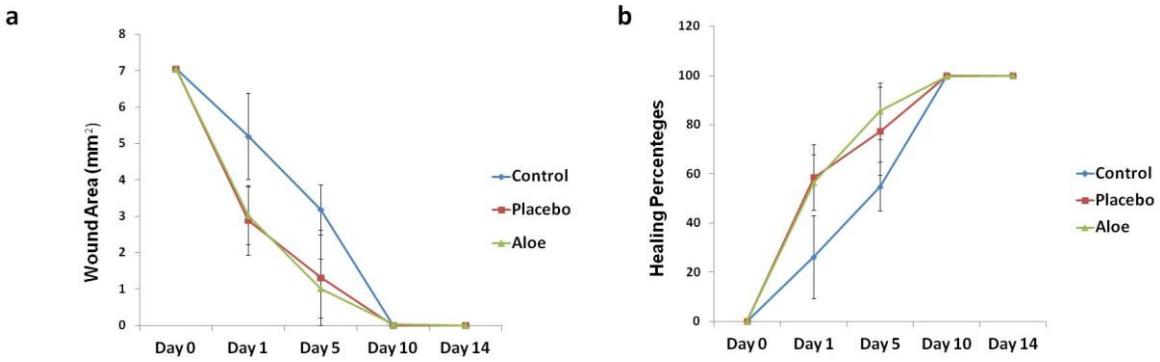

Figure 1 (a) Clinical evaluation of mean and standard error of ulcer area (in mm^2) in all groups; (d) Clinical evaluation of percentage of wound healing (%)in all groups during the experimental time.

Histopathological analysis

Descriptive analysis

On day 1 an ulcer with exposure of connective tissue was observed in all groups. Discrete or no migration of epithelial cells in the center of the wound was found in all groups. Intense diffuse acute inflammation (polymorphonuclear infiltrate) and hemorrhage was noticed in the wound area.

On Day 5, reepithelialization covering less than half of the wound was found in the three groups, with some animals exhibiting reepithelization in more than half of the wound. Some focal points of acute inflammation were still present in most animals, but the number of mononuclear cells was higher than on Day 1. Mononuclear infiltrate, neovascularization and fibroblast proliferation were evident.

On Day 10, all groups exhibited reepithelialization covering the entire wound. The three groups exhibited epithelium with irregular thickness. The three groups had chronic inflammation, and some animals had complete healing and resolution of the inflammatory process.

On Day 14, all groups showed reepithelialization across the wound, some with irregular thickness, others normal. All groups showed sparse chronic inflammation with new muscle fibers differentiation.

Semi-quantitative analysis

Table 3 and figure 2a demonstrates the mean results of the grading system analysis of reepithelialization, with no statistically significant differences between groups in all periods.

Table 3. Histopathological evaluation of degree of reepitelialization (mean and standard error).

Group	Day 1	Day 5	Day 10	Day 14
Control	0,75 ±0,95 aA	2,75 ±0,5 aA	3,5 ±0,5 aA	3,5± 0,5 aA
Placebo	1,0 ±0,81 aA	2,0 ±0,81 aA	3,75 ±0,5 aA	4,0± 0,0 aA
Aloe Vera	1,25 ±0,95 aA	1,75 ±0,5 aA	3,25 ±0,5 aA	3,75± 0,5 aA

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test).

Table 4 and figure 2b demonstrates the mean values of the grading system analysis of inflammation, no statistically significant differences between groups, across all periods.

Table 4. Histopathological evaluation of degree of inflammation (mean and standard error).

Group	Day 1	Day 5	Day 10	Day 14
Control	1,0 ±0,0 aA	2,5 ±0,5 aA	3.75 ±0,5 aA	3,75± 0,5 aA
Placebo	1,25 ±0,5 aA	1,75 ±0,95 aA	4,0 ±0,0 aA	4,0± 0,0 aA
Aloe Vera	1,5 ±0,5 aA	2,5 ±1,0 aA	3,25 ±0,5 aA	3,5± 0,5 aA

Different lowercase letters on lines (intra-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test); Different uppercase letters in columns (inter-group analysis) denote significant difference ($p < 0.05$, Kruskal-Wallis test).

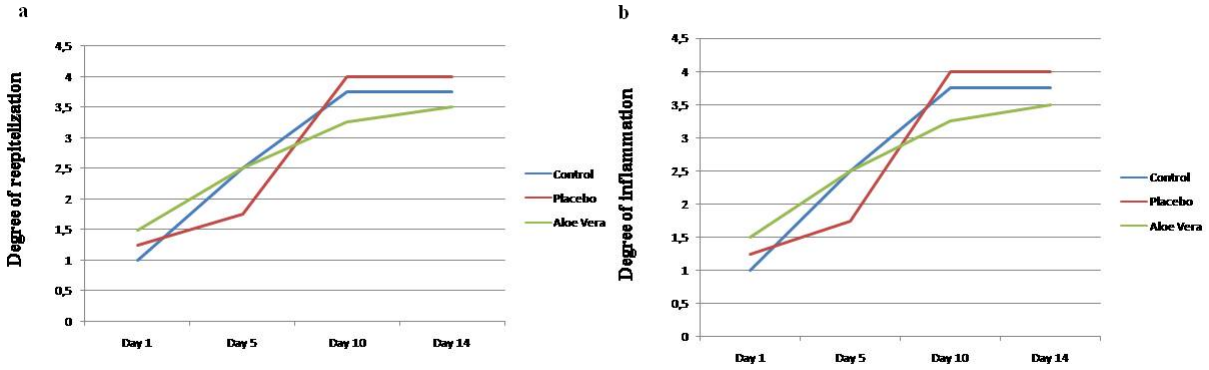

Figure 2 (a) Histopathological evaluation of degree of reepitelialization; (b) Histopathological evaluation of degree of inflammation.

DISCUSION

A wide range of therapies to accelerate oral wound healing of oral ulcers have been suggested in recent years (Chavan *et al*., 2012; Felix *et al*., 2012; Leão *et al*., 2007). The most used treatments for these conditions include corticosteroids that could cause to several side effects. In an attempt to discover potential replacement drugs, this study analyzed Aloe Vera in oral ulcer wound healing in rats. In our study, the clinical and histopathological evaluations showed that the topical treatment with 0.5% Aloe Vera hydroalcoholic extract was not effective to accelerate oral wound healing.

Several studies have investigated the effects of different formulations of Aloe Vera (concentrations and vehicles) in the treatment of various inflammatory diseases in order to speed up healing. Some of these studies show that topic Aloe Vera accelerated the healing process of wounds resulting from burns (Khorasani *et al.*, 2009), psoriasis vulgaris (Syed *et al.*, 1996), gynecological surgical interventions (Schmidt and Greenspoon 1991) and oral lichen planus (Choonhakarn *et al.*, 2007). However, other investigations revealed that the use of Aloe Vera delayed the healing process (Paulsen *et al.*, 2005; Schmidt and Greenspoon, 1991). Taken together, these studies do not afford to establish a comparison, since the methods to obtain Aloe Vera, its final concentration and tested vehicles were different across

the experimental protocols. Some of these studies even fail to provide information about the final concentration of Aloe Vera tested (table 5).

In our study, the Aloe Vera extract tested revealed a moderate inflammation process in the initial days of ulcer healing followed by the reepithelialization, formation of granulation tissue and remodeling of the connective tissue. However, these effects were similar in the control and placebo groups. Although the 0.5% Aloe Vera extract hydroalcoholic did not promote an increase in wound healing, it did not present any side effect. One possible explanation for the non-healing effect of Aloe Vera tested in our study could be associated with product manufacture methods and concentration of the phytotherapic agent, when compared to the literature. One of the most discussed component of Aloe Vera that could lead to a different response are polysaccharides (Paulsen *et al*., 2005). Whatever the reason, commercial Aloe Vera products have been shown to contain widely varying levels of polysaccharides that have been attributed to differing climatic conditions, different gel preparation techniques and product storage. A low polysaccharide content would make the Aloe Vera gel more similar to the placebo gel. Unfortunately, our Aloe Vera gel was not tested for its content.

Other important point that could influence tissue response is Aloe Vera concentration. We decided to use the 0.5% Aloe Vera concentration based on some studies that previously indicate positive results (Eshghi *et al.*, 2010; Khorasani *et al*., 2009; Syed et al., 1996) and because local side effects like erythema and desquamation were evident when the highest concentration (98%) was used. However, based on our results, we believe that 0.5% Aloe Vera for oral wound healing was not sufficient to induce faster healing. This could be explained based on the fact that the healing of oral mucosa is faster than in the skin, and that perhaps low concentrations of Aloe Vera are not sufficient to stimulate the oral epithelium, which usually has higher proliferative index. Whatever the explanation is, however, the

implication is that the 0.5% Aloe Vera extract hydroalcoholic tested did not add significantly to the therapeutic effect in oral wounds. Further studies comparing different concentrations of Aloe Vera in oral ulcers are recommended before use in humans with the aim to achieve the best formulation for treatment of oral lesions.

Table 5. Studies using Topical Aloe Vera form.

Author	**Treated condition**	**Sample**	**Concentration of Aloe Vera**	**Vehicle**	**Results**
Khorasani et al. (2008)	Second degree burns	Humans	0,5%	Spray powder	Accelerated wound healing
Syed et al. (1996)	Psoriasis vulgaris	Humans	0,5%	Hydrophilic cream	Accelerated wound healing
Schmidt, Greenspoon (1991)	Healing by second intention	Humans	Not Specified	Commercial Aloe Vera Gel (Carrington Dermal Wound Gel)	Delayed healing
Choonhakarn et al. (2007)	Lichen planus	Humans	70%	Mucilage, sorbitol, potassium sorbate, sodium metabisulphite, hydroxyethylcellulose	Accelerated wound healing
Paulsen et al. (2004)	Psoriasis vulgaris	Humans	98%	Commercial Aloe Vera Gel^{R} (Aloe Vera Group ApS, Soborg, Denmark)	Delayed healing
Heggers et al. (1997)	Excisional wound model	Rats	Not Specified	Commercial Aloe Vera Gel (Dermaide Aloe)	Accelerated wound healing
Chithra et al. (1997)	Excisional wound model	Rats	Not Specified	Aloe Vera Gel (Water and lyophilized Aloe Vera powder)	Accelerated wound healing
Eshghi et al. (2010)	Posthemorrhoidectomy	Humans	0,5%	Cream with propylene glycol, sodium lauryl sulfate and methylparaben	Accelerated wound healing
Mendonça et al. (2009)	Excisional wound model	Rats	Not Specified	Mucilage	Accelerated wound healing

Conclusion

In conclusion, the findings of the present study show that the 0.5% Aloe Vera hydroalcoholic extract does not promote any local side effect, but it is not useful to improve the healing of oral ulcers.

Acknowledgments

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References

1. Araújo LU, Guimarães AG, Mosqueira VCF, Carneiro CM, Barcellos NMS. 2010. Profile of wound healing process induced by allantoin. *Acta Cirúrgica Brasileira*. **25:** 460-466.
2. Camacho-Alonso F, Lopez-Jornet P. 2007. Clinical-pathological study of the healing of wounds provoked on the dorso-lingual mucosa in 186 albino rats. *Otolaryngol. Head Neck Surg*. **136:** 119-24.
3. Chavan M, Jain H, Diwan N, Khedkar S, Shete A, Durkar S. 2012. Recurrent aphthous stomatitis: a review. *J Oral Pathol Med*. **8:** 577-83.
4. Chithra P, Sajithlal GB, Chandrakasan G. 1998. Influence of Aloe vera on collagen characteristics in healing dermal wound in rats. *Molecular And Cellular Biochemistry*. **181:** 71-76.
5. Choonhakarn C, Busaracome P, Sripanidkulchai B, Sarakarn P. 2007. The efficacy of aloe vera gel in the treatment of oral lichen planus: a randomized controlled trial. *British Journal Of Dermatology*. **158:** 573-577.
6. Eming SA, Krieg T, Davidson JM. 2007. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol.* **27:** 514–25.

7. 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. *The Journal Of Alternative And Complementary Medicine*. **16:** 647-650.
8. Felix DH, Luker J, Scully C. 2012. Oral medicine: 1. Ulcers: aphthous and other common ulcers. *Dent Update*. **39:** 513-6, 518-9.
9. Fernandes KPS, Bussadori SK, Marques MM, Wadt NSY, Bach E, Martins MD. 2010. Healing and cytotoxic effects of Psidiumguajava (Myrtaceae) leaf extracts. *Brazilian Journal of Oral Sciences*. **9:** 449-454.
10. Heggers JP, Elzaim H, Garfield R, Goodheart R, Listengarten D, Zhao J, Phillips LG. 1997. Effect of the Combination of Aloe Vera, Nitroglicerin, and L-NAME on Wound Healing in the Rat Excisional Model. *The Journal Of Alternative And Complementary Medicine*. **3:** 149-153.
11. Herlihy JT, Bertrand HA, Kim JD, Ikeno Y, Yu BP. 1998. Effects of Aloe Vera Ingestion in the Rat I. Growth, Food and Fluid Intake and Serum Chemistry. *Phytotherapy Research*. **12:** 183-188.
12. Khorasani G, Hosseinimehr SJ, Azadbakht M, Zamani A, Mahdavi MR. 2009. Aloe Versus Silver Sulfadiazine Creams for Second-Degree Burns: A Randomized Controlled Study. *Surgery Today*. **39:** 587-591.
13. Kumar V, Cotran RS, Robbins SL. 2003. *Robbins basic pathology.* (7nd edn). Imprenta Philadelphia, PA: Saunders.
14. Leão JC, Gomes VB, Porter S. 2007. Ulcerative Lesions of the Mouth: an update for the general medical practitioner. *Clinics* **62:** 769-780.
15. Mandelbaum SH, Santis EPD, Mandelbaum, MHS. 2003. Cicatrization: current concepts and auxiliary resources -Part I. *An Bras Dermatol*. **78:** 393-408.

16. Martins MD, Marques MM, Bussadori SK, Martins MA, Pavesi VC, Mesquita-Ferrari RA, *et al*. 2009. Comparative analysis between Chamomilla recutita and corticosteroids on wound healing. An in vitro and in vivo study. *Phytother Res*. **23:** 274-278.
17. Martins MD, Fernandes KPS, Pavesi VC, França CM, Mesquita RA, Bussadori SK. 2011. Healing properties of papain-based gel on oral ulcers. *Braz J Oral Sci*. **10:** 120-123.
18. Mendonça RJD, Coutinho-Netto, J. 2009. Cellular aspects of wound healing. *An Bras Dermatol*. **84:** 257-262.
19. Mendonça FAS, Junior JRP, Esquisatto MAM, Mendonça JS, Franchini CC, Santos GMT. 2009. Effects os the application of Aloe vera and microcurrent on the healing of wounds surgically induced in Wistar rats. *Acta Cirúrgica Brasileira*. **24:** 150-156.
20. Mogosanu GD, Popescu FC, Busuio CJ, Lascar I, Mogoanta L. 2013. Comparative study of microvascular density in experimental third-degree skin burns treated with topical preparations containing herbal extracts. *Rom J Morphol Embryol*. **54:** 107–113.
21. O'hara M, Kiefer D, Farrell K, Kemper K. 1998. A Review of 12 Commonly Used Medicinal Herbs. *Arch Fam Med.* **7:** 523-536.
22. Paulsen E, Korsholm L, Brandrupt F. 2005. A double-blind, placebo-controlled study of a commercial Aloe vera gel in the treatment of slight to moderate psoriasis vulgaris. *European Academy Of Dermatology And Venereology*. **19:** 326-331.
23. Schmidt JM, Greenspoon JS. 1991. Aloe Vera Dermal Wound Gel Is Associated With a Delay in Wound Healing. *Obstetrics e Gynecology.* **78:** 647-650.
24. Sinha UK, Gallagher LA. 2003. Effects of steel scalpel, ultrasonic scalpel, CO2 laser, and monopolar and bipolar electrosurgery on wound healing in guinea pig oral mucosa. *Laryngoscope*. **113:** 228-36.

25. Syed TA *et al*. 1996. Management of psoriasis with Aloe vera extract in a hydrophilic cream: a placebo controlled, double-blind study. *Tropical Medicine And International Health*. **1:** 505-509.

26. Vogler BK, Ernest E. 1999. Aloe Vera: a systematic review of its clinical effectiveness. *British Journal Of General Practice*. **49:** 823-828.

3 CONSIDERAÇÕES FINAIS

Baseado no resultado do presente estudo podemos concluir que o uso tópico do extrato hidroalcoólico de Aloe Vera 0,5% não foi capaz de acelerar o reparo de úlceras bucais em ratos tanto clínica quanto histopatologicamente.

REFERÊNCIAS

ARAÚJO, L.U. et al. Profile of wound healing process induced by allantoin. **Acta Cirúrgica Brasileira**, Ouro Preto, v. 25, p. 460-466, 2010.

BLAZSÓ, G. et al. Pycnogenol accelerates wound healing and reduces scar formation. **Phytotherapy Research**, Malden, v. 18, p. 579-581, 2004.

CAMACHO-ALONSO, F.; LOPEZ-JORNET, P. Clinical-pathological study of the healing of wounds provoked on the dorso-lingual mucosa in 186 albino rats. **Journal of Otolaryngology - Head & Neck Surgery**. London, v. 136, p. 119-24, 2004.

CERVERA, A. et al. Ação do Agaricus blazei no processo de cicatrização de úlceras. In: 25a Reunião Anual da SBPqO, 2008, Águas de Lindóia. **Brazilian Oral Research**, 2008. v. 22, p. 92-92.

CHAUDHARI, M.; MENGI, S. Evaluation of phytoconstituents of terminalia arjuna for wound healing activity in rats. **Phytotherapy Research**, Malden, v. 20, p. 799-805, 2006.

CHAVAN, M. et al. Recurrent aphthous stomatitis: a review. **Journal of Oral Pathology & Medicine**, Malden, v. 8, p. 577-83, 2012.

CHITHRA, P.; SAJITHLAL, G.B.; CHANDRAKASAN, G. Influence of Aloe vera on collagen characteristics in healing dermal wound in rats. **Molecular And Cellular Biochemistry**, Madras, v. 181, p. 71-76, 1998.

CHOONHAKARN, C. et al. The efficacy of aloe vera gel in the treatment of oral lichen planus: a randomized controlled trial. **British Journal Of Dermatology**, Malden, v. 158, p. 573-577, 2007.

EMING, S.A.; KRIEG, T.; DAVIDSON, J.M. Inflammation in wound repair: molecular and cellular mechanisms. **Journal of Investigative Dermatology**, Chapel Hill, v. 27, p. 514–25, 2007.

ESHGHI, F. et al. Effects of aloe vera cream on posthemorrhoidectomy pain and wound healing: results of a randomized, blind, placebo-control study. **The Journal Of Alternative And Complementary Medicine**, New York, v. 16, p. 647-650, 2010.

FELIX, D.H.; LUKER, J.; SCULLY, C. Oral medicine: 1. ulcers: aphthous and other common ulcers. **Dental Update**, Surrey, v. 39, p. 513-6, 518-9, 2012.

FERNANDES, K.P.S. et al. Biocompatibility analysis of MTA, Portland cement and modified Portland cement on cultured fibroblast cells and subcutaneous tissue. **ConScientiae Saúde**, São Paulo, v. 9, p. 11-16, 2010.

FIELD, E.A.; ALLAN, R.B. Review article: oral ulceration – etiopathogenesis, clinical diagnosis and management in the gastrointestinal clinic. **Alimentary, Pharmacology & Therapeutics**, Malden, v. 18, p. 949-962, 15 ago. 2003.

HEGGERS, J.P. et al. Effect of the combination of aloe vera, nitroglicerin, and L-NAME on wound healing in the rat excisional model. **The Journal Of Alternative And Complementary Medicine**, Texas, v. 3, p. 149-153, 1997.

HERLIHY, J.T. et al. Effects of aloe vera ingestion in the rat I: growth, food and fluid intake and serum chemistry. **Phytotherapy Research**, Malden, v. 12, p. 183-188, 1998.

INAN, A. et al. Effects of aloe vera on colonic anastomoses of rats. **Surgical Practice**, Hong Kong, v. 11, p. 60-65, 2007.

JURGE, S. et al. Recurrent aphthous stomatitis. **Oral Diseases**, London, v. 12, p. 1-21, 10 jan. 2005.

KHORASANI, G. et al. Aloe versus silver sulfadiazine creams for second-degree burns: a randomized controlled study. **Surgery Today**, Tokyo, v. 39, p.587-591, 2009.

KUMAR, V.; COTRAN, R.S.; ROBBINS, S.L. **Robbins basic pathology**. 7th ed. Philadelphia, PA: Saunders, 2003.

LEÃO, J.C.; GOMES, V.B.; PORTER, S. Ulcerative Lesions of the mouth: an update for the general medical practitioner. **Clinics**, Recife, v. 62, p. 769-780, 2007.

LUSBY, P.E.; COOMBES, A.L.; WILKINSON, J.M. A comparasion of wound healing following treatment with lavandula x allardii honey or essential oil. **Phytotherapy Research**, Malden, v. 20, p. 755-757, 2006.

MARGARET, I. et al. Wound healing activity of gliricidiamaculata. **Phytotherapy Research**, Malden, v. 12, p. 586-588, 1998.

MANDELBAUM, S.H.; SANTIS, E.P.D.; MANDELBAUM, M.H.S. Cicatrização: conceitos atuais e recursos auxiliares - Parte I. **Anais Brasileiros Dermatologia**, São Paulo, v. 78, p. 393-408, 2003.

MARTINS, M.D. et al. Comparative analysis between chamomilla recutita and corticosteroids on wound healing: an in vitro and in vivo study. **Phytotherapy Research**, Malden, v. 23, p. 274-278, 2009.

MARTINS, M.D. et al. Healing properties of papain-based gel on oral ulcers. **Brazilian Journal of Oral Sciences**, v. 10, Piracicaba, p. 120-123, 2011.

MENDONÇA, R.J.D.; COUTINHO-NETTO, J. Aspectos celulares da cicatrização. **Anais Brasileiros Dermatologia**, São Paulo, v. 84, p. 257-262, 2009.

MENDONÇA, F.A.S. et al. Efeitos da aplicação de Aloe Vera e microcorrente no reparo de lesões cirúrgicas induzidas em ratos Wistar. **Acta Cirúrgica Brasileira**, São Paulo, v. 24, p. 150-156, 2009.

MENSAH, A.Y. et al. In vitro evaluation of effects of two ghanaian plants relevant to wound healing. **Phytotherapy Research**, Malden, v. 20, p. 941-944, 2006.

MIZIARA, I.D. O tratamento da estomatite aftóide recorrente ainda intriga. **Revista da Associação Médica Brasileira**, São Paulo, v. 55, p. 95-107, 2009.

MOGOSANU, G.D. et al. Comparative study of microvascular density in experimental third-degree skin burns treated with topical preparations containing herbal extracts. **Romanian Journal of Morphology and Embryology**, Craiova, v. 54, p. 107–113, 2013.

MUKHERJEE, P.K. et al. Evaluation of wound healing activity of some herbal formulations. **Phytotherapy Research**, Malden, v. 17, p. 265-268, 2003.

O'HARA, M. et al. A review of 12 commonly used medicinal herbs. **Archives of Family Medicine**, Washington, v. 7, p. 523-536, 1998.

PAIVA, L.A.F. et al. Investigation on the wound healing activity of oleo-resin from copaifera langsdorffi in rats. **Phytotherapy Research**, Malden, v. 16, p. 737-739, 2002.

PAULSEN, E.; KORSHOLM, L.; BRANDRUPT, F. A double-blind, placebo-controlled study of a commercial aloe vera gel in the treatment of slight to moderate psoriasis vulgaris. **European Academy Of Dermatology And Venereology**, Los Angeles, v. 19, p. 326-331, 2005.

SCHMIDT, J.M.; GREENSPOON, J.S. Aloe vera dermal wound gel is associated with a delay in wound healing. **Obstetrics & Gynecology**, Los Angeles, v. 78, p. 647-650, 1991.

SCULLY, C.; SHOTTS, R. Mouth ulcers and other causes of orofacial soreness and pain. **Bmj**, London, v. 174, p. 162-165, 15 jul. 2000.

SINHA, U.K.; GALLAGHER, L.A. Effects of steel scalpel, ultrasonic scalpel, CO2 laser, and monopolar and bipolar electrosurgery on wound healing in guinea pig oral mucosa. **Laryngoscope**, New York, v. 113, 228-36, 2003.

SHETTY, S.; UDUPA, S. UDUPA, L. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of ocimum sanctum linn in rats. **Evidence-Based Complementary and Alternative Medicine**, New York, v. 5, p. 95-101, 4 abr. 2007.

SHUKLA, A.; RASIK, A.M.; DHAWAN, B.N. Asiaticoside-induced elevation of antioxidant levels in healing wounds. **Phytotherapy Research**, Malden, v. 13, p. 50-54, 1999.

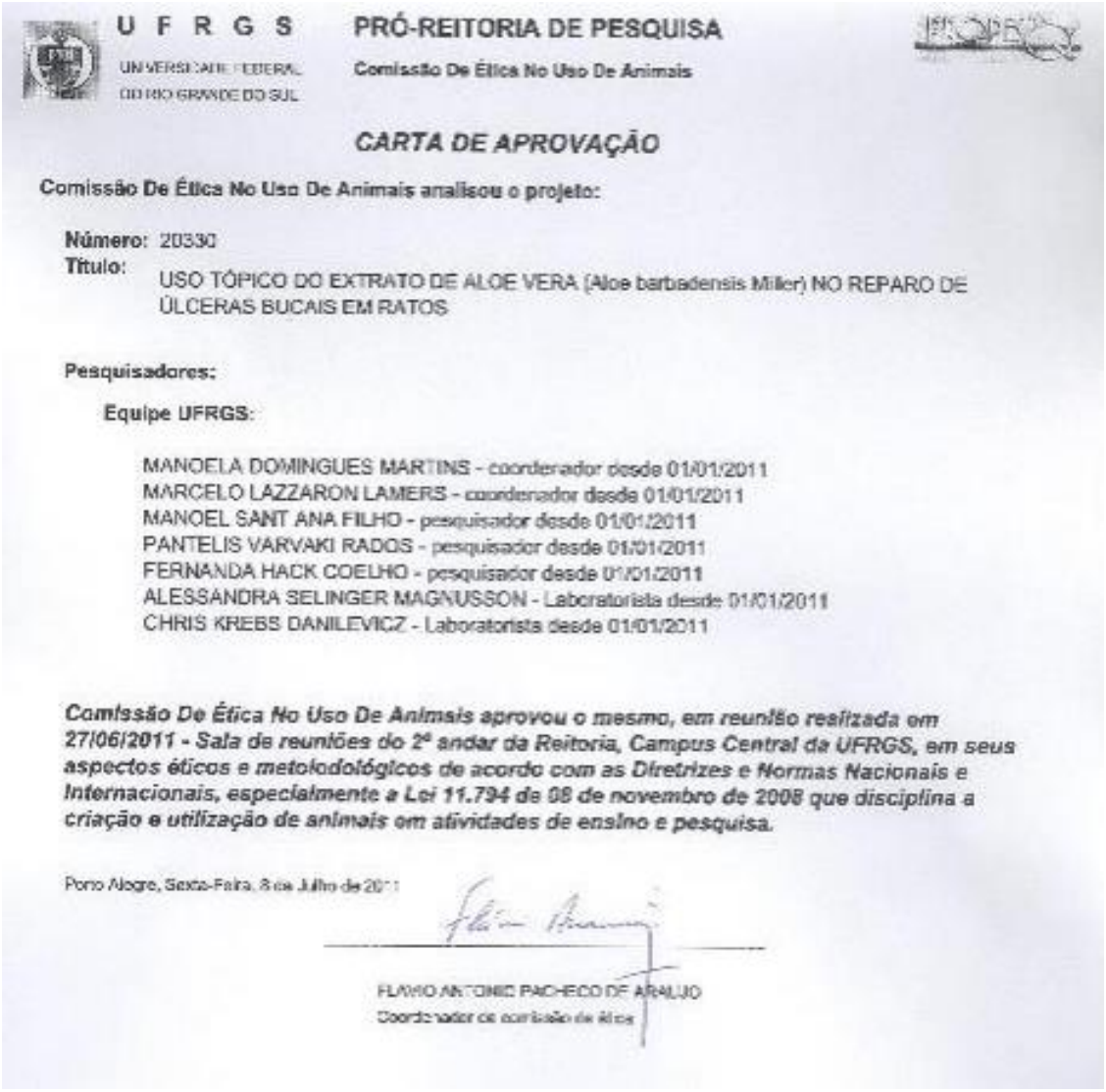
SPERONI, E. et al. Effects of differential extraction of verbena officinalis on rat models of inflammation, cicatrization and gastric damage. **Planta Medica**, Bologna, v. 73, p. 227-235, 2007.

SUGUNA, L. et al. Influence of terminalia chebula on dermal wound healing in rats. **Phytotherapy Research**, v. 16, Malden, p. 227-231, 2002.

SYED, T.A. et al. Management of psoriasis with aloe vera extract in a hydrophilic cream: a placebo controlled, double-blind study. **Tropical Medicine And International Health**, Malmo, v. 1, p. 505-509, 1996.

VOGLER, B.K.; ERNEST, E. Aloe Vera: a systematic review of its clinical effectiveness. **British Journal Of General Practice**, London, v. 49, p. 823-828, 1999.

ANEXO A – CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA



U F R G S
UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 20330
Título: USO TÓPICO DO EXTRATO DE ALOE VERA (Aloe barbadensis Miller) NO REPARO DE ÚLCERAS BUCAIS EM RATOS

Pesquisadores:

Equipe UFRGS:

MANOELA DOMINGUES MARTINS - coordenador desde 01/01/2011
MARCELO LAZZARON LAMERS - coordenador desde 01/01/2011
MANOEL SANT ANA FILHO - pesquisador desde 01/01/2011
PANTELIS VARVAKI RADOS - pesquisador desde 01/01/2011
FERNANDA HACK COELHO - pesquisador desde 01/01/2011
ALESSANDRA SELINGER MAGNUSSON - Laboratorista desde 01/01/2011
CHRIS KREBS DANILEVICZ - Laboratorista desde 01/01/2011

Comissão De Ética No Uso De Animais aprovou o mesmo, em reunião realizada em 27/06/2011 - Sala de reuniões do 2º andar da Reitoria, Campus Central da UFRGS, em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008 que disciplina a criação e utilização de animais em atividades de ensino e pesquisa.

Porto Alegre, Sexta-Feira, 8 de Julho de 2011

FLAVIO ANTONIO PACHECO DE ARAUJO
Coordenador da comissão de ética