PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
ESCOLA DE CIÊNCIAS DA SAÚDE
ESTOMATOLOGIA CLÍNICA

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EFEITO DA APLICAÇÃO TÓPICA DO EXTRATO DE CAMOMILA E DA CURCUMINA EM ÚLCERAS TRAUMÁTICAS EM LÍNGUA DE RATOS: ANÁLISE CLÍNICA E HISTOLÓGICA

Porto Alegre
2018

PÓS-GRADUAÇÃO - *STRICTO SENSU*

Pontifícia Universidade Católica
do Rio Grande do Sul

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Dissertação apresentada à Escola de Ciências da Saúde da Pontifícia Universidade Católica do Rio Grande do Sul como parte dos requisitos para obtenção do título de Mestre em Odontologia, área de concentração em Estomatologia Clínica.

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Porto Alegre

2018

EPÍGRAFE

“A verdadeira viagem do descobrimento não consiste em procurar novas paisagens,

mas em ter novos olhos”.

Marcel Proust

AGRADECIMENTOS

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Agradeço a ***Deus***, quem me deu o dom da vida e me dá forças para seguir em frente a cada novo desafio.

Agradeço à minha família, *meus pais*, ***Jaime e Cristiane***, e minha irmã, ***Jaciane***, pelo apoio incondicional, pelas palavras de incentivo e de carinho a cada nova jornada por mim escolhida, e pelos valores passados, pela troca constante de amor e sabedoria. Para eles, todo meu amor e meu Muito Obrigada!

Aos ***meus colegas da Estomatologia Clínica***, pela amizade construída ao longo desses dois anos de curso, e pela troca de angústias e alegrias na convivência diária.

Aos ***amigos e familiares,*** pelo apoio e por compreenderem minha ausência.

Ao ***Serviço de Estomatologia***. Às ***professoras Maria Antônia Z. de Figueiredo e Karen Cherubini***, por todo ensinamento e conhecimento transmitidos. Pela disponibilidade em todos os momentos, e pelos cafezinhos pós-ambulatórios ou almoços compartilhados. São profissionais exemplares e que deixam em mim a saudade e a certeza que o trabalho em equipe faz toda a diferença. Muito obrigada! Também à ***Márcia*** por todo auxilio prestado.

À ***professora Maria Martha Campos***, que contribuiu muito para este trabalho, esclarecendo dúvidas e compartilhando conhecimentos em suas aulas.

Agradeço à ***equipe do CEMBE,*** pelo auxílio e orientação diária com os animais. Pelo zelo com os animais, vocês são um exemplo de responsabilidade.

Agradeço àqueles que me cederam seu tempo e me auxiliaram na realização da fase experimental da minha pesquisa. Particularmente, as minhas colegas ***Mariana, Dieni e Valesca***, as quais se dedicaram integralmente ao experimento,

sempre se mostrando dispostas, mesmo durante feriados ou finais de semana. Aos colegas ***Gabriel e Noronha***, que auxiliaram em momentos não menos importantes, como a eutanásia dos animais. Sem vocês a conclusão desta etapa não seria possível.

Ao ***professor Fábio Luiz Dal Moro Maito*** por disponibilizar os equipamentos do Laboratório de Patologia Oral da Faculdade de Odontologia da PUCRS. À ***Janaína***, pelo auxílio que me deu na confecção e captura de todas as lâminas, sempre disposta a auxiliar-me, quando necessário.

Por fim, agradeço principalmente à ***professora Fernanda Gonçalves Salum*** pela orientação deste trabalho, e por todo aprendizado compartilhado. Obrigada pela disponibilidade e por toda paciência nestes dois anos, pois sem isso este trabalho não seria concluído.

RESUMO

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A mucosa bucal é um sítio frequente para a ocorrência de úlceras traumáticas, as quais podem ser causadas por agentes mecânicos ou químicos. Até o momento, não existe um tratamento padrão-ouro para o manejo dessas lesões, as quais podem ser dolorosas e ter impacto negativo na qualidade de vida dos pacientes. Apesar de estudos sugerirem a utilização da camomila e da curcumina no tratamento de lesões ulceradas da mucosa bucal, não há consenso sobre a sua eficácia no manejo dessas lesões. A literatura tem demonstrado que ambos os fitoterápicos modulam os níveis de citocinas pró-inflamatórias como TNF-α e IL-1β. No primeiro artigo desta dissertação foi realizada uma revisão integrativa da literatura investigando a eficácia das intervenções tópicas com camomila e curcumina no manejo da dor e na cicatrização de lesões ulceradas da mucosa bucal. Poucos ensaios clínicos controlados foram encontrados e estes sugerem um efeito benéfico da camomila e da curcumina no processo cicatricial e na redução da sintomatologia em pacientes com mucosite oral, ulceração aftosa recorrente e líquen plano oral. Estudos em modelos animais investigando os efeitos desses fitoterápicos em úlceras traumáticas bucais demonstraram redução da fase inflamatória do processo de cicatrização, maior deposição de fibras colágenas e epitelização. O segundo artigo desta dissertação apresenta um estudo experimental, que foi realizado com o objetivo de avaliar o efeito do extrato de camomila 10% e curcumina 2% no reparo de úlceras traumáticas em língua de ratos. Trinta e nove ratos *Wistar* foram distribuídos em três grupos: controle, extrato de camomila e curcumina. Uma úlcera de 5 mm de diâmetro foi confeccionada no ventre lingual de cada animal. As substâncias foram aplicadas na forma de gel, duas vezes ao dia, durante cinco dias. Os animais foram eutanasiados sete dias após

a confecção das lesões. Na avaliação macroscópica, não houve diferença entre os grupos camomila, curcumina e controle quanto à área de úlcera remanescente (P=0.20). A análise histológica também não demonstrou diferença entre os grupos quanto à área de tecido epitelial neoformado (P=0.97) ou intensidade do infiltrado inflamatório (P=0.72). Considerando o modelo experimental empregado, os achados do presente estudo demonstraram que extrato de camomila 10% e a curcumina 2% não aceleraram o reparo de úlceras mecanicamente induzidas na mucosa bucal nem reduziram a intensidade do infiltrado inflamatório, contrariando achados prévios na literatura.

Palavras-chave: Extrato de Camomila, Curcumina, Cicatrização, Úlceras orais, Fitoterápicos.

ABSTRACT

ABSTRACT

The oral mucosa is a frequent site for traumatic ulcers, which can be caused by mechanical or chemical agents. There is no a gold standard treatment for the management of these lesions, which can be painful and have a negative impact on quality of life. Although studies suggest the use of chamomile and curcumin in the treatment of ulcerated lesions of the oral mucosa, there is no a consensus as to its efficacy in the management of these lesions. The literature has shown that both phytotherapics modulate the levels of inflammatory cytokines such as TNF-α and IL-1β. Therefore, in the first article of this dissertation, an integrative literature review was conducted investigating the efficacy of topical interventions with chamomile and curcumin in the management of pain and in the healing of ulcerated lesions of the oral mucosa. Few controlled clinical trials have been found and they suggest a beneficial effect of chamomile and curcumin in the healing process and in the reduction of symptoms in patients with oral mucositis, recurrent aphthous ulceration and oral lichen planus. Studies in animal demonstrated a reduction in the inflammatory wound healing phase, increased deposition of collagen fibers and epithelization. The second article of this dissertation presents an experimental study, which was carried out to evaluate the effect of the 10% chamomile extract and 2% curcumin in the repair of traumatic ulcers on tongue of rats. Thirty-nine Wistar rats were divided into three groups: control, chamomile extract and curcumin. A 5-mm-diameter excisional wound was made in the center of ventral tongue. The substances were applied as a gel twice daily for five days. The animals were euthanized 7 days after the lesions were made. In the macroscopic evaluation, there was no significant difference among chamomile, curcumin and control groups regarding to area of residual ulcer (P= 0.20). Histological analysis also

showed no difference among the groups regarding newly formed epithelium area (P=0.97) or intensity of inflammatory infiltrate (P=0.72). Considering the experimental model, the findings of the present study showed that 10% chamomile extract and 2% curcumin did not accelerate the repair of traumatic oral ulcers or reduced the intensity of the inflammatory infiltrate, contradicting previous findings in the literature.

Keywords: Chamomile extract, Curcumin, Wound healing, Oral Ulcer, Natural compounds.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

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BMT Bone marrow transplantation

COX Cyclooxygenase

EGF Epidermal Growth fator

IL-1β Interleukin-1beta

OLP Oral lichen planus

OM Oral mucositis

PDGF Platelet-derived growth factor

RAS Recurrent aphthous stomatitis

ROS Reactive oxygen species

TNF- α Tumor necrosis factor-alfa

TGF-β Transforming Growth factor-beta

SUMÁRIO

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1 INTRODUÇÃO

1 INTRODUÇÃO

A mucosa bucal é constantemente submetida a agressões de natureza física ou química, sendo um sítio frequente para a ocorrência de lesões ulceradas (GILVETTI; PORTER; FEDELE, 2010). Próteses mal adaptadas, traumatismos dentários, mordiscação, traumas durante a escovação dentária e aparelhos ortodônticos são os agentes mecânicos mais comumente associados à ocorrência dessas lesões (BARONI et al., 2006; JIVANESCU et al., 2015; RENNICK et al., 2016). Dentre os agentes químicos, destacam-se os produtos de natureza alcalina ou ácida como ácido acetilsalicílico, tabletes de potássio, suplemento pancreático e bisfosfonatos (BASCONEZ-MARTÍNEZ; FIGUERO-RUIZ; ESPARZA-GÓMEZ, 2005; MUNÕZ-CORCUERA et al., 2009a).

A mucosa bucal é formada por tecido epitelial pavimentoso estratificado, que pode ser queratinizado ou não, suportado por tecido conjuntivo. Ela constitui uma barreira protetora para o organismo, impedindo a penetração de agentes externos prejudiciais à saúde. As ulcerações bucais traumáticas caracterizam-se pela descontinuidade da superfície epitelial e são encontradas com mais frequência na mucosa não queratinizada (CAVALCANTE et al., 2011). A permeabilidade mais elevada deste tecido e a suscetibilidade a danos são maiores devido à diferença da composição lipídica na membrana de revestimento (SHEIKH et al., 2013). Os locais mais comuns para ocorrência de úlceras traumáticas são as bordas da língua, mucosa labial, mucosa jugal, assoalho bucal e palato mole (BARRONS, 2001; CAVALCANTE et al., 2011; HAMEDI et al., 2016). Clinicamente tais leões apresentam-se como uma área amarelada circundada por halo eritematoso, entretanto, quando adquirem caráter crônico, o halo torna-se esbranquiçado (MUNÕZ-CORCUERA et al., 2009a;

CAVALCANTE et al., 2011). As úlceras orais são geralmente dolorosas, comprometendo a nutrição, fonação e higiene bucal, aumentando assim os riscos de infecções (FIDLER et al., 1996; LIM et al., 2015; THORAT et al., 2015).

O reparo dessas lesões ocorre por segunda intenção e uma série de eventos inflamatórios ocorre em cascata contemplando os quatro estágios da cicatrização: hemostasia, inflamação, proliferação e remodelamento (ENOCH; LEAPER, 2007; YOUNG; MCNAUGHT, 2011). Diversas células inflamatórias são atraídas para o local da ferida por meio da liberação de citocinas, incluindo neutrófilos, macrófagos, linfócitos e plasmócitos (YOUNG; MCNAUGHT, 2011).

Quando o dano tecidual ocorre há uma lesão microvascular e extravasamento de sangue para dentro da ferida. A perda de integridade estrutural inicia a cascata de coagulação, com agregação plaquetária e formação do coágulo. As plaquetas liberam grânulos alfa, que secretam vários fatores de crescimento, incluindo o fator de crescimento derivado de plaquetas (PDGF), fator-1 de crescimento semelhante à insulina (IGF-1), fator de crescimento epidérmico (EGF), fator transformador de crescimento β (TGF- β) e fator plaquetário- IV (ENOCH; LEAPER, 2007; CHIQUET; KATSAROS; KLETSAS, 2015).

A constituição do infiltrado inflamatório varia de acordo com a fase de evolução da úlcera (CAVALCANTE et al., 2011). Neutrófilos são atraídos para o local da ferida dentro de 24-48 horas após o trauma por meio da liberação de fragmentos de proteínas da matriz extracelular, TGF-β, componentes do sistema complemento (por exemplo, C3a, C5a) e produtos peptídicos formil-metionil de bactérias. Uma vez no ambiente da ferida eles fagocitam bactérias e outras partículas estranhas, promovendo a liberação de enzimas degradantes e radicais livres derivados de oxigênio, o que minimiza a contaminação e favorece o processo normal de

cicatrização. Com a progressão do processo inflamatório, os neutrófilos sofrem apoptose e são progressivamente substituídos por monócitos. Os fatores quimiotáticos para neutrófilos e monócitos são ativados durante os diferentes períodos de inflamação, mantendo a migração de monócitos após a cessação da migração de neutrófilos (ENOCH; LEAPER, 2007; YOUNG; MCNAUGHT, 2011). Nas 48-72 horas seguintes, os monócitos sofrem uma alteração fenotípica, chegando ao local da ferida como macrófagos teciduais. Os macrófagos são as células reguladoras fundamentais para o reparo. Eles atuam como células fagocíticas, além de serem os principais produtores de fatores de crescimento responsáveis pela proliferação celular. Além disso, os macrófagos liberam enzimas proteolíticas (por exemplo, colagenase), que ajudam a debridar a ferida (ENOCH; LEAPER, 2007; YOUNG; MCNAUGHT, 2011).

Cerca de 72 h após o trauma, o perfil histológico da úlcera apresenta alterações, a infiltração celular torna-se predominantemente linfoplasmocitária, característica de inflamação crônica. Nesta fase há proliferação de vasos sanguíneos e fibroblastos, sinalizando o início do reparo. Há migração de fibroblastos, deposição de matriz extracelular e formação de tecido de granulação. Uma vez no interior da ferida, os fibroblastos proliferam e produzem fibronectina, proteínas da matriz, ácido hialurônico e, posteriormente, colágeno e proteoglicanos. Estes componentes ajudam a construir a nova matriz extracelular, que suporta ainda mais o crescimento celular interno e é essencial para o processo de remodelamento. A epitelização da ferida representa a etapa final da fase proliferativa, requer um ambiente úmido, adequada nutrição e controle bacteriológico (ENOCH; LEAPER, 2007; CAVALCANTE et al., 2011; CHIQUET; KATSAROS; KLETSAS, 2015). O remodelamento inicia simultaneamente com o desenvolvimento do tecido de granulação e continua por longo período. Há contínua síntese e degradação do colágeno, a matriz extracelular é

constantemente remodelada até 21 dias após o ferimento. A contração da ferida ocorre por meio de interações entre os fibroblastos e a matriz extracelular circundante e é influenciada por uma série de citocinas (ENOCH; LEAPER, 2007; CAVALCANTE et al., 2011; CHIQUET; KATSAROS; KLETSAS, 2015).

Considerando-se que a lesão tecidual faz com que haja início quase imediato da inflamação aguda, o controle do processo inflamatório é desejável e pode otimizar a reparação de feridas. Apesar da variedade de agentes tópicos para o tratamento de úlceras traumáticas bucais, especialmente agentes anti-inflamatórios e antissépticos, não há consenso sobre a eficácia desses fármacos e muitos não foram ainda devidamente avaliados e testados, sendo utilizados empiricamente. Nos últimos anos tem havido um interesse crescente no uso de fitoterápicos no processo da cicatrização (MARTINS et al., 2009; HAMEDI et al., 2016). Açafrão, aloe vera, cravo, canela, camomila, curcumina entre outros estão entre os produtos utilizados para tal finalidade (DUARTE et al., 2011; FARJANA; CHANDRASEKARAN; BAGAVAD, 2014).

A camomila, também conhecida como *Chamomilla recutita* (L.) Rauschert (sinônimo de *Matricaria recutita* (L.) Rauschert, e *Matricaria Chamomilla* L.), da família Asteraceae, é um dos componentes de chá mais consumidos no mundo. É uma planta medicinal que tem sido utilizada durante séculos para tratamento de enfermidades inflamatórias como febre, diarréia, dor menstrual, tumores hepáticos e intestinais (MARTINS et al., 2009; SINGH et al., 2011). A flor de camomila contém várias compostos biologicamente ativos, incluindo óleos essenciais e polifenóis que são responsáveis por praticamente todos os efeitos farmacológicos conhecidos. Os principais componentes do óleo essencial extraído das flores de camomila são os terpenóides α-bisabolol e seus óxidos, azulenos, incluindo camazuleno, e derivados de acetileno. Dentre os flavonóides (compostos fenólicos), destaca-se a apigenina

(SRIVASTAVA; GUPTA, 2009).

A apigenina é responsável pelos efeitos ansiolítico e quimiopreventivo da camomila (MCKAY; BLUMBERG, 2006; SRIVASTAVA; GUPTA, 2009). O camazuleno possui reconhecida atividade anti-inflamatória, que é reforçada pelo α-bisabolol. Os terpenos, bisabolol e camazuleno apresentam atividades anti-inflamatórias, antialérgicas, antiespasmódicas, antibacteriana, antipirética, antiulcerogênica e antifúngica. A camomila tem se mostrado eficaz na redução da mucosite oral produzida por metotrexato, na remoção de *smear layer* oriunda do preparo de canais radiculares, redução da gengivite e como agente acelerador na cicatrização de úlceras em animais e humanos (MAZOKOPAKIS et al., 2005; RAMOS-e-SILVA et al., 2006; SADR LAHIJANI et al., 2006; MARTINS et al., 2009; GOES et al., 2016). Em estudos *in* vitro, o óleo essencial demonstrou atividade antibacteriana sobre algumas espécies de *Sthaphylococcus e Candida (*WYGANOWSKA-SWIATKOWSKA, 2016).

A *Curcuma longa* ou açafrão-da-terra é uma planta da família *zingiberaceae,* originária do sudeste asiático, cultivada e utilizada na Índia e China há mais de 2.500 anos. A polpa laranja contida no interior da raiz do açafrão constitui a fonte do pó medicinal cúrcuma. Os componentes do açafrão são denominados curcuminóides e incluem, principalmente, a curcumina (diferuloilmetano), desmetoxicurcumina e bisdemetoxicurcumina (TOPMAN; LIN; GEFEN, 2013; FARJANA; CHANDRASEKARAN; BAGAVAD, 2014; AKBIK et al., 2014). A curcumina, composta em sua estrutura química por [1,7-bis- (4-hidroxi-3-metoxifenil) -1,6-heptadieno-3,5-dione], é um polifenol lipofílico de baixo peso molecular, considerado o constituinte mais ativo (GOEL; AGGARWAL, 2010; CHEPPUDIRA et al., 2013).

A pasta derivada da *Curcuma* é tradicionalmente usada na medicina indiana para tratamento de enfermidades das vias biliares, tosse, úlceras diabéticas,

perturbações hepáticas, reumatismo e sinusite (KRISHNASWAMY, 2008; AKBIK et al., 2014*).* Acredita-se que a curcumina têm potencial antimicrobiano, anti-inflamatório, propriedades antioxidantes e até mesmo anticancerígenas (TOPMAN; LIN; GEFEN, 2013; THORAT et al., 2015). Segundo Thorat et al. (2015), a curcumina também apresenta efeitos na cicatrização de úlceras orais, por meio do aumento da proliferação celular e da síntese de colágeno no local da ferida. Acredita-se que nas fases proliferativa e de remodelamento, o uso tópico de produtos com curcumina acelera a cicatrização das feridas, aumentando a migração de fibroblastos, a formação de tecido de granulação, deposição de colágeno e a angiogênese no local da lesão, favorecendo a re-epitelização e contração da ferida (AKBIK et al., 2014). Dados pré-clínicos sugerem que a curcumina pode inibir a atividade da COX (cicloxigenase) sobre neutrófilos e plaquetas humanas, como também remover espécies reativas de oxigênio (ROS) e inibir a produção de TNF-α (fator de necrose tumoral-alfa) e da IL-1 (interleucina-1), citocinas relevantes para o crescimento tumoral. Deste modo, acredita-se que a curcumina possui um potencial importante na prevenção de doenças cardiovasculares e na carcinogênese (SHARMA; GESCHER, 2005).

Entre as várias propriedades atribuídas à camomila e à curcumina, destacam-se seus efeitos anti-inflamatório e antioxidante (HERNÁNDEZ-CERUELOS, 2010; HAMMAD et al., 2011; JABRI et al., 2016; MIRAJ; ALESAEIDI, 2016). Ambos os produtos fitoterápicos modulam os níveis de citocinas pró-inflamatórias tais como TNF-a e IL-1β (interleucina 1-beta) (CURRA et al., 2012; OLIVEIRA et al., 2016). Os níveis aumentados dessas citocinas estão diretamente envolvidos no atraso do processo cicatricial (CURRA et al., 2013, OLIVEIRA et al., 2016). Considerando a ocorrência frequente de lesões ulceradas na mucosa oral, a investigação do efeito de agentes tópicos não tóxicos, de baixo custo e facilmente acessíveis, como os

medicamentos à base de plantas, é uma alternativa interessante. Consequentemente, o objetivo deste estudo foi investigar o efeito do gel de extrato de camomila 10% e do gel de curcumina 2% no reparo de úlceras traumáticas na língua de ratos.

2 PROPOSIÇÃO

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2.1 Objetivo Geral

Avaliar o efeito da aplicação tópica de extrato de camomila (10%) e curcumina (2%) em úlceras traumáticas produzidas no ventre da língua de ratos.

2.2 Objetivos Específicos

- Realizar uma revisão integrativa da literatura, investigando a eficácia das intervenções tópicas com camomila e curcumina no manejo da dor e na cicatrização de lesões ulceradas da mucosa bucal.
- Avaliar macroscopicamente o efeito da aplicação tópica de extrato de camomila (10%) e curcumina (2%) no reparo de úlceras traumáticas induzidas no ventre da língua de ratos.
- Avaliar, por meio de análise histológica, o efeito desses agentes nos componentes celulares do processo inflamatório e na neoformação de tecido epitelial em úlceras traumáticas induzidas no ventre lingual de ratos.

3 ARTIGO DE REVISÃO DA LITERATURA

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CHAMOMILE EXTRACT AND CURCUMIN AS AN ALTERNATIVE TO MANAGEMENT OF ORAL ULCERS: INTEGRATIVE LITERATURE REVIEW

Artigo submetido ao periódico: *Phytotherapy Research*

Qualis Capes Odontologia 2017: A2

Fator de Impacto: 3.092 (2016)

CHAMOMILE EXTRACT AND CURCUMIN AS AN ALTERNATIVE TO MANAGEMENT OF ORAL ULCERS: INTEGRATIVE LITERATURE REVIEW

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ABSTRACT

Purpose: The objective of this study was to perform an integrative review of the literature, investigating the efficacy of topical interventions with chamomile and curcumin in pain management and healing of ulcerative lesions of the oral mucosa.

Materials and Methods: An electronic search of the databases Pubmed/Medline, Cochrane Central Register of controlled clinical trials, Web of Science, Bireme/LILACS and Scopus was undertaken, using a combination of the terms: *Curcumin, Chamomile, Stomatitis Herpetic, Lichen Planus, Aphthous Stomatitis, Oral Ulcer, Oral Mucositis* and their MeSh/Dec´s terms.

Results and Conclusion: Twenty studies investigating the effect of chamomile and curcumin on the healing of oral wounds were included. Fifteen of the studies were clinical trials and five were experimental studies. Recurrent Aphthous Stomatitis (RAS), Oral Mucositis (OM), Oral Lichen Planus (OLP) and mechanically induced ulcers were treated topically with the phytotherapeutic agents in different formulations and concentrations. The topical application of chamomile and curcumin reduced symptoms and clinical signs of OM, RAS, OLP, as well as accelerate the healing of oral wounds. Topical extract of chamomile and curcumin are effective on pain reduction and tissue repair of ulcerative oral lesions.

Keywords: Chamomile, Curcumin, Oral ulcer, Wound healing, Natural compounds.

INTRODUCTION

The oral mucosa is frequently affected by ulcerative lesions caused by physical or chemical injuries, microbial infection and immunological disorders among others (Siu, Landon, & Ramos, 2015; Thorat, Sarvagod, Kulkarni,& Hosmani, 2015). Mechanical injuries can occur due to poor-fitting dentures, dental trauma, cheek-chewing, trauma caused by brushing and the use of orthodontic appliances (Baroni, Capristo, Rossiello, Faccenda, & Satriano, 2006; Rennick, Campbell, Naidu, Taylor, & Buschang, 2016). Oral mucositis, lichen planus, recurrent aphthous stomatitis are disorders that can result in painful ulcers in the oral cavity, affecting nutrition and oral hygiene, thus increasing the risk of infections (Fidler *et al.*, 1996; Lim, Haung, Wang, & Hua, 2015; Thorat *et al.,* 2015).

Clinically, oral ulcers are painful, appear as a yellowish area, surrounded by an erythematous halo; however, when they become chronic, the halo turns whitish (Muñoz-Corcuera, Esparza-Gómez, González-Moles, & Bascones-Martínez, 2009; Cavalcante *et al.,* 2011). The repair of this type of injury occurs by secondary intention, and events occur in a cascade, comprising the stages of inflammation, proliferation and remodeling, culminating with healing (Enoch & Leaper, 2007; Young & McNaugth, 2011). The management of oral ulcers with substances that promote local antisepsis and support the healing process is recommended (Mariano *et al.,* 2015; Hamedi *et al.,* 2016). The use of topical medications has been proposed to accelerate the healing process and provide symptomatic relief from the ulcers. In the literature, studies have suggested the use of corticosteroids, antibiotics, anesthetics, antiinflammatory drugs, antihistamines, antiseptics and medicinal plants (Schemel-Suárez, López-López, & Chimenos-Kustner, 2015; Rennick *et al.,* 2016). Currently, there is growing interest in

the use of plant-derived medications because of their low cost and easy availability. Studies have investigated the antibacterial, antiinflammatory, and wound-healing effects of herbal products derived from aloe vera, propolis, honey, chamomile and curcumin, among others, on lesions of the oral mucosa (Mazokopakis, Vrentzos, Papadakis, Babalis, & Ganotakis, 2005; Skaba *et al.*, 2013; Coelho *et al*., 2015).

Matricaria recutita Linn (chamomile) is a phytotherapeutic plant commonly used owing to its antioxidant, antimicrobial, anxiolytic, antiinflammatory, antidiarrheal, angiogenic, anticarcinogenic, hepatoprotective, and antidiabetic effects (Mazokopakis *et al.,* 2005; McKay & Blumberg, 2006; Miraj & Alesaeidi, 2016). Chamomile belongs to the family Asteraceae, and it is known to possess various active flavonoids and to be rich in terpenoids such as α-bisabolol, azulene, matricin and chamazulene. These components account for the antioxidant, antiinflammatory and antibacterial activities of chamomile (McKay & Blumberg, 2006; Srivastava & Gupta, 2009; Hernández-Ceruelos *et al,* 2010).

Curcuma longa is a plant derived of the turmeric spice, and it belongs to the ginger family and has been widely used throughout history in Eastern cooking (Elad *et al.,* 2013; Thorat *et al.,* 2015). Its main component, curcumin, is an yellowish-orange polyphenol, which has both antioxidant and antiinflammatory effects. Recent studies demonstrated its antiinflammatory, antimicrobial, antioxidant, antiaging and anticancer activities, which may aid in the wound healing process, whether oral or cutaneous (Singer, McClain, Romanov, Rooney, & Zimmerman, 2007; Tuorkey & Karolin, 2009; Goel & Aggarwal, 2010; Rao *et al.,* 2014; Lim *et al*., 2016). A systematic review by Vaughn, Branum and Sivamani (2016) showed evidence of the use of turmeric or curcumin and its formulations for the treatment of skin lesions such as eczema, acne,

psoriasis and vitiligo, pointing out its benefits in treating these diseases, topically as well as orally.

There are several products available for patients with oral ulcers, and there is a need to establish an adequate and accessible treatment. Although several studies, both experimental and clinical, suggest the use of chamomile and curcumin in the treatment of ulcerative lesions of oral mucosa, there is no consensus about their efficacy in the management of these lesions. Therefore, the objective of this study was to perform an integrative review of the literature, investigating the efficacy of topical interventions with chamomile and curcumin in pain management and healing of ulcerative lesions of the oral mucosa.

MATERIAL AND METHODS

An integrative review was performed to bring together and summarize the published literature on topical chamomile and curcumin for the treatment of oral wounds such as oral lichen planus, aphthous stomatitis, oral mucositis, oral herpes simplex and oral traumatic ulcers. An electronic search of the databases Pubmed, National Library of Medicine's Medline, Cochrane Central Register of controlled clinical trials, Web of Science, Bireme/LILACS and Scopus up to September 2017 was undertaken, using the terms: *"Curcumin" OR "Chamomile" AND " Stomatitis, Herpetic" OR "Oral Lichen Planus" OR " Stomatitis, Aphthous" OR " Oral Ulcer" OR "Oral Mucositis" and their MeSh/Dec´s terms.*

Inclusion and Exclusion Criteria

Publications written in English, Spanish and Portuguese were included. The search was limited to human and animal studies, which were analyzed separately. We included controlled studies that used chamomile or curcumin topically for the treatment of ulcerative lesions of the oral mucosa. The substances used and their frequency of application needed to be described. The controls could be a placebo or any other comparative treatment. To be included in this review, the study should report the pain relief (human studies) and/or the effect on wound healing.

In the selection of the articles we excluded reviews, case reports, series of cases, author debates or letters to the editor.

Study design

Initially, the research of the articles was carried out by two independent researchers in the databases described, using the search key described above. After the initial search, the exclusion of articles was carried out by reading the titles and abstracts. At this time, duplicate articles were also removed. If there was insufficient information provided in the abstract or if there was a disagreement between the reviewers, the authors reviewed the full article before reaching an agreement.

Data extraction was then completed in duplicate by the same two independent reviewers. The following data were collected: sample, study groups, intervention (topical agents), type of oral lesion, length of follow-up and outcome. The search strategy and the flow diagram of article selection are shown in Figures 1 and 2 for chamomile and curcumin, respectively.

After analysis of the results, one article referring to chamomile (Martins *et. al,* 2009), which has not been located by the search key was included in this review. In

the indexation of this article, the term “wound healing” was employed, with no reference to the mouth or oral mucosa.

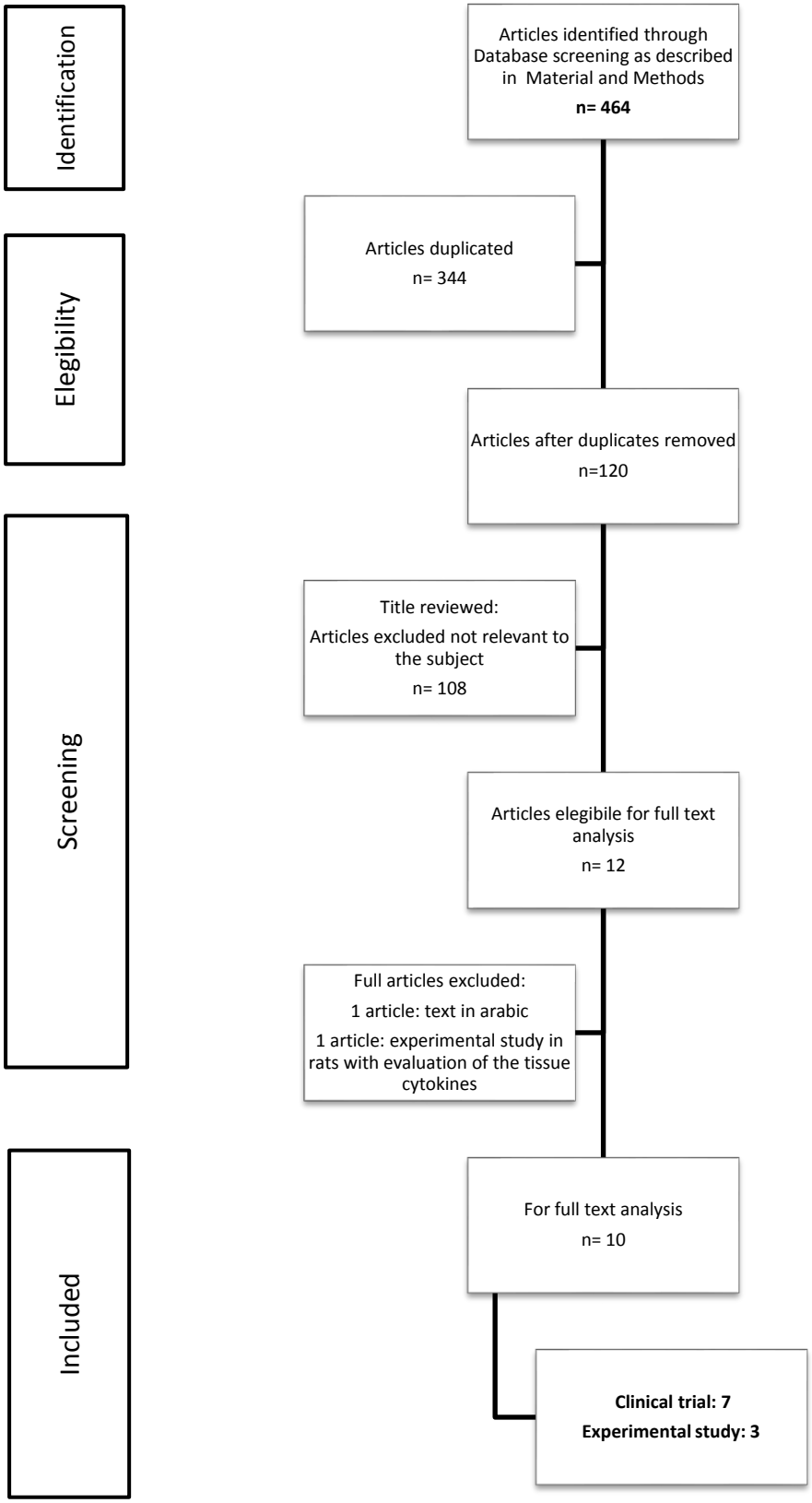

Figure 1- Flow diagram of Chamomile article selection.

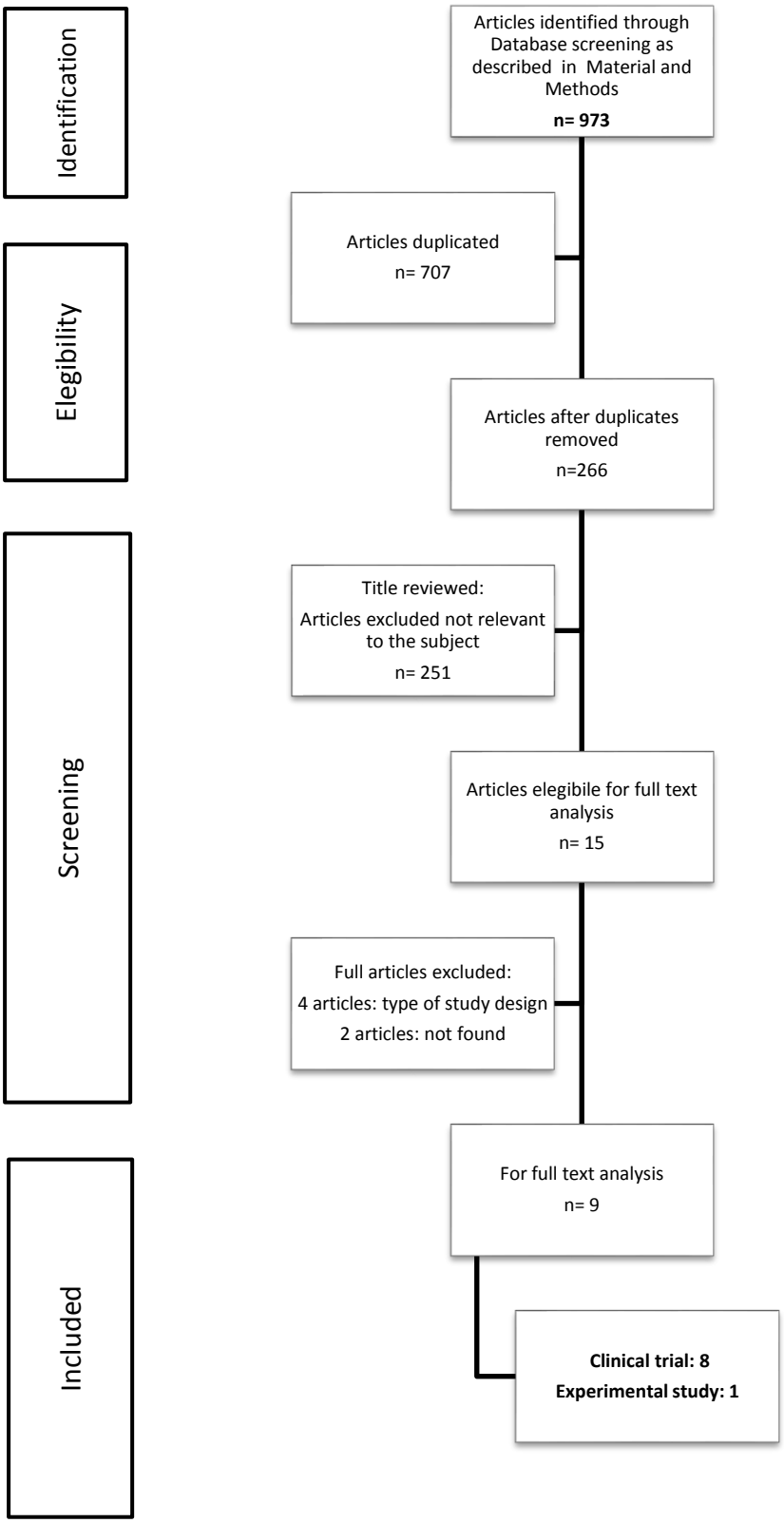

Figure 2- Flow diagram of Curcumin article selection.

RESULTS

Chamomile

Table 1 describes randomized clinical trials using topical chamomile, as mouthwash or gel, in the treatment of oral mucositis, oral lichen planus or recurrent aphthous stomatitis. No studies were found on patients with herpetic stomatitis or traumatic mouth ulcers. Controls consisted of the use of placebo agents, allopurinol, saline, or the omission of any treatment.

Table 2 describes four experimental studies in animal models determining the topical effect of chamomile on oral traumatic ulcers (mechanically induced) or chemo-induced oral mucositis. *Wistar* rats and hamsters were the two models used in these studies. Chamomile was used topically in orabase (10%) and corticosteroids in cream (betamethasone, triamcinolone acetonide and clobetasol propionate) were used in the control groups.

Of the selected studies, only one evaluated the topical use of chamomile in the control of minor recurrent aphthous stomatitis (Tadbir *et al.,* 2015). Chamomile showed results superior to those of placebo and similar to those of triamcinolone in reducing ulcers and pain intensity. However, in the triamcinolone group, clinical resolution was faster.

Lopez Jornet and Aznar-Cayuela (2016) evaluated the topical use of chamomile in the oral lichen planus and observed improvement in the symptoms and clinical appearance of the lesions after four weeks. In the whole sample there was histopathological confirmation of the diagnosis, but the authors noted that a possible limitation of the study was the use of non-standardized criteria for assessing the

severity of the disease. This was also the only controlled clinical study to use chamomile in patients with oral lichen planus.

Five of the seven selected clinical trials evaluated the effect of chamomile on oral mucositis, totaling 395 patients. In all cases, mucositis was a result of chemotherapy, and in two studies, the patients were subsequently submitted to bone marrow transplantation. All studies obtained positive results with the use of chamomile in oral mucositis, with the exception of Fidler *et al.* (1996), who did not observe differences in mucositis scores between the chamomile and placebo groups. These authors reported the use of 30 drops of chamomile, diluted in 100 ml of water, three times a day, but the concentration of the phytotherapeutic drops was not specified in the study.

Dos Reis *et al.* (2016) evaluated the effect of 2.5% chamomile, used by means of cryotherapy, in patients submitted to chemotherapy. The occurrence of oral mucositis was lower in patients in the chamomile group than in the control group. However, the authors reported some limitations such as the small sample size, and the impossibility of using a double-blind design due to the taste and color of the ice chips.

The use of chamomile to treat oral mucositis in patients submitted to bone marrow transplantation was evaluated by Ardakani *et al.* (2016) and Braga *et al.* (2015). Despite the high dose of chemotherapy, the studies reported reductions in the incidence and intensity of oral mucositis with the use of chamomile-containing mouthwashes. In addition, the need for parenteral nutrition and opioid medications was lower in these subjects, and none of them showed adverse effects from chamomile. Braga *et al.* (2015) determined that when used at a concentration of 1%, chamomile showed the best results in the control of oral mucositis. Ardakani *et al.* (2016) reported

that the use of mouthwashes with sodium chloride, chlorhexidine and nystatin, by all patients during hospitalization, was a possible confounding factor. In addition, their mouthwash consisted of a combination of chamomile with another herbal product (*Mentha piperita)*.

Table 1. Randomized controlled clinical trials on the topical use of Chamomile in the management of oral wounds

Author/Year	Sample	Type of lesion	Topical agent	Length of study/ Follow up	Outcome
Fidler *et al.* (1996)	164 patients	OM (chemo-induced)	- Chamomile, 30 drops in 100 ml water - Placebo	14 days	No substantial difference in OM scores between the two protocol arms (P= 0.43).
Shabanloei and Ahmadi (2009)	83 patients	OM (chemo-induced)	- Chamomile flower (8 g/50 ml) - Allopurinol, 5 mg/ml - Saline	16 days	Significant differences were found between allopurinol, chamomile and saline groups in OM scores (P=0.017), pain intensity (P=0.027) and OM persistence (P=0.00). Chamomile group showed a decrease in OM intensity (P=0.01) and pain (P=0.02) when compared to saline group in the periods evaluated. No significant difference was found between allopurinol and chamomile groups (P=0.82).
Braga *et al.* (2015)	40 patients	OM (BMT)	- 0.5% Chamomile mouthwash - 1% Chamomile mouthwash - 2% Chamomile mouthwash - Control group (without mouthwash)	From the 1st day of treatment until BMT	Incidence of OM was significantly less in group that used mouthwash containing 1% chamomile (3.30%) compared to the control group (9.90%) (P =0.01).
Tadbir *et al.* (2015)	45 patients	RAS	- Chamomile extract Orabase (C) - Triamcinolone Orabase (T) - Placebo Orabase (O)	6 days	In the triamcinolone and chamomile groups, ulcer size and pain intensity were significantly less than in placebo group (P=0.001). There was no statistically significant difference between the triamcinolone and chamomile groups (P>0.05). Complete resolution of symptoms occurred significantly sooner in the triamcinolone group.
Ardakani *et al.* (2016)	70 patients	OM (BMT)	- Chamomile(1 ml/50 ml), with *Mentha piperita* - Placebo	Treatment was continued until complete healing of OM or hospital discharge.	Duration (P<0.0001) and severity (P=0.009) of OM and pain were significantly reduced in chamomile group.
Dos Reis *et al.* (2016)	38 patients	OM (chemo-induced)	- Cup of ice chips made with 2.5% chamomile infusion - Cup of ice chips made with water	5 days of the first course	Chamomile group had less mouth pain than control group on day 8 (P=0.02), with a tendency on day 15 (P=0.09), but not on day 22 (P=0.14).
Lopez Jornet and Aznar-Cayuela (2016)	55 patients	OLP	- 2% Chamomile gel - Placebo	4 weeks	Patients treated with chamomile showed significant improvements with regard to pain (P<0.001), burning rmouth sensation (P<0.001), itching (P=0.011), quality of life (P<0.001) and clinical characteristics (P=0.001).

OLP-oral lichen planus/OM-oral mucositis/ RAS- recurrent aphthous stomatitis/BMT-bone marrow transplantation.

In the four experimental studies selected, chamomile was applied in orabase at a concentration of 10% (Martins *et* al.,2009; Duarte, Quirino, Patrocínio & Anbinder, 2011; Pavesi *et al*., 2011; Oliveira *et al.,* 2016). Martins *et al*. (2009), Duarte *et al.* (2011) and Oliveira *et a*l. (2016) evaluated the repair of mechanically induced oral ulcers. Martins *et al.* (2009) demonstrated the clinical and histopathological improvement of oral ulcers in rats. The epithelium exhibited complete repair after five days of herbal use. Duarte *et al.* (2011) observed, after 10 days, acceleration of lesion epithelialization and increased deposition of collagen fibers with topical use of chamomile. Oliveira *et al.* (2016) also reported that chamomile hastened the repair process, differing from the corticosteroid and control groups. There was greater collagen fiber deposition and smaller ulcer size after 10 days of treatment in both diabetic and non-diabetic rats. In the chamomile-treated animals, there was a reduction in the apoptosis of epithelial cells and lower expression of TNF-α as well.

Pavesi *et al.* (2011) observed a reduction in oral mucositis scores in hamsters with topical use of 10% chamomile extract. The group treated with chamomile exhibited the lowest degree of mucositis throughout the experiment compared to the control and corticoid groups. In the histopathological analysis, the chamomile group had mild hyperemia, mild inflammatory infiltration and absence of ulceration, both at 5 and 14 days after chemotherapy infusion.

Table 2. Experimental studies on the topical application of chamomile in the management of oral wounds.

Author/Year	Sample	Type of lesion	Topical agent	Length of study/ Follow up	Outcome
Martins *et al*. (2009)	120 Wistar rats	Oral ulcer (tongue)	- Control (no drugs) - 10% Chamomile fluid extract, - 1 mg/g Triamcinolone acetonide, - 0.05% Clobetasol propionate cream - 0.05% Clobetasol propionate paste	1,3,5,7 and 14 days	The animals treated with chamomile displayed faster wound healing compared to the animals treated with corticosteroids and control group. At 5 days, the chamomile group showed complete epithelium repair with connective tissue underneath along with fibrosis and few or no inflammatory cells. In the experimental groups, treated with clobetasol, there was complete tissue repair only after 14 days of treatment. Wound healing in the clobetasol groups was significantly slower than in the control group ($P < 0.05$).
Duarte *et al*. (2011)	36 Wistar rats	Oral ulcer (tongue)	- 10% Chamomile fluid extract - Control group (without treatment)	3, 7 and 10 days	Treated animals showed greater re-epithelialization compared to control animals (P=0.008) and a greater percentage of collagen fibers (P=0.022) after 10 days of treatment.
Pavesi *et al.* (2011)	105 Hamsters	OM (chemo-induced)	- 10% Chamomile fluid extract - 5 ml/0.5 mg Betamethasone elixir, - Control group (without treatment)	2,5,8,10,12,14 and 16 days	There was a significant difference between the chamomile and control groups (P<0.0001) in clinical scores of OM. The group treated with chamomile had a 12-fold greater chance of scoring zero (absence of mucositis) than the corticoid group (P=0.0001).
Oliveira *et al.* (2016)	Diabetic Wistar rats (number of animals not available)	Oral ulcer (left buccal mucosa)	- 10% Chamomile fluid extract ointment, (normoglycemic) - 10% Chamomile fluid extract ointment, (diabetic) - 1mg/g Triamcinolone Orabase (diabetic) - Saline (normoglycemic): negative control - Saline (diabetic): positive control	5 and 10 days	All experimental groups showed a decrease in ulcer area from the 5th day to the 10th day, except the triamcinolone group (P=0.082). Animals treated with chamomile extract showed a change in chronic inflammatory profile on the 5th experimental day, unlike the other groups. Additionally, on the 10th day, the histological and clinical findings for the chamomile groups were similar to those for the negative control. On the 10th experimental day, only the group treated with triamcinolone showed a significantly greater ulcer size compared with the positive control group.

OM-oral mucositis.

Curcumin

Table 3 describes clinical trials using curcumin topically in the treatment of oral mucositis, oral lichen planus and recurrent aphthous stomatitis. There were also no studies using curcumin in patients with herpetic stomatitis or oral traumatic ulcers. From the eight randomized controlled trials described, triamcinolone, chlorhexidine and iodopovidone were the substances used as controls.

In addition to clinical studies, we included an experimental study evaluating the effect of topical 1% curcumin on acetic acid-induced ulcers on rabbit upper lip mucosa (Lim *et al*., 2016). A cream with 1% curcumin was applied twice daily. At 7 and 14 days, animals were sacrificed and specimens were taken. After 14 days, curcumin enhanced wound healing, leading to a significant difference in the size of ulcer and restoring the integrity of the epithelium (P values not available).

Topical effect of curcumin for recurrent aphthous stomatitis was evaluated by Manifar, Obwaller, Gharehgozloo, Boorboor Shirazi Kordi and Akhondzadeh (2012) and Deshmukh and Bagewadi (2014), who used different concentrations of the agent in gel form. Manifar *et al*. (2012) observed a decrease in ulcer size and pain at 4 and 7 days compared to placebo. Deshmukh and Bagewadi (2014) also reported improvement in recurrent aphthous stomatitis with curcumin, obtaining results similar to those with triamcinolone at 7 days. Both studies totalized a sample of 117 patients with recurrent aphthous stomatitis and suggested that curcumin has a beneficial effect in the treatment of this oral condition, which is comparable to that of the corticosteroid commonly used.

Rao *et al.* (2014) and Mansourian, Amanlou, Shirazian, Jahromi and Amirian (2015) investigated the topical effect of curcumin on radioinduced oral mucositis, while Patil,Guledgud, Kulkarni, Keshari and Tayal (2015) included patients submitted

concomitantly to radio- and chemotherapy. The degree of oral mucositis was variable, but curcumin showed better results when compared to iodopovidone, chlorhexidine and placebos agents, fostering a decrease in pain, oral erythema and burning mouth sensation.

The effect of topical curcumin in the form of gel, paste and mouthwash on atrophic and erosive lesions of oral lichen planus was evaluated by Kia, Shirazian, Mansourian, Khodadadi and Ashnagar (2015), Amirchaghmaghi *et al.* (2016) and Thomas *et al.* (2017). The last authors reported a reduction in burning mouth sensation, erythema and ulceration scores, with the use of *Curcuma longa* extract. However, the improvement in these parameters was less than in the group that used topical corticosteroid therapy. Among the groups that received the herbal medicine, the results were proportional to the frequency of topical application of curcumin gel.

On the other hand, Amirchaghmaghi *et al. (*2016) did not observe improvement in the clinical signs of oral lichen planus and burning mouth sensation with the use of topical curcumin compared to placebo. The patients of both groups received treatment with dexamethasone and nystatin oral suspension three times a day. Kia *et al.* (2015) compared the effects of curcumin and triamcinolone on oral lichen planus symptoms and obtained similar results for the two groups.

Table 3. Randomized controlled clinical trials on the topical use of curcumin in management of oral wounds.

Author/Year	Sample	Type of lesion	Topical agents	Length of study/ Follow up	Outcome
Manifar *et al*. (2012)	57 patients	RAS	- 2% Curcumin gel - Placebo gel	14 days	Curcumin decreased ulcer size on day 4 (P=0.02) and day 7 (P=0.04) and pain as well on day 4 (P=0.03).
Deshmukh and Bagewadi (2014)	60 patients	RAS	- 10mg/g Curcumin gel - 0.1% Triamcinolone acetonide gel	7 days	There were no significant differences in pain (P=0.500) and duration (P=0.516), size (P=0.630) and number (P=0.193) of ulcers between groups. The reduction in size and number of ulcers was statistically significant in both groups from day 0 to day 7.
Rao *et al.* (2014)	79 patients	OM (radio-induced)	-Turmeric gargle, 400 mg curcumin dissolved in 80 ml of boiling water and cooled (mouthwashes) -Povidone-iodine gargle, 1:100 mL	6 weeks	There was a significant difference in OM grade between groups. In the turmeric group, 14 of 39 patients developed intolerable mucositis (score 3 and 4), while in the povidone-iodine group, 34 of the 40 patients developed intolerable mucositis (P<0.0001). Weight loss was also less in the turmeric group (P<0.001).
Mansourian *et al.* (2015)	37 patients	OM (radio-induced)	- 0.5% *Curcuma longa* gel - Placebo	21 days	There was a significant difference in OM grades between the two groups (P<0.001). No grade-3 mucositis was observed in patients who used *Curcuma*. The mean size of oral lesions, oral erythema and burning mouth sensation in the *Curcuma* group were significantly less compared to control group (P<0.001).
Kia *et al.* (2015)	50 patients	OLP	- Curcumin paste, 5% - Triamcinolone paste, 0.1%	4 weeks	There was no significant difference in pain reduction (36% in the curcumin group and 32% in triancinolone group) between the groups (P=0.31). Complete remission of atrophia and ulcerations was observed in one patient (4%) in each group.
Patil *et al.* (2015)	20 patients	OM (radio-induced and chemo- induced)	- 0.004% curcumin mouthrinse (1:5) - 0.2% chlorhexidine (1:1)	20 days	Significant difference was noted in pain (P< 0.001), erythema (P=0.05), ulceration (P<0.001) and OM grade (P=0.003) between the two groups. The curcumin group showed a greater reduction in parameters in comparison with chlorhexidine.
Amirchaghmaghi *et al.* (2016)	20 patients	OLP	- 2000 mg curcumin tablets/day - Placebo	4 weeks	No significant difference between groups in the remission of atrophic/erosive lesions (P=0.77).
Thomas *et al.* (2017)	75 patients	OLP	- 1% *Curcuma longa* extract- three times daily - 1% *Curcuma longa* extracts - six times daily. - 0.1% triamcinolone acetonide	3 months	Significant reduction in burning mouth sensation, erythema and ulceration (P<0.001) in all three groups. Triamcinolone group showed the greatest reduction in burning mouth sensation, erythema and ulceration score (P<0.001) after three months of application. *Curcuma* six times daily showed a greater reduction in burning mouth sensation, erythema and ulceration score (P<0.001) compared to *Curcuma* three times daily.

OLP-oral lichen planus/OM-oral mucositis/ RAS- recurrent aphthous stomatitis.

DISCUSSION

Among the different properties attributed to chamomile and curcumin, their antiinflammatory and antioxidant effects stand out (Akbik, Ghadiri, Chrzanowski & Rohanizadeh, 2014; Jabri *et al.,* 2016; Oliveira *et al*., 2016). The literature shows that both phytotherapeutic products modulate the levels of proinflammatory cytokines such as TNF-α and IL-1β (Curra *et al.,* 2013; Oliveira *et al*., 2016). The increase in these cytokines accounts directly for the delay in wound healing during the inflammatory process (Curra *et al.,* 2013). In addition to reducing TNF-α levels, chamomile decreased apoptosis of epithelial cells of the oral mucosa, favoring tissue repair (Oliveira *et al.,* 2016). Considering these properties and the growing interest in herbal medicine, we conducted an integrative literature review evaluating the effect of chamomile extract and curcumin on ulcerative lesions of the oral mucosa. We found that the number of randomized controlled trials using chamomile and curcumin in the management of these lesions was low, as well as the number of controlled experimental studies in animal models.

The selected studies demonstrated a reduction of symptoms, size and duration of recurrent aphthous stomatitis with the use of chamomile extract and curcumin (Manifar *et al*., 2012; Deshmukg & Bagewadi, 2014; Tadbir *et al.,* 2015). The effects seemed similar to those of triamcinolone, and these herbals may be an alternative in the management of this lesion.

Oral lichen planus is a mucocutaneous condition of autoimmune nature, which may present with painful and persistent ulcerative lesions. Only one of the selected studies compared chamomile to placebo, demonstrating reduced symptoms and

improved clinical appearance of lesions (Lopez Jornet & Aznar-Cayuela, 2016). Regarding curcumin, the results also showed beneficial effects. However, Kia *et al.* (2015) observed no differences between curcumin and triamcinolone, while Thomas *et al.* (2017) found that the corticosteroid was superior to the herbal medicine in the improvement of signs and symptoms of oral lichen planus. This difference could have been attributed to the different scales used to evaluate oral lichen planus signs, as well as the difference in drug concentration. In the study by Amirchaghmaghi *et al.* (2016), who evaluated the effect of curcumin on oral lichen planus, patients in both groups continued to receive dexamethasone treatment, which made it impossible to evaluate the actual effect of the herbal remedy.

Most studies, both clinical and experimental, evaluated the effect of chamomile and curcumin on chemo- or radio-induced oral mucositis (Fidler *et al.,* 1996; Shabanloei *et al.*, 2009; Rao *et al*., 2004; Braga *et al*., 2015; Patil *et al*., 2015; Mansourian *et al*., 2015; Ardakani *et al*. 2016; Dos Reis *et al.,* 2016). The studies have shown that these herbal medicines reduce the incidence, severity and duration of oral mucositis. Rao *et al.* (2014) pointed out a lower incidence of intolerable mucositis (grades 3 and 4) in patients who underwent head and neck radiotherapy with the use of curcumin-containing mouthwash.

Experimental studies have evaluated the effect of chamomile and curcumin on mechanically or chemically induced oral traumatic ulcers (Martins *et al.,* 2009; Duarte *et al*., 2011; Oliveira *et al*., 2016). Both phytotherapeutic products appeared to exert a positive effect on the healing of these lesions, reducing the initial (inflammatory) healing phase, with a higher deposition of collagen fibers, accelerating epithelization.

Most of the selected studies highlight the lack of adverse effects with the use of these herbal (Rao *et al*., 2014; Mansorian *et al.,* 2015; Tadbir *et al*., 2015;

Amirchaghmaghi *et al*, 2016; Ardakani *et al.,* 2016; Dos Reis *et al*., 2016; Thomas *et al*., 2017). However, Braga *et al.* (2015) described the occasional occurrence of burning mouth sensation, nausea and vomiting with the use of chamomile-based mouthwash at concentrations of 1 and 2%. According to Kia *et al.* (2015), a few patients complained of burning sensation, itching, mild swelling and xerostomia, which were gone by the end of the first week of using curcumin. Most of the patients complained of undesirable yellow color, particularly on the gums.

Healing of oral wounds is complex because of the various structures involved and the commensal microorganisms to which these lesions are exposed. In this article, we discussed studies that used chamomile and curcumin to treat traumatic ulcers, recurrent aphthous stomatitis, lichen planus and oral mucositis. These lesions exhibit different etiology and nature, so the results for each of them were discussed separately. Chamomile and curcumin appear to be effective in controlling pain at different concentrations and also have a positive influence on the tissue repair process of oral wounds. The low risk of systemic toxicity or allergies makes these herbal medicines a good alternative for the management of these lesions.

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4 ARTIGO DE PESQUISA

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EFFECT OF TOPICAL CHAMOMILE EXTRACT AND CURCUMIN ON THE REPAIR OF TRAUMATIC ULCERS ON THE TONGUE OF RATS

Artigo submetido ao periódico: *Phytotherapy Research*

Qualis Capes Odontologia 2017: A2

Fator de Impacto: 3.092 (2016)

EFFECT OF TOPICAL CHAMOMILE EXTRACT AND CURCUMIN IN TRAUMATIC ULCERS ON THE TONGUE OF RATS

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ABSTRACT

Purpose: To investigate the effect of 10% chamomile extract gel and 2% curcumin gel on the repair of traumatic ulcers on tongue of rats.

Materials and Methods: Thirty-nine male Wistar rats were divided in three groups: Control, Chamomile e Curcumin. A circular excisional wound, 5 mm in diameter and 1 mm deep, was made in the center of the ventral tongue. The substances were used in gel and applied twice daily for five days. All animals were euthanized at 6 days postoperative.

Results and conclusion: There was no significant difference among the groups in relation to area of residual ulcer (P=0.20), newly formed epithelium (P=0.97) or intensity of inflammatory response (P=0.724). Despite the antiinflammatory and antioxidant properties of the phytotherapeutic products tested, the present study showed that 10% chamomile extract and 2% curcumin did not accelerate the repair of traumatic oral ulcers or decrease the intensity of the inflammatory infiltrate.

Key words: Oral ulcer, Chamomile, Curcumin, Wound healing, Natural compounds.

INTRODUCTION

The oral mucosa is a frequent site for ulcerated lesions, which can be caused by mechanical, chemical or infectious agents or even immunological diseases (Siu, Landon, & Ramos, 2015). Mechanical trauma, due to orthodontic appliances, implant microscrews, cheek-chewing and poor-fitting dentures, is one of the main causes of traumatic ulcers (Rennick,Campbell, Naidu, Taylor; & Buschang, 2016). There is a break in the mucous membrane with a loss of surface tissue. These lesions cause pain and discomfort and compromise nutrition and oral hygiene, affecting the life quality of patients (Duarte, Quirino, Patrocínio, & Anbinder, 2011; Lim, Huang, Wang,& Hua, 2015).

Oral ulcers heal by second intention. These processes involve four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Some important cells and growth factors (cytokines) are involved during various stages of the wound healing process (Young, & McNaught, 2011). At 24-48 h after the injury, the inflammatory phase begins, wich important for the debridement of the wound and favors the normal process of wound healing. However, excessive inflammation, induced by overexpression of interleukin 1-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), can lead to delay in the repair process of these lesions (Oliveira *et al.,* 2016). In the proliferative phase, which begins about 72 h afterwards, there is proliferation of the blood vessels and fibroblasts, with deposition of the extracellular matrix and formation of granulation tissue, signaling the beginning of repair (Young; & McNaught, 2011). Wound epithelialization represents the final step of the proliferative phase. Wound remodeling starts simultaneously with the development of granulation tissue and continues for a long period (Cavalcante *et al*., 2011).

Different topical therapies have been used for oral ulcers to promote adequate healing and to decrease painful symptoms and the risk of infection as well. To date, many therapeutic modalities have been used empirically, since there have not been any gold standard agent for this purpose. These therapies include antiinflammatory medications, antimicrobial agents, anesthetics, analgesics, corticosteroids and antiseptics. They can be used alone or in combination (Martins *et al*., 2009; Rennick *et al.,* 2015).

Natural products have also been used as an alternative treatment for oral wounds (Martins *et al.,* 2009; Curra *et al*, 2013; Deshmukh & Bagewadi, 2014). Among these, *Matricaria recutita* L., popularly known as chamomile, is a medicinal plant that has been used for centuries for the treatment of inflammatory disorders, fever, diarrhea, menstrual pain, liver disease and intestinal tumors. It is believed to have antiinflammatory, antimicrobial, antispasmodic and antioxidant activities, besides sedative effects (Martins *et al.,* 2009; Duarte *et al.,* 2011; Jabri *et al.,* 2016). In the mouth, chamomile has shown good clinical results in the management of gingivitis, reducing the bacterial load (Carcamo, Oliva & González, 2011). Besides, it has been shown to accelerate the healing of linear incisional wounds on skin in rats (Jarrahi *et al*., 2010) and to reduce oral pain and accelerate the healing process in oral ulcers in humans (Ardakani *et al*., 2016).

Curcuma longa Linn or turmeric is an herb belonging to the ginger family (Zingiberaceae). Curcumin, the active ingredient in turmeric, is a polyphenol that is responsible for the bright yellow color (Goel, & Aggarwal, 2010). Curcumin is commonly used in Indian traditional medicine in the treatment of biliary disorders, cough, diabetic ulcers, hepatic disorders, rheumatism and sinusitis (Akbik, Ghadiri, Chrzanowski, & Rohanizadeh, 2014). This medicinal plant is known to have potent

antioxidant, antiinflammatory, antifungal and antimicrobial properties (Goel, & Aggarwal, 2010; Akbik *et al*., 2014).

Among the various properties attributed to chamomile and curcumin, the most notable are their antiinflammatory and antioxidant effects (Hernández-Ceruelos *et al.,* 2010; Jabri *et al.,* 2016). Both phytotherapeutic products modulate the levels of proinflammatory cytokines such as TNF-α and IL-1β. The increased levels of these cytokines are directly involved in delayed healing during the inflammatory process (Curra *et al*., 2013, Oliveira *et al.,* 2016). Considering the frequent occurrence of ulcerated lesions in the oral mucosa, the investigation of the effect of non-toxic, low-cost and easily accessible topical agents such as herbal medicines is an interesting alternative. Accordingly, the purpose of this study was to investigate the effect of 10% chamomile extract gel and 2% curcumin gel on the repair of traumatic ulcers on tongue of rats.

MATERIAL AND METHODS

The study was approved by the Ethics Committee on the Use of Animals of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil (protocol No. 7697).

Thirty-nine male *Wistar* rats (*Rattus norvegicus*), weighing 300 – 400 g were used in this study. The animals were kept in the Center for Experimental Biological Models in temperature-controlled (23±1°C) chambers equipped with input and output air filters and with a 12-h light-dark cycle. They were provided with food and water ad

libitum and housed in cages appropriate for rodents. The animals were randomly divided into:

- Control group (n= 13): vehicle
- Chamomile group (n=13): 10% chamomile
- Curcumin group (n=13): 2% curcumin

The test substances were prepared in gel form (Manifar *et al.,* 2012; Martins *et al.,* 2009; Curra *et al.,* 2013), with carbopol as the vehicle. The rats were anesthetized with intramuscular injection of ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (3 mg/kg). After sedation a circular excisional wound, 5 mm in diameter and 1 mm deep, was made in the center of the ventral tongue. It was made 3 mm from the apex, using a punch biopsy tool. Mucosa specimens were removed by sharp dissection exposing a circular area for secondary healing. The topical application of medication began after 24 h, with application twice daily (morning and evening) for five days. Animals were restrained by hand and 0.1 mL of the substance used for the treatment was applied to the ulcer area with a sterile swab. Water and food were removed for thirty minutes after each application to maintain the gel in contact with the region as long as possible. All animals were euthanized at 6 days postoperative. Euthanasia was performed using a carbon dioxide chamber (Figure 1).

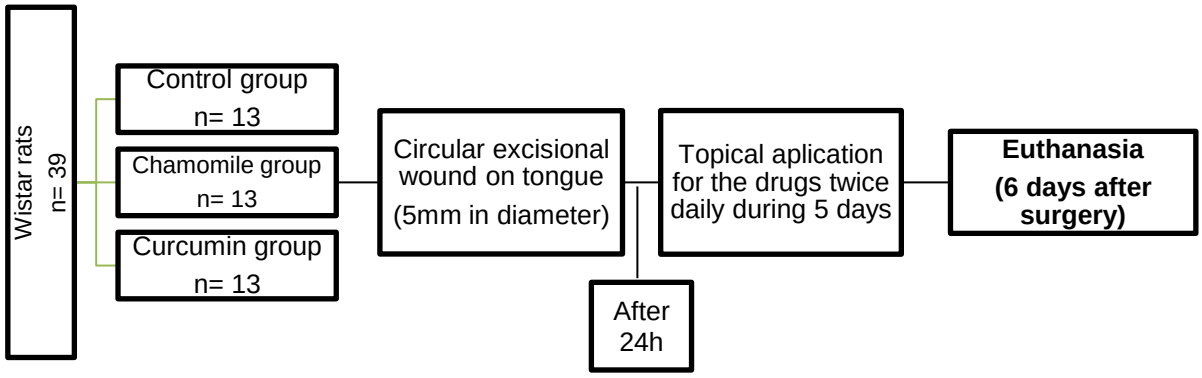

Figure 1. Flowchart representing the stages of the study.

Macroscopic Analysis

Immediately after euthanasia, a clinical evaluation of the repair was performed through visual inspection. Briefly, the images of the tongues were captured with a digital camera (Canon 60D, Tokyo, Japan). The images of the ulcers were outlined manually (polygonal tool) using ImageJ Pro Plus 7.0 1.50i software, and area was obtained in mm^2 (Teixeira *et al.,* 2018).

Sample Processing and Histological Analysis

Following clinical analysis, the tongue of each rat was surgically removed and fixed in 10% formalin for 24 h. After 24 h, specimens were cross-sectioned in the central region of the wound and subjected to routine histological processing. The specimens were embedded in paraffin and 5-µm-thick sections were obtained and stained with hematoxylin and eosin (HE).

All HE-stained slides were examined by a single examiner under a light microscope. We selected a field such that the wound could be histomorphometrically evaluated throughout its width. Images were captured with a digital camera (Retiga 2000R CCD - Surrey, Canada) connected to a binocular light microscope (Axioskop 40 - Carl Zeiss, Göttingen, Germany) with an original magnification of 20 X (objective lens - Carl Zeiss, Göttingen, Germany) and saved in TIFF format. The measurement of epithelial tissue area was performed with the aid of ImageJ Pro Plus 7.0 1.50i software. Briefly, a line about 5 mm long was drawn so that the ulcerated area would occupy the central portion. Epithelial tissue contained in this region was outlined by the area measurement tool (polygonal shape) to obtain the epithelial area in mm^2 of each edge. The values found were added to obtain total area of epithelium formed (Teixeira *et al.,* 2018).

For the analysis of inflammation, the field with the greatest intensity of inflammatory infiltrate was selected and captured with an original magnification of 200X and 400X (objective lens - Carl Zeiss, Göttingen, Germany). The intensity of inflammatory response was scored on a scale from 0 to 3 according to the following criteria (Figueiredo *et al*., 2001): 0- Absent (absence of inflammation); 1-Mild (sparse mononuclear cells), 2-Moderate (mononuclear infiltrate and/or sparse neutrophils and eosinophils); 3- Intense (polymorphonuclear infiltrate of neutrophils and eosinophils).

Intra-examiner calibration was achieved by examination in duplicate of 10 histological slides with an interval of seven days between each evaluation. Intra-class correlation coefficient was used for ulcer and epithelial areas and Kappa was used for inflammatory infiltrate. The values obtained were 0.9 for epithelial and ulcer areas and 0.6 for inflammatory infiltrate.

Statistical Analysis

Data were initially analyzed using descriptive statistics. To compare the epithelial tissue area and residual ulcer area among the different groups, we used one-way ANOVA and Kruskal-Wallis test, respectively. To compare the intensity of the inflammatory response between the groups, we used the Pearson chi-square test. $P \leq 0.05$ was used to reject the null hypothesis. SPSS version 20.0 and GraphPad Prism 5.0 were used to perform the statistical analysis.

RESULTS

During the experimental period, the animals gained weight, indicating that the tongue wound did not harm their feeding.

Macroscopic and Histological Analysis

In all groups, there was clinically visible residual ulcer at the end of experimental period. The lesions showed well-defined outlines, were covered by a yellow pseudomembrane and surrounded by whitish halo (Figure 2). There was no significant difference among the groups in relation to area of residual ulcer after five days of treatment ($P=0.20$), that is, six days after making the wound (Table 1).

The measurement of the area was used to determine the amount of epithelial tissue formed in each specimen. At five days of treatment, there was no significant difference in mean values between the groups ($P=0.97$) (Table 1).

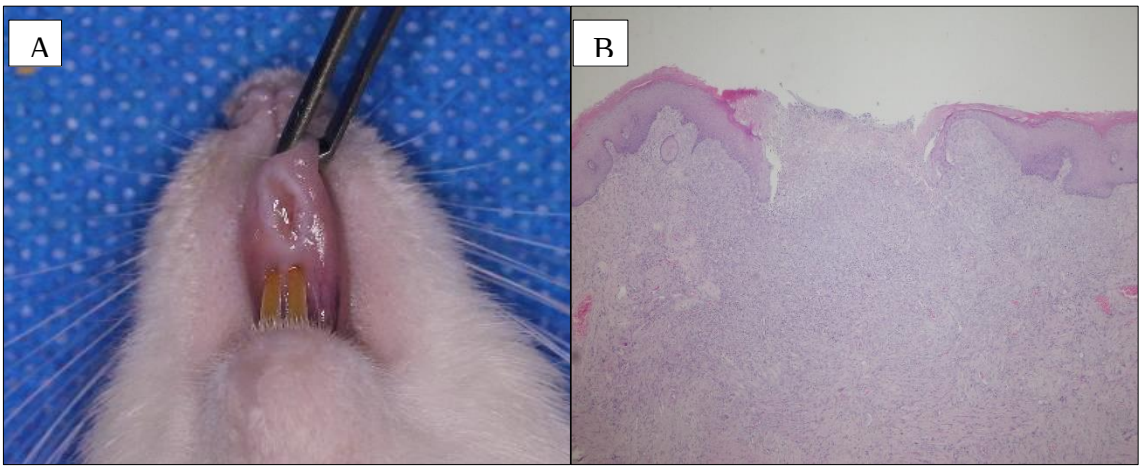

Figure 2- Residual ulcer in the curcumin group. A- Clinical picture showing the lesion covered by a pseudomebrana and surrounded by a whitish halo. B- Necrotic connective tissue and epithelium proliferation from the wound edges at 6 days (hematoxylin and eosin staining, original magnification 10x).

Table 1. Ulcer area (mm^2) and epithelial area (mm^2) after treatment with chamomile, curcumin and placebo.

	Chamomile Group (n=13)	**Curcumin Group (n=13)**	**Control Group (n=13)**	**P**
Ulcer Area (median, 25-75)	7.41 (2.59-10.62)	4.14 (2.00-11.38)	4.08 (1.98-12.24)	0.20*
Epithelial Area (media ±SD)	0.69 (±0.20)	0.67 (±0.21)	0.69 (±0.31)	0.97**

*Kruskal–Wallis test.
** *One-way* ANOVA.

In the histological analysis, the presence of residual ulcer was observed in all the specimens, covered by a fibrinopurulent membrane and surrounded by orthokeratinized squamous epithelial tissue of variable thickness. Connective tissue was either fibrous and well organized, or loose and disorganized. Most of the specimens (82%) of the chamomile, curcumin and control groups had moderate inflammatory infiltrate (score 2) without significant differences (P=0.724) (Fig.3 and 4). A mononuclear inflammatory infiltrate, consisting predominantly of

macrophages and interspersed by sparse polymorphonuclear cells (neutrophils), was observed.

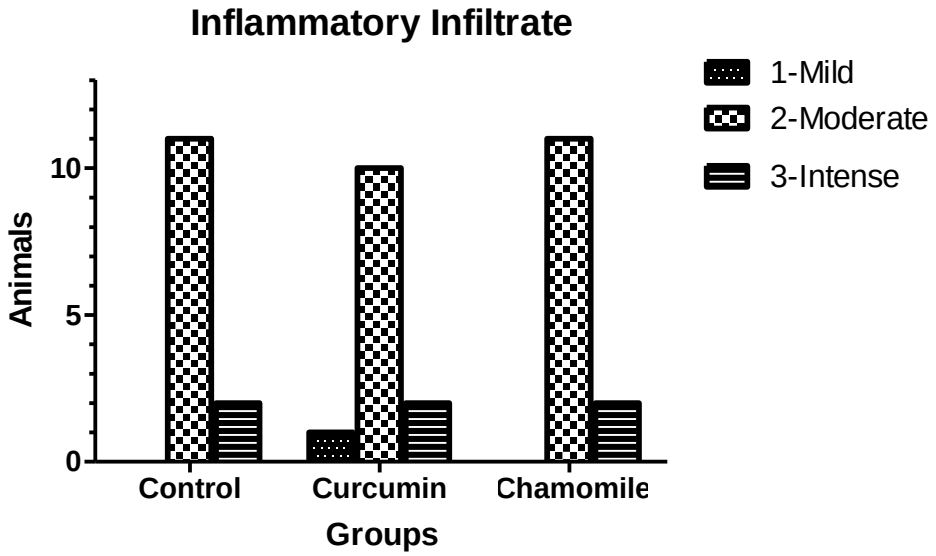

Figure 3. Intensity of inflammatory infiltrate in control, curcumin and chamomile groups, according to the criteria used in the study.

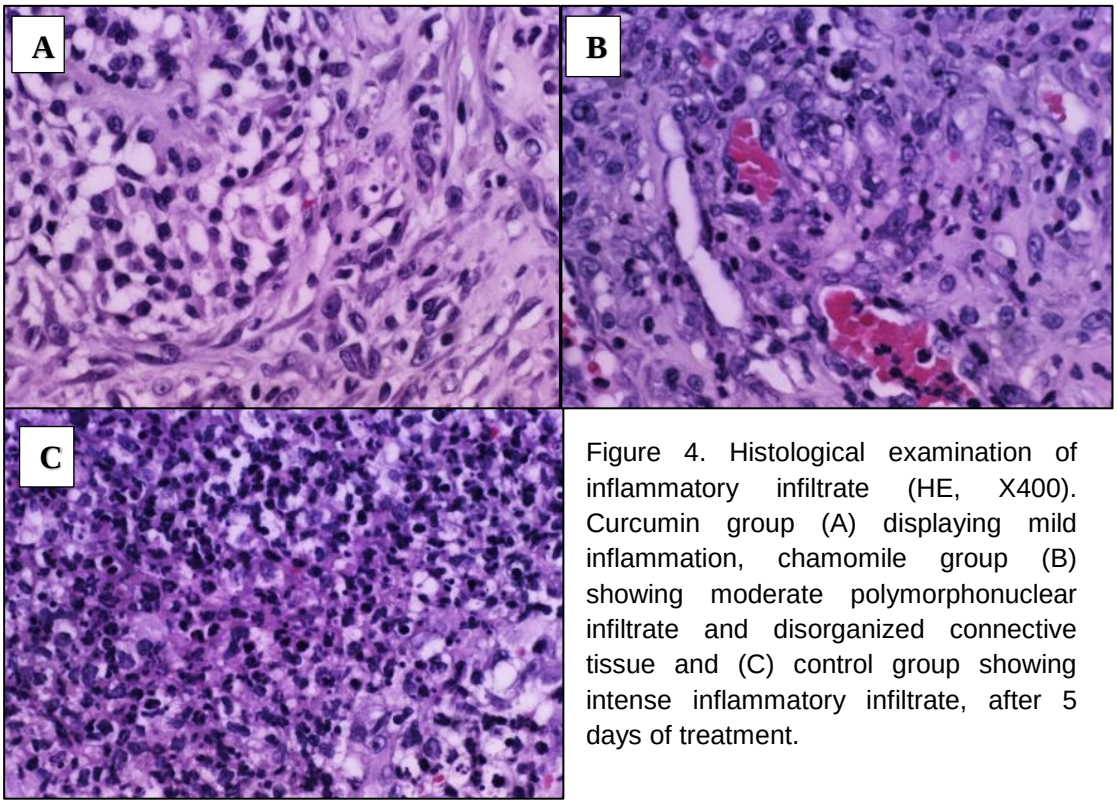

Figure 4. Histological examination of inflammatory infiltrate (HE, X400). Curcumin group (A) displaying mild inflammation, chamomile group (B) showing moderate polymorphonuclear infiltrate and disorganized connective tissue and (C) control group showing intense inflammatory infiltrate, after 5 days of treatment.

DISCUSSION

Modulation of the inflammatory response during the healing process of oral lesions is essential for tissue repair to occur properly and in a shorter period. Considering the properties of chamomile and curcumin, we studied here the effect of these phytotherapeutic products on the repair of mechanically induced ulcers on the rat tongue. The residual ulcer area, the newly formed epithelium area, and the intensity of inflammatory infiltrate in the chamomile and curcumin groups did not differ compared to the placebo. Therefore, chamomile and curcumin had no effect on accelerating the healing of these ulcerated lesions or reducing the inflammatory process.

The literature demonstrates that chamomile and curcumin have antiinflammatory effects (Pavesi *et al.,* 2011; Oliveira *et al.,* 2016; Lim *et al.*, 2016), acting directly on the wound-healing process. Curcumin inhibits the production of TNF-α and lL-1β, cytokines released by monocytes and macrophages, which decreases the duration of the inflammatory phase of healing (Akbik *et al.,* 2014). The antiinflammatory effect of chamomile is due to apigenin, a flavonoid present in this plant, which assists in the reduction of TNF-α production (Oliveira *et al*., 2016). Chamomile has also shown a stimulatory effect on the growth of keratinocytes *in vitro* (Alerico, Beckenkamp, Vignoli-Silva, Buffon, & Von Poser, 2015) and inhibited the apoptosis of these cells in a study in ulcerated lesions on the oral mucosa of rats (Oliveira *et al.,* 2016). In addition, other authors have pointed out the antioxidant properties of these phytotherapeutic products as being responsible for the reduction of cellular oxidative stress, which favors the wound healing process (Hernández-Ceruelos *et al.,* 2010; Jabri *et al.,* 2016; Lim *et al*., 2016). Considering these properties, we expected to see

significant improvements in the parameters evaluated in the chamomile and curcumin groups.

When applying chamomile on oral ulcers of rats, Duarte *et al.* (2011) did not observe differences in re-epithelialization or in the percentage of collagen fibers after three and seven days of treatment. However, after 10 days of chamomile use, the treated group displayed significantly greater epithelial neoformation and deposition of collagen fibers than in the control group. Lim *et al.* (2016) and Oliveira *et al.* (2016), in studies in animal models, also observed a decrease in residual ulcer area after 10 days of topical application of 1% curcumin and 10% chamomile, respectively. In our study, the evaluations were performed after five days of treatment, that is, six days after the mechanical induction of the ulcers. This difference in evaluation period may explain the different results in relation to the residual ulcer and wound epithelialization. We chose to perform the clinical and histological evaluations six days later to observe the wound healing process in the proliferative phase and in the initial phase of remodeling, evaluating the inflammatory infiltrate and neoformation of epithelial tissue.

Martins *et al.* (2009) investigated the reduction in size and epithelialization of ulcerated lesions in rat tongue after topical application of chamomile gel and observed that this substance accelerated the healing of wounds as early as the 5th day after the ulcer was made. This result might have been associated with the size of the lesions, which was 3 mm, differing from our study using a 5-mm punch. In addition, in the study by Martins *et al. (*2009), the assessment of ulcer healing was performed using scores, considering the epithelium and microscopic aspects of the connective tissue. Duarte *et al.* (2011) also evaluated the epithelialization and size of the residual ulcer by means of scores. In the present study, evaluations of the residual ulcer area and epithelial

tissue area were performed with the aid of software, reducing the subjectivity of the analysis, which may also explain the different results in relation to these parameters.

Histopathological analysis showed that most of the samples (82%), independent of the evaluated group, showed moderate inflammatory infiltrate, with predominance of mononuclear cells and/or neutrophils and sparse eosinophils. On the other hand, Duarte *et al.* (2011) detected the presence of mild inflammatory infiltrate with the application of the chamomile extract on ulcerated lesions in the tongue of rats after seven days. The authors described a moderate number of mononuclear cells, few polymorphonuclear cells, and an immature granulation tissue. The difference in the intensity of the inflammatory infiltrate between both studies could not be explained with the data available, since the chamomile formulation, drug application, and methods of analysis were similar.

When evaluating chemo-induced oral mucositis in hamsters, Pavesi *et al.* (2013) also observed lower inflammatory scores with topical use of chamomile compared to placebo and betamethasone after the 8th day. Lim *et al.* (2016) in a study using topical curcumin on rabbit oral ulcers observed a reduction in inflammatory infiltrate in the treatment group over placebo group after seven days. In the study of Lim *et al.* (2016), however, the criteria used for scoring inflammatory activity were not mentioned. The inflammatory alterations were detailed in a descriptive way, without statistical comparison between the groups.

Controlled, randomized clinical trials have demonstrated beneficial effects of chamomile and curcumin applied topically in oral mucosal diseases such as recurrent aphthous ulceration (Manifar *et al*., 2012; Deshmukh & Bagewadi, 2014), lichen planus (Amirchaghmaghi *et al*.,2016; Lopez Jornet & Aznar-Cayuela, 2016; Thomas *et al*., 2017) and oral mucositis(Mansourian, Amanlou, Shirazian, Jahromi, & Amirian ,2015;

Ardakani *et al.,* 2016; Dos Reis *et al.,* 2016). Due to the completely different etiopathogenesis of these lesions compared to that of the traumatic ulcers described in this study, it is difficult to compare results. In addition, it should be noted that in our study, traumatic ulcers were induced in healthy animal models with no morbidities such as diabetes or immunosuppression. It is possible that under different systemic conditions we could observe a superior effect of the phytotherapeutic agents investigated in relation to placebo.

The medications used in our study were prepared in the gel form, so that their higher viscosity would increase adhesion to the oral mucosa. Curcumin was formulated at 2% and chamomile at 10% on the basis of the literature (Manifar *et al.,* 2012; Martins *et al.,* 2009; Curra *et al.,* 2013). In each application, 0.1 ml of each substance was used, because of the size of the oral cavity of the animal, where a larger amount would not be possible. The application of the substances in each group started 24 hours after the ulcer was made to allow the formation of the clot and fibrin matrix, which is essential for tissue repair, preserving the epithelial margins and releasing growth factors close to the edges (Young & McNaught, 2011). In addition, this period was important for the recovery of the animals after the surgical procedure (Kovalik *et al*., 2014).

The substances tested in our study did not show adverse effects considering the clinical evaluation performed. Studies indicated the apparent safety of curcumin and chamomile extract (Martins *et al*., 2009, Lim *et al*., 2015). Toxicity analysis, *in vitro*, revealed positive cell viability in cultures treated with chamomile extract. However, this viability was significantly lower when compared to control and corticosteroid groups. This result may lie in the antimicrobial effect of chamomile, which may have influenced cell survival (Martins *et al.,* 2009). Regarding curcumin, Chainani-Wu *et al.* (2012) observed good tolerability when using a dose of 600 mg/day in patients with oral lichen

planus. There was no difference in hepatic enzyme levels between the curcumin and placebo groups. However, diarrhea was an adverse effect reported by the patients who used curcumin. This side effect was not observed in the animals in our study.

The repair of oral ulcers is complex, due to the peculiarities of the oral cavity (commensal microbiota), local trauma and the need for an adequate initial inflammatory response. Despite the antiinflammatory and antioxidant properties of the phytotherapeutic products tested, the present study showed that 10% chamomile extract and 2% curcumin did not accelerate the repair of traumatic oral ulcers or reduce the intensity of the inflammatory infiltrate, considering the experimental model used.

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5 DISCUSSÃO COMPLEMENTAR

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A mucosa bucal é constantemente afetada por lesões ulceradas, sejam elas de natureza química, física ou decorrentes de doenças imunológicas. Até o momento não existe um tratamento padrão-ouro para o manejo dessas lesões, as quais podem ser dolorosas e ter impacto negativo na qualidade de vida dos pacientes.Em vista disso, torna-se necessário o uso de medicações que ajudem a minimizar os danos, auxiliando no controle da dor e facilitando o processo de reparo tecidual.Nos últimos anos tem havido um interesse crescente no uso de fitoterápicos no processo da cicatrização (MARTINS et al., 2009, CHINSEMBU, 2016; HAMEDI et al., 2016). Dentre eles, podemos destacar a camomila e a curcumina por suas propriedades anti-inflamatórias e antioxidantes, favorecendo o processo de reparo tecidual (GOEL; AGGARWAL, 2010; HERNÁNDEZ-CERUELOS et al., 2010).

Assim, no primeiro artigo desta dissertação foi realizada uma revisão integrativa da literatura com a finalidade de abordar o efeito do uso tópico da camomila e da curcumina no reparo de lesões ulceradas da mucosa bucal. Constatou-se que estas substâncias utilizadas topicamente em diferentes formulações, seja como enxaguatório bucal, gel, creme/pasta, apresentaram efeito benéfico na cicatrização de lesões da mucosa bucal como úlceras traumáticas, mucosite radio ou quimioinduzida, ulceração aftosa recorrente e líquen plano (TADBIR et al., 2015;THOMAS et al., 2017). Além de diminuir a liberação de radicais livres, essas substâncias podem reduzir a fase inflamatória da cicatrização, podendo acelerar a epitelização das feridas (PAVESI et al.,2013; OLIVEIRA et al., 2016).

Tais evidências incentivaram a realização de um estudo no qual foi avaliado o efeito da aplicação tópica do extrato de camomila e da curcumina no reparo de úlceras mecanicamente induzidas na língua de ratos. Porém, conforme descrito no segundo artigo, a avaliação macroscópica das lesões, bem como a área de tecido epitelial neoformado não mostraram diferenças significativas dos grupos camomila e curcumina em relação ao controle após cinco dias de tratamento.Também não foi observada diferença entre os grupos quanto à intensidade do infiltrado inflamatório. Esses achados divergem da literatura, que relata que ambos os fitoterápicos causam redução da liberação de citocinas pró-inflamatórias (TNF-α e IL-1β), com maior deposição de fibras colágenas e proliferação de células epiteliais (AKBIK et al., 2014; OLIVEIRA et al., 2016).

No presente estudo, as avaliações da área de úlcera remanescente e área de tecido epitelial foram realizadas com auxílio de um software, diminuindo a subjetividade da análise, o que pode justificar os resultados distintos aos dos estudos anteriores em relação a estas variáveis. Deve-se considerar que as condições experimentais controladas deste estudo como o uso de animais saudáveis, que não apresentavam morbidades, a confecção das úlceras bucais com *punch* estéril e individualizado, favoreceram o reparo tecidual. Talvez em situações adversas como em animais diabéticos ou imunossuprimidos, por exemplo, ou ainda em quadros inflamatórios exacerbados como na mucosite oral, fosse possível observar resultados distintos.

Assim como em outros estudos (MARTINS et al., 2009, DUARTE et al., 2011) o infiltrado inflamatório foi avaliado por meio de escores, considerando o tipo e a quantidade aproximada das células inflamatórias presentes. É possível que a utilização de outros métodos, como análise por meio de marcadores

imunoistoquímicos específicos para macrófagos (CD68) e linfócitos (CD3) trouxesse dados adicionais sobre o processo inflamatório nas lesões tratadas com camomila e curcumina. Além disso, novos estudos podem ser realizados com o uso de marcadores de proliferação celular, angiogênese e neoformação de colágeno, ligados diretamente ao processo de reparo tecidual.

No presente estudo, as formulações do extrato de camomila e da curcumina e a frequência de aplicação dessas substâncias foram semelhantes às de estudos anteriores (MARTINS et al, 2009; MANIFAR et al., 2012). Além disso, o extrato de camomila foi manipulado na mesma concentração de um produto comercialmente disponível (AdMuc®). Entretanto, pelo tamanho da boca do animal, a quantidade de produto empregado em cada aplicação era muito pequena. Apesar de recobrir a totalidade da ferida, pode não ter sido suficiente para causar o efeito benéfico desejado.

A lesão foi induzida no ventre lingual dos ratos, considerando que a língua é a localização mais comum de úlceras traumáticas (BASCONES-MARTÍNEZ et al., 2005). A porção ventral foi escolhida por ser menos suscetível a danos mastigatórios e permitir a manutenção da substância sobre a ferida por um tempo maior. A úlcera foi confeccionada com um *punch* estéril de 5mm, seguindo estudos anteriores, que utilizaram esta metodologia no mesmo modelo animal (JASPER et al., 2016, TEIXEIRA et al.,2018). Podemos sugerir que com a utilização de um *punch* de menor tamanho, talvez resultados diferentes pudessem ter sido obtidos no mesmo tempo experimental.

Em nosso estudo, o extrato de camomila e a curcumina não se mostraram eficazes no tratamento das lesões bucais mecanicamente induzidas. Apesar da ausência clínica de efeitos adversos com o uso tópico dos fármacos e de suas

propriedades anti-inflamatórias e antioxidantes, estudos complementares fazem-se necessários, para que estes fitoterápicos possam ser considerados uma alternativa no tratamento de lesões traumáticas bucais.

6 CONCLUSÕES

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Com base nos resultados deste estudo, pode-se concluir que:

- O extrato de camomila e a curcumina não foram efetivos no processo de reparo de úlceras mecanicamente induzidas na mucosa bucal de ratos.
- Os agentes fitoterápicos utilizados, apesar de suas propriedades anti-inflamatórias e antioxidantes, não reduziram a intensidade do processo inflamatório nos grupos estudados, sem influenciar o reestabelecimento da arquitetura tecidual de maneira mais rápida.

7 REFERÊNCIAS COMPLEMENTARES

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ANEXO A

APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA PUCRS

SIPESQ

Sistema de Pesquisas da PUCRS

Código SIPESQ: 7697 Porto Alegre, 7 de dezembro de 2016

Prezado(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou o Projeto de Pesquisa "EFEITO DA APLICAÇÃO TÓPICA DE EXTRATO DE CAMOMILA, CURCUMINA E DEXAMETASONA NO REPARO DE ÚLCERAS TRAUMÁTICAS EM VENTRE LINGUAL DE RATOS: ANÁLISE CLÍNICA, HISTOLÓGICA E IMUNOISTOQUÍMICA" coordenado por FERNANDA GONCALVES SALUM.

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está autorizada a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Duração do Projeto: 07/12/2016 - 07/03/2017

Nº de Animais	Espécie
52	Rattus norvegicus
Total de Animais: 52	

Atenciosamente,

Comissão de Ética no Uso de Animais(CEUA)

APÊNDICE 1

FICHA DE COLETA DE DADOS

FICHA DE COLETA DE DADOS

ESTUDOS EM PACIENTES

- CONTROLADO/RANDOMIZADO

-MÉTODO PARA GERAR A SEQUÊNCIA ALEATÓRIO

-CEGAMENTO: PARTICIPANTE/QUEM FAZ A INTERVENÇÃO-QUEM ANALISA OS RESULTADOS

-PACIENTES PERDIDOS DURANTE O ESTUDO

NÚMERO DE PARTICIPANTES	
GRUPOS	
DOSES	
TEMPO DE USO	
ESQUEMA DE ADMINISTRAÇÃO	
QUAL ERA O CONTROLE	
QUAL LESÃO	
MÉTODO DE AVALIAÇÃO(TAMANHO DAS LESÕES/ESCALA DE DOR)	
RESULTADOS	
CONCLUSÃO	

ESTUDOS EM ANIMAIS

-CONTROLADO-RANDOMIZADO

-MÉTODO PARA GERAR A SEQUÊNCIA ALEATÓRIO

-DESCRIÇÃO DAS PERDAS

NÚMERO DE ANIMAIS	
GRUPOS	
DOSES	
TEMPO D USO	
ESQUEMA DE ADMINISTRAÇÃO	
QUAL ERA O CONTROLE	
QUAL LESÃO	
MÉTODO DE AVALIAÇÃO(TAMANHO DAS LESÕES/ANÁLISE HISTOLÓGICA)	
RESULTADOS	
CONCLUSÃO	

APÊNDICE 2

FICHA DE ANÁLISE CLÍNICA E HISTOLÓGICA

FICHA DE ANÁLISE CLÍNICA E HISTOLÓGICA

Número da Lâmina:.....

Grupo:.....

Tamanho da úlcera (mm):.....

Área da úlcera (mm²): .....

Infiltrado Inflamatório

( ) Ausente (ausência de inflamação)

( ) Leve (células mononucleares esparsas)

( ) Moderada (infiltrado mononuclear e/ou neutrófilos e eosinófilos esparsos)

( ) Intensa (infiltrado polimorfonuclear de neutrófilos e eosinófilos)

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