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RENATA DI CREDDO BRUM

ANÁLISE HISTOLÓGICA DO PERÓXIDO DE CARBAMIDA ASSOCIADO OU NÃO COM *ALOE VERA* NO REPARO DE ÚLCERAS EM MUCOSA ORAL DE RATOS

CURITIBA

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do Título de Mestre em Odontologia – Área de Concentração em Dentística.

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CURITIBA

2009

O verdadeiro filósofo não busca o reconhecimento de si,

mas sim de suas idéias.

(Lucian Rodrigues Cardoso)

DEDICO ESTE TRABALHO

Aos meus pais João e Rosângela.

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SUMÁRIO

LISTA DE SIGLAS E ABREVIATURAS

S – Solução Salina.

PC – Peróxido de carbamida 16%.

A – Gel de *Aloe vera* 0,5%.

PCA – Peróxido de Carbamida 16% + gel de *Aloe vera* 0,5%.

Fig. – Figura.

HE – Hematoxilina e Eosina.

AT – Azul de Toluidina.

SR – Picrosírius.

LISTA DE FIGURAS

LISTA DE TABELAS

1. ARTIGO EM PORTUGUÊS

ANÁLISE HISTOLÓGICA DO PERÓXIDO DE CARBAMIDA ASSOCIADO OU NÃO COM *ALOE VERA* NO REPARO DE ÚLCERAS EM MUCOSA ORAL DE RATOS

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RESUMO

Os agentes clareadores apresentam efeitos transitórios como sensibilidade dental, irritação e edema dos tecidos orais. A *Aloe vera* tem propriedade anti-inflamatória, analgésica, antimicrobiana e cicatrizante. O objetivo deste trabalho foi avaliar histologicamente o processo de reparo em úlceras na mucosa oral de ratos, tratadas pelo gel de peróxido de carbamida associado ou não com a *Aloe vera* 0,5%. Foram induzidas úlceras com NaOH 40%, por 7 dias em 112 ratos *Wistar* e posteriormente foram aleatoriamente divididos em 4 grupos (n=28): Salina (S), Peróxido de Carbamida 16% (PC), Gel de *Aloe vera* 0,5% (A) e Peróxido de Carbamida 16% + gel de *Aloe vera* 0,5% (PCA). Os animais foram mortos nos períodos de 2, 7, 14 e 21 dias após o início do tratamento. Os espécimes foram processados histologicamente e corados em hematoxilina-eosina, azul de toluidina e picrosírius. Foram quantificados na área epitélio/conjuntivo e submucosa da úlcera o número de mastócitos, e sob luz polarizada foi avaliada a percentagem da área e densidade de colágeno maturo e imaturo. Os dados foram analisados pelos testes de Análise de Variância, Tukey HSD e Games-Howell ($p<0,05$). Os resultados obtidos demonstraram diferenças estatisticamente significantes no grupo A no período de 2 dias para percentagem de área de colágeno maturo (18,52±8,07) e imaturo (81,48±8,07) e na densidade de colágeno maturo (44,99±8,69) e imaturo (55,01±8,69) para epitélio/conjuntivo. No grupo PCA as diferenças foram no período de 2 dias para o número de mastócitos (0,12±0,16) na região epitélio/conjuntivo e aos 7 dias na região submucosa (0,51±0,09), para percentagem de colágeno maturo (16,99±13,81) e imaturo (83,01±13,81). Concluiu-se que a *Aloe vera* 0,5% associada ou não a agentes clareadores demonstrou efeito anti-inflamatório e cicatrizante em úlceras de mucosa oral de ratos, diminuindo o número de mastócitos na região mais superficial da lesão e estimulando a fibroplasia.

Palavras- chave: clareamento dental, *aloe vera*, peróxido de carbamida, mastócito, colágeno, processo de reparo, úlcera.

INTRODUÇÃO

Os produtos utilizados para o clareamento dental são os peróxidos de carbamida e de hidrogênio. O mecanismo do clareamento dental é um processo de oxidação complexo que gera radicais livres após a quebra desses peróxidos. O peróxido de carbamida se dissocia na saliva em peróxido de hidrogênio e uréia. A uréia dissocia-se em amônia e dióxido de carbono, já o peróxido de hidrogênio, considerado ingrediente ativo do clareamento, penetra na estrutura dental resultando em oxigênio e água.[1] Embora o organismo humano apresente mecanismos de proteção como o epitélio da mucosa oral e constituintes salivares, além de várias outras formas de defesa para metabolizar o peróxido de hidrogênio intra e extracelularmente, os agentes clareadores possuem a capacidade de invadir e gerar efeitos tóxicos ao tecidos orais e suas células.[2] A técnica de clareamento caseiro, introduzida por Haywood e Heymann[3] contém na sua composição peróxido de carbamida variando sua concentração de 10 a 22%.[4] É uma técnica simples, conservadora e que apresenta bons resultados; porém não é um tratamento inócuo, pois gera efeitos adversos que afetam os tecidos duros e moles da cavidade oral. Dentre estes efeitos estão sensibilidade dental,[5] irritação gengival,[6] modificações na superfície do esmalte[7,8] e dentina[9] e ulcerações.[10] Dentro da literatura, diversos trabalhos avaliaram o efeito dos peróxidos de carbamida e hidrogênio nos tecidos duros e moles da cavidade oral.[6,7,8,9,10] Kirsten *et al.*[6] realizaram a técnica de clareamento com moldeira e concluíram que o peróxido de carbamida 16% causou inflamação gengival que persistiu até 45 dias depois do término do tratamento. Pinto *et al.*[8] e Cavalli *et al.*[7] demonstraram redução da microdureza e aumento da rugosidade de superfície do esmalte submetido à diversas concentrações de peróxido de carbamida e de hidrogênio. Arcari *et al.*[9] demonstraram redução da

microdureza da dentina submetida ao peróxido de carbamida 10% por 7 horas e Rees & Orth[10] concluíram que o peróxido de hidrogênio 3% usado em bochechos pode gerar úlceras no tecido oral.

As ulcerações são lesões que geram perda de epitélio com exposição do tecido conjuntivo subjacente e clinicamente mostram-se arredondadas ou ovaladas, rasas, com membrana branca-acinzentada no centro e halo eritematoso circundante.[11,12]

O processo inflamatório está relacionado com uma série de eventos patofisiológicos que geram diferentes efeitos em cada organismo. Existem vários mediadores trabalhando neste processo, como as aminas com destaque para a histamina, peptídeos, lipídeos como as prostaglandinas e leucotrienos.[13] A aspirina e os salicilatos são considerados potentes agentes anti-inflamatórios, a dose de 3g de cada uma dessas drogas reduziram a produção de prostaglandinas em torno de 95% em humanos.[14] Durante a inflamação há uma quantidade variável de mastócitos, que são células originárias na medula óssea, com núcleos ovais contendo grânulos citoplasmáticos metacromáticos que desempenham importante papel no processo inflamatório liberando mediadores químicos.[15]

No processo de reparo de úlceras ocorre uma série de eventos inflamatórios e imunológicos originados das células além da ação de mediadores químicos que visam restabelecer o tecido lesado. Durante esse processo o colágeno é produzido e depositado pelos fibroblastos sendo fundamental na reparação tecidual.[16]

A *Aloe vera* é um cactus tropical, constituída por mais de 75 agentes ativos incluindo vitaminas, enzimas, minerais, lignina, saponina, ácido salicílico e aminoácidos.[17] Dentre estes constituintes destacam-se o acemanan, o lactato de magnésio, a aloína, a barbaloína e a emodina.[17] Esta planta produz substâncias com

diferentes composições e propriedades terapêuticas, tais como umidificantes, anti-inflamatórias, analgésicas e cicatrizantes,[18] as quais foram demonstradas em estudos clínicos[19,20] e experimentais.[21,22]

O uso de produtos farmacológicos à base de plantas medicinais pode ser indicado com a finalidade de diminuir o potencial inflamatório dos géis clareadores dentais. Desta forma, o objetivo deste trabalho foi avaliar histologicamente o processo de reparo em úlceras da mucosa oral tratadas pelo gel de peróxido de carbamida associado ou não à *Aloe vera* 0,5%, em ratos.

MATERIAL E MÉTODO

O estudo foi aprovado pelo Comitê de Ética no Uso de Animais (CEUA) da Pontifícia Universidade Católica do Paraná (PUCPR), nº 226/07.

Foram utilizados 112 ratos machos adultos do tipo *Rattus novergicus albinus*, da linhagem *Wistar*, pesando cerca de 180g. Os animais foram obtidos junto ao biotério da PUCPR, mantidos em gaiolas plásticas e alimentados com ração e água *ad libitum*, respeitando-se o ciclo diurno-noturno.

A anestesia geral foi induzida por via intraperitoneal com tiopental sódico® - 20mg/kg (Cristália, Itapira, SP, Brasil). Na porção mais anterior da face dorsal da língua dos animais foram induzidas, quimicamente, úlceras com hidróxido de sódio (NaOH) 40%, pela aplicação tópica e diária, com auxílio de hastes de algodão durante um minuto, por 7 dias. Após a constatação clínica visual da formação de úlcera na língua de cada animal, os mesmos foram aleatoriamente divididos em 4 grupos (n=28) e tratados com salina (S), peróxido de carbamida 16% (PC), gel de *Aloe vera* 0,5% (A), e gel composto de peróxido de carbamida 16% + *Aloe vera* 0,5% (PCA).

Os géis de Peróxido de Carbamida 16% e de *Aloe vera* 0,5% (Galena Indústria farmacêutica – São Paulo, SP, Brasil) ou a associação de ambos foram preparados em farmácia de manipulação e aplicados diariamente, por uma única vez, com hastes de algodão, na região da úlcera, por 2 minutos.

Os animais foram mortos por superdosagem de tiopental sódico®, por via intraperitoneal, nos períodos de 2, 7, 14 e 21 dias após o início do tratamento. As línguas foram removidas e seccionadas no sentido longitudinal, fixadas em formalina neutra 10% por 24 horas.

De cada animal foram confeccionadas três lâminas coradas com hematoxilina-eosina (HE), azul de toluidina 0,2% (AT) e picrosírius (SR). Nas secções coradas em HE, as lâminas foram observadas nos aumentos de 50, 100, 200 e 400X com o microscópio de luz binocular OLYMPUS BX50 (OLYMPUS, Tóquio, Japão), visando descrever subjetivamente o processo de reparo na área ulcerada, sem a intenção de realizar uma análise quantitativa. Foi observado a presença ou não de ulceração e de vasos sanguíneos e o predomínio do tipo de infiltrado inflamatório (agudo, misto ou crônico).

Nas secções coradas com AT foi quantificado o número de mastócitos, identificados pela sua cor púrpura. Em cada lâmina foram obtidas quatro imagens da área da lesão ulcerada e tratada, correspondente a 2 campos superficiais – epitélio/ conjuntivo e 2 campos profundos - submucosa. As imagens foram capturadas com aumento de 200X pela câmara de vídeo (Sony Íris CCD, Tóquio, Japão) acoplada ao microscópio e enviadas ao computador. Em cada imagem do campo histológico com o auxílio do programa Image Pro Plus versão 4.5 (Media Cibernetics, Silver Spring, MD, EUA), foi confeccionado um retículo virtual (3x4) de $1mm^2$ cada para a contagem do número de mastócitos.[23]

A percentagem da área e a densidade de colágeno maturo e imaturo foram mensuradas nas secções coradas pelo SR usando luz polarizada, onde o colágeno maturo apresenta birrefringência intensa, de coloração amarelo e vermelho, enquanto o colágeno imaturo produz uma coloração esverdeada.[24] Essa avaliação foi realizada em um campo na área epitélio/conjuntivo e outro na submucosa, mensuradas pelo mesmo programa Image Pro Plus.

Os testes estatísticos de Normalidade de Shapiro–Wilk e Homogeneidade de Levene foram aplicados na análise quantitativa. O teste de Análise de Variância

($p<0,05$) foi aplicado para comparar as médias dos grupos, nas áreas epitélio/conjuntivo e submucosa das úlceras, para o número de mastócitos e para a percentagem da área e densidade do colágeno maturo e imaturo com o software SPSS 15.0 para Windows® (SPSS Inc., Chicago, Illinois, EUA).

Nas variáveis em que o teste Análise de Variância acusou diferença nos valores médios entre grupos e, quando houve homogeneidade de variâncias entre os grupos pelo teste de Levene, as comparações múltiplas foram feitas pelo teste de Tukey HSD. Caso contrário foi utilizado o teste de comparações múltiplas para variâncias heterogêneas de Games-Howell.

RESULTADOS

As lâminas avaliadas em HE para o grupo S mostraram, aos 2 dias pós-tratamento, uma área de úlcera na mucosa oral com interrupção do epitélio e exposição do tecido conjuntivo (Fig.1A). Esta área exibiu um infiltrado inflamatório agudo com predominância de polimorfonucleares (PMN) subjacentes à área da úlcera. Na porção mais profunda observou-se um infiltrado inflamatório misto, além de um número variável de vasos sanguíneos e menor quantidade de fibras colágenas. No período de 7 dias, a área da úlcera estava epitelizada, porém o tecido epitelial se encontrava mais espesso. No tecido conjuntivo adjacente à área da úlcera observou-se um infiltrado inflamatório misto e deposição inicial das fibrilas de colágeno (Fig.1B). Aos 14 dias, o epitélio mostrou-se regenerado e o tecido conjuntivo subjacente à área ulcerada permanecia com infiltrado inflamatório crônico, exibindo um número variável de vasos sanguíneos e fibroblastos dispersos pelo tecido (Fig.1C). As células mononucleares (MN) apresentaram-se em número reduzido em relação ao sétimo dia e ainda foi observada a deposição e início da remodelação do colágeno. No tecido conjuntivo mais profundo, aos 14 dias, havia a predominância de fibroblastos e de vasos sanguíneos com poucas e dispersas células mononucleares. No vigésimo primeiro dia, o tecido conjuntivo na porção epitélio/conjuntivo e na submucosa mostrou-se reparado (Fig.1D), porém ainda em remodelação (maior número de fibroblastos). O infiltrado inflamatório estava ausente, no entanto, células mononucleares dispersas foram observadas.

A descrição histológica do processo de reparo nos períodos de 2, 7, 14, e 21 dias para os grupos PC, A e PCA mostrou características similares ao grupo S, exceto em alguns aspectos: aos 7 dias, no grupo PC, constatou-se visualmente um maior número de vasos sanguíneos. No grupo A, aos 14 dias, observou-se um

número maior de fibroblastos tanto na área subjacente ao epitélio quanto na submucosa. No grupo PCA, aos 2 dias, observou-se um infiltrado inflamatório misto com predomínio de células MN e alguns PMN. No mesmo grupo, aos 7 e 14 dias, notou-se uma maior quantidade de vasos sangüíneos no tecido conjuntivo subjacente ao epitélio e na submucosa.

Os resultados obtidos aos 14 dias, pelos testes estatísticos, não demonstraram diferenças para o número de mastócitos, percentagem de colágeno maturo e imaturo, para área e densidade, nas regiões epitélio/conjuntivo e submucosa.

Os resultados para a contagem do número de mastócitos apresentaram diferenças estatisticamente significantes para os períodos de 2 e 7 dias. Na região epitélio/conjuntivo, o maior valor foi para o PC (0,86±0,20) (Fig.2A) e o menor valor foi encontrado no PCA (0,12±0,16) (Fig.2B) para 2 dias. Na região submucosa, no período de 7 dias, o maior valor encontrado foi para S (1,13±0,32) (Fig.2C) e o menor valor para PCA (0,51±0,09) (Fig.2D).

Os valores médios da contagem do número de mastócitos da região epitélio/conjuntivo e submucosa ($p<0,05$) estão dispostos na tabela 1.

Tabela 1 – Média e desvio padrão (SD) da contagem de mastócitos da região epitélio/conjuntivo e submucosa.

Mastócitos	Dias	GRUPOS			
		S	PC	A	PCA
Epitélio/ conjuntivo	2	$0,79±0,70^{A}$	$0,86±0,20^{A}$	$0,25±0,39^{A,B}$	$0,12±0,16^{B}$
	7	$0,46±0,24^{A}$	$0,21±0,16^{A}$	$0,26±0,25^{A}$	$0,32±0,11^{A}$
	14	$0,39±0,31^{A}$	$0,16±0,37^{A}$	$0,48±0,23^{A}$	$0,26±0,24^{A}$
	21	$0,52±0,38^{A}$	$0,13±0,17^{A}$	$0,60±0,39^{A}$	$0,47±0,49^{A}$
Submucosa	2	$0,93±0,51^{A}$	$1,21±0,58^{A}$	$0,79±0,71^{A}$	$0,60±0,42^{A}$
	7	$1,13±0,32^{A}$	$0,61±0,33^{B}$	$1,06±0,34^{A}$	$0,51±0,09^{B}$
	14	$1,13±0,48^{A}$	$0,85±0,64^{A}$	$1,01±0,23^{A}$	$0,95±0,61^{A}$
	21	$1,02±0,50^{A}$	$0,76±0,54^{A}$	$0,64±0,31^{A}$	$0,72±0,42^{A}$

Letras diferentes na mesma linha mostram diferença significativa entre os grupos ($p<0,05$).

Os valores para a percentagem da área de colágeno maturo/imaturo da região epitélio/conjuntivo apresentaram diferenças estatisticamente significantes para os períodos de 2 e 7 dias. Para o colágeno maturo, o maior valor foi para o S tanto aos 2 dias (56,36±16,19) (Fig.3A) como aos 7 dias (55,24±24,86) (Fig.3C); já o menor valor foi encontrado no A (18,52±8,70) (Fig.3B) aos 2 dias e no PCA (16,99±13,81) (Fig.3D) aos 7 dias. Para o colágeno imaturo o maior valor encontrado aos 2 dias foi no A (81,48±8,07) e o menor no S (43,64±16,19). Entretanto, aos 7 dias, o maior valor foi para o PCA (83,01±13,81) e o menor para o S (44,76±24,86). Esses dados estão demonstrados na tabela 2.

Tabela 2 – Média e desvio padrão (SD) da percentagem da área de colágeno maturo e imaturo da região epitélio/conjuntivo.

Área de Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	56,36±16,19^{A}	20,08±19,51^{B}	18,52±8,07^{B}	36,23±25,58^{A,B}
	7	55,24±24,86^{A}	41,45±28,17^{A,B}	36,63±25,70^{A,B}	16,99±13,81^{B}
	14	58,62±29,95^{A}	55,62±23,16^{A}	58,16±21,70^{A}	54,14±24,42^{A}
	21	59,08±22,48^{A}	63,34±38,86^{A}	37,82±19,88^{A}	38,69±23,14^{A}
IMATURO	2	43,64±16,19^{B}	79,92±19,51^{A}	81,48±8,07^{A}	63,77±25,58^{A,B}
	7	44,76±24,86^{B}	58,55±28,17^{A,B}	63,37±25,70^{A,B}	83,01±13,81^{A}
	14	41,38±29,95^{A}	44,38±23,16^{A}	41,84±21,70^{A}	45,86±24,42^{A}
	21	40,92±22,48^{A}	36,66±38,86^{A}	62,28±19,88^{A}	61,31±23,14^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos ($p<0,05$).

Os valores para a percentagem da densidade de colágeno maturo/imaturo na região epitélio/conjuntivo apresentaram diferenças estatisticamente significantes para o períodos de 2 dias. Para o colágeno maturo, o maior valor foi para o S (72,55± 11,20) (Fig.3A) já o menor valor foi encontrado no PC (40,04±17,12) (Fig.3E). Para o colágeno imaturo o maior valor encontrado foi no PC (59,96±17,02) e o menor no PCA (38,88±16,85) (Fig.3F). Os dados estão dispostos na tabela 3.

Tabela 3 – Média e desvio padrão (SD) da percentagem de densidade de colágeno maturo e imaturo da região epitélio/conjuntivo.

Densidade de Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	72,55± 11,20^{A}	40,04±17,12^{B}	44,99±8,69^{B}	61,12±16,85^{A,B}
	7	59,51±18,25^{A}	52,62±17,13^{A}	51,51± 14,42^{A}	43,33±11,62^{A}
	14	55,45±14,27^{A}	56,61±13,32^{A}	61,24±11,19^{A}	49,11±14,84^{A}
	21	64,41±17,64^{A}	63,72±31,16^{A}	42,71±13,58^{A}	46,61±18,52^{A}
IMATURO	2	27,45± 11,20^{B}	59,96±17,02^{A}	55,01±8,69^{A,B}	38,88±16,85^{B}
	7	40,49±18,25^{A}	47,38±17,13^{A}	48,49±14,42^{A}	56,67±11,62^{A}
	14	44,55±14,27^{A}	43,39±13,32^{A}	38,76±19,11^{A}	50,89±14,84^{A}
	21	35,59±17,64^{A}	36,28±31,16^{A}	57,29±13,58^{A}	53,39±18,52^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

Os resultados para a percentagem da área de colágeno maturo/imaturo da região submucosa apresentaram diferenças estatisticamente significantes para o períodos de 21 dias. Para o colágeno maturo, o maior valor foi para o PC (69,92±16,76) (Fig.4A) já o menor valor foi encontrado no PCA (21,35 ±10,98) (Fig.4B) aos 2 dias. Para o colágeno imaturo o maior valor encontrado foi no PCA (78,65±10,98) e o menor no PC (30,08±16,76). Esses dados estão dispostos na tabela 4.

Tabela 4 – Média e desvio padrão (SD) da percentagem da área de colágeno maturo e imaturo da região submucosa.

Área de Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	54,86±32,72^{A}	26,95±17,43^{A}	29,10±21,52^{A}	47,06±30,28^{A}
	7	47,62±30,26^{A}	39,88±30,51^{A}	18,67± 12,08^{A}	35,27±20,70^{A}
	14	76,73±23,02^{A}	45,71±25,10^{A}	52,15±24,98^{A}	44,14± 24,06^{A}
	21	41,24±25,11^{A,B}	69,92±16,76^{A}	36,73± 26,07^{B}	21,35 ±10,98^{B}
IMATURO	2	45,14±32,72^{A}	73,05±17,43^{A}	70,90±21,52^{A}	52,94±30,28^{A}
	7	52,38±30,26^{A}	60,12±30,51^{A}	81,33±12,08^{A}	64,73±20,70^{A}
	14	23,27±23,02^{A}	54,29±25,10^{A}	47,85±24,98^{A}	55,86±24,06^{A}
	21	54,76±25,11^{A,B}	30,08±16,76^{B}	63,27±26,07^{A}	78,65±10,98^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

Os resultados para a percentagem da densidade de colágeno maturo/imaturo na região submucosa apresentaram diferenças estatisticamente significantes para o períodos de 21 dias. Para o colágeno maturo, o maior valor foi para o S (60,11±19,70) (Fig.4C) já o menor valor foi encontrado no A (45,97±12,35) (Fig.4D).

Para o colágeno imaturo o maior valor encontrado foi no PCA (61,20±14,83) (Fig.4B) e o menor no PC (32,09±10,12) (Fig.4A). Os dados estão dispostos na tabela 5.

Tabela 5 – Média e desvio padrão (SD) da percentagem de densidade de colágeno maturo e imaturo da região submucosa.

Densidade de Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	60,11±19,70^{A}	46,69±19,75^{A}	45,97±12,35	57,15±19,79^{A}
	7	55,84±18,35^{A}	46,82±22,89^{A}	32,10±8,83	46,77±12,36^{A}
	14	69,25±15,71^{A}	53,49±14,29^{A}	57,82±15,22	49,10±11,86^{A}
	21	56,29±13,11^{A,B}	67,91±10,12^{A}	51,96±17,21^{A,B}	38,80±14,83^{B}
IMATURO	2	39,89±19,70^{A}	53,31±19,75^{A}	54,03±12,35^{A}	42,85±19,79^{A}
	7	44,16±18,35^{A}	53,18±22,89^{A}	67,90±8,83^{A}	53,23±12,36^{A}
	14	30,75±15,71^{A}	46,51±14,29^{A}	42,18±15,22^{A}	50,90±11,86^{A}
	21	43,71±13,11^{A,B}	32,09±10,12^{B}	48,04±17,21^{A,B}	61,20±14,83^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos ($p<0,05$).

DISCUSSÃO

A úlcera é uma lesão comum na mucosa oral e sua cicatrização é dividida em fases específicas como a inflamatória (2 dias), proliferativa com a síntese de matriz extracelular (7 dias) e a remodelação (21 dias).[16]

O presente estudo avaliou um extrato medicinal (*Aloe vera*) e o peróxido de carbamida, associados ou não, nas ulcerações induzidas na mucosa oral de ratos observando a quantidade dos mastócitos durante o processo de reparo. A distribuição dos mastócitos não foi homogênea nas áreas epitélio/conjuntivo e na submucosa no período de 2, 7, 14 e 21 dias.

Os mastócitos são células móveis do sistema imunológico, que desempenham um papel crítico na indução da inflamação e no processo de reparo, respondendo a estímulos imunológicos e não imunológicos. Nas lesões orais, a desgranulação destas células pode ser identificada pela exteriorização dos grânulos e diminuição dos estoques intracelulares de mediadores, sendo determinado pela coloração histoquímica do azul de toluidina[25].

Um número de fatores, tais como drogas, hormônios, viroses, toxinas e traumas podem induzir os mastócitos a sintetizar e/ou secretar seus mediadores[23] como a catepsina G, histamina, serotonina, heparina, hidrolases ácidas e as citocinas como o fator de necrose tumoral-α (TNF) e interleucina.[25] A histamina e o TNF exercem papel fundamental na resposta inflamatória. A histamina causa o aumento da permeabilidade vascular devido à contração das células endoteliais e formação de espaços entre as mesmas. O TNF induz a expressão endotelial, principalmente da selectina-E, aumentando a oferta de moléculas de adesão na superfície do endotélio induzindo a uma maior migração de neutrófilos, linfócitos-T, monócitos e leucócitos para área inflamada.[25]

Neste estudo, aos 2 e 7 dias da aplicação dos fármacos, houve diferença estatística significante na região epitélio/conjuntivo e submucosa, respectivamente em relação ao número de mastócitos. Aos 2 dias no grupo PCA, comparado ao grupo S e ao PC, houve uma maior desgranulação dos mastócitos, pois seu número reduziu significativamente.

A partir do 7º dia inicia-se a fase proliferativa do processo de reparo da úlcera, com a cronificação da lesão e espera-se que o número de mastócitos aumente em relação ao 2º dia. Isto foi constatado na submucosa para os grupos S e A, porém, para os grupos que continham peróxido de carbamida, os mastócitos ainda apresentavam-se em baixo número indicando a continuidade da desgranulação. Sugere-se que o peróxido de carbamida em contato com a mucosa foi degradado em peróxido de hidrogênio e esse em oxigênio e água. Esta reação pode ter sido um fator catalisador, pela liberação de radicais livres que sensibilizaram os mastócitos acentuando sua desgranulação.[4]

Na região submucosa o número de mastócitos aos 2, 7, 14 e 21 dias foi observado maior em relação à região epitélio/conjuntivo. Este fato, provavelmente, pode ter ocorrido devido à proximidade desta região com o tecido conjuntivo circunjacente normal, pois o processo de reparo é iniciado na área mais profunda da úlcera em direção a superfície. Na região epitélio/conjuntivo observou-se que a desgranulação dessas células permaneceu maior nesses mesmos períodos. Isto pode ser devido a não penetração dos fármacos até a região mais profunda pela presença da barreira do coágulo formado durante o processo inflamatório.

O método de polarização com picrosirius é um procedimento histoquímico específico para detecção de colágeno onde o colágeno intersticial apresenta diferentes cores e intensidade de birrefringência. Áreas birrefringentes amarelas ao

vermelho são indicativas do colágeno maturo e as verdes indicam o colágeno imaturo, o primeiro tipo a aparecer durante o processo de neoformação tecidual.[26] A cor e a intensidade da birrefringência do colágeno podem também variar dependendo do diâmetro da fibra, espessura do corte tecidual ou ambos[26]. A percentagem da área de colágeno imaturo na região epitélio conjuntivo aos 2 e 7 dias no grupo S foi menor que nos grupos PC, A e PCA e o inverso foi encontrado para o colágeno maturo, isso também ocorreu aos 21 dias na região submucosa. Aos 2 e 7 dias, houve diferença estatística significante, na região epitélio/conjuntivo, para os grupos PC e A quando comparados ao S, já na região submucosa a diferença estatística ocorreu aos 21 dias no PC em relação ao S, A e PCA. No presente estudo sugere-se que nos grupos que possuem *Aloe vera* pode ter ocorrido uma maior síntese e deposição de colágeno. Isto foi verificado em trabalhos com diferentes metodologias, usando cultura de células[27] e a estimativa da quantidade de colágeno através da solubilidade do tecido de granulação.[28]

A *Aloe vera* tem capacidade de estimular a cicatrização de úlceras em pele de ratos pelo aumento da síntese de colágeno e de glicosaminoglicanas da matriz extracelular[28]. A maior fração de carboidrato derivada desta planta é o acemanan, que possui habilidade de induzir a proliferação de fibroblastos gengivais em cultura[27] e promover a cicatrização de úlceras aftosas em humanos.[29]

Na fase inflamatória da cicatrização da úlcera inicia-se uma sequência de eventos com a deposição das plaquetas, formação do coágulo e posterior liberação de mediadores químicos pelas plaquetas formando um gradiente quimiotático que orienta a migração dos neutrófilos.[30] Estas células são responsáveis pela fagocitose, com eliminação de microorganismos e restos teciduais como o colágeno. Aos 2 dias, no presente estudo, observou-se uma alta percentagem do colágeno maturo em

relação à área e densidade no grupo S na região epitélio/conjuntivo, provavelmente porque o colágeno pré-existente no tecido conjuntivo não foi completamente degradado. Em contrapartida, no grupo PC a densidade do colágeno imaturo foi maior que no grupo controle mostrando que os fibroblastos estão em processo de síntese de colágeno. Esse resultado diverge dos achados em estudo de cultura de fibroblastos humanos gengivais em que o peróxido de hidrogênio é citotóxico e causa dano na membrana celular externa ou no interior da célula, além de ter efeitos sobre a viabilidade/morfologia, proliferação e atividades funcionais que são importantes para a manutenção do tecido e para a cicatrização.[2] No entanto, outros tipos de fibroblastos podem ser mais resistentes à lise pelo peróxido de hidrogênio,[31] concordando com os achados do presente estudo. No grupo A, observou-se uma maior porcentagem de área e densidade de colágeno imaturo. Estes achados corroboram aos de Thompson[32] que demonstrou alta atividade proliferativa de fibroblastos e síntese de colágeno.

Aos 7 dias, a percentagem de colágeno maturo se manteve maior no grupo A. Foi verificado que a *Aloe vera* estimula a angiogênese e um aumento da permeabilidade capilar facilitando a regeneração tecidual em queimadura de *guinea pig* tratada pela aplicação tópica.[33] A maioria dos mediadores envolvidos na fibroplasia também possuem atividade angiogênica, foi verificado que os fibroblastos produzem ou secretam o fator de crescimento endotelial vascular (VGF) devido a um dos seus principais constituintes químicos, o acemanan.[34,15]

A fibroplasia tem início aos 7 dias com a formação de tecido de granulação na região da ferida, juntamente com uma matriz frouxa de colágeno constituída por fibronectina, ácido hialurônico e células, como os macrófagos e fibroblastos, além de vasos recém formados e exsudatos. Aos 14 e 21 dias continua a fibroplasia e a

remodelação do colágeno neoformado. Nos grupos tratados com a *Aloe vera*, a percentagem da área e densidade de colágeno imaturo foi maior em relação ao grupo PC, sugerindo que a *Aloe vera* possa ter promovido uma continuidade na neoformação de colágeno, devido à indução da proliferação de fibroblastos.[29] A associação da *Aloe vera* com peróxido de carbamida no clareamento dental mostrou efetividade terapêutica no processo de reparo de úlceras na mucosa oral de ratos reforçando a hipótese da sua atividade cicatrizante.

CONCLUSÃO

Dentro das limitações deste trabalho, as conclusões obtidas foram que a *Aloe vera* 0,5% associada ou não ao peróxido de carbamida, demonstrou atividade anti-inflamatória e cicatrizante em úlceras de mucosa oral de ratos:

– diminuindo o número de mastócito na região mais superficial da úlcera na fase inflamatória do processo de reparo aos 2 dias;

– estimulando a fibroplasia e remodelação, sugerindo uma continuidade na neoformação de colágeno.

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TABELAS

Tabela 1 – Médias e desvio padrão (SD) da contagem de mastócitos da região epitélio/conjuntivo e submucosa.

Mastócitos	Dias	GRUPOS			
		S	PC	A	PCA
Epitélio/ conjuntivo	2	0,79±0,70^{A}	0,86±0,20^{A}	0,25±0,39^{A,B}	0,12±0,16^{B}
	7	0,46±0,24^{A}	0,21±0,16^{A}	0,26±0,25^{A}	0,32±0,11^{A}
	14	0,39±0,31^{A}	0,16±0,37^{A}	0,48±0,23^{A}	0,26±0,24^{A}
	21	0,52±0,38^{A}	0,13±0,17^{A}	0,60±0,39^{A}	0,47±0,49^{A}
Submucosa	2	0,93±0,51^{A}	1,21±0,58^{A}	0,79±0,71^{A}	0,60±0,42^{A}
	7	1,13±0,32^{A}	0,61±0,33^{B}	1,06±0,34^{A}	0,51±0,09^{B}
	14	1,13±0,48^{A}	0,85±0,64^{A}	1,01±0,23^{A}	0,95±0,61^{A}
	21	1,02±0,50^{A}	0,76±0,54^{A}	0,64±0,31^{A}	0,72±0,42^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

Tabela 2 – Média e desvio padrão (SD) da percentagem da área de colágeno maturo e imaturo da região epitélio/conjuntivo.

Área de Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	56,36±16,19^{A}	20,08±19,51^{B}	18,52±8,07^{B}	36,23±25,58^{A,B}
	7	55,24±24,86^{A}	41,45±28,17^{A,B}	36,63±25,70^{A,B}	16,99±13,81^{B}
	14	58,62±29,95^{A}	55,62±23,16^{A}	58,16±21,70^{A}	54,14±24,42^{A}
	21	59,08±22,48^{A}	63,34±38,86^{A}	37,82±19,88^{A}	38,69±23,14^{A}
IMATURO	2	43,64±16,19^{B}	79,92±19,51^{A}	81,48±8,07^{A}	63,77±25,58^{A,B}
	7	44,76±24,86^{B}	58,55±28,17^{A,B}	63,37±25,70^{A,B}	83,01±13,81^{A}
	14	41,38±29,95^{A}	44,38±23,16^{A}	41,84±21,70^{A}	45,86±24,42^{A}
	21	40,92±22,48^{A}	36,66±38,86^{A}	62,28±19,88^{A}	61,31±23,14^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

Tabela 3 – Média e desvio padrão (SD) da percentagem de densidade de colágeno maturo e imaturo da região epitélio/conjuntivo.

Densidade Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	72,55± 11,20^{A}	40,04±17,12^{B}	44,99±8,69^{B}	61,12±16,85^{A,B}
	7	59,51±18,25^{A}	52,62±17,13^{A}	51,51±14,42^{A}	43,33±11,62^{A}
	14	55,45±14,27^{A}	56,61±13,32^{A}	61,24±11,19^{A}	49,11±14,84^{A}
	21	64,41±17,64^{A}	63,72±31,16^{A}	42,71±13,58^{A}	46,61±18,52^{A}
IMATURO	2	27,45± 11,20^{B}	59,96±17,02^{A}	55,01±8,69^{A,B}	38,88±16,85^{B}
	7	40,49±18,25^{A}	47,38±17,13^{A}	48,49±14,42^{A}	56,67±11,62^{A}
	14	44,55±14,27^{A}	43,39±13,32^{A}	38,76±19,11^{A}	50,89±14,84^{A}
	21	35,59±17,64^{A}	36,28±31,16^{A}	57,29±13,58^{A}	53,39±18,52^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

Tabela 4 – Média e desvio padrão (SD) da percentagem da área de colágeno maturo e imaturo da região submucosa.

Área de Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	54,86±32,72^{A}	26,95±17,43^{A}	29,10±21,52^{A}	47,06±30,28^{A}
	7	47,62±30,26^{A}	39,88±30,51^{A}	18,67± 12,08^{A}	35,27±20,70^{A}
	14	76,73±23,02^{A}	45,71±25,10^{A}	52,15±24,98^{A}	44,14± 24,06^{A}
	21	41,24±25,11^{A,B}	69,92±16,76^{A}	36,73± 26,07^{B}	21,35 ±10,98^{B}
IMATURO	2	45,14±32,72^{A}	73,05±17,43^{A}	70,90±21,52^{A}	52,94±30,28^{A}
	7	52,38±30,26^{A}	60,12±30,51^{A}	81,33±12,08^{A}	64,73±20,70^{A}
	14	23,27±23,02^{A}	54,29±25,10^{A}	47,85±24,98^{A}	55,86±24,06^{A}
	21	54,76±25,11^{A,B}	30,08±16,76^{B}	63,27±26,07^{A}	78,65±10,98^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

Tabela 5 – Média e desvio padrão da percentagem de densidade de colágeno maturo e imaturo da região submucosa.

Densidade Colágeno	Dias	GRUPOS			
		S	PC	A	PCA
MATURO	2	60,11±19,70^{A}	46,69±19,75^{A}	45,97±12,35	57,15±19,79^{A}
	7	55,84±18,35^{A}	46,82±22,89^{A}	32,10±8,83	46,77±12,36^{A}
	14	69,25±15,71^{A}	53,49±14,29^{A}	57,82±15,22	49,10±11,86^{A}
	21	56,29±13,11^{A,B}	67,91±10,12^{A}	51,96±17,21^{A,B}	38,80±14,83^{B}
IMATURO	2	39,89±19,70^{A}	53,31±19,75^{A}	54,03±12,35^{A}	42,85±19,79^{A}
	7	44,16±18,35^{A}	53,18±22,89^{A}	67,90±8,83^{A}	53,23±12,36^{A}
	14	30,75±15,71^{A}	46,51±14,29^{A}	42,18±15,22^{A}	50,90±11,86^{A}
	21	43,71±13,11^{A,B}	32,09±10,12^{B}	48,04±17,21^{A,B}	61,20±14,83^{A}

Letras diferentes na mesma linha mostram diferença significativa entre os grupos (p<0,05).

FIGURAS

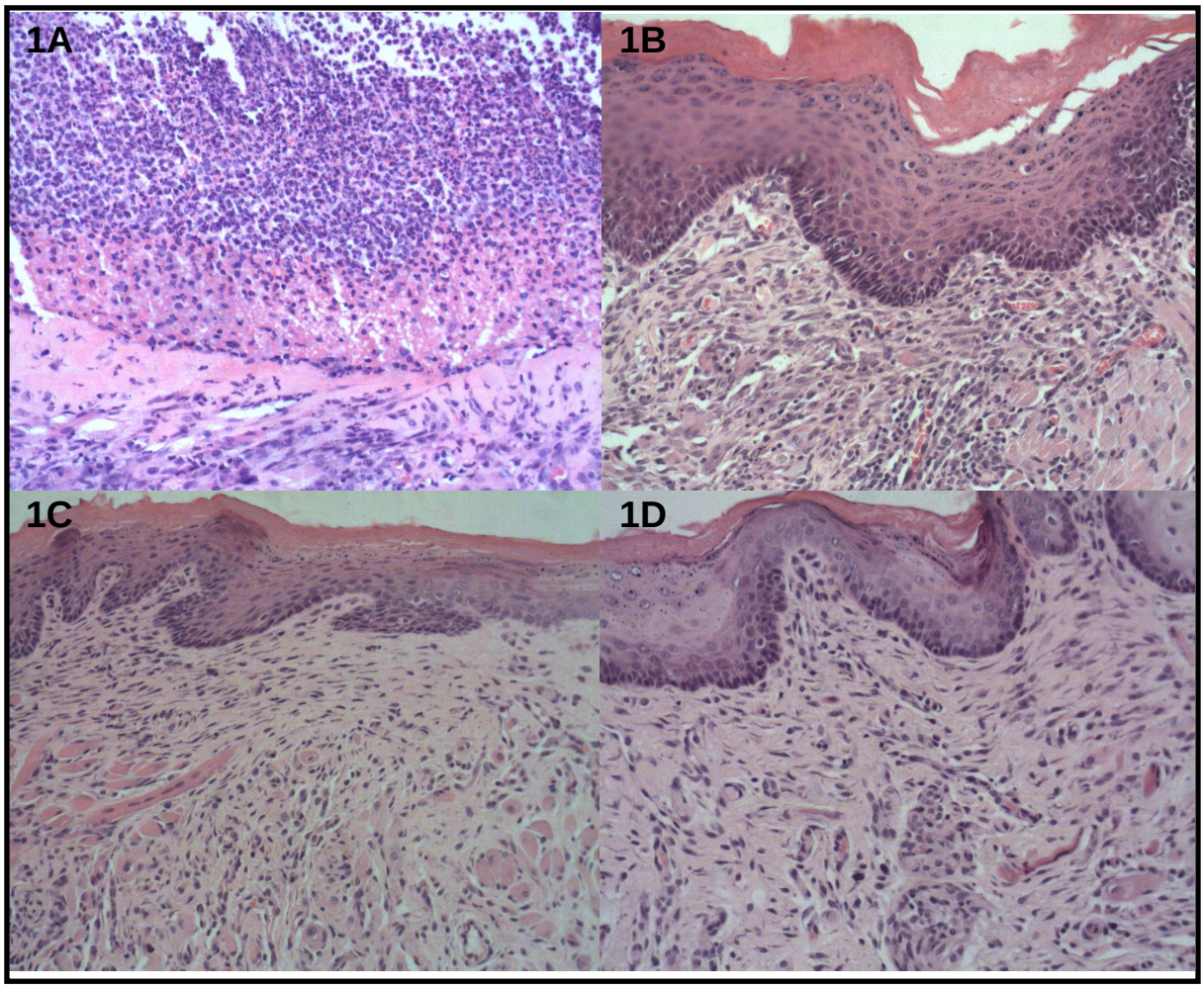

Figura 1 – Grupo Salina 1A, 1B, 1C e 1D correspondendo aos períodos de 2, 7, 14 e 21 dias, respectivamente. Coloração de HE, aumento de 20X.

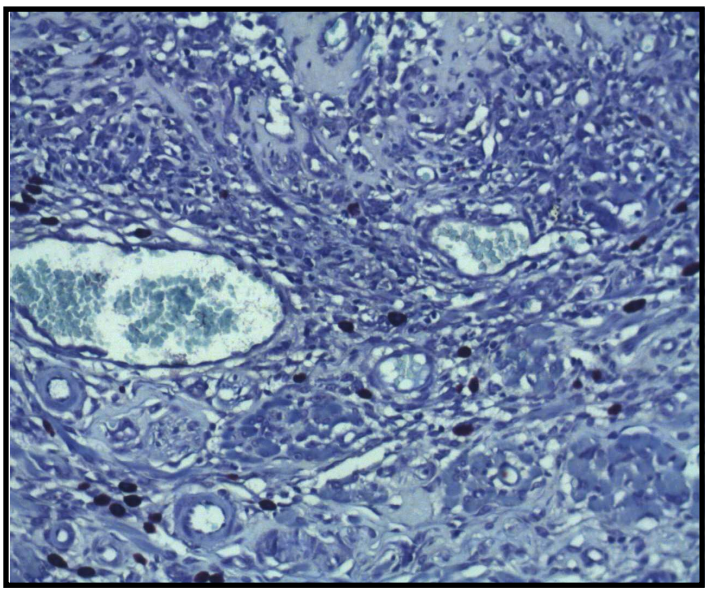

Figura 2A: Disposição dos mastócitos no grupo PC (epitélio/conjuntivo) aos 2 dias. Coloração de AT, aumento de 20X.

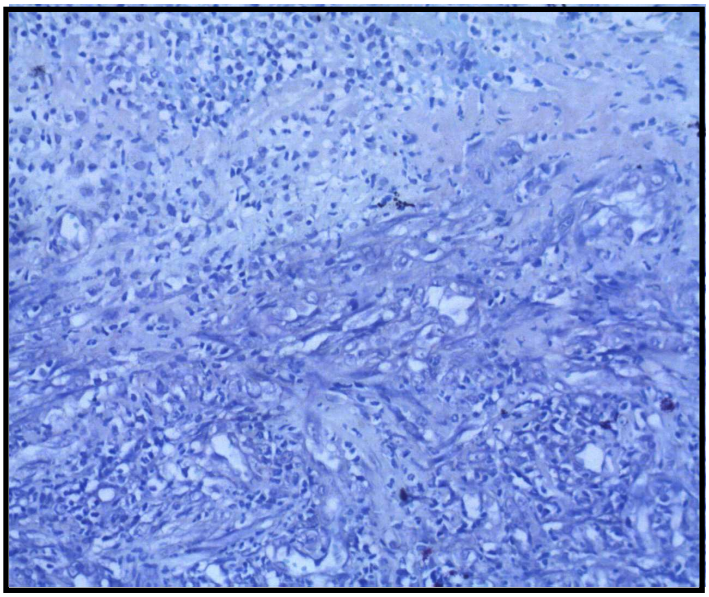

Figura 2B: Disposição dos mastócitos no grupo PCA (epitélio/conjuntivo) aos 2 dias. Coloração de AT, aumento de 20X.

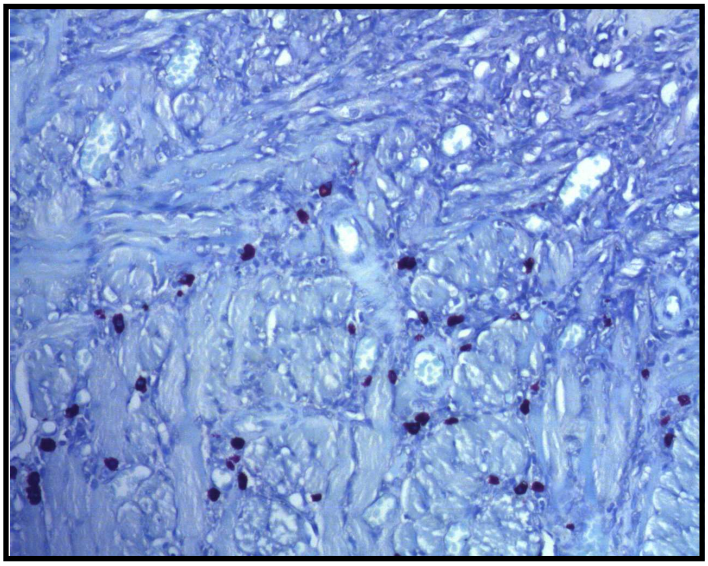

Figura 2C: Disposição dos mastócitos no grupo S (submucosa) aos 7 dias. Coloração de AT, aumento de 20X.

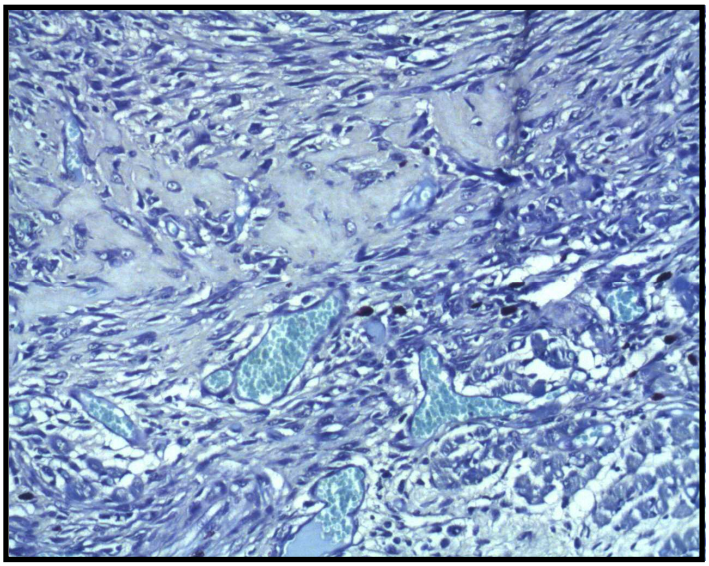

Figura 2D: Disposição dos mastócitos no grupo PCA (submucosa) aos 7 dias. Coloração de AT, aumento de 20X.

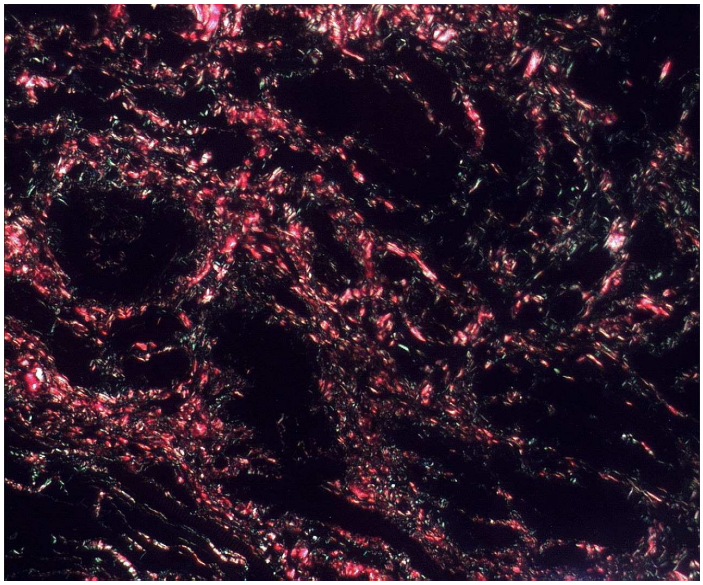

Figura 3A: Disposição do colágeno maturo/imaturo da região epitélio/conjuntivo em relação à área e densidade para o grupo S aos 2 dias.

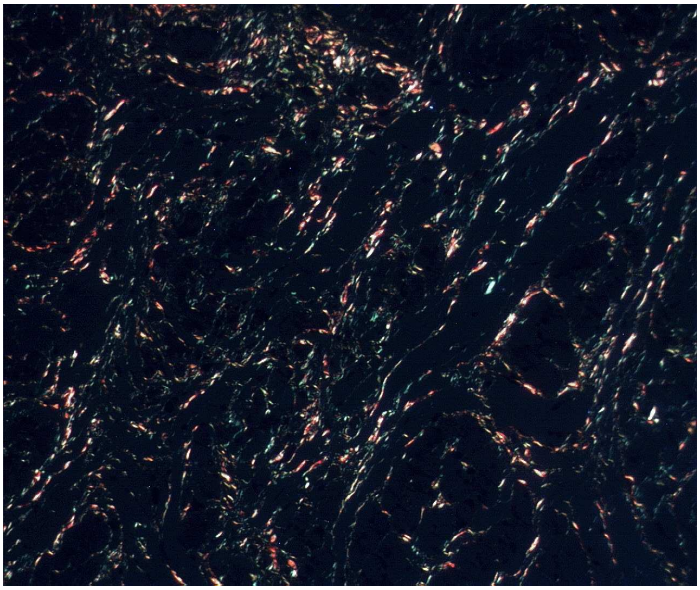

Figura 3B: Disposição do colágeno maturo/imaturo da região epitélio/conjuntivo em relação à área para o grupo A aos 2 dias.

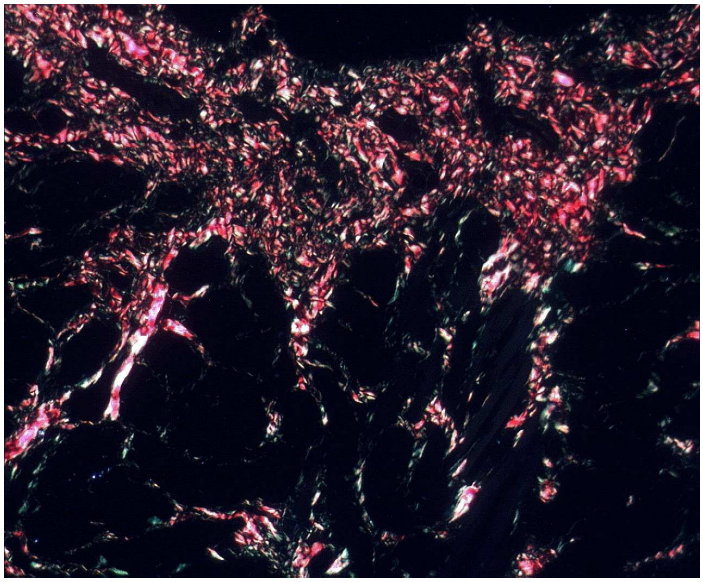

Figura 3C: Disposição do colágeno maturo/imaturo da região epitélio/conjuntivo em relação à área para o grupo S aos 7 dias.

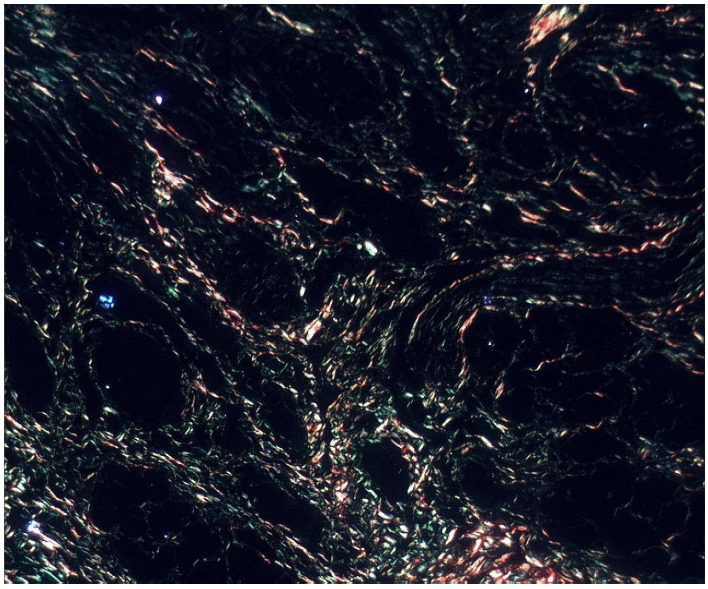

Figura 3D: Disposição do colágeno maturo/imaturo da região epitélio/conjuntivo em relação à área para o grupo PCA aos 7 dias.

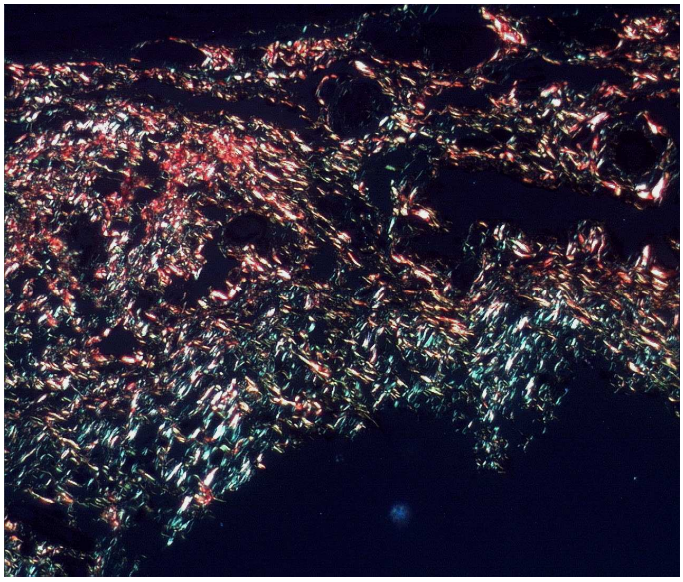

Figura 3E: Disposição do colágeno maturo/imaturo na região epitélio/conjuntivo em relação à densidade para o grupo PC aos 2 dias.

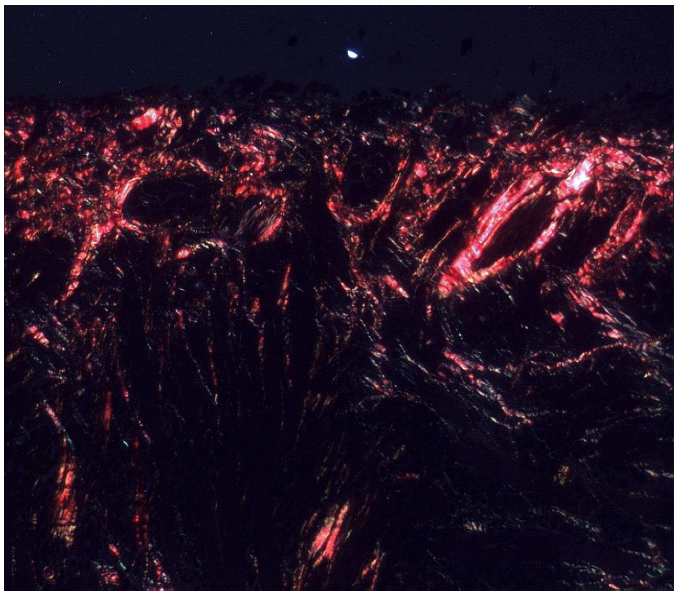

Figura 3F: Disposição do colágeno maturo/imaturo na região epitélio/conjuntivo em relação à densidade para o grupo PCA aos 2 dias.

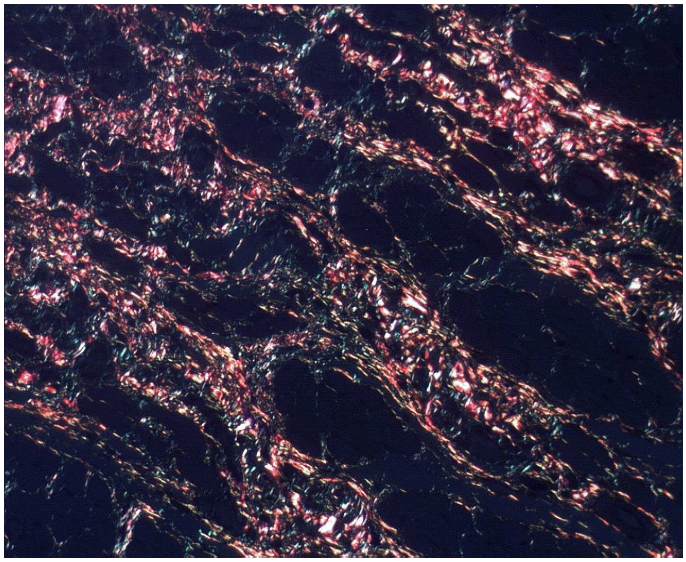

Figura 4A: Disposição do colágeno maturo/imaturo na região submucosa em relação à densidade para o grupo PC aos 21 dias.

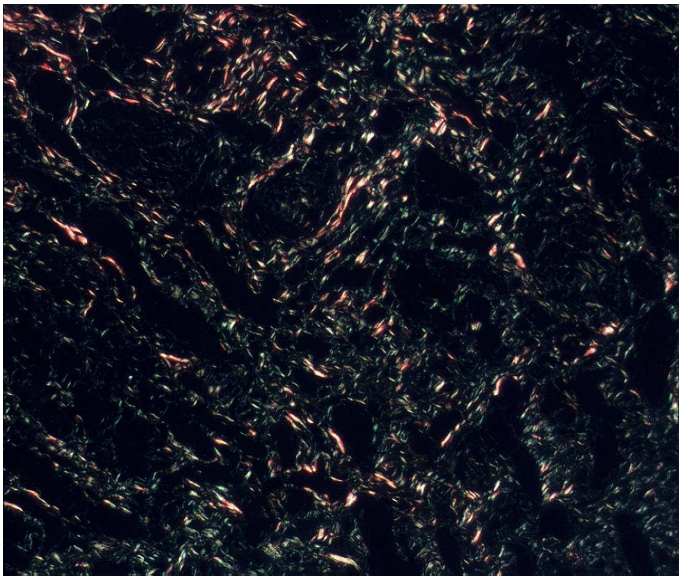

Figura 4B: Disposição do colágeno maturo/imaturo na região submucosa em relação à densidade para o grupo PCA aos 21 dias.

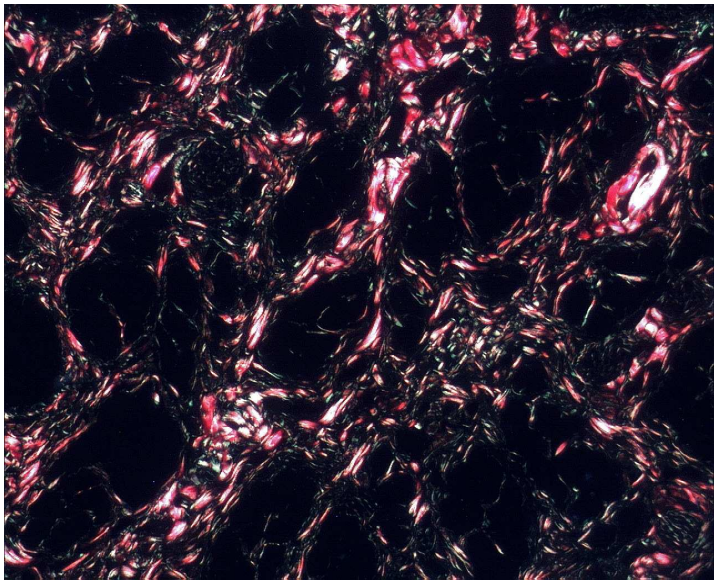

Figura 4C: Disposição do colágeno maturo/imaturo na região submucosa em relação à densidade para o grupo S aos 21 dias.

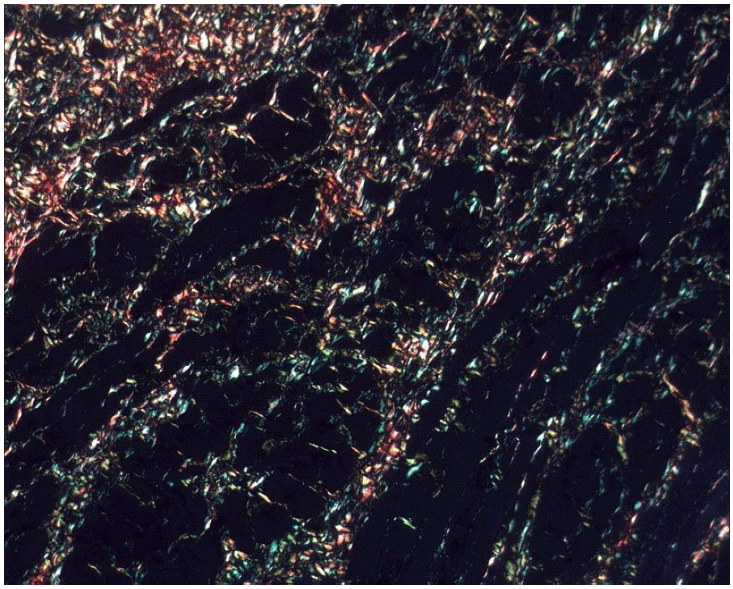

Figura 4D: Disposição do colágeno maturo/imaturo na região submucosa em relação à densidade para o grupo A aos 21 dias.

2. ARTIGO EM INGLÊS

HISTOLOGICAL ANALYSIS OF CARBAMIDE PEROXIDE ASSOCIATED OR NOT WITH ALOE VERA ON ULCER REPAIR IN RATS ORAL MUCOSA

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ABSTRACT

Bleaching agents have transitory effects, such as dental sensitivity, irritation and edema of the oral tissues. *Aloe vera* has anti-inflammatory, analgesic, antimicrobial and healing properties. The aim of this study was to make a histological evaluation on the repair process of ulcers in rats oral mucosa, treated with carbamide peroxide gel, associated or not with 0,5% *Aloe vera*. Ulcers were induced with 40% NaOH, for 7 days in 112 *Wistar* rats, which were afterwards randomly divided into 4 groups (n=28): Saline (S), 16% Carbamide Peroxide (PC) , 0.5% *Aloe vera* Gel (A) and 16% Carbamide Peroxide + 0.5% *Aloe vera* Gel (PCA). The animals were killed in the periods of 2, 7, 14 and 21 days after the treatment began. The specimens were histologically processed and stained with Hematoxylin-Eosin, Toluidine Blue and Picrosirius. In the epithelium/conjunctive and sub mucosa areas of the ulcer, the number of mastocytes was quantified, and the percentage of area and density of mature and immature collagen was evaluated under polarized light. The data were tabulated and the ANOVA, Tukey HSD and Games-Howell ($p<0.05$) tests were applied. The results obtained demonstrated statistically significant differences in Group A in the period of 2 days for the percentage area of mature (18.52±8.07) and immature collagen (81.48±8.07) and in the density of mature (44.99±8.69) and immature collagen (55.01±8.69) for the epithelium/conjunctive region. In the Group PCA the differences were in the period of 2 days for the number of mastocytes (0.12±0.16) in the epithelium/conjunctive region and at 7 days in the sub mucosa region (0.51±0.09), for the percentage of mature (16.99±13.81) and immature (83.01±13.81) collagen. It was concluded that the 0.5% *Aloe vera* gel, whether or not associated with bleaching agents, demonstrated an anti-inflammatory and healing effect on ulcers in rats' oral mucosa, reducing the number of mastocytes in the more superficial region of the lesion and stimulating fibroplasia.

Key Words: Dental bleaching, *aloe vera*, carbamide peroxide, mastocyte, collagen, repair process, ulcer.

INTRODUCTION

The products used for dental bleaching are carbamide and hydrogen peroxide. The dental bleaching mechanism is a complex process of oxidation, which generates free radicals after the breakdown of the carbamide or hydrogen peroxides. Carbamide peroxide dissociates in the saliva into hydrogen peroxide and urea. The urea dissociates into ammonia and carbon dioxide, whereas the hydrogen peroxide, considered the active ingredient of bleaching, penetrates into the dental structure resulting in oxygen and water.[1] Although the human body has protective mechanisms such as the epithelium of the oral mucosa and salivary constituents, in addition to various other forms of defense for metabolizing the hydrogen peroxide intra and extracellular, the bleaching agents have the capacity to invade and generate toxic effects on the oral tissues and their cells.[2] The at-home bleaching technique, introduced by Haywood and Heymann[3] contains carbamide peroxide on its composition, at a concentration ranging from 10 to 22%.[4] It is a simple and conservative technique that presents good results; nevertheless, it is not an innocuous treatment, as it generates adverse effects that affect both hard and soft tissues in the oral cavity. Among these effects are dental sensitivity[5], gingival irritation[6], changes in the enamel[7,8] and in the dentin surfaces[9] and ulcerations[10]. In the literature, various studies have evaluated the effect of carbamide and hydrogen peroxides on hard and soft tissues of the oral cavity.[6,7,8,9,10] Kirsten *et al.*[6] performed a bleaching technique with a tray and concluded that the 16% carbamide peroxide yielded gingival inflammation that persisted for up to 45 days after the conclusion of the treatment. Pinto *et al.*[8] and Cavalli *et al.*[7] demonstrated a reduction in the microhardness and an increase in the surface roughness of enamel submitted to various concentrations of carbamide and hydrogen peroxide. Arcari *et al*[9]

demonstrated a reduction in the microhardness of dentin submitted to 10% carbamide peroxide for 7 hours and Rees & Orth[10] concluded that the 3% hydrogen peroxide used in mouthrinses could generate ulcers in the oral tissue.

Ulcerations are lesions that generate loss of the epithelium with exposure of the subjacent conjunctive tissue, and clinically, is shown to be rounded or oval, shallow, with a grayish-white membrane in the center of the surrounding erythomatous halo.[11,12]

The inflammatory process is associated with a series of pathophysiological events that generate different effects in each organism. There are many mediators available, amines such as histamine, peptides, lipids such as prostaglandins and leukotrienes.[13] Aspirin and salicylate are considered to be equally potent as anti-inflammatory agents, the dose 3g of aspirin or salicylate reduced the urinary output of prostaglandins metabolites in humans by 85-95%.[14] During inflammation there is a variable quantity of mastocytes, which are cells originated in the bone marrow, with oval nucleus containing metachromatic cytoplasmatic granules that play an important role in the inflammatory process, releasing chemical mediators.[15]

In the ulcer repair process, a series of inflammatory and immunologic steps originating from the cells and chemical mediators with the aim of reestablishing the health of the tissues take place. During this process, collagen is produced and deposited by fibroblasts and it is essential to tissue repair.[16]

Aloe vera is a tropical cactus, constituted of over 75 active agents including vitamins, enzymes, minerals, lignin, saponin, salicylic acid and aminoacids.[17] Among these constituents acemanan, magnesium lactate, aloin, barbaloin and emodin are outstanding.[17] This plant produces substances with different compositions and therapeutic properties, such as humidifiers, anti-inflammatory agents, analgesics and

healing agents,[18] which have been proved in clinical[19,20] and experimental studies.[21,22]

The use of medicinal plant-based pharmacological products can be indicated for the purpose of reducing the inflammatory potential of dental bleaching gels. Thus, the aim of this study was to make a histological evaluation of the repair process of ulcers in rats oral mucosa, treated with carbamide peroxide gel, associated or not with 0,5% *Aloe vera*.

MATERIAL AND METHOD

The project of this study was approved by the Ethics Committee on the Use of Animals of the Pontifical Catholic University of Parana (PUCPR), under the protocol Nº 226/07.

One hundred and twelve adult male rats of the *Rattus novergicus albinus* type, *Wistar* breed, weighing approximately 180 g each were used. The animals were obtained from the PUCPR vivarium, kept in plastic cages, and fed on rations and water *ad libitum*, respecting the diurnal-nocturnal cycle.

General anesthesia was induced intraperitoneally with sodium thiopenthal® - 20mg /kg (Cristália, Itapira, SP, Brazil). In the most anterior part of the dorsal face of the animals' tongues, ulcers were chemically induced by daily topical application of 40% sodium hydroxide (NaOH), using a cotton pellet, for one minute, for 7 days. After clinical visual finding of the ulcer formation on the tongue of each animal, they were randomly divided into 4 groups (n =28) and treated as follows: Saline (S), 16% Carbamide Peroxide (PC), 0.5% *Aloe vera* Gel (A), and 16% Carbamide Peroxide + 0.5% *Aloe vera* Gel (PCA).

The 16% Carbamide Peroxide and 0.5% *Aloe vera* Gels (Galena Indústria Farmacêutica – São Paulo, SP, Brazil) or the association of the two were prepared by a dispensing pharmacy, and applied daily, once only in the ulcer region, for 2 minutes, using cotton pellets.

The animals were killed by overdose of sodium thiopental®, administered intraperitoneally, in the periods of 2, 7, 14 and 21 days after the treatment began. The tongues were removed, sectioned in the longitudinal direction and fixed in 10% neutral formalin for 24 hours.

Of each animal´s tongue, three slides were made, stained with Hematoxylin-

Eosin (HE), 0.2% Toluidine Blue (TB) or Picrosirius (SR). In the HE stained sections, the slides were observed at magnifications of 50, 100, 200 and 400X, with a binocular microscope OLYMPUS BX50 (Olympus, Tokyo, Japan) aiming to, in a subjective manner, to describe the repair process in the ulcerated area, without the intention of making a quantitative analysis. The presence or absence of ulceration, quantity of blood vessels and the predominance of the type of inflammatory infiltrate (acute, mixed or chronic) were observed.

In the sections stained with TB, the number of mastocytes, identified by their purple color, was quantified. In each slide, four images were obtained of the ulcerated and treated lesion areas, corresponding to 2 superficial fields – epithelium/conjunctive, and 2 deep fields – sub mucosa. The images were captured at a 200X magnification by a video camera (Sony Íris CCD, Tokyo, Japan) coupled to a microscope and sent to the computer. In each image of the histologic field, with the use of the Image Pro-Plus Program version 4.5 (Media Cibernetics, Silver Spring, MD, USA), a virtual reticule (3 x 4) of 1 mm^2 each was made for counting the number of mastocytes.[23]

The percentage of the areas and density of the mature and the immature collagen were measured in the SR stained sections using polarized light, in which the mature collagen presented intense birefringence, of yellow and red color, whereas the immature collagen produced a greenish color.[21] One of this evaluation was made in a field in the epithelium/conjunctive area and the other in the sub mucosal area, measured by the same Image Pro-Plus program.

The statistical tests of Normality Shapiro – Wilk, and Homogeneity Levene were applied in the quantitative analysis. The ANOVA test ($p<0.05$) was applied to compare the means of the groups, in the epithelium/conjunctive and sub mucosal

areas of the ulcers, for the number of mastocytes, and for the percentage of areas and density of mature and immature collagen, with SPSS 15.0 for Windows® software (SPSS Inc., Chicago, Illinois, USA).

In the variables in which the ANOVA test showed difference in the mean values between the groups, and when there was homogeneity of variances among the groups by the Levene test, multiple comparisons were made by the Tukey HSD test. Otherwise, the Games-Howell multiple comparisons test for heterogeneous variances was used.

RESULTS

The slides evaluated in HE for Group S at 2 days post-treatment showed an ulcer in the oral mucosa with interruption of the epithelium and exposure of the conjunctive tissue (Fig.1A). This area exhibited an acute inflammatory infiltrate with predominance of polymorphonuclear cells (PMN) subjacent to the area of the ulcer. In the deeper portion a mixed inflammatory infiltrate was observed, in addition to a variable number of blood vessels, and lower quantity of collagen fibers. In the period of 7 days, the ulcer area was epithelized, but the epithelial tissue was thicker. In the conjunctive tissue adjacent to the area of the ulcer, a mixed inflammatory infiltrate and initial deposition of collagen fibrils was observed (Fig.1B). At 14 days, the epithelium was shown to be regenerated and the conjunctive tissue subjacent to the ulcerated area remained with chronic inflammatory infiltrate, exhibiting a variable number of blood vessels and fibroblasts dispersed throughout the tissue (Fig.1C). The mononuclear cells (MN) were presented in reduced number in comparison with the seventh day, and the deposition and beginning of collagen remodeling was also observed. In the deeper conjunctive tissue, at 14 days there was predominance of fibroblasts and blood vessels with few and dispersed mononuclear cells. On the twenty-first day, the conjunctive tissue in the epithelium/conjunctive portion and in the sub mucosa was shown to be repaired (Fig.1D), but still in a remodeling stage (larger number of fibroblasts). The inflammatory infiltrate was absent, however, dispersed mononuclear cells were observed.

The histological description of the repair process in the periods of 2, 7, 14, and 21 days for Groups PC, A and PCA showed characteristics similar to those of the group S, except for some aspects: at 7 days, in Group PC, a larger number of blood vessels were visually found. In Group A, at 14 days, a larger number of fibroblasts

were observed both in the area subjacent to the epithelium and in the sub mucosa. In the group PCA, at 2 days, in some slides, a mixed inflammatory infiltrate with predominance of MN cells and some PMN cells were observed. In the same group, at 7 and 14 days, a larger quantity of blood vessels was noted in the conjunctive tissue subjacent to the epithelium and in the sub mucosa.

The results obtained by the statistical tests at 14 days, showed no differences for the number of mastocytes, percentage of mature and immature collagen, area and density, in the epithelium/conjunctive areas and sub mucosa.

The results for the counts of the number of mastocytes presented statistically significant differences for the periods of 2 and 7 days. In the epithelium/conjunctive region, the highest value was for PC (0.86±0.20) (Fig.2A) and the lowest value was found for the PCA group (0.12±0.16) (Fig.2B) for 2 days. In the sub mucosa region, in the period of 7 days, the highest value found was for S (1.13±0.32) (Fig.2C) and the lowest value for PCA (0.51±0.09) (Fig.2D).

The mean values for the number of mastocytes in the epithelium/conjunctive region and sub mucosa ($p<0.05$) are shown in Table 1.

Table 1 – Mean values and standard deviation for the number of mastocytes in the epithelium/conjunctive region and sub mucosa area.

		GROUPS			
Mastocytes	Days	S	PC	A	PCA
Epithelium/ Conjunctive	2	0.79 ± 0.70^{A}	0.86 ± 0.20^{A}	$0.25\pm0.39^{A,B}$	0.12 ± 0.16^{B}
	7	0.46 ± 0.24^{A}	0.21 ± 0.16^{A}	0.26 ± 0.25^{A}	0.32 ± 0.11^{A}
	14	0.39 ± 0.31^{A}	0.16 ± 0.37^{A}	0.48 ± 0.23^{A}	0.26 ± 0.24^{A}
	21	0.52 ± 0.38^{A}	0.13 ± 0.17^{A}	0.60 ± 0.39^{A}	0.47 ± 0.49^{A}
Submucosa	2	0.93 ± 0.51^{A}	1.21 ± 0.58^{A}	0.79 ± 0.71^{A}	0.60 ± 0.42^{A}
	7	1.13 ± 0.32^{A}	0.61 ± 0.33^{B}	1.06 ± 0.34^{A}	0.51 ± 0.09^{B}
	14	1.13 ± 0.48^{A}	0.85 ± 0.64^{A}	1.01 ± 0.23^{A}	0.95 ± 0.61^{A}
	21	1.02 ± 0.50^{A}	0.76 ± 0.54^{A}	0.64 ± 0.31^{A}	0.72 ± 0.42^{A}

Different letters in the same line show significant differences between groups ($p<0,05$).

The values for the percentage of the area of mature/immature collagen in the epithelium/conjunctive region presented statistically significant differences for the

periods of 2 and 7 days. For mature collagen, the highest value was for S both at 2 days (56.36±16.19) (Fig.3A) and at 7 days (55.24±24.86) (Fig.3C); whereas the lowest value was found in A (18.52±8.70) (Fig.3B) at 2 days and in PCA (16.99±13.81) (Fig.3D) at 7 days. For immature collagen the highest value found at 2 days was in A (81.48±8.07) and the lowest in S (43.64±16.19). However, at 7 days, the highest value was for PCA (83.01±13.81) and the lowest for S (44.76±24.86). These data are decicted in Table 2.

Table 2 – Mean values and standard deviation for the percentage of the area of mature/immature collagen in the epithelium/conjunctive region.

Area of Collagen	Days	GROUPS			
		S	PC	A	PCA
MATURE	2	56.36±16.19^{A}	20.08±19.51^{B}	18.52±8.07^{B}	36.23±25.58^{A,B}
	7	55.24±24.86^{A}	41.45±28.17^{A,B}	36.63±25.70^{A,B}	16.99±13.81^{B}
	14	58.62±29.95^{A}	55.62±23.16^{A}	58.16±21.70^{A}	54.14±24.42^{A}
	21	59.08±22.48^{A}	63.34±38.86^{A}	37.82±19.88^{A}	38.69±23.14^{A}
IMMATURE	2	43.64±16.19^{B}	79.92±19.51^{A}	81.48±8.07^{A}	63.77±25.58^{A,B}
	7	44.76±24.86^{B}	58.55±28.17^{A,B}	63.37±25.70^{A,B}	83.01±13.81^{A}
	14	41.38±29.95^{A}	44.38±23.16^{A}	41.84±21.70^{A}	45.86±24.42^{A}
	21	40.92±22.48^{A}	36.66±38.86^{A}	62.28±19.88^{A}	61.31±23.14^{A}

Different letters in the same line show significant differences between groups (p<0,05).

The values for the percentage of density of mature/immature collagen in the epithelium/conjunctive region presented statistically significant differences for the periods of 2 days. For mature collagen, the highest value was for S (72.55± 11.20) (Fig.3A) whereas the lowest value was found in PC (40.04±17.12) (Fig.3E). For immature collagen the highest value found was in PC (59.96±17.02) and the lowest in PCA (38.88±16.85) (Fig.3F). The data are shown in Table 3.

Table 3 – Mean values and standard error for the percentage of density of mature/immature collagen in the epithelium/conjunctive region.

Density of Collagen	Days	GROUPS			
		S	PC	A	PCA
MATURE	2	72.55± 11.20^{A}	40.04±17.12^{B}	44.99±8.69^{B}	61.12±16.85^{A,B}
	7	59.51±18.25^{A}	52.62±17.13^{A}	51.51±14.42^{A}	43.33±11.62^{A}
	14	55.45±14.27^{A}	56.61±13.32^{A}	61.24±11.19^{A}	49.11±14.84^{A}
	21	64.41±17.64^{A}	63.72±31.16^{A}	42.71±13.58^{A}	46.61±18.52^{A}
IMMATURE	2	27.45± 11.20^{B}	59.96±17.02^{A}	55.01±8.69^{A,B}	38.88±16.85^{B}
	7	40.49±18.25^{A}	47.38±17.13^{A}	48.49±14.42^{A}	56.67±11.62^{A}
	14	44.55±14.27^{A}	43.39±13.32^{A}	38.76±19.11^{A}	50.89±14.84^{A}
	21	35.59±17.64^{A}	36.28±31.16^{A}	57.29±13.58^{A}	53.39±18.52^{A}

Different letters in the same line show significant differences between groups (p<0,05).

The results for the percentage of area of mature/immature collagen in the submucosa region presented statistically significant differences for the periods of 21 days. For mature collagen, the highest value was for PC (69.92±16.76) (Fig.4A) whereas the lowest value was found in PCA (21.35 ±10.98) (Fig.4B) at 2 days. For immature collagen the highest value found was in PCA (78.65±10.98) and the lowest in PC (30.08±16.76). The data are shown in Table 4.

Table 4 – Mean values and standard error for the percentage of area of mature/immature collagen in the sub mucosa region.

Area of Collagen	Days	GROUPS			
		S	PC	A	PCA
MATURE	2	54.86±32.72^{A}	26.95±17.43^{A}	29.10±21.52^{A}	47.06±30.28^{A}
	7	47.62±30.26^{A}	39.88±30.51^{A}	18.67± 12.08^{A}	35.27±20.70^{A}
	14	76.73±23.02^{A}	45.71±25.10^{A}	52.15±24.98^{A}	44.14± 24.06^{A}
	21	41.24±25.11^{A,B}	69.92±16.76^{A}	36.73± 26.07^{B}	21.35 ±10.98^{B}
IMMATURE	2	45.14±32.72^{A}	73.05±17.43	70.90±21.52^{A}	52.94±30.28^{A}
	7	52.38±30.26^{A}	60.12±30.51	81.33±12.08^{A}	64.73±20.70^{A}
	14	23.27±23.02^{A}	54.29±25.10	47.85±24.98^{A}	55.86±24.06^{A}
	21	54.76±25.11^{A,B}	30.08±16.76^{B}	63.27±26.07^{A}	78.65±10.98^{A}

Different letters in the same line show significant differences between groups (p<0,05).

The results for the percentage of density of mature/immature collagen in the submucosa region presented statistically significant differences for the periods of 21 days. For mature collagen, the highest value was for S (60.11± 19.70) (Fig.4C)

whereas the lowest value was found in A (45.97±12.35) (Fig.4D). For immature collagen the highest value found was in PCA (61.20±14.83) (Fig.4B) and the lowest in PC (32.09±10.12)(Fig.4A). The data are shown in Table 5.

Tabela 5 – Mean values and standard error for the percentage of density of mature/immature collagen in the submucosa region.

Density of Collagen	Dias	GROUPS			
		S	PC	A	PCA
MATURE	2	60.11±19.70^{A}	46.69±19.75^{A}	45.97±12.35^{A}	57.15±19.79^{A}
	7	55.84±18.35^{A}	46.82±22.89^{A}	32.10±8.83^{A}	46.77±12.36^{A}
	14	69.25±15.71^{A}	53.49±14.29^{A}	57.82±15.22^{A}	49.10±11.86^{A}
	21	56.29±13.11^{A,B}	67.91±10.12^{A}	51.96±17.21^{A,B}	38.80±14.83^{B}
IMMATURE	2	39.89±19.70^{A}	53.31±19.75^{A}	54.03±12.35^{A}	42.85±19.79^{A}
	7	44.16±18.35^{A}	53.18±22.89^{A}	67.90±8.83^{A}	53.23±12.36^{A}
	14	30.75±15.71^{A}	46.51±14.29^{A}	42.18±15.22^{A}	50.90±11.86^{A}
	21	43.71±13.11^{A,B}	32.09±10.12^{B}	48.04±17.21^{A,B}	61.20±14.83^{A}

Different letters in the same line show significant differences between groups ($p<0,05$).

DISCUSSION

The ulcer is a common lesion in the oral mucosa and its healing is divided into specific phases such as the inflammatory stage (2 days), proliferative with synthesis of the extracellular matrix (7 days) and remodeling (21 days).[16]

The present study evaluated a medicinal extract (*Aloe vera*) and carbamide peroxide, associated or not, on ulcerations induced in the oral mucosa of rats, altering the quantity of mastocytes during the repair process. The distribution of mastocytes was not homogeneous in the epithelium/conjunctive areas and sub mucosa in the periods of 2, 7, 14 and 21 days.

The mastocytes are mobile cells of the immunological system, which perform a critical role in the induction of inflammation and repair process, responding to immunological and non immunological stimuli. In oral lesions the degranulation of these cells may be identified by the exteriorization of the granules and diminishment of intracellular stocks of mediators, being determined by the histochemical staining of Toluidine blue[25].

A number of factors, such as drugs, hormones, viruses, toxins and traumas may induce the mastocytes to synthesize and/or secrete their mediators[23] such as cathepsin G, histamine, serotonin, heparin, acid hydrolases and cytokines, such as the tumoral-α necrosis factor (TNF) and interleukin.[25] Histamine and TNF play a fundamental role in the inflammatory response. Histamine causes an increase in the vascular permeability due to the contraction of the endothelial cells and to the formation of spaces between them. TNF induces endothelial expression, mainly of E-selectin, increasing the offer of molecules of adhesion on the surface of the endothelium, inducing greater migration of neutrophils, T-lymphocytes, monocytes and leukocytes to the inflamed area.[25]

In this study, at 2 and 7 days after application of the drugs, there was statistically significant difference in the epithelium/conjunctive region and sub mucosa, respectively, with regard to the number of mastocytes. At 2 days in the PCA group, compared to S and PC groups, there was greater degranulation of the mastocytes, as their number was significantly reduced.

As from the 7th day the proliferative stage in the ulcer repair process begins, with the chronification of the lesion, and it is expected that the number of mastocytes would increase in comparison to the 2nd day. This was found in the sub mucosa for the groups S and A, but for the groups that contained carbamide peroxide, the mastocytes were still shown to be low in number, indicating continuity of degranulation. It can be hypothesized that the carbamide peroxide in contact with the mucosa was degraded into hydrogen peroxide and this into oxygen and water. This reaction could have been a catalyzer factor for the release of free radicals that sensitized the mastocytes, accentuating their degranulation.[4]

In the sub mucosa region the number of mastocytes at 2, 7, 14 and 21 days was numerically higher in comparison to the number in the epithelium/conjunctive region. This fact may have occurred due to the proximity of this region to the normal circumjacent conjunctive tissue, as the repair process begins in the deeper area of the ulcer in the direction toward the surface. In the epithelium/conjunctive region, it was observed that degranulation of those cells continued to be higher in the same periods. This may not be due to penetration of drugs until the deeper region by the presence of a barrier clot formed during the inflammatory process.

The polarization method with Picrosirius is a histochemical procedure specifically for detecting collagen in which the interstitial collagen presents different colors and intensities of birefringence. Yellow to red birefringent areas are indicative

of mature collagen and the green areas indicate immature collagen, the first type to appear during the tissue neo-formation process.[26] The color and intensity of collagen birefringence may also vary depending on the diameter of the fiber, thickness of the tissue cut, or both[26]. The percentage of area of immature collagen in the epithelium/ conjunctive region at 2 and 7 days in Group S was numerically lower than in Groups PC, A and PCA, and the inverse was found for mature collagen, this also occurred at 21 days in the sub-mucosa region. At 2 and 7 days, there was statistically significant difference in the epithelium/conjunctive region for the PC and A groups when compared to S, whereas in the submucosa region the statistical difference occurred at 21 days for the PC in comparison to S, A and PCA.

In the present study, it can be suggested that in the groups that had *Aloe vera*, a greater synthesis and deposition of collagen could have occurred. This was verified in studies with different methodologies, using cell cultures[27] and the estimate of the quantity of collagen by means of the solubility of granulation tissue.[28]

Aloe vera has the capacity to stimulate ulcer healing on the skin of rats by increasing the synthesis of collagen and glycosaminolgycans of the extracellular matrix[28]. The major fraction of carbohydrate derived from this plant is acemanan, which has the ability to induce the proliferation of gingival fibroblasts in culture[27] and promote healing of aphthous ulcers in humans.[29]

In the inflammatory phase of ulcer healing a sequence of events begins with the deposition of platelets, formation of thrombus and later release of chemical mediators by the platelets forming a chemiotactic gradient that orients the migration of neutrophils[30]. These cells are responsible for phagocytosis, with the elimination of microorganisms and tissue rests, such as collagen. In the present study, at 2 days, a high percentage of mature collagen was observed in relation to the area and density

in Group S in the epithelium/conjunctive region, probably because the pre-existent collagen in the conjunctive tissue was not completely degraded. Whereas, in the PC group, the density of immature collagen was greater than it was in the control group, showing that the fibroblasts were in a process of collagen synthesis. This result differs from the findings in a culture study of human gingival fibroblasts, in which hydrogen peroxide is cytotoxic and causes damage to the external cellular membrane or inside the cell, in addition to having effects on the viability/morphology, proliferation and functional activities that are important for the maintenance of the tissue and for healing.[2]. Nevertheless, other types of fibroblasts could be more resistant to lyses by hydrogen peroxide[31], in agreement with the findings of the present study. In the group A, a larger percentage of area and density of immature collagen was observed. These findings corroborate those of Thompson[32], who demonstrated high proliferative activity of fibroblasts and collagen synthesis.

At 7 days, the percentage of mature collagen was maintained higher in group A. It was verified that *Aloe vera* stimulated angiogenesis and an increase in capillary permeability, facilitating tissue regeneration in burns in guinea pigs treated by the topical application.[33] The majority of mediators involved in fibroplasia also have angiogenic activity, and it was verified that the fibroblasts produce or secrete the vascular endothelial growth factor (VGF) due to one of its main chemical constituents, acemanan.[34,15]

Fibroplasia beings at 7 days with the formation of granulation tissue in the injured region, together with a loose collagen matrix constituted by fibronectin, hyaluronic acid and cells, such as macrophages and fibroblasts, in addition to recently formed vessels and exudates. At 14 and 21 days fibroplasia and neo-formed collagen remodeling continue. In the groups treated with *Aloe vera*, the percentage of

the area and density of immature collagen was higher in comparison to the PC group, suggesting that *Aloe vera* may have promoted a continuity in collagen neo-formation, due to the induction of fibroblast proliferation.[29]

The association of *Aloe vera* with carbamide peroxide in dental bleaching showed therapeutic effectiveness in the process of ulcer repair in the oral mucosa of rats, reinforcing the hypothesis of its healing activity.

CONCLUSION

Within the limitations of this *in vivo* study, it can be concluded that 5% *Aloe vera* associated or not to carbamide peroxide, demonstrated anti-inflammatory and healing activity in ulcers in the oral mucosa of rats by:

- reducing the number of mastocytes in the most superficial region of the ulcer in the inflammatory stage of the repair process at 2 days.

- stimulating fibroplasia and remodeling, suggesting a continuity in the collagen neo - formation.

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TABLES

Table 1 – Mean values and standard deviation for the number of mastocytes in the epithelium/conjunctive region and sub mucosa area.

Mastocytes	Days	GROUPS S	PC	A	PCA
Epithelium/ Conjunctive	2	0.79±0.70^{A}	0.86±0.20^{A}	0.25±0.39^{A,B}	0.12±0.16^{B}
	7	0.46±0.24^{A}	0.21±0.16^{A}	0.26±0.25^{A}	0.32±0.11^{A}
	14	0.39±0.31^{A}	0.16±0.37^{A}	0.48±0.23^{A}	0.26±0.24^{A}
	21	0.52±0.38^{A}	0.13±0.17^{A}	0.60±0.39^{A}	0.47±0.49^{A}
Submucosa	2	0.93±0.51^{A}	1.21±0.58^{A}	0.79±0.71^{A}	0.60±0.42^{A}
	7	1.13±0.32^{A}	0.61±0.33^{B}	1.06±0.34^{A}	0.51±0.09^{B}
	14	1.13±0.48^{A}	0.85±0.64^{A}	1.01±0.23^{A}	0.95±0.61^{A}
	21	1.02±0.50^{A}	0.76±0.54^{A}	0.64±0.31^{A}	0.72±0.42^{A}

Different letters in the same line show significant differences between groups (p<0,05).

Table 2 – Mean values and standard deviation for the percentage of the area of mature/immature collagen in the epithelium/conjunctive region .

Area of Collagen	Days	GROUPS S	PC	A	PCA
MATURE	2	56.36±16.19^{A}	20.08±19.51^{B}	18.52±8.07^{B}	36.23±25.58^{A,B}
	7	55.24±24.86^{A}	41.45±28.17^{A,B}	36.63±25.70^{A,B}	16.99±13.81^{B}
	14	58.62±29.95^{A}	55.62±23.16^{A}	58.16±21.70^{A}	54.14±24.42^{A}
	21	59.08±22.48^{A}	63.34±38.86^{A}	37.82±19.88^{A}	38.69±23.14^{A}
IMMATURE	2	43.64±16.19^{B}	79.92±19.51^{A}	81.48±8.07^{A}	63.77±25.58^{A,B}
	7	44.76±24.86^{B}	58.55±28.17^{A,B}	63.37±25.70^{A,B}	83.01±13.81^{A}
	14	41.38±29.95^{A}	44.38±23.16^{A}	41.84±21.70^{A}	45.86±24.42^{A}
	21	40.92±22.48^{A}	36.66±38.86^{A}	62.28±19.88^{A}	61.31±23.14^{A}

Different letters in the same line show significant differences between groups (p<0,05).

Table 3 – Mean values and standard error for the percentage of density of mature/immature collagen in the epithelium/conjunctive region.

Density of Collagen	Days	GROUPS S	PC	A	PCA
MATURE	2	72.55± 11.20^{A}	40.04±17.12^{B}	44.99±8.69^{B}	61.12±16.85^{A,B}
	7	59.51±18.25^{A}	52.62±17.13^{A}	51.51±14.42^{A}	43.33±11.62^{A}
	14	55.45±14.27^{A}	56.61±13.32^{A}	61.24±11.19^{A}	49.11±14.84^{A}
	21	64.41±17.64^{A}	63.72±31.16^{A}	42.71±13.58^{A}	46.61±18.52^{A}
IMMATURE	2	27.45± 11.20^{B}	59.96±17.02^{A}	55.01±8.69^{A,B}	38.88±16.85^{B}
	7	40.49±18.25^{A}	47.38±17.13^{A}	48.49±14.42^{A}	56.67±11.62^{A}
	14	44.55±14.27^{A}	43.39±13.32^{A}	38.76±19.11^{A}	50.89±14.84^{A}
	21	35.59±17.64^{A}	36.28±31.16^{A}	57.29±13.58^{A}	53.39±18.52^{A}

Different letters in the same line show significant differences between groups (p<0,05).

Table 4 – Mean values and standard error for the percentage of área of mature/immature collagen in the submucosa region.

Area of Collagen	Days	GROUPS			
		S	PC	A	PCA
MATURE	2	54.86±32.72^{A}	26.95±17.43^{A}	29.10±21.52^{A}	47.06±30.28^{A}
	7	47.62±30.26^{A}	39.88±30.51^{A}	18.67± 12.08^{A}	35.27±20.70^{A}
	14	76.73±23.02^{A}	45.71±25.10^{A}	52.15±24.98^{A}	44.14± 24.06^{A}
	21	41.24±25.11^{A,B}	69.92±16.76^{A}	36.73± 26.07^{B}	21.35 ±10.98^{B}
IMMATURE	2	45.14±32.72^{A}	73.05±17.43	70.90±21.52^{A}	52.94±30.28^{A}
	7	52.38±30.26^{A}	60.12±30.51	81.33±12.08^{A}	64.73±20.70^{A}
	14	23.27±23.02^{A}	54.29±25.10	47.85±24.98^{A}	55.86±24.06^{A}
	21	54.76±25.11^{A,B}	30.08±16.76^{B}	63.27±26.07^{A}	78.65±10.98^{A}

Different letters in the same line show significant differences between groups (p<0,05).

Tabela 5 – Mean values and standard error for the percentage of density of mature/immature collagen in the submucosa region.

Density of Collagen	Dias	GROUPS			
		S	PC	A	PCA
MATURE	2	60.11±19.70^{A}	46.69±19.75^{A}	45.97±12.35^{A}	57.15±19.79^{A}
	7	55.84±18.35^{A}	46.82±22.89^{A}	32.10±8.83^{A}	46.77±12.36^{A}
	14	69.25±15.71^{A}	53.49±14.29^{A}	57.82±15.22^{A}	49.10±11.86^{A}
	21	56.29±13.11^{A,B}	67.91±10.12^{A}	51.96±17.21^{A,B}	38.80±14.83^{B}
IMMATURE	2	39.89±19.70^{A}	53.31±19.75^{A}	54.03±12.35^{A}	42.85±19.79^{A}
	7	44.16±18.35^{A}	53.18±22.89^{A}	67.90±8.83^{A}	53.23±12.36^{A}
	14	30.75±15.71^{A}	46.51±14.29^{A}	42.18±15.22^{A}	50.90±11.86^{A}
	21	43.71±13.11^{A,B}	32.09±10.12^{B}	48.04±17.21^{A,B}	61.20±14.83^{A}

Different letters in the same line show significant differences between groups (p<0,05).

FIGURES

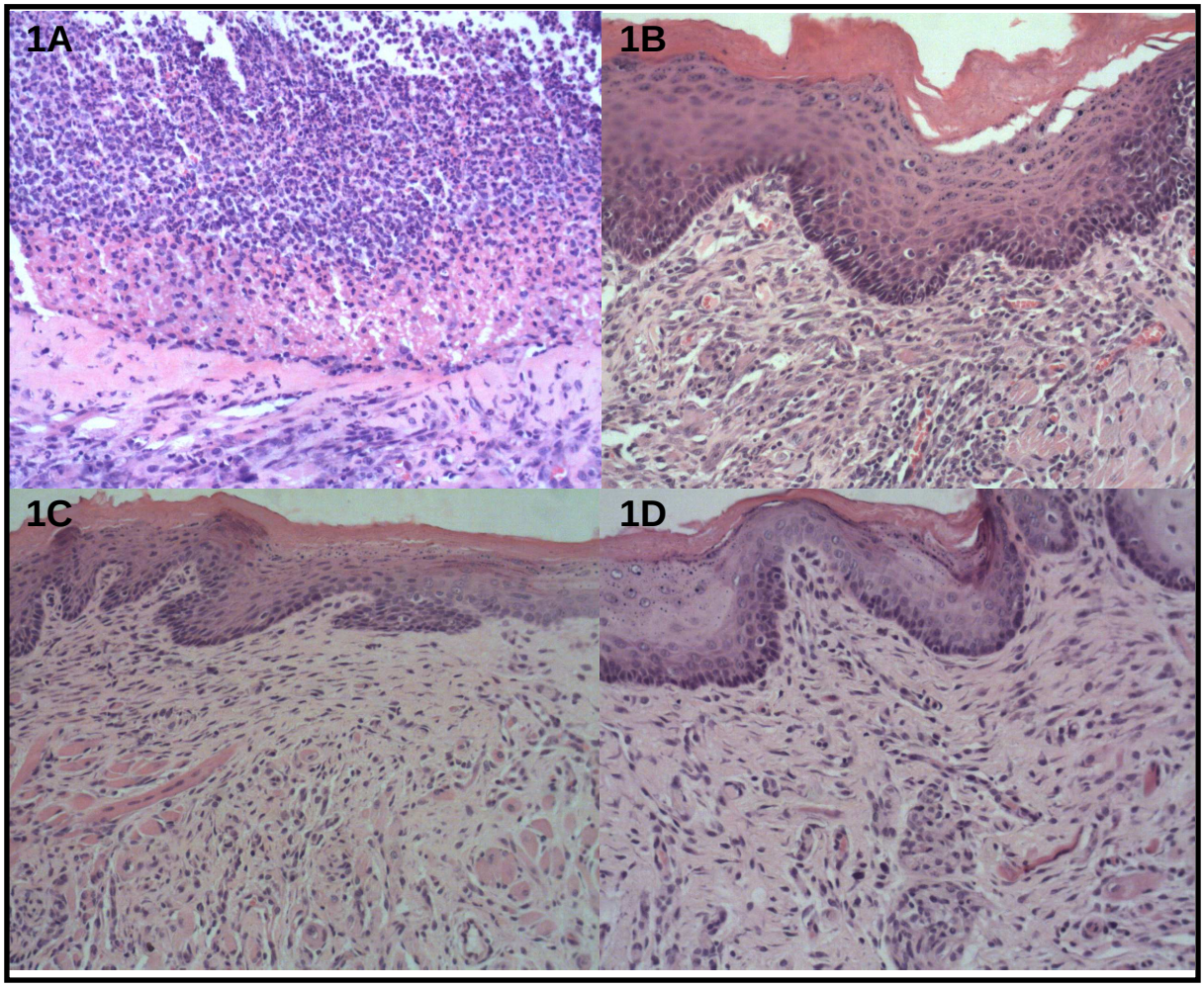

Figure 1 – Group Saline 1A, 1B, 1C e 1D corresponding to the periods of 2, 7, 14 and 21 days, respectively. HE coloration, 20X magnification.

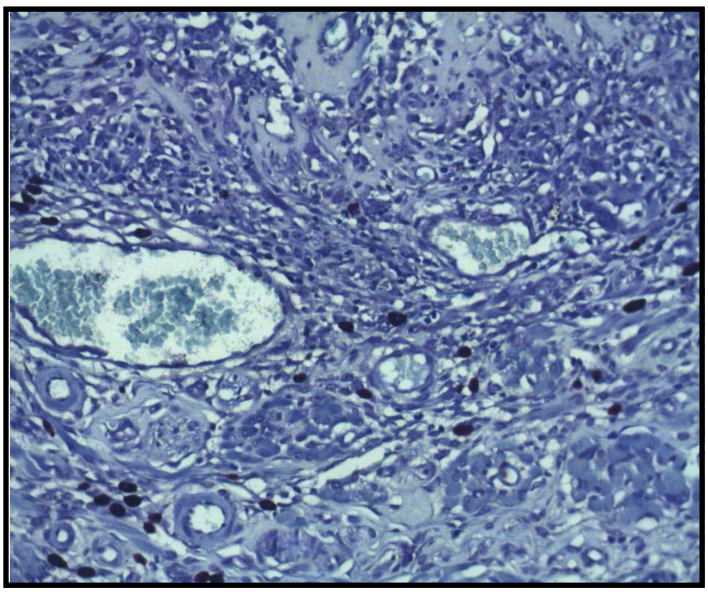

Figure 2A: Mastocytes in PC group in 2 days, in purple color disperses in conjunctive tissue (BT 20X).

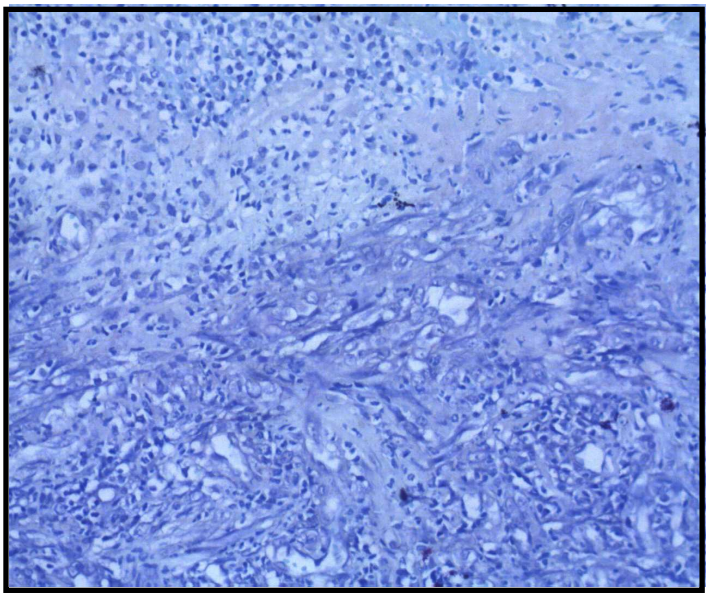

Figure 2B: Mastocytes in PCA group in 2 days, in purple color disperses in conjunctive tissue (BT 20X).

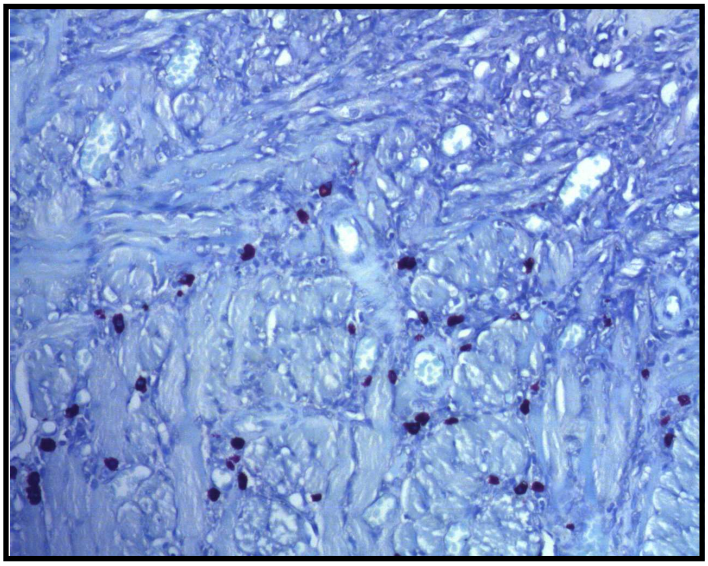

Figure 2C: Mastocytes in S group in 7 days, in purple color disperses in conjunctive tissue (BT 20X).

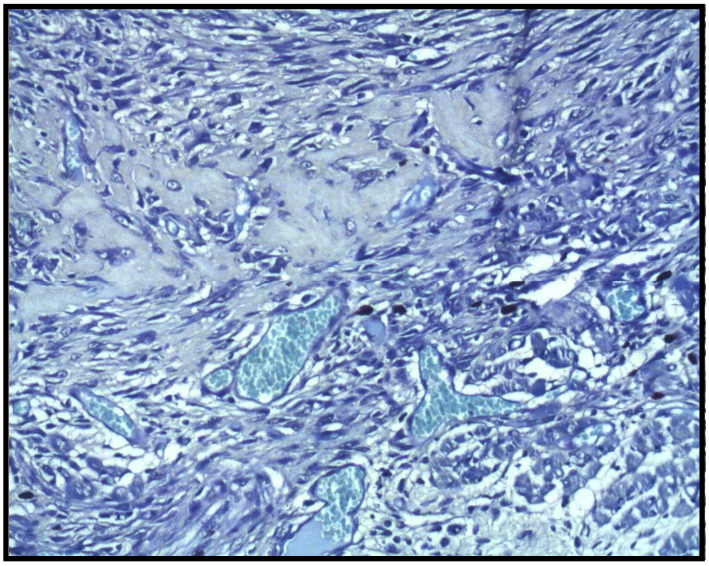

Figure 2D: Mastocytes in PCA group in 7 days, in purple color disperses in conjunctive tissue (BT 20X)

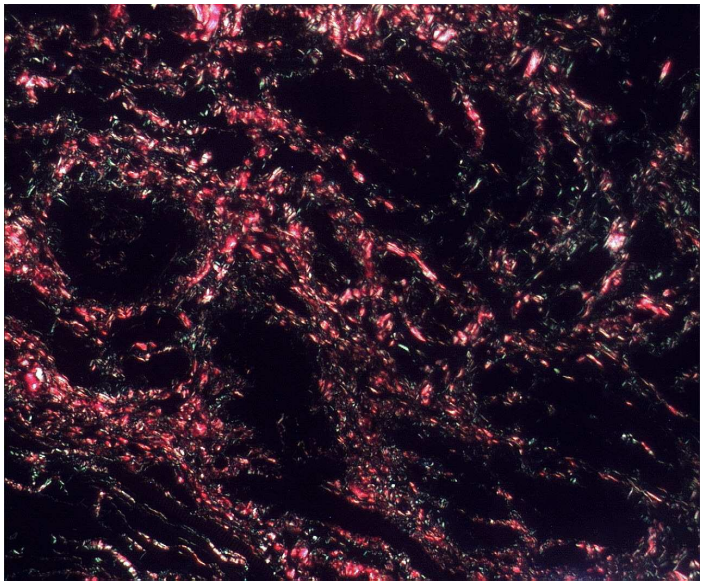

Figure 3A: Immature/mature of collagen disposition to S group for 2 days.

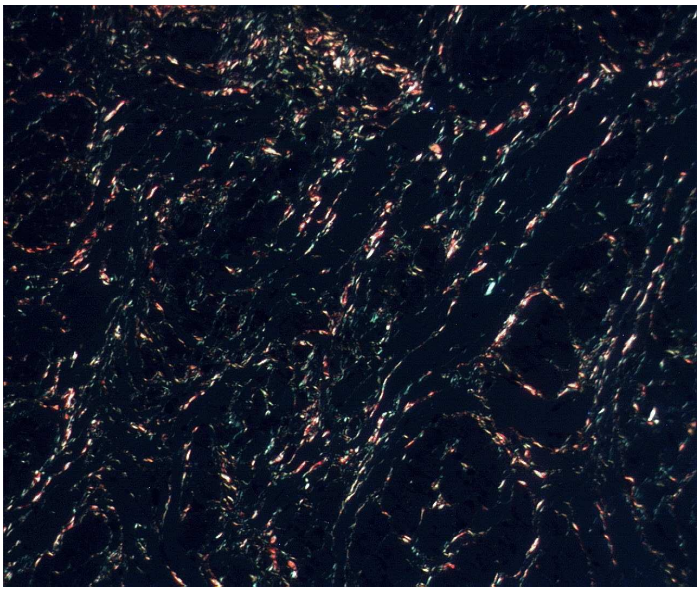

Figure 3B: Immature/mature of collagen disposition to A group for 2 days.

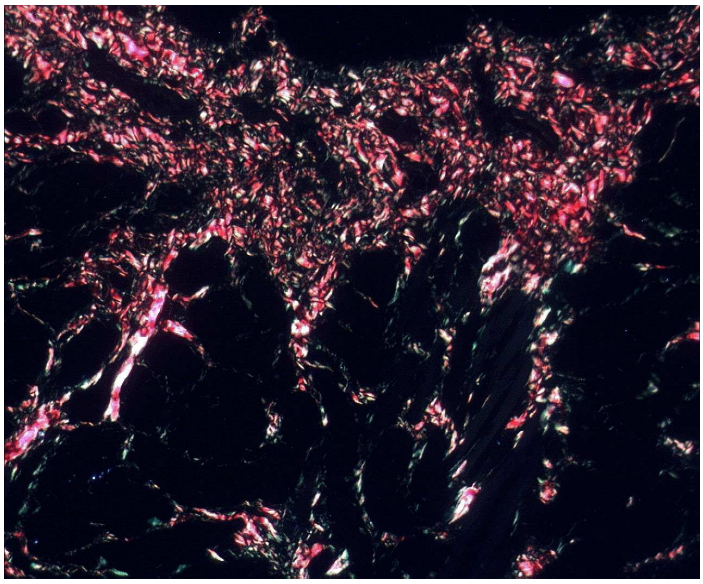

Figure 3C: Immature/mature of collagen disposition to S group for 7 days.

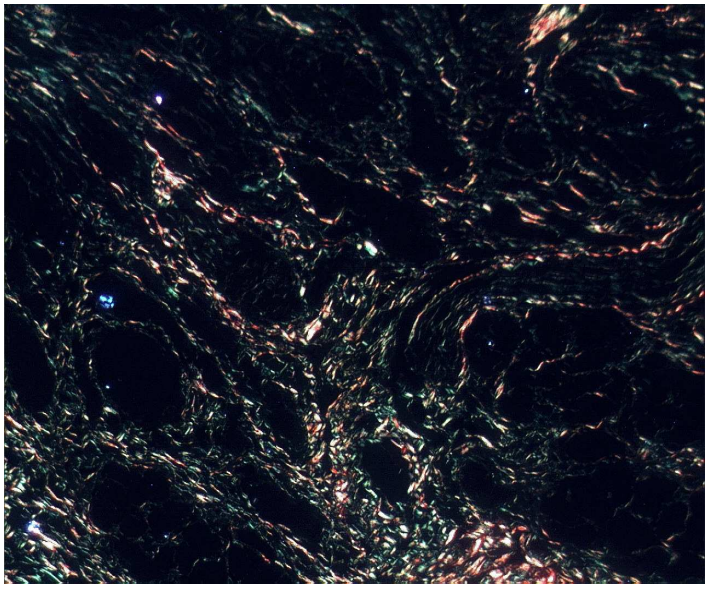

Figure 3D: Immature/mature of collagen disposition to PCA group for 7 days.

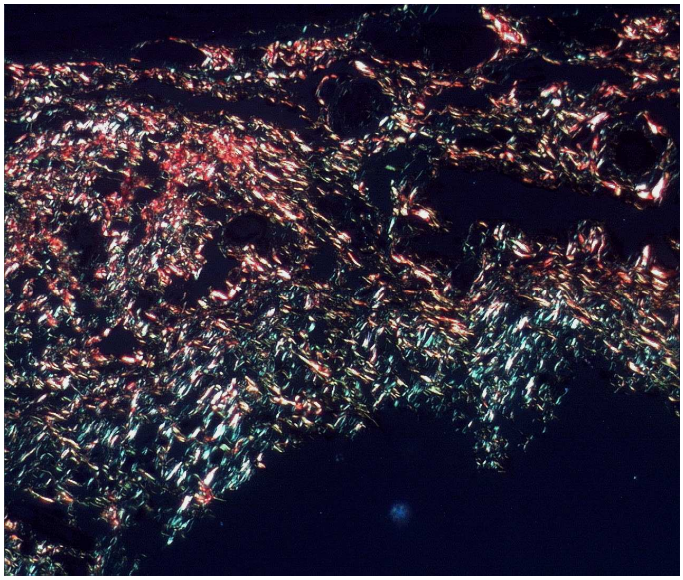

Figure 3E: Immature/mature of collagen disposition to PC group for 2 days.

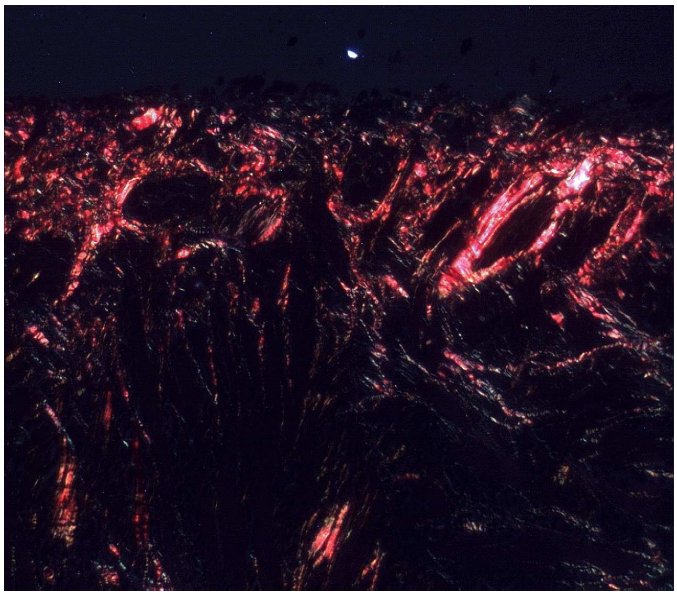

Figure 3F: Immature/mature of collagen disposition to PCA group for 2 days.

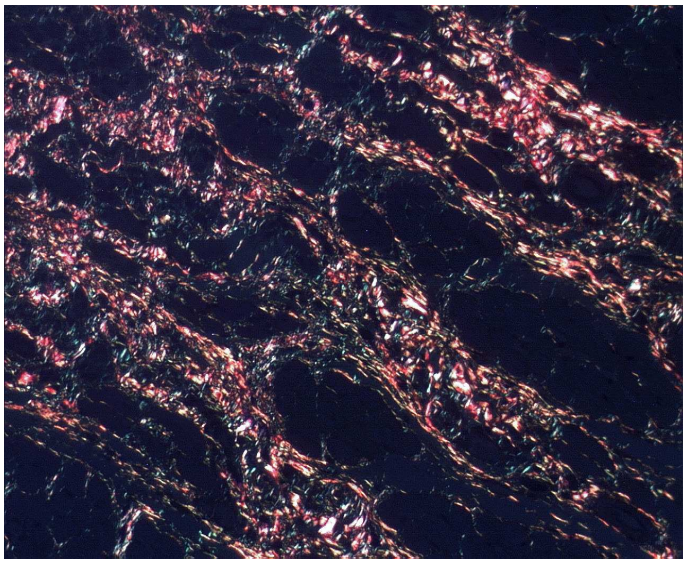

Figure 4A: Immature/mature of collagen disposition to PC group for 21 days.

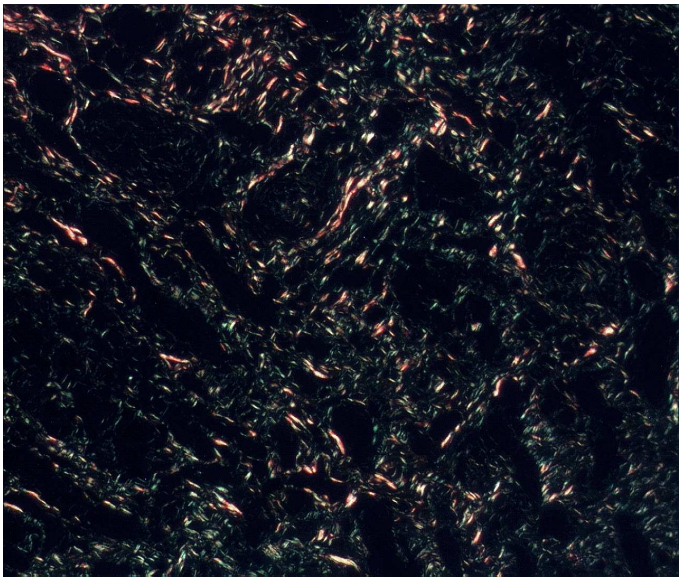

Figure 4B: Immature/mature of collagen disposition to PCA group for 2 days.

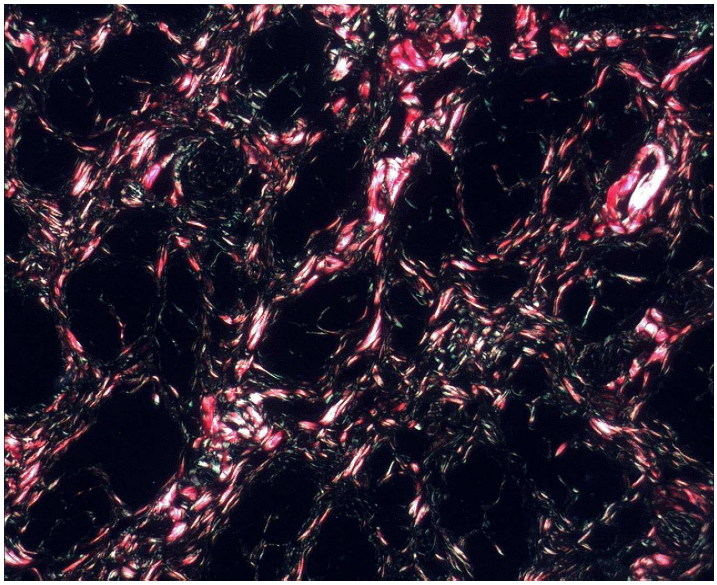

Figure 4C: Immature/mature of collagen disposition to S group for 21 days.

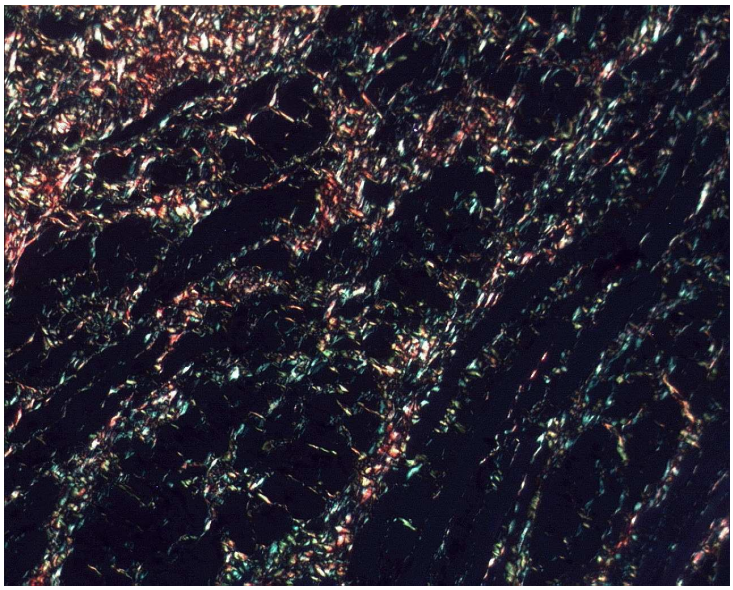

Figure 4D: Immature/mature of collagen disposition to A group for 21 days.

ANEXOS

APROVAÇÃO DO COMITÊ DE ÉTICA

PUCPR

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

NÚCLEO DE BIOÉTICA
COMITÊ DE ÉTICA NO USO DE ANIMAIS

PARECER DE PROTOCOLO DE PESQUISA

Parecer nº: 226/07 CEUA PUCPR
Registro do projeto no CEUA: 289 2ª versão

Data do parecer: 10/12/2007

Título do Projeto:

Associação de plantas medicinais (Zingiber oficinalle, Aloe Vera e Arnica montana) com gel clareador sobre o reparo tecidual de lesões ulceradas durante o processo de clareamento caseiro.

Pesquisador responsável:

Rui Fernando Mazur

Equipe da Pesquisa:

Ana Maria Grégio; Janaína Bertoncelo de Almeida; Renata Di Creddo Brum; Camila Paloma Pinto.

Instituição:

PUCPR

Categoria do Experimento - Categoria B

Espécie de Animal	Sexo	Idade ou peso	Quantidade
Rattus norvegicus albinus, Rodentia, Mammalia da linhagem Wistar	Macho	180g	240

O colegiado do CEUA em reunião no dia 06/12/2007, avaliou o projeto e emite o seguinte parecer: **APROVADO.**

MATERIAL E MÉTODO

O projeto deste estudo foi aprovado pelo Comitê de Ética no Uso de Animais (CEUA) da Pontifícia Universidade Católica do Paraná (PUCPR), nº 226/07, seguindo os Princípios Éticos na Experimentação Animal, de acordo com a Lei 6.638 de 08 de Maio de 1979.

Foram utilizados 112 ratos machos adultos do tipo *Rattus novergicus albinus*, Rodentia Mammalia da linhagem *Wistar*, pesando cerca de 180g. Os animais foram obtidos junto ao biotério da PUCPR, mantidos em gaiolas plásticas e alimentados com ração e água *ad libitum*, respeitando-se o ciclo diurno-noturno (Figura 1).

A anestesia geral foi induzida por via intraperitoneal com tiopental sódico® - 20mg/Kg (Cristália, Itapira, SP, Brasil) – Figura 2. Na porção mais anterior da face dorsal da língua dos animais foram induzidas, quimicamente, úlceras com hidróxido de sódio (NaOH) 40%, pela aplicação tópica e diária, com auxílio de cotonetes durante um minuto, por 7 dias. Após a constatação clínica visual da formação de úlcera na língua de cada animal, os mesmos foram aleatoriamente divididos em 4 grupos (n=28) e tratados com os fármacos: S – Salina (n=7), PC – peróxido de carbamida 16% (n=7), A – Gel de *Aloe vera* 0,5% (n=7), e PCA – peróxido de carbamida 16% + gel de *Aloe vera* 0,5% (n=7) - Tabela 1.

TABELA 1 - Divisão dos grupos de acordo com o tratamento, período de tratamento e via de administração.

Grupos	Tratamento	Período de tratamento	Via de administração Dose 100µL = 1gota
1- Controle	Salina 0,9%	02 dias	Aplicação tópica
		07 dias	
		14 dias	
		21 dias	
2-	Peróxido de carbamida 16%	02 dias	Aplicação tópica
		07 dias	
		14 dias	
		21 dias	
3	*Aloe vera* 0.5%	02 dias	Aplicação tópica
		07 dias	
		14 dias	
		21 dias	
4	Peróxido de carbamida 16% + *Aloe vera* 0.5%	02 dias	Aplicação tópica
		07 dias	
		14 dias	
		21 dias	

Os géis de Peróxido de Carbamida 16% e de *Aloe vera* 0,5% (Galena Industria farmaceutica – São Paulo, SP, Brasil) ou a associação de ambos foram preparados em farmácia de manipulação, aplicados diariamente e por uma única vez com cotonetes embebidos nos produtos e friccionados nas úlceras por 2 minutos (Figura 3).

Os animais foram mortos por overdose de tiopental sódico®, por via intraperitoneal, nos períodos de 2, 7, 14 e 21 dias após o início do tratamento. As línguas foram removidas e seccionadas no sentido longitudinal (Figura 4), fixadas em formalina neutra 10% por 24 horas e processadas em laboratório.

Decorrido o período mínimo de 24 horas da fixação da formalina, os espécimes foram seccionados transversalmente seguindo o sentido epitélio-tecido conjuntivo, e essas porções foram armazenadas em cassetes previamente

identificados (Figura 5). Para a desidratação foram realizadas imersões dos cassetes em álcool 70, 80 e 90% e em seguida 3 imersões em álcool absoluto. Posteriormente, os espécimes foram diafanizadas em xilol PA e incluídas em parafina sob forma de blocos.

De cada animal foram confeccionadas três lâminas, coradas com Hematoxilina-Eosina (HE), Azul de toluidina 0,2% (AT) e Picrosírius (SR). Nas secções coradas em HE as lâminas foram observadas nos aumentos de 50, 100, 200 e 400X, visando descrever subjetivamente o processo de reparo na área ulcerada, sem a intenção de realizar uma análise quantitativa. Foi observado, de forma geral, a presença ou não de ulceração, a quantidade de vasos sanguíneos e o predomínio do tipo de infiltrado inflamatório (agudo, misto ou crônico).

Nas secções coradas com AT foi quantificado o número de mastócitos, identificados pela sua cor púrpura. Em cada lâmina foram obtidas quatro imagens da área da lesão ulcerada e tratada, correspondente a 2 campos superficiais – epitélio/ conjuntivo (região subjacente à camada basal do tecido epitelial) e 2 campos profundos - submucosa (região dos tecidos mais profundos da língua do animal). As imagens foram capturadas com aumento de 200X pela câmara de vídeo (Sony Íris CCD, Tóquio, Japão) acoplada ao microscópio e enviadas ao computador. Em cada imagem do campo histológico com o auxílio do programa Image Pro-Plus versão 4.5 (Media Cibernetics, Silver Spring, MD, EUA), foi confeccionado um retículo virtual (3 x 4) de 1 mm^2 cada, para a contagem do número de mastócitos[19].

A percentagem da área e a densidade de colágeno maturo e imaturo foram mensuradas nas secções coradas pelo SR usando luz polarizada, onde o colágeno maturo apresenta birrefringência intensa, de coloração amarelo e vermelho, enquanto o colágeno imaturo produz uma coloração esverdeada[17]. Essa avaliação

foi realizada em um campo na área epitélio/conjuntivo e outro na submucosa, mensuradas pelo mesmo programa Image Pro-Plus.

Os testes estatísticos de Normalidade de Shapiro – Wilk e Homogeneidade de Levene foram aplicados na análise quantitativa. O teste de ANOVA ($p<0,05$) foi aplicado para comparar as médias dos grupos, nas áreas epitélio/conjuntivo e submucosa das úlceras, para o número de mastócitos e para a percentagem da área e densidade do colágeno maturo e imaturo com o software SPSS 15.0 para Windows® (SPSS Inc., Chicago, Illinois, EUA).

Nas variáveis em que o teste ANOVA acusou diferença nos valores médios entre grupos e, quando houve homogeneidade de variâncias entre os grupos pelo teste de Levene, as comparações múltiplas foram feitas pelo teste de Tukey HSD. Caso contrário foi utilizado o teste de comparações múltiplas para variâncias heterogêneas de Games-Howell.

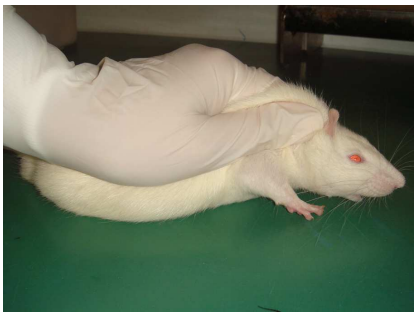

Figura 1 – Estabilização do rato da linhagem *Wistar* albinos (*Rattus norvergicus*).

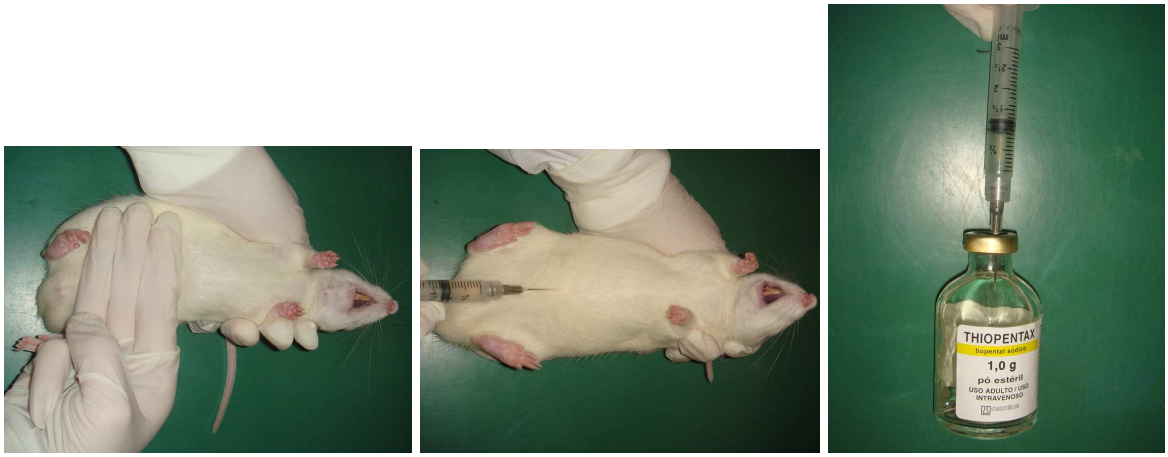

Figura 2 – Analgesia geral por via intraperitoneal com o tiopental sódico®.

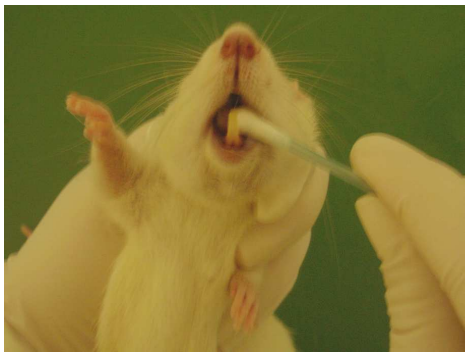

Figura 3 – Aplicação tópica dos fármacos.

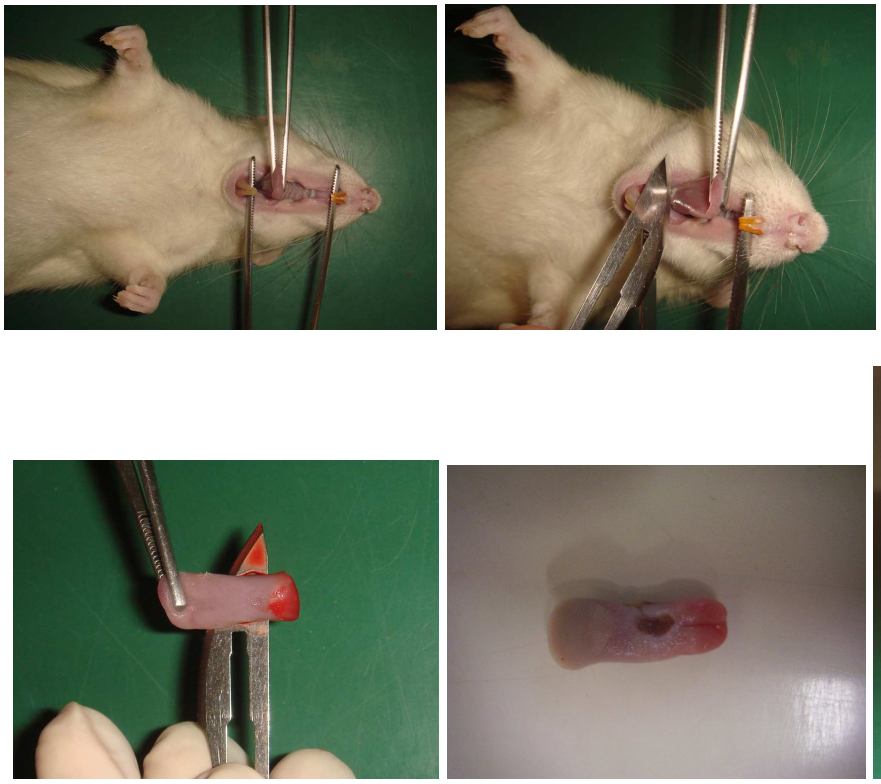

Figura 4 – Remoção da língua do animal e armazenamento em Formalina 10 %.

Figura 5 - Corte da língua e armazenamento nos cassetes.

REVISÃO DE LITERATURA

1. Hewlett, E. R. Etiology and management of whitening-induced tooth hypersensitivity. J Calif Dent Assoc 2007; 35(7): 499-506.

Tooth hypersensitivity has long been, and continues to be, the most commonly reported adverse effect of vital tooth whitening with peroxide gels. The complex etiology of whitening-induced tooth hypersensitivity has been a major obstacle in developing a definitive strategy for its prevention. This article reviews the multiple etiologic factors implicated in whitening-induced tooth hypersensitivity and the evidence for efficacy of various strategies for its management.

2. Tipton, D. A., Braxton, S. D., Dabbous, M. Kh. Effect of a bleaching agent on human gingival fibroblasts. J Periodontol 1995; 66:7-13.

Mild oxygenating agents generating low concentrations of hydrogen peroxide (H2O2) are effective alternatives to heat-activated 30% H2O2 in bleaching discolored, vital teeth. There are concerns about possible pathological effects of long-term exposure to bleaching agents, and irritation and ulceration of the gingiva and other oral soft tissues can occur. The objective of this study was to determine the effect of one of these agents on gingival fibroblasts in vitro. Microscopic examination revealed that concentrations of 0.05% to 0.025% of the agent appeared to kill most of the cells. At concentrations of 0.025% to 0.017% some morphological changes were noted; the cells appeared normal at concentrations of < or = 0.0125%. The agent significantly (P < or = 0.002) decreased proliferation (measured by incorporation of [3H]-thymidine into cellular DNA) at concentrations as low as 0.006%. The agent also had a dose-dependent effect on fibronectin production, measured by ELISA, causing significant (P < or = 0.03) decreases at concentrations as low as 0.017%. The agent significantly decreased the production of types I (P < or = 0.01) and III (P < or = 0.04) collagens

(measured by ELISA) at concentrations as low as 0.0125%. Type V collagen was not detected under any conditions. Catalase, which catalizes the breakdown of H2O2, abolished toxic effects of a 0.05% solution. The results show that in vitro, the agent is toxic to human gingival fibroblasts, inhibiting several cellular functions.

3. Haywood, V. B.; Heymann, H. O. Nightguard vital bleaching. Quintessence Int 1989; 20:173-176.

With the current interest in esthetic dentistry, vital bleaching is a viable option to consider when treating intrinsically stained or discolored teeth whose form and integrity are deemed acceptable. Nightguard vital bleaching offers an apparently safe and effective means of bleaching mildly discolored teeth using a soft nightguard worn by the patient at night. The majority of the bleaching is accomplished outside the office, which provides significant savings of cost and time for the patient. The paper relates a technique about nightguard vital bleaching.

4 Tam, L. The Safety of Home Bleaching Techniques. J Can Dent Assoc 1999;65:453-5. How safe is home tooth bleaching? Home bleaching is a popular dental procedure used to whiten teeth. The first clinical study of nightguard vital tooth bleaching using a carbamide peroxide product was published in 1989. Carbamide peroxide is the most commonly used active ingredient in home bleaching systems. It breaks down into hydrogen peroxide and urea in aqueous solution. Although concentrations of 10% carbamide peroxide (equivalent to approximately 3% hydrogen peroxide) are most commonly used, bleaching systems containing up to 22% carbamide peroxide are available for home use. The safety of home bleaching has been questioned. This article looks at the minor or transient adverse effects of home bleaching on oral tissues as well as the potential major long-term or systemic risks of the technique on dental and soft tissues.

5. Kirsten, G.A.; Freire, A.; de Lima, Adilson A.; Aparecido Ignácio, S.; Souza, E.M. Effect of reservoirs on gingival inflammation after home dental bleaching. Quintessence Int 2009, 40:195-202.

OBJECTIVE: To evaluate the influence of reservoirs on the gingival mucosa of patients submitted to at-home bleaching with 16% carbamide peroxide. METHOD AND MATERIALS: Nineteen nonsmoking male patients, 18 to 25 years of age, were submitted to home bleaching with a 16% carbamide peroxide gel for 2 consecutive hours for 21 days. The custom-made mouth trays were made with a reservoir on only the left side and cut anatomically 1 mm beyond the gingival margin. Smears of the gingival mucosa were obtained by the exfoliation cytology in liquid media technique before (control), immediately after, and 30 and 45 days after treatment. The samples were processed in the laboratory and evaluated according to Papanicolaou's criteria of malignity. Statistical analysis was carried out by McNemar test, 2 proportions test, and Wilcoxon test with a level of significance of 1%. RESULTS: The presence of a reservoir in the custom tray resulted in an increase of inflammation only immediately after the bleaching procedure. After 30 and 45 days, the difference between inflammation on the sides with and without a reservoir was not statistically significant. Significant differences were found in the degree of inflammation, classified as predominantly mild on the nonreservoir side and moderate on the reservoir side ($P < .01$). CONCLUSIONS: A 16% carbamide peroxide bleaching gel caused gingival inflammation immediately after the procedure and persisted until 45 days after the bleaching treatment. The use of a reservoir in the custom tray for home bleaching resulted in higher rates and higher intensity of gingival inflammation.

6. Cavalli, V.; Arrais, C. A. G.; Giannini, M.; Ambrosano, G. M. B. High-concentrated carbamide peroxide bleaching agents effects on enamel surface. J Oral Rehabil 2004; 31(2): 155- 9.

Concern has been expressed regarding the adverse effects of peroxide-containing tooth bleaching agents on enamel surface. This study examined enamel average surface roughness before (baseline) and after an in-office bleaching protocol and investigated the influence of high concentrations of carbamide peroxide gels on its surface staining and morphology. Flat enamel surfaces were submitted to 35 and 37% carbamide peroxide or to no bleaching treatment (n = 10) and evaluated with a profilometer. Eight specimens from each group were randomly selected and immersed in a 2% methylene blue solution. Afterwards, specimens were ground into powder and prepared for the spectrophotometric analysis. Two remained specimens of each group were examined using a scanning electron microscope. Data were subjected to analysis of variance and Tukey test (P > 0.05). Baseline roughness average was statistically similar for all groups, however, 35% carbamide peroxide produced the roughest enamel surfaces. Different concentrations of carbamide peroxide produced similar staining means and enamel surface morphological alterations.

7. Pinto, C. F.; Oliveira, R.; Cavalli, V.; Giannini, M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. Braz oral Res 2004; 18(4): 306-11.

The aim of this study was to evaluate the surface roughness, microhardness and morphology of human enamel exposed to six bleaching agents (at baseline and post-treatment). Human dental enamel samples were obtained from human third molars and randomly divided into seven groups (n = 11): control, Whiteness Perfect--10%

carbamide peroxide (10% CP), Colgate Platinum--10% CP, Day White 2Z--7.5% hydrogen peroxide (7.5% HP), Whiteness Super--3% CP, Opalescence Quick--35% CP and Whiteness HP--35% HP. Bleaching agents were applied according to manufacturers' instructions. The control group remained not treated and stored in artificial saliva. Microhardness testing was performed with a Knoop indentor and surface roughness was analyzed with a profilometer. Morphologic observations were carried out with scanning electron microscopy (SEM). Results were statistically analyzed by two-way analysis of variance and Tukey's test (5%), and revealed a significant decrease in microhardness values and a significant increase in surface roughness post-bleaching. Changes in enamel morphology after bleaching were observed under SEM. It was concluded that bleaching agents can alter the microhardness, roughness and morphology of dental enamel surface

8. Arcari, G. M.; Baratieri, L. N.; Maia, H. P.; De Freitas, S. F.T. Influence of the duration of treatment using a 10% carbamide peroxide bleaching gel on dentin surface microhardness: an in situ study. Quintessence Int 2005; 36(1): 15-24.

This in situ study evaluated the influence of two home-use tooth bleaching regimes (1 hour/day and 7 hours/day) using 10% carbamide peroxide (Nite White Excel 2Z) on the surface microhardness of dentin over a 21-day period. Nine blocks of dentin derived from the teeth of subjects, each with at least two thirds molar in need of extraction, were obtained from the cervical region and submitted to surface-hardness analysis (Shimadsu HMV/2000), fixed to an intraoral palate device fabricated for each subject, and positioned (3 left side, 3 right side, 3 center) according to experimental group. During the bleaching period, casts bearing the whitening agent were applied to the blocks for 7, 1, and 0 hours, respectively (7 left side, 3 right side, 0 center). After the treatment period, the specimens were once again submitted to microhardness

analysis, using the same equipment as before, and then analysis of variance and the Scheffé test were applied to the mean differences between the initial and final evaluations. The results demonstrated that the statistical difference between the 1-hour and 7-hour groups was not significant. However, the 7-hour group, when compared with the control group, demonstrated statistically significant differences with a reduction in microhardness. Despite the occurrence of mineral loss in the 1-hour and 7-hour groups, this difference was only 3.1% and 5.4%, respectively, which allows one to conclude that these values probably have no clinical significance.

9. Rees, T. D., Orth, C. F. Oral ulcerations with use of hydrogen peroxide. J Periodontol 1986; 57(11): 689-692.

Hydrogen peroxide has been advocated for many years as an oral rinse useful in control of various oral conditions. Several authorities, however, have suggested that this material may be harmful to oral tissues, especially if the tissues have been previously injured. This article presents two case reports demonstrating harmful oral effects from hydrogen peroxide rinses. The findings suggest that oral hygiene techniques emphasizing the use of 3% hydrogen peroxide in periodontal therapy may require reevaluation.

10. Mimura, M. A. M.; Hirota, S. K.; Sugaya, N. N.; Sanches Jr, J. A.; Migliari. Systemic treatment in severe cases of recurrent aphthous stomatitis: an open trial. Clinics 2009; 64(3): 193-98.

PURPOSE: This study aimed to evaluate the efficacy of the systemic drugs thalidomide, dapsone, colchicine, and pentoxifylline in the treatment of severe manifestations of RAS. METHODS: An open, 4-year clinical trial was carried out for 21 consecutive patients with severe RAS. Initially, patients were given a 2-week course of prednisone to bring them to a baseline status. Simultaneously, one of the

four test drugs was assigned to each patient to be taken for a period of 6 months. During the course of the trial, patients were switched to one of the other three drugs whenever side effects or a lack of satisfactory results occurred, and the 6-month limit of the treatment was then reset. RESULTS: The most efficient and best-tolerated drug was thalidomide, which was administered to a total of eight patients and resulted in complete remission in seven (87.5%). Dapsone was prescribed for a total of nine patients, of whom eight (89%) showed improvement in their symptoms, while five showed complete remission. Colchicine was administered to a total of ten patients, with benefits observed in nine (90%), of whom four showed complete remission. Pentoxyfilline was administered to a total of five patients, with benefits observed in three (60%), of whom one patient showed complete remission. CONCLUSION: The therapeutic methods used in this trial provided significant symptom relief. Patients experienced relapses of the lesions; however, this occurred after withdrawal of their medication during the follow-up period.

11. Scully, C.; Porter, S. Oral mucosal disease: recurrent aphthous stomatitis. Br Oral Maxillofac Surg. 2008; 46(3): 198-206.

Recurrent aphthous stomatitis (RAS; aphthae; canker sores) is common worldwide. Characterised by multiple, recurrent, small, round, or ovoid ulcers with circumscribed margins, erythematous haloes, and yellow or grey floors, it usually presents first in childhood or adolescence. Its aetiology and pathogenesis is not entirely clear, but there is genetic predisposition, with strong associations with interleukin genotypes, and sometimes a family history. Diagnosis is on clinical grounds alone, and must be differentiated from other causes of recurrent ulceration, particularly Behçet disease - a systemic disorder in which aphthous-like ulcers are associated with genital ulceration, and eye disease (particularly posterior uveitis). Management remains

unsatisfactory, as topical corticosteroids and most other treatments only reduce the severity of the ulceration, but do not stop recurrence.

12. Vane J & Botting R (1987) Inflammation and the mechanism of action of anti-inflammatory drugs The FASEB Journal: official publication of Federation of American Societies for Experimental Biology **1(2)** 89-96.

Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, and, more recently, platelet-activating factor (PAF) and interleukin-1. Evidence for their involvement comes from studies with competitive antagonists for their receptors and inhibitors of their synthesis. H_1 histamine antagonists are effective for hay fever and some skin allergies such as urticaria, which indicates the importance of histamine in these conditions. Symptoms of rheumatoid arthritis are alleviated by the aspirinlike anti-inflammatory drugs, which inhibit the cyclo-oxygenase enzyme and reduce synthesis of prostanoids. Corticosteroids prevent the formation of both PGs and LTs by causing the release of lipocortin, wich by inhibition of phospholipase.

14. Prussin C.; Metcalfe D. D. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2006; 117(2): 450-6.

IgE, mast cells, basophils, and eosinophils constitute essential elements in allergic inflammation. Allergen-specific IgE, synthesized in response to allergens in the environment, becomes fixed to FcepsilonRI on the membranes of mast cells and basophils. Aggregation of receptor-bound IgE molecules on re-exposure to specific allergen results in the production of mediators that produce the allergic response. Principal among the cells drawn to sites of mediator release is the eosinophil.

15. Aukhil I, Biology of Wound Healing, Periodontology 2000. 2000; 22:44-50.

Periodontal tissues represent a unique system in the human body where epithelial, soft and mineralized connective tissues come together to form a junction. This junction, referred to as the dentogingival junction, is a complex structure, and maintenance of the integrity of this junction is critical for the preservation of underlying bone and periodontal ligament. As per the classic description of wound healing, initially there is temporary repair characterized by the formation of a clot in the wounded tissues. Inflammatory cells followed by fibroblasts and endothelial cells then invade the clot to form a granulation tissue, while the epithelial cells migrate to cover the denuded surfaces (or form a junction at the tooth interface). Finally, maturation of the healing tissue matrix is seen along with contraction or scarring. This paper relates the biology of wound healing.

16. Vogler, B.K.; Ernst, E. Aloe vera: a systematic review of its clinical effectiveness. Br J Gen Pract. 1999; 49:823-828.

BACKGROUND: The use of aloe vera is being promoted for a large variety of conditions. Often general practitioners seem to know less than their patients about its alleged benefits. AIM: To define the clinical effectiveness of aloe vera, a popular herbal remedy in the United Kingdom. METHOD: Four independent literature searches were conducted in MEDLINE, EMBASE, Biosis, and the Cochrane Library. Only controlled clinical trials (on any indication) were included. There were no restrictions on the language of publication. All trials were read by both authors and data were extracted in a standardized, pre-defined manner. RESULTS: Ten studies were located. They suggest that oral administration of aloe vera might be a useful adjunct for lowering blood glucose in diabetic patients as well as for reducing blood lipid levels in patients with hyperlipidaemia. Topical application of aloe vera is not an effective preventative for radiation-induced injuries. It might be effective for genital

herpes and psoriasis. Whether it promotes wound healing is unclear. There are major caveats associated with all of these statements. CONCLUSION: Even though there are some promising results, clinical effectiveness of oral or topical aloe vera is not sufficiently defined at present.

17. Wynn, R.L. Aloe vera gel: update for dentistry. Gen Dent. 2005; 53(1): 6-9.

The fresh gel or mucilage from *Aloe barbadensis* Mill (family Liliaceae)- otherwise known as *aloe vera* – is a handy homegrown remedy that can be used both as a moisturizing agent and for the treatment of minor burns, skin abrasions, and irritations. It has been suggested that external application of aloe vera gel promotes wound healing. It has been reported that acemannan hydrogel accelerates the healing of aphthous ulcers and reduces the pain associated with them. This article is a review of the properties and effects of aloe vera.

18. Langmead, L.; Feakins, R. M.; Goldthorpe, S.; Holt, H.; Tsironi, E.; De Silva, A.; Jewell, D. P.; Rampton, D. S. Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. Aliment Pharmacol Ther 2004; 19: 739-47.

BACKGROUND: The herbal preparation, aloe vera, has been claimed to have anti-inflammatory effects and, despite a lack of evidence of its therapeutic efficacy, is widely used by patients with inflammatory bowel disease. AIM: To perform a double-blind, randomized, placebo-controlled trial of the efficacy and safety of aloe vera gel for the treatment of mildly to moderately active ulcerative colitis. METHODS: Forty-four evaluable hospital out-patients were randomly given oral aloe vera gel or placebo, 100 mL twice daily for 4 weeks, in a 2 : 1 ratio. The primary outcome measures were clinical remission (Simple Clinical Colitis Activity Index </= 2), sigmoidoscopic remission (Baron score </= 1) and histological remission

(Saverymuttu score </= 1). Secondary outcome measures included changes in the Simple Clinical Colitis Activity Index (improvement was defined as a decrease of >/= 3 points; response was defined as remission or improvement), Baron score, histology score, haemoglobin, platelet count, erythrocyte sedimentation rate, C-reactive protein and albumin. RESULTS: Clinical remission, improvement and response occurred in nine (30%), 11 (37%) and 14 (47%), respectively, of 30 patients given aloe vera, compared with one (7%) [P = 0.09; odds ratio, 5.6 (0.6-49)], one (7%) [P = 0.06; odds ratio, 7.5 (0.9-66)] and two (14%) [P < 0.05; odds ratio, 5.3 (1.0-27)], respectively, of 14 patients taking placebo. The Simple Clinical Colitis Activity Index and histological scores decreased significantly during treatment with aloe vera (P = 0.01 and P = 0.03, respectively), but not with placebo. Sigmoidoscopic scores and laboratory variables showed no significant differences between aloe vera and placebo. Adverse events were minor and similar in both groups of patients. CONCLUSION: Oral aloe vera taken for 4 weeks produced a clinical response more often than placebo; it also reduced the histological disease activity and appeared to be safe. Further evaluation of the therapeutic potential of aloe vera gel in inflammatory bowel disease is needed.

19. Langmead, L.; Makins, R.J.; Rampton, D. S. Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro. Aliment Pharmacol Ther 2004; 19: 521-27.

BACKGROUND: Oral aloe vera gel is widely used by patients with inflammatory bowel disease and is under therapeutic evaluation for this condition. AIM: To assess the effects of aloe vera in vitro on the production of reactive oxygen metabolites, eicosanoids and interleukin-8, all of which may be pathogenic in inflammatory bowel disease. METHODS: The anti-oxidant activity of aloe vera was assessed in two cell-free, radical-generating systems and by the chemiluminescence of incubated

colorectal mucosal biopsies. Eicosanoid production by biopsies and interleukin-8 release by CaCo2 epithelial cells in the presence of aloe vera were measured by enzyme-linked immunosorbent assay. RESULTS: Aloe vera gel had a dose-dependent inhibitory effect on reactive oxygen metabolite production; 50% inhibition occurred at 1 in 1000 dilution in the phycoerythrin assay and at 1 in 10-50 dilution with biopsies. Aloe vera inhibited the production of prostaglandin E2 by 30% at 1 in 50 dilution ($P = 0.03$), but had no effect on thromboxane B2 production. The release of interleukin-8 by CaCo2 cells fell by 20% ($P < 0.05$) with aloe vera diluted at 1 in 100, but not at 1 in 10 or 1 in 1000 dilutions. CONCLUSION: The anti-inflammatory actions of aloe vera gel in vitro provide support for the proposal that it may have a therapeutic effect in inflammatory bowel disease.

20. Cutle, L.; Kempf, M.; Kravchuck, O.; George, N.; Liu, P-Y.; Chang, H-E.; Mill, J.; Wang, X-Q.; Kimble, R. M. The efficacy of Aloe vera, tea tree oil and saliva as first aid treatment for partial thickness burn injuries. Burns 2008; 34: 1176-82.

Many alternative therapies are used as first aid treatment for burns, despite limited evidence supporting their use. In this study, Aloe vera, saliva and a tea tree oil impregnated dressing (Burnaid) were applied as first aid to a porcine deep dermal contact burn, compared to a control of nothing. After burn creation, the treatments were applied for 20 min and the wounds observed at weekly dressing changes for 6 weeks. Results showed that the alternative treatments did significantly decrease subdermal temperature within the skin during the treatment period. However, they did not decrease the microflora or improve re-epithelialisation, scar strength, scar depth or cosmetic appearance of the scar and cannot be recommended for the first aid treatment of partial thickness burns.

21. Somboonwong, J.; Jariyapongskul, A.; Thanamittramanee, S.; Patumraj, S. Therapeutic effects of *Aloe vera* on cutaneous microcirculation and wound healing in second degree burn model in rats. J Med Assoc Thai 2000; 83: 417-25.

OBJECTIVE: To demonstrate the microcirculatory and wound healing effects of Aloe vera on induced second degree burn wounds in rats. METHOD: A total of 48 male Wistar rats were equally divided into 4 groups as follows: sham controls, untreated burn-wound rats, those treated with once-daily application of normal saline (NSS) and those treated with once-daily application of lyophilized Aloe vera gel. The animals in each group were equally subdivided into 2 subgroups for the study of cutaneous microcirculation and wound healing on day 7 and 14 after burn. Dorsal skinfold chamber preparation and intravital fluorescence microscopic technique were performed to examine dermal microvascular changes, including arteriolar diameter, postcapillary venular permeability and leukocyte adhesion on postcapillary venules. RESULTS: On day 7, the vasodilation and increased postcapillary venular permeability as encountered in the untreated burn were found to be reduced significantly ($p < 0.05$) in both the NSS- and Aloe vera-treated groups, but to a greater extent in the latter. Leukocyte adhesion was not different among the untreated, NSS- and Aloe vera-treated groups. On day 14, vasoconstriction occurred after the wound had been left untreated. Only in the Aloe vera-treated groups, was arteriolar diameter increased up to normal condition and postcapillary venular permeability was not different from the sham controls. The amount of leukocyte adhesion was also less observed compared to the untreated and NSS- treated groups. Besides, the healing area of the Aloe vera-treated wound was better than that of the untreated and NSS- treated groups during 7 and 14 days after burn.

CONCLUSION: Aloe vera could exhibit the actions of both anti-inflammation and wound healing promotion when applied on a second degree burn wound.

22. Natah, S.S.; Hietanen, J.; Häyrinen-Immonen, R.; Jungell, P.; Malmström, M.; Konttinen, Y. T. Expression of cell proliferation-associated nuclear antigen (Ki-67) in recurrent aphthous ulcers (RAU). Oral Med Pathol. 1998; 3: 29-34.

The histological features of recurrent aphthous ulcer (RAU) are consistent with increased number and activation of T cells in the diseased areas. A previous study suggested that 13-23% of the cells express IL-2 and transferring receptors, which implies potential for local proliferation. The aim of the study was to evaluate the proliferation activity in 10 RAU lesions compared to 9 oral traumatic ulcer (TU) lesions. Demonstration of proliferating cells was performed by application of affinity-purified rabbit antin-human Ki-67 antibodies in avidin-biotin-peroxidase complex (ABC) staining to both RAU and TU biopsies. We found that the basal/suprabasal layers of RAU epithelium away from the ulcer contained 30% more proliferating cells (127 ± 24 vs 97 ± 19 cells/0.2 mm^2,$P<0.01$) than the epithelium of TU. Furthermore, $9\pm4\%$ (115 ± 29 cells/0.2 mm^2) of the infiltrating inflammatory cells in RAU lesions were Ki-67-positive proliferating cells, compared to only $3\pm1\%$ (37 ± 14 cells/0.2mm^2) in TU inflammatory infiltrates.These findings suggested that the mitotic activity in increased in RAU epithelium and that the relatively longer persistence of RAU cannot be explained by a lack of new epithelial cell production. Furthermore, mononuclear inflammatory cells replication *in situ* represent one mechanism for the expansion of the inflammatory cell infiltrates at sites of RAU and TU lesions.

23. Montes, G.S. Structural biology of the fibres of the collagenous and elastic systems. Cell Biol Int. 1996; 20(1): 15-27.

The different types of fibres of the collagenous and elastic systems can be demonstrated specifically in tissue sections by comparing the typical ultrastructural picture of each of the fibre types with studies using selective staining techniques for light microscopy. A practical modus operandi, which includes the recommended staining procedures and interpretation of the results, is presented. Micrographs and tables are provided to summarize the differential procedures. Reticulin fibres display a distinct argyrophilia when studied by means of silver impregnation techniques, and show up as a thin meshwork of weakly birefringent, greenish fibres when examined with the aid of the Picrosirius-polarization method. In addition, electron-microscopic studies showed that reticulin fibres are composed of a small number of thin collagen fibrils, contrasting with the very many thicker fibrils that could be localized ultrastructurally to the sites where non-argyrophilic, coarse collagen fibres had been characterized by the histochemical methods used. The three different fibre types of the elastic system belong to a continuous series: oxytalan-elaunin-elastic (all of the fibre types comprising collections of microfibrils with, in the given sequence, increasing amounts of elastin). The three distinct types of elastic system fibres have different staining characteristics and ultrastructural patterns. Ultrastructurally, a characteristic elastic fibre consists of two morphologically different components: a centrally located solid cylinder of amorphous and homogeneous elastin surrounded by tubular microfibrils. An oxytalan fibre is composed of a bundle of microfibrils, identical to the elastic fibre microfibrils, without amorphous material. In elaunin fibres, dispersed amorphous material (elastin) is intermingled among the microfibrils.

24. Walsh, L.J. Mast cells and inflammation. Crit Ver oral Biol Méd. 2003; 14(3): 188-98.

Mast cells are mobile granule-containing secretory cells that are distributed preferentially about the microvascular endothelium in oral mucosa and dental pulp. The enzyme profile of mast cells in oral tissues resembles that of skin, with most mast cells expressing the serine proteases tryptase and chymase. Mast cells in oral tissues contain the pro-inflammatory cytokine tumour necrosis factor-α in their granules, and release of this promotes leukocyte infiltration during envolving inflammation in several conditions, including lichen planus, gingivitis, pulpitis, and periapical inflammation, through induction of endothelial-leukocyte adhesion molecules. Mast cell synthesis and release of other mediators exerts potent immunoregulatory effects on other cell types, while several T-lymphocyte-derived cytokines influence mast cell migration and mediator release. Mast cell proteases may contribute to alterations in basement membranes in inflammation in the oral cavity, such as the disruptions that allow cytotoxic lymphocytes to enter the epithelium in oral lichen planus. A close relationship exists among mast cells, neural elements, and laminin, and this explains the preferential distribution of mast cells in tissues. Mast cells are responsive to neuropeptides and, through their interaction with neural elements, form a neural immune network with Langerhans cells in mucosal tissues. This facilitates mast cell degranulation in response to a range of immunological and non-immunological stimuli. Because mast cells play a pivotal role in inflammation, therapies that target mast cell functions could have value in the treatment of chronic inflammatory disorders in the oral cavity.

25. Junqueira, L.C.U.; Montes, G.S.; Sanchez, E.M. The influence of tissue section thickness on the study of collagen by the Picrosirius polarization method. Histochemistry. 1982, 74:153-156.

The influence of tissue section thickness on the color and intensity of birefringence displayed by collagen in tissue sections studied by means of the Picrosirius-polarization method, is reported in this paper. When dermal collagen sections of different thicknesses (ranging from 0.25 to 11 micrometers) were studied by this method, it became evident that not only did the intensity of birefringence increase proportionally to tissue section thickness, as was to be expected, but also a gradual shift in color from green through a yellow to red could be observed as tissue section thickness increased. The limitations of the Picrosirius-polarization method for the localization of collagen types I, II, and III in routinely used histological slides is discussed, showing that this method is useful for the study of the distribution of the different types of interstitial collagen in normal adult vertebrate organs.

26. Jettanacheawcheawchankit, S.; Sasithanasate, S.; Sangvanich, P.; Banlunara, W. Acemannan stimulates gingival fibroblast proliferation; expressions of keratinocyte growth factor-1, vascular endothelial growth factor, and type I collagen; and wound healing. J Pharmacol Sci. 2009, 109:525-531.

Aloe vera has long been used as a traditional medicine for inducing wound healing. Gingival fibroblasts (GFs) play an important role in oral wound healing. In this study, we investigated the effects of acemannan, a polysaccharide extracted from Aloe vera gel, on GF proliferation; keratinocyte growth factor-1 (KGF-1), vascular endothelial growth factor (VEGF), and type I collagen production; and oral wound healing in rats. [(3)H]-Thymidine incorporation assay and ELISA were used. Punch biopsy wounds were created at the hard palate of male Sprague Dawley rats. All treatments (normal saline; 0.1% triamcinolone acetonide; plain 1% Carbopol; and Carbopol containing 0.5%, 1%, and 2% acemannan (w/w)) were applied daily. Wounded areas and histological features were observed at day 7 after treatment. From our studies,

acemannan at concentrations of 2, 4, 8, and 16 mg/ml significantly induced cell proliferation ($P<0.05$). Acemannan concentrations between 2 - 16 mg/ml significantly stimulated KGF-1, VEGF, and type I collagen expressions ($P<0.05$). Wound healing of animals receiving Carbopol containing 0.5% acemannan (w/w) was significantly better than that of the other groups ($P<0.05$). These findings suggest that acemannan plays a significant role in the oral wound healing process via the induction of fibroblast proliferation and stimulation of KGF-1, VEGF, and type I collagen expressions.

27. Chithra, P.; Sajithlal, G.B.; Chandrakasan, G. Influence of Aloe vera on collagen characteristics in healing dermal wounds in rats Mol Cell Biochem. 1998, 181:71-76.

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. This end is achieved mainly by the synthesis of the connective tissue matrix. Collagen is the major protein of the extracellular matrix, and is the component which ultimately contributes to wound strength. In this work, we report the influence of Aloe vera on the collagen content and its characteristics in a healing wound. It was observed that Aloe vera increased the collagen content of the granulation tissue as well as its degree of crosslinking as seen by increased aldehyde content and decreased acid solubility. The type I/type III collagen ratio of treated groups were lower than that of the untreated controls, indicating enhanced levels of type III collagen. Wounds were treated either by topical application or oral administration of Aloe vera to rats and both treatments were found to result in similar effects.

28. Grindlay, D., Reynolds, T. The Aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. J Ethnopharmacol. 1986, 16: 117-51.

The mucilaginous gel from the parenchymatous cells in the leaf pulp of Aloe vera has been used since early times for a host of curative purposes. This gel should be

distinguished clearly from the bitter yellow exudate originating from the bundle sheath cells, which is used for its purgative effects. Aloe vera gel has come to play a prominent role as a contemporary folk remedy, and numerous optimistic, and in some cases extravagant, claims have been made for its medicinal properties. Modern clinical use of the gel began in the 1930s, with reports of successful treatment of X-ray and radium burns, which led to further experimental studies using laboratory animals in the following decades. The reports of these experiments and the numerous favourable case histories did not give conclusive evidence, since although positive results were usually described, much of the work suffered from poor experimental design and insufficiently large test samples. In addition some conflicting or inconsistent results were obtained. With the recent resurgence of interest in Aloe vera gel, however, new experimental work has indicated the possibility of distinct physiological effects. Chemical analysis has shown the gel to contain various carbohydrate polymers, notably either glucomannans or pectic acid, along with a range of other organic and inorganic components. Although many physiological properties of the gel have been described, there is no certain correlation between these and the identified gel components.

30. O´Donnell-Torney, J., DeBoer, C., Nathan C. Resistance of human tumor cells in vitro to oxidative cytolysis. Clin Inv. 1985; 76:80-86.

Nine human cell types, six of them malignant, displayed a marked resistance to lysis by hydrogen peroxide (LD50, 2-20 mM). Of the reactive oxygen intermediates generated extracellularly, only H2O2 lysed all the cell types. OH was lytic to one of four, OI- to one of one, and O-2 to none of four cell types tested. Resistance to oxidative lysis did not correlate with specific activity of catalase, glutathione (GSH) peroxidase, other peroxidases, or glutathione disulfide reductase, or with specific

content of GSH. Resistance to H2O2 seemed to occur via mechanisms distinct from those responsible for cellular consumption of H2O2. Consumption was inhibitable by azide and was probably due to catalase in each cell type. In contrast, resistance to oxidative lysis occurred via distinct routes in different cells. One cell type used the GSH redox cycle as the primary defense against H2O2, like murine tumors previously studied. Other cells seemed to utilize catalase as the major defense against H2O2. Nonetheless, with both catalase and the GSH redox cycle inhibited, all the human cells tested exhibited an inherent resistance to oxidative lysis, that is, resistance independent of detectable degradation of H2O2.

33. Almas, K.; Al-Harbi, M. The effect of a 10% carbamide peroxide home bleaching system on the gingival health. J Contemp Dent Pract. 2003; (4)1:32-41. Esthetics plays an important role in dentistry today. Because of an increased emphasis on beauty and health, cosmetic dentistry has been the thrust to the forefront of many practices. Many health products are used for bleaching teeth, but all side effects are not known. Tooth sensitivity and gingival irritation are the most common side effects, but they are typically mild and transient in nature. The aim of this investigation was to evaluate objectively the effect of 10% carbamide peroxide gel (Opalescence Utradent Inc, USA) on gingival health by measuring changes in Bleeding Index, Plaque Index, and Gingival Index. Eighteen subjects, 11 female and 7 male, age range 15-30 years (mean 24 years), were selected for the study as a convenient sample. Their teeth had either fluorosis, dental stains, smoking, or tetracycline staining. The Opalescence system was used as office monitored, at-home bleaching for three weeks. There was a statistically significant reduction in bleeding on probing (1% - 37%, p < or = 0.003), Plaque Index (4% - 50%, p < or = 0.000), and Gingival Index (2.5% - 34%, p < or = 0.002). Only two subjects reported tooth hypersensitivity, and none of the subjects

complained about gingival irritation. There was a positive change in tooth color as far as staining was concerned. Further research including randomized controlled, double blind clinical trials is needed to confirm these findings and to examine other factors related to bleaching of teeth.

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- Journal article: special issue/supplement

  Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **(Supplement 6)** 119-144.

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