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EFEITO ANTINOCICEPTIVO, ANTIINFLAMATÓRIO E ANTIOXIDANTE DA *Aloe saponaria* Haw EM MODELOS DE QUEIMADURA EM RATOS

TESE DE DOUTORADO

Mariane Arnoldi da Silva

Santa Maria, RS, Brasil,

2013

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Por

Mariane Arnoldi da Silva

Tese apresentada no curso de Doutorado do Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutor em Ciências Biológicas: Bioquímica Toxicológica**.

Orientador: Prof. Dr. Juliano Ferreira

Santa Maria, RS, Brasil

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Universidade Federal de Santa Maria
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A comissão examinadora, abaixo assinada, aprova a Tese de Doutorado

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elaborada por

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como requisito parcial para obtenção do grau de

Doutor em Bioquímica Toxicológica

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Santa Maria, 19 de Dezembro de 2013.

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“Viver no mundo sem tomar consciência do significado do mundo é como vagar por uma imensa biblioteca sem tocar os livros.”

Os ensinamentos Secretos de Todos os Tempos

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria, RS, Brasil

EFEITO ANTINOCICEPTIVO, ANTIINFLAMATÓRIO E ANTIOXIDANTE DA *Aloe saponaria* Haw EM MODELOS DE QUEIMADURA EM RATOS

AUTOR: Mariane Arnoldi da Silva
ORIENTADOR: Juliano Ferreira
LOCAL E DATA DA DEFESA: Santa Maria, 19 de dezembro de 2013.

Apesar da planta *Aloe saponaria* Haw, conhecida popularmente como "babosa pintadinha", ser utilizada na medicina popular brasileira devido ao seu efeito benéfico sobre lesões por queimaduras de pele, não existem dados científicos que confirmem o seu uso popular. Dessa forma o objetivo do presente estudo foi investigar os efeitos da *A. saponaria* em relação aos parâmetros nociceptivos, inflamatórios e oxidantes em modelos de lesão térmica em ratos. Para isso foram utilizados ratos Wistar machos adultos submetidos ou não à lesão térmica (causada pela imersão da pata em água a 37 ou 70 ºC durante 8 ou 5 segundos em animais anestesiados) ou queimaduras solares (induzida por radiação ultravioleta B (UVB) em animais anestesiados). Os animais foram topicamente tratados com o veículo (creme base - Lanete), sulfadiazina de 1% (controle positivo) ou com extrato de *A. saponaria* (0,3-30%) uma vez por dia, durante 2 ou 6 dias. Cada dia, 30 minutos antes do próximo tratamento, foram avaliados parâmetros nociceptivos (alodínia mecânica estática e dinâmica, alodínia térmica e dor espontânea), parâmetros inflamatórios (edema da pata) e estresse oxidativo (aumento dos níveis de peróxido de hidrogênio-H_2O_2, proteína carbonilada ou peroxidação lipídica e redução dos níveis de tióis). Além disso, em alguns tempos após os diferentes estímulos também foi avaliado a infiltração de leucócitos no tecido lesado (por análise histológica ou pela medida das atividades da mieloperoxidase - MPO, N-acetil-glucosaminidase - NAGase e eosinoperoxidase - EPO para neutrófilos, macrófagos e eosinófilos, respectivamente). Com diferentes eficácias temporais de ação, o tratamento tópico com o creme de *Aloe saponaria* (10%) ou de sulfadiazina (1%) reduziu a nocicepção, o edema e a infiltração de leucócitos nos animais com lesão térmica induzida tanto por água aquecida, quanto por radiação UVB. Além disso, o tratamento com *A. saponaria* reduziu o estresse oxidativo da pele de animais

irradiada com UVB, um efeito que parece ser devido a ação antioxidante produzida tanto pelo extrato de *A. saponaria*, quanto por alguns de seus constituintes (aloína e rutina). Dessa forma, os nossos resultados demonstram que a aplicação tópica de *A. saponaria* apresentou efeitos antinociceptivos, antiinflamatórios e antioxidantes em modelos de lesão térmica (água aquecida ou radiação solar), o que confirma os benefícios do seu uso tradicional para lesões por queimaduras.

Palavras-chave: Aloe saponaria, aloína, queimadura, inflamação, nocicepção, estresse oxidativo, sulfadiazina de prata.

ABSTRACT

PhD Thesis
Graduate Course in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF *Aloe saponaria Haw* IN A MODEL OF BURN IN RATS

AUTHOR: Mariane Arnoldi da Silva
ADVISOR: Juliano Ferreira
PLACE AND DATE OF THE DEFENSE: Santa Maria, December, 19th, 2013.

In Brazil, the plant *Aloe saponaria* Haw, popularly known as “babosa pintadinha”, has been empirically used for its potential effect on skin burn injury, there are no scientific data confirming its popular use. Thus, the aim of the present study was to investigate the effects of *Aloe saponaria* on nociceptive, inflammatory and antioxidants parameters in rat models of thermal injury. Adult male Wistar rats were subjected to a thermal injury (immersion in water at 70 or 37 ºC, respectively, for 5 or 8 seconds) or sunburn (induced by UVB irradiation), in both experiments the animals were anesthetized. Burned animals were topically treated with vehicle (base cream), sulfadiazine 1% (positive control) or *Aloe saponaria* cream (0.3-30%) once a day for 2 or 6 days. Each day, 30 min before the treatment, we measured nociceptive (static and dynamic mechanical allodynia, thermal allodynia and spontaneous pain), inflammatory (paw edema) parameters and oxidative stress (increases in H_2O_2, protein carbonyl levels and lipid peroxidation and a decrease in thiol content). In addition, was also evaluated infiltration of leukocytes in injured tissue (for histology or by measuring the activity of myeloperoxidase (MPO), N - acetyl - glucosaminidase (NAGase) and eosinoperoxidase (EPO), for neutrophils, macrophages, and eosinophils infiltration, respectively), were also determined 2 or 6 days after the thermal injury. With different efficacies time of action, the topical treatment with the *Aloe saponaria* cream (10%) and sulfadiazine (1%) decreased nociception, edema and leukocyte infiltration in animals induced by either thermal injury scald burn), or by UVB radiation. Moreover, the treatment with *A. saponaria* reduced the oxidative stress of the skin of animals irradiated with UVB, an effect that appears to be due to the antioxidant action produced by both the extract of *A. saponaria*, and by some of these constituents (rutin and aloin).

Thus, our results demonstrate that topical application of *A. saponaria* showed antinociceptive, anti-inflammatory and antioxidant models of thermal injury (scald burn or solar radiation), which confirms the benefits of its traditional use for burn injuries effects.

Keywords: *Aloe saponaria,* aloin, burn, inflammation, nociception, oxidative stress, silver sulfadiazine.

LISTA DE FIGURAS E TABELAS

ARTIGO 1.

MANUSCRITO.

LISTA DE ABREVIATURAS

DNA	Ácido desoxirribonucléico
EPO	Eosinoperoxidase
ER's	Espécies reativas
ERO's	Espécies reativas de oxigênio
H_2O_2	Peróxido de hidrogênio
IASP	Associação Internacional para o Estudo da Dor (IASP)
MPO	Mieloperoxidase
NAGase	N-acetil-glucosaminidase
$O_2^{-\cdot}$	Radical superóxido
$OH^{\cdot}$	Radical hidroxila
UVA	Radiação ultravioleta A
UVB	Radiação ultravioleta B
UVC	Radiação ultravioleta C

SUMÁRIO

1. APRESENTAÇÃO

No item **INTRODUÇÃO** consta uma revisão sucinta da literatura sobre os temas abordados nesta tese.

A metodologia realizada e os resultados obtidos que compõem esta tese estão apresentados sob a forma de artigo científico publicado, o qual se encontra no item **ARTIGOS**. As seções Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no próprio artigo e representam a íntegra deste estudo.

O item **CONCLUSÕES** é encontrado no final desta tese e apresenta interpretações e comentários gerais sobre os artigos científicos contido neste trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem no item **INTRODUÇÃO** desta tese.

2. INTRODUÇÃO

2.1. Dor inflamatória

A dor é um dos principais sintomas clínicos que levam os indivíduos a procurarem os serviços primários de saúde. Esta é considerada uma resposta fisiologicamente benéfica ao organismo, pois é capaz de alertar os indivíduos para a ocorrência de alterações na sua integridade e funcionabilidade, permitindo que sejam adotados mecanismos de defesa ou de fuga (este tipo de dor é chamada de nociceptiva). Porém, a dor também possui características deletérias e desagradáveis associadas, como: sofrimento, estresse, prejuízo nas relações sociais e econômicas, tornando-se um problema que deve ser rapidamente e efetivamente tratado (BRENANN et al., 2007).

A Associação Internacional para o Estudo da Dor define a dor, como sendo: “uma experiência sensorial e emocional desagradável, associada a uma lesão tecidual real ou potencial, ou descrita em termos que sugerem tal lesão” (MERSKEY e BOGDUK, 1995; LOESER e TREEDE, 2008). Além disso, é importante definir, que mesmo o paciente sendo incapaz de comunicar-se verbalmente, isso não exclui a possibilidade do mesmo estar enfrentando um quadro doloroso, sendo necessário um tratamento adequado para o alívio da dor (CRAIG, 2009).

Enquanto a dor envolve a percepção de um estímulo aversivo, a nocicepção é um termo fisiológico usado para descrever o processo neural de codificação e processamento do estímulo nocivo. A nocicepção é a progenitora da dor, experiência complexa e subjetiva que, por sua vez, causa o sofrimento. Contudo, a nocicepção não é uma sensação uniforme, e a qualidade da dor e o início das respostas protetoras são determinados por muitos fatores na medula espinhal e em estruturas supra-espinhais envolvidas na integração e modificação dos sinais nociceptivos (LOESER e TREEDE, 2008).

A capacidade do sistema somatossensorial em detectar estímulos nocivos e estímulos que potencialmente lesionam os tecidos é um importante mecanismo protetor que envolve múltiplos mecanismos centrais e periféricos, sendo definidos como nocicepção (LOESER e TREEDE, 2008). Além desses efeitos sensoriais, a percepção e a experiência subjacente da “dor” são multifatoriais e influenciadas por fatores físicos e psicológicos. Fatores psicológicos que influenciam a experiência da dor incluem os processos de atenção e outros processos cognitivos (memória/aprendizado, processamento do pensamento, crenças e humor), respostas comportamentais, e interações com outras pessoas. A dor pode ser um indicador de dano tecidual, mas pode também ser experimentada na ausência de alguma causa identificável (MACINTYRE, 2010).

A detecção de estímulos nocivos, sendo eles: térmicos, mecânicos e químicos requerem que ocorra a ativação dos nociceptores, os quais são amplamente distribuídos por todo o corpo (pele, músculos, articulações, vísceras, meninges e etc.) e que agem transmitindo, tanto a informação nociceptiva, quanto a informação não nociceptiva para a medula espinal (JULIUS e BASBAUM, 2001; GOLD e GEBHART, 2010; KUNER, 2010).

A dor pode ser causada por diferentes fatores. A dor inflamatória é um tipo de dor adaptativa e protetora, porém nessa situação a sensibilidade sensorial é acrescida após o dano aos tecidos, de maneira a auxiliar a recuperação do local lesado (LOESER e TREEDE, 2008; WOOLF, 2010). A inflamação é uma reação do organismo (tecido) frente a diferentes tipos de lesões, caracterizando-se classicamente pelo desenvolvimento de edema (tumor), dor, vermelhidão (rubor), calor, e em alguns casos, também pela perda de função do tecido afetado (LOESER e TREEDE, 2008; WOOLF, 2010). Neste caso, a hipersensibilidade aos estímulos

potencialmente nocivos auxilia a reduzir danos posteriores e também promove a recuperação, assim estímulos anteriormente inócuos podem ser percebidos como dolorosos. Dessa forma, os pacientes relatam a sensação de dor a estímulos anteriormente inócuos (dor a estímulos não nociceptivos denominada alodínia), ou o aparecimento de hiperalgesia que é considerada como a percepção exacerbada da dor a estímulos anteriormente descritos como dolorosos. Finalmente, a dor pode ainda aparecer espontaneamente e sem necessidade de estimulação externa, podendo ser descrita como dor em queimação ou choque (LOESER e TREEDE, 2008; COSTIGAN et al., 2009; WOOLF, 2010).

Esses comportamentos podem ser resultados da ativação de células residentes, como os mastócitos, da infiltração de células inflamatórias (neutrófilos e macrófagos), e da liberação de inúmeros mediadores inflamatórios (cininais, aminas, prostanóides, fatores de crescimento e citocinas, que em conjunto com prótons e trifosfato de adenosina compõem uma “sopa inflamatória” que promove redução do limiar e amplificação na resposta dos nociceptores que inervam o tecido inflamado (sensibilização periférica). Além destas alterações periféricas, pode também ocorrer uma resposta aumentada dos neurônios nociceptivos no sistema nervoso central pelos estímulos aferentes normais ou sublimiares (sensibilização central) (SCHOLZ e WOOLF, 2001; LOESER e TREEDE, 2008; LATREMOLIERE e WOOLF, 2009; WOOLF, 2010).

A dor inflamatória é causada pela ativação do sistema imune após lesão tecidual ou infecção, e esta forma de dor é de fato uma das características principais da inflamação. Mesmo que esta dor seja adaptativa, ainda deve ser reduzida em pacientes acometidos por essa lesão, como no caso de pacientes acometidos por

processos térmicos (queimaduras) e em casos de lesão extensa ou grave (CHANDRATRE et al., 2013; WOOLF, 2010).

2.2. Queimadura

No Brasil, as queimaduras representam um agravo significativo à saúde pública. São observados em torno de um milhão de casos de lesões térmicas a cada ano, sendo que desses casos, 200 mil são atendidos em serviços de emergência e 40 mil são conduzidos para a hospitalização, segundo a Sociedade Brasileira de Cirurgia Plástica (PICCOLO et al., 2008). Em meio a essas estatísticas, as queimaduras mais comuns, tendo as crianças como vítimas, estão às decorrentes de escaldamentos (manipulação de líquidos quentes, como água fervente, pela curiosidade característica da idade) e as que ocorrem em casos de violência doméstica. Por sua vez, entre os adolescentes e adultos jovens (17 a 35 anos) o agente térmico é o contato direto com as chamas e queimaduras solares (DUPONT, 2012).

As queimaduras são conceituadas como lesões dos tecidos orgânicos em decorrência de trauma de origem térmica resultante da exposição ou contato com chamas, líquidos e superfícies quentes, eletricidade, frio, substâncias químicas, radiação, atrito ou fricção. Dentre os agentes causadores de queimaduras, as causas mais comuns, são pelo contato com líquidos superaquecidos, chamas e radiação solar (BISHOP et al., 2007; 2009; EVERS et al., 2010).

A queimadura solar, causada pela exposição à radiação ultravioleta, é principalmente desenvolvida pela radiação UVB (BISHOP et al., 2009). A luz ultravioleta é subdividida em três faixas de comprimento de onda denominadas radiação ultravioleta A (UVA - 320-400 nm), que é capaz de penetrar mais

profundamente atingindo a derme, causa indução de necrose de células endoteliais, danos nos vasos sanguíneos e degradação de colágeno. Já a radiação ultravioleta C (UVC - 100-280 nm), a qual são eficientemente bloqueadas pela atmosfera, e dessa forma não atingem a pele, e por último temos a radiação ultravioleta B (UVB - 290-320 nm), afeta a camada basal da epiderme, provocando lesões no ácido desoxirribonucléico (DNA) e danos as proteínas (PORTUGAL-COHEN et al., 2009). Dentre as três bandas, a UVB é a responsável pela maioria dos danos agudos e crônicos, resultando em uma variedade de respostas na pele (PORTUGAL-COHEN et al., 2009; SWALWELL et al., 2012).

As lesões são altamente variáveis em termos de tecido afetado, gravidade e complicações resultantes. Dentre os órgãos afetados, a pele é o principal e o mais atingido nesse tipo de lesão. A pele é considerada o maior órgão do corpo humano, recobrindo e resguardando a superfície corporal contra agentes físicos, químicos ou bacterianos, e da perda de água pelo corpo. Além disso, possui também funções imunitárias, e é o principal órgão responsável pela manutenção da temperatura corporal e também nervosas, detectando diferentes sensações corporais, como as manifestações táteis e das dolorosas. A pele é composta por epiderme (camada mais externa), derme (camada subjacente a epiderme), e hipoderme (camada mais profunda) (CORMACK, 2008).

A principal classificação das queimaduras é referente à profundidade da ferida, sendo dividida em três tipos: queimaduras de primeiro, segundo e terceiro grau.

- A queimadura de primeiro grau (espessura superficial) – eritema solar: é caracterizada por atingir apenas a epiderme, não há formação de bolhas no local. A

região afetada apresenta-se hiperemiada, com desenvolvimento de edema e dor, sendo também observado uma descamação em 3 a 7 dias após a queimadura;

- Segundo grau (espessura parcial – superficial e profunda): afeta a epiderme e parte da derme, observa-se o desenvolvimento de bolhas no local lesionado. As bolhas são divididas em dois tipos de acordo com a profundidade da lesão: superficial (base da bolha é rósea, úmida e dolorosa) ou profunda (base da bolha é branca, seca, indolor e menos dolorosa). A recuperação ocorre num período que pode variar entre 7 a 21 dias após a lesão;
- Terceiro grau (espessura total): são lesões que afetam todas as camadas da pele, e em muitos pacientes ocorrem lesões em estruturas mais profundas, como: músculos, nervos e até mesmo tecido ósseo. É um dano tecidual indolor e com aspecto esbranquiçado ou em alguns casos enegrecida e rígido (redução da elasticidade tecidual), além disso, é um dano tecidual em que não ocorrerá reepitelização (EVERS et al., 2010; GRAVANTE et al., 2006).

A profundidade da lesão depende do agente térmico envolvido e da duração que o paciente ficar exposto a este agente. No caso, de queimaduras por líquidos quentes e radiação solar, são geralmente lesões superficiais, ao passo que em lesões pelo contato com chamas tendem a ser mais profundas (SUMMER et al., 2007; RiICHARDSON et al., 2009).

As queimaduras por escaldamento ou solar geram enormes reações fisiológicas no organismo, sendo a principal a resposta imunológica no organismo. Lesões térmicas geram um grande processo inflamatório, que inicia-se poucos minutos após a lesão e que estão associados com a liberação de mediadores inflamatórios, os quais causam tanto efeitos locais, quanto sistêmicos (PARIHAR et al., 2008; RICHARDSON e MUSTARD, 2009). Sabe-se que o contato com água

quente, em uma temperatura acima de 40ºC, leva a desnaturação de proteínas e perda da integridade da membrana plasmática das células (EVERS et al., 2010). É também possível encontrar uma grande variedade de tipos celulares nessas lesões, tais como: plaquetas, linfócitos, neutrófilos, macrófagos e fibroblastos (HORTON et al., 2003). Esse processo inflamatório é também composto, pela liberação de inúmeros mediadores locais, como: serotonina, bradicinina, óxido nítrico, produtos da cascata do ácido eicosanóide (prostaglandinas e tromboxanos), fator de necrose tumoral, interleucinas, histamina e ERO e radicais livres de nitrogênio (HORTON et al., 2003; PARIHAR et al., 2008).

Em pacientes com queimaduras já foi observado à presença de um desequilíbrio do estado redox celular, caracterizado como estresse oxidativo. Neste caso, as espécies reativas (ER) são mecanismos importantes na manutenção e desenvolvimento do processo patológico. Em lesões térmicas, foi observado que a liberação de ERO nos tecidos lesados inicia-se com a ativação da NADPH oxidase, que reduz o oxigênio em $O_2^{-\cdot}$. Apesar de desempenharem um papel na morte bacteriana, as espécies reativas de oxigênio, também causam danos ao tecido, devido à produção excessiva durante as condições inflamatórias (HORTON et al., 2003).

As espécies reativas de oxigênio são resultado da ativação de neutrófilos e, além disso, a histamina presente é responsável, pelo aumento da permeabilidade vascular progressiva, o que está relacionado com o desenvolvimento de edema nesses pacientes (HOSNUTER et al., 2004; PARIHAR et al., 2008). Esse aumento da produção e liberação de ERO pode ocasionar uma exacerbação nessa lesão, já que esses radicais livres causam dano tecidual, principalmente devido à ocorrência de peroxidação lipídica. O que é facilitado, pela redução das defesas antioxidantes

do organismo, tornando a lesão mais suscetível as espécies reativas de oxigênio (HOSNUTER et al., 2004; PARIHAR et al., 2008).

Além disso, em inúmeros tecidos, como pele, plasma, fígado, pulmão, a queimadura está associada ao desenvolvimento de peroxidação lipídica, que mostrou-se uma importante causa de dano oxidativo das membranas celulares e, eventualmente morte celular (SENER et al., 2002). E Bertin – Maghit e colaboradores (2002), demonstraram o envolvimento do estresse oxidativo na lesão por queimadura em modelos animais e estudos em humanos, mais uma vez evidenciando a importância do estresse oxidativo, no processo pós lesão térmica.

Um fator importante observado, nesses pacientes é o aparecimento de um quadro doloroso. Essa dor é percebida no momento e local da lesão, devido à estimulação dos nociceptores locais e transmissão do impulso nervoso para a medula espinhal, além disso, quando a dor da queimadura aguda não for controlada pode ocorrer um aumento na incidência da dor crônica (RICHARDSON e MUSTARD, 2009). A dor é um dos principais e o mais desagradável sintoma observado pelos pacientes com lesões térmicas, sendo observado o desenvolvimento de alodínia mecânica estática e dinâmica nesses pacientes, os quais são observados quando em contato com um estímulo que antes era inócuo. Também são observados quadros de dor espontânea, ou seja, o paciente sente dor, mesmo sem entrar em contato com nenhum estímulo (BISHOP et al., 2009). Além dos parâmetros citados acima, são também observados um aumento de mediadores inflamatórios e edema (TANAKA et al., 1999; SASAKI et al., 2011). A participação de ERO na produção da dor foi inicialmente evidenciada em estudos *in vitro,* onde o peróxido de hidrogênio (H_2O_2), radical superóxido ($O_2^{-}\cdot$) e radical hidroxila ($OH^{\cdot}$) estimularam nociceptores (terminações periféricas livres de fibras sensoriais

sensíveis a estímulos nocivos) cardíacos e cutâneos, especialmente após isquemia ou aplicação de mediadores inflamatórios (KRESS et al., 1995; HUANG et al, 1995).

Devido a isso, os sistemas celulares desenvolveram uma série de mecanismos fisiológicos capazes de neutralizar as espécies reativas, convertendo-a em outras moléculas menos reativas, representando os mecanismos antioxidantes do organismo animais (PEREZ et al., 2003; HACIMUFTUOGLU et al., 2006). Em relação aos mecanismos de defesa do organismo, já foi evidenciado que o tratamento com substâncias antioxidantes promovem uma melhora do quadro clínico de processos inflamatório, e pós queimadura, tanto em humanos, bem como em modelos animais, apresentando efeitos benéficos na redução do edema causado pela queimadura, sugerindo uma relação de causa e efeito (YOUN et al., 1992; PEREZ et al., 2003; HACIMUFTUOGLU et al., 2006). E estudos *in vivo* demonstraram que o tratamento sistêmico com antioxidantes reduz a nocicepção produzida por diferentes estímulos agressivos (GUEDES et al., 2006; KIM et al., 2004). Outros estudos também apresentaram, um efeito benéfico após o uso de vitamina C em modelo animal em ratos, em que o edema pós-queimadura foi reduzido (TANAKA et al., 1999), evidenciando os benefícios da utilização de antioxidantes durante o estresse oxidativo após queimaduras (ROCK et al., 1997).

Como observado, o processo patofisiológico que é originado a partir de uma queimadura, é extremamente complexo, e envolve vários fatores. A dor (aguda) é provavelmente a mais difícil de tratar, principalmente, porque estudos demonstram que o próprio tratamento curativo causam dor, podendo muitas vezes se equiparar a dor causada pela própria lesão, além disso, essa dor também está de certa forma relacionada com todo o estresse que o paciente foi acometido e todos os transtornos pós-traumáticos (de JONG et al., 2007). O tratamento desses pacientes é

extremamente complexo e envolve diferentes terapêuticas, sendo elas desde a utilização de analgésicos simples, analgésicos opióides, até mesmo a necessidade de intervenção cirúrgica (RICHARDSON e MUSTARD, 2009; EVERS et al., 2010). Tratamentos esses, que devem ser bem avaliados pelos clínicos, devido a toda alteração patofisiológica que ocorre devido ao processo de queimadura, o que dificulta ainda mais o tratamento desses pacientes (RICHARDSON e MUSTARD, 2009).

Assim, a utilização de substâncias exógenas, com capacidade antioxidante, em quadros de dor patológica pode ser extremamente benéfica na modulação desse desequilíbrio do estado redox celular, responsável pelo quadro doloroso nesses pacientes. Dessa forma, um aumento da disponibilidade das terapias antioxidantes seria de considerável interesse para os clínicos, no tratamento da dor e inflamação observadas em queimaduras.

2.3. *Aloe saponaria*

Atualmente, a procura por novas substâncias que possam ser utilizadas para a melhoria da qualidade de vida da sociedade tem despertado o interesse principalmente das indústrias farmacêuticas por plantas empregadas na medicina popular. Desta forma, a abordagem etnofarmacológica é de grande importância, pois combina informações adquiridas nas comunidades locais que fazem uso da flora medicinal com estudos fitoquímicos e/ou farmacológicos, o que torna também mais fácil determinar a real eficácia desses produtos utilizados pela população.

A utilização dos remédios tradicionais e plantas medicinais no tratamento de queimaduras e feridas é um aspecto importante do tratamento e como forma de reduzir os encargos financeiros. Vários produtos naturais têm sido relatados como

eficazes para o tratamento de doenças de pele, incluindo lesões ocasionadas por queimaduras, como o mel (DAVIS e PEREZ, 2009; BILSEL et al., 2002). Outro produto extremamente relacionado com o tratamento de queimaduras são as da espécie *Aloe* (KHORASANI et al., 2009, BEDI e SHENFELT, 2002, DAVIS e PEREZ, 2009).

Desde 1986, a *Aloe vera* (*Aloe barbadensis* Miller), pertencente à família *Xanthorraceae*, tem sido usada como um produto da medicina tradicional para diferentes doenças, principalmente relacionadas à pele, e como ingrediente em muitos produtos cosméticos (DAS et al., 2010;), podendo ser incorporada em géis e/ou cremes (GALLHAGH et al., 2003). Essas preparações vêm sendo utilizadas desde a antiguidade para o tratamento de diabetes, níveis elevados de lipídeos sanguíneos em humano, e em lesão causada por queimaduras (DAS et al., 2010). Muitos estudos vêm ao longo dos tempos relatando uso e os benefícios do tratamento com *Aloe*. Relatos de casos e estudos em animais têm demonstrado que a *Aloe vera* reduz lesões térmicas, prurido e cicatrizes associadas com dermatite por radiação (KLEIN e PENNEYS, 1988; BEDI e SHENEFELT, 2002).

Os produtos da *Aloe* têm sido utilizados para aliviar o processo inflamatório e acelerar a cicatrização causa por lesões térmicas (PARK et al., 2008). São também encontrados como substratos ativos nas *Aloe,* principalmente antraquinonas (aloína) e polissacarídeos (PARK et al., 2008). Além disso, estudos sugerem que *Aloe vera,* ou um dos seus componentes, promovem a melhora de lesões em diferentes tipos de modelos animais (MAENTHAISONG et al., 2007). Dentre os efeitos observados da *Aloe vera* estão: ação antimicrobiana, antifúngica, antiinflamatória e antioxidante (ZAPATA et al., 2013, PARK et al., 2009). Em continuação, Roberts e Trevis, em 1995, demonstraram que o tratamento com *Aloe vera* proporciona um efeito

cicatrizante em lesões, provenientes da exposição a radiação (ROBERTS e TREVIS, 1995).

Trabalhos *in vivo* e *in vitro* com animais demonstram na *Aloe vera* a presença salicilatos, que agem como agentes analgésicos e antiinflamatórios pela inibição da produção de prostaglandinas. Foi também observado a presença de lactato de magnésio, que está relacionado com o seu efeito anti–prurido, pela inibição da descarboxilase histidina, controlando a transformação em histamina. Também têm sido ressaltadas atividades bactericidas e antifúngicas da planta *in vitro* (STEPHEN et al., 2009).

Em 1996, Bunyapraphatsara e coloboradores, demonstraram que o tratamento com creme de *Aloe vera* (50%) foi mais efetivo em tratar camundongos com queimaduras de 1º, 2º e 3º grau, do que os que foram tratados com o gel fresco de *Aloe vera.* Provavelmente, porque o creme base aumente a absorção da droga e a atividade, tenha sido mais bem preservada. Efeito este, que foi reforçado com o estudo clínico desenvolvido por Khorasani e colaboradores, em 2009 onde se observou claramente, uma maior eficiência do tratamento com o creme de *Aloe vera* em pacientes com queimaduras de segundo grau, quando comparados com os tratados com sulfadiazina de prata 1% (KHORASANI et al., 2009).

O dano tissular causado por queimaduras inicia uma cascata de eventos, dentre elas está à ativação e migração de leucócitos da corrente sanguínea para o local da lesão. Em relação a estudos com a *Aloe vera,* tem-se demonstrações de que planta causa uma redução na adesão leucocitária no endotélio de ratos submetidos a modelos de queimaduras (DUANSAK et al., 2003). O que demonstra mais uma vez os efeitos antiinflamatórios da espécie *Aloe.* As lesões térmicas como já citado, geram um grande processo inflamatório, observado também pelo aumento

da adesão leucocitária, e a produção de citocinas inflamatórias como o TNF-α e a IL-6 (DUANSAK et al., 2003; PARIHAR et al., 2008; RICHARDSON e MUSTARD, 2009), e estes parâmetros foram reduzidos após, o uso tópico da planta *Aloe vera* (DUANSAK et al., 2003).

Além do processo inflamatório gerado pela lesão causada por agente térmico, foi observado um aumento da produção e liberação de radicais livres os quais podem ocasionar uma exacerbação nessa lesão, e que é facilitado, pela redução das defesas antioxidantes do organismo (HOSNUTER et al., 2004; PARIHAR et al., 2008). Trabalhos anteriores demonstraram que tanto a *Aloe vera* quanto a *Aloe ferox*, reduzem o estresse oxidativo, demonstrando seu potencial antioxidante (HU et al., 2003; LOOTS et al., 2007).

No entanto, apesar da grande quantidade de trabalhos demonstrando os efeitos do uso da *Aloe vera*, poucos são os que demonstram os efeitos da *Aloe saponaria*. Pertencente a mesma família, e conhecida popularmente como: “babosa pintadinha”, tem sido utilizada popularmente como tratamento para queimaduras (SOARES et al., 2004). A *Aloe saponaria* é uma planta originária do sul da África, encontrada também no sul do Brasil, e primeiramente, tende a ser cultivada como uma planta ornamental (SIMÕES et al., 1989; SOARES et al., 2004). Em relação às formas de utilização da *Aloe saponaria,* são encontradas diferentes tipos de preparos e para diferentes utilizações. Essas preparações vão desde cremes, pomadas, até mesmo chás (SOARES et al., 2004). Porém nenhum estudo científico comprovou o seu efeito para esse tipo de lesão, como já foi demonstrado com as outras plantas da mesma espécie. Yoo e colaboradores em 2008, que o extrato da *Aloe* saponaria apresenta efeitos antioxidantes, antinociceptivo e antiinflamatório, em animais submetidos a neuropatia por administração de cisplatina. Do mesmo

modo, a planta também apresenta efeitos antitumorais, inibindo tanto a ativação celular, como sua proliferação (SAMPEDRO et al., 2004).

Devido à quantidade escassa de trabalhos envolvendo a planta *Aloe saponaria*, e a sua utilização para tratamentos de enfermidades pela população, tornam-se extremamente relevantes, estudos que consigam demonstrar os seus efeitos em diversos tipos de lesões.

3. OBJETIVOS

3. OBJETIVOS

3.1 Objetivo Geral

Avaliar o efeito anti-nociceptivo, anti-inflamatório e antioxidante do extrato da *Aloe saponaria* em modelos de queimaduras em ratos.

3.2 Objetivos Específicos

1. Verificar os efeitos anti-nociceptivos e anti-inflamatórios do extrato da *Aloe saponaria* em modelos de queimaduras causada por água quente e irradiação UVB na pata de ratos;
2. Analisar o efeito antioxidante do extrato e dos principais compostos presentes na *Aloe saponaria* em modelo de queimadura por irradiação UVB na pata de ratos *in vitro* e *in vivo*;

4. ARTIGO E MANUSCRITO

Os resultados inseridos nesta tese apresentam-se sob a forma de artigo científico e de um manuscrito, o qual se encontra aqui estruturado. Os itens Introdução, Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se no artigo. O primeiro artigo está disposto conforme aceito para publicação na revista Journal of Ethnopharmacology, e o primeiro manuscrito está estruturado para publicação na revista Journal of Photochemistry and Photobiology.

4.1. Artigo 1

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Antinociceptive and anti-inflammatory effects of *Aloe saponaria* Haw on thermal injury in rats

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ABSTRACT

Ethnopharmacological relevance: In Brazil, the plant *Aloe saponaria* Haw, popularly known as "babosa pintadinha", has been empirically used for its potential effect on thermal injury. Because there are no scientific data confirming its popular use, the aim of the present study was to investigate the effects of *Aloe saponaria* on nociceptive and inflammatory parameters in a rat model of thermal injury.
Materials and methods: Adult male Wistar rats were subjected to a thermal injury or sham procedure (immersion in water at 70 or 37 °C, respectively, for 5 or 8 s). Burned animals were topically treated with vehicle (base cream), sulfadiazine 1% (positive control) or *Aloe saponaria* cream (0.3%–30%) once a day for 2 or 6 days. Each day, 30 min before the treatment, we measured nociceptive (static and dynamic mechanical allodynia, thermal allodynia and spontaneous pain) and inflammatory (paw edema) parameters. Moreover, enzymatic indicators of leukocyte infiltration into burned tissue were also determined 2 or 6 days after the thermal injury.
Results: The thermal injury (fist and second-degree) procedure, but not the sham procedure, induced nociception and inflammation from 1 to 6 days after the injury. The topical treatment with *Aloe saponaria* cream (10%) reduced nociceptive behaviors from day 1 to 6 (peak at day 2), edema at days 5 and 6 (peak at day 6) and myeloperoxidase, N-acetyl-glucosaminidase and eosinoperoxidase activities at day 6. The antinociceptive and anti-inflammatory effects of *Aloe saponaria* were obtained with doses of 3%–30%, with maximal inhibition obtained with a dose of 10% (reductions of 39 ± 9%, 41 ± 9%, 31 ± 7%, 83 ± 7% and 23 ± 2% for static and dynamic mechanical allodynia, thermal allodynia, spontaneous pain and paw edema, respectively).
Conclusion: Our results demonstrate that topically applied *Aloe saponaria* presented antinociceptive and anti-inflammatory effects in rats subjected to a thermal injury, which supports its traditional use for burn injuries.

1. Introduction

A thermal injury is a posttraumatic inflammatory wound caused by contact with heat, cold, electricity, chemicals, radiation or friction, and it is accompanied by both local and systemic effects (Evers et al., 2010). Most burns are the result of exposure to flame and are induced by scalding (characterized by hot pouring liquids into the body). Burn injuries are accompanied by intense inflammation, tissue damage, infection and a significant incidence of death and disability (Summer et al., 2007; Parihar et al., 2008). Moreover, uncontrolled acute burn pain increases the incidence of chronic pain and associated depression, the need for multiple operative procedures and prolonged hospitalization and rehabilitation, leading to high health care costs (Richardson and Mustard, 2009).

According to folk medicine, species from the genus *Aloe* belong to the *Xanthorrhoeaceae* Family (The Angiosperm Phylogeny Group, 2009), especially *Aloe vera*, can be used to treat burn wounds and to promote other healing processes (Capasso et al., 1998). The *Aloe* species are most likely native to South and East Africa, although they are widespread throughout the world (Iwu, 1993). Pre-clinical studies have demonstrated that some *Aloe* species, such as *Aloe vera*, *Aloe spicata* and *Aloe ferox*, have anti-inflammatory and burn-healing properties (Chithra et al., 1998, Barros et al., 2007;

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Loots et al., 2007). Moreover, clinical studies have shown that *Aloe vera* has an efficacy superior to that of silver sulfadiazine to treat burn injuries (Maenthaisong et al., 2007; Khorasani et al., 2009).

Similar to other *Aloe* species, *Aloe saponaria* Haw, popularly known as "babosa-pintadinha", is also commonly used to treat pain and burn injuries in southern Brazil, where the current study was carried out (Soares et al., 2004). However, to the best of our knowledge, there has not been a chemical composition investigation or a pre-clinical study confirming the putative analgesic and anti-inflammatory activities of *Aloe saponaria* on burn injuries. Thus, the goal of this study was to perform a chemical characterization and to analyze the antinociceptive and anti-inflammatory effects of *Aloe saponaria* in the treatment of thermal injury.

2. Materials and methods

2.1. Plant material

The plant was collected in July 2008, at Cunhaporã, in southern Brazil. A voucher specimen number SMDB 8749 was deposited at the Herbarium of the Botany Department, Federal University of Santa Maria (UFSM), Brazil.

2.2. Extraction and pharmaceutical formulation

Fresh leaves were cut in small pieces and macerated with ethanol (70%) at room temperature for seven days with daily agitation. The crude hydroethanolic extracts from leaves were concentrated to dryness in a rotary evaporator (at a temperature below 50 °C). For the tests, the dry extract (0.3%–30%) or silver sulfadiazine (1%, used as a positive control) were incorporated into Lanette cream (Lanette® wax 12.0 g; Solid Vaseline 11.0 mL, propylene glycol 7.0 mL; parabens solution preservative 3.3 g; imidazolidinyl urea preservative solution 50%; distilled water for 100 g) manufactured by the Pharmacy of the Federal University of Santa Maria. Lanette cream without drugs was used as the vehicle.

Some of physicochemical characterization of creams was carried out, in relation to pH, viscosity and spreadability. pH values were determined after dispersion of the creams in ultrapure water (10%, w/v) using a calibrated potentiometer (Mettler Toledo, São Paulo, Brazil). The viscosity was evaluated using a rotational viscosimeter (Brookfield LVDVII+Pro model, USA) and spindle SC4-25 with a small sample adapter. The spreadability of formulations was determined according to the methodology previously described by Borghetti and Knorst (2006).

2.3. Animals

Experiments were performed on adult male Wistar rats (weighing 250–300 g) bred in our animal house. The animals were housed in a controlled temperature (22 ± 2 °C) with a 12 h light/dark cycle. They were given standard lab food and water ad libitum. The animals were habituated in the experimental room for at least 30 min before the experiments. The experiments were performed in accordance with the current ethical guidelines for the investigation of experimental pain in conscious animals from Zimmermann (1983). Animals were randomly assigned to individual treatment groups and all subsequent behavioral tests were performed blindly. Moreover, to verify the reproducibility of our data, the experiments were performed at least in two blocks. The intensities of the noxious stimuli were previously defined by pilot stimuli–response curves. The used noxious stimuli and number of animals were the minimum necessary to demonstrate the consistent and statistically significant effects of the procedures and the drug treatments. The Committee on the Use and Care of Laboratory Animals at our university approved this study (no. 117/2010).

2.4. Reagents, equipment and general procedures for HPLC-DAD

All chemicals were of analytical grade. Methanol, acetic acid, gallic acid and caffeic acid were purchased from Merck (Darmstadt, Germany). Quercetin, rutin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan) equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and the LC solution 1.22 SP1 software.

2.4.1. Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using a C_{18} column (4.6 mm × 250 mm) packed with 5 μm diameter particles. The mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition of the gradient was 5% of B for 2 min and increased to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 100 min, respectively (Evaristo and Leitão, 2001). The extract of the plant was dissolved in ethanol at a concentration of 8 mg/mL and analyzed. The presence of six phenolic compounds, namely, gallic, chlorogenic and caffeic acids and the flavonoids quercetin, rutin and kaempferol, was investigated. Identification of these compounds was performed by comparing their retention times and UV absorption spectra with those of commercial standards. The flow rate was 0.6 mL/min, the injection volume was 40 μl and the wavelengths were 254 nm for gallic acid, 325 nm for caffeic acid, and 365 nm for quercetin, rutin and kaempferol. All of the samples and mobile phases were filtered through 0.45 μm membrane filters (Millipore) and then degassed with an ultrasonic bath prior to use. Stock solutions of the reference standards were prepared in the HPLC mobile phase at a concentration range of 0.031–0.250 mg/ml for kaempferol, quercetin and rutin and 0.006–0.250 mg/ml for gallic and caffeic acids. The chromatography peaks were confirmed by comparing the retention times with those of the reference standards and by the DAD spectra (200–500 nm). The calibration curves are as follows: gallic acid, $Y=12407x+1059.8$ ($r=0.9993$); caffeic acid, $Y=16862x+1126.3$ ($r=0.9997$); rutin, $Y=18973x+1575.7$ ($r=0.9989$); quercetin, $Y=20134x+1492.2$ ($r=0.9995$); and kaempferol, $Y=17923x+1853.9$ ($r=0.9978$). All chromatography operations were carried out at ambient temperature and in triplicate.

2.5. Experimental design

To investigate the effects of *Aloe saponaria* on thermal injury, adult male Wistar rats were subjected to a thermal injury or sham procedure (immersion in water at 70 or 37 °C, respectively, for 5 and 8 s (s)). Burned animals were topically treated with vehicle (base cream) or *Aloe saponaria* (0.3%–30%) immediately after thermal procedure and once a day for 2 or 6 days. The doses range used in our study were based in previous studies using *Aloe vera* leaf extract topical formulations to treat burns that ranged from 0.5% to 50% (Khorasani et al., 2009; Lv et al., 2006; Bunyapraphatsara et al., 1996). As a positive control, we have treated a separated group of animals with silver sulfadiazine (1%). Each day, we measured nociceptive (static and dynamic mechanical allodynia, thermal allodynia and spontaneous pain) and inflammatory (paw edema) parameters 30 min before the treatment. Moreover, enzymatic indicators of

leukocyte infiltration into the burned tissue were also determined 6 days after the burn.

2.6. Thermal injury models

Rats received a scald burn as described previously by Gao et al. (2010). Following the measurement of baseline responses, the animals were anaesthetized with 2% isofluorane, and the right hind paw was placed and held in 70 °C water for 5 or 8 s. In the sham procedure group, the right hind paw was exposed to 37 °C water for 5 or 8 s. After the stimulus was applied, the rat was returned to the individual testing compartment and allowed to recover from anesthesia within 2–3 min. Hot-water immersion for 5 and 8 s was considered first and second-degree burn, respectively (Gao et al. 2010). Since second-degree thermal injury produced a more severe tissue lesion than the first-degree injury, we assessed nociception and inflammation for two and six days after burn, respectively, to avoid unnecessary discomfort of animals.

2.7. Nociception assessment

2.7.1. Static mechanical allodynia

Static mechanical allodynia was measured as described previously by Chaplan et al. (1994). Rats were individually placed in clear Plexiglas boxes (9 cm × 7 cm × 11 cm) on elevated, wire mesh platforms to access the ventral surface of the hind paws. The paws were touched with a series of seven von Frey hairs (6–100 g). The von Frey hairs were applied perpendicular to the plantar surface of the paws with sufficient force to cause a slight buckling against the paws and were held for approximately 2 s. The 50% withdrawal threshold was determined using the up-and-down method of Dixon (1980). In this paradigm, testing was initiated with the 15-g hair. Stimuli were always presented consecutively; either ascending or descending. Withdrawal thresholds were verified at several time points after thermal injury (from1 to 6 days) and were compared with the baseline values (before thermal injury).

2.7.2. Dynamic mechanical allodynia

The dynamic response to a non-noxious mechanical stimulus was measured with modifications as described previously by Jaggi and Singh (2011). The response to a smooth paintbrush has been described as allodynia because naive rats rarely withdraw from this stimulus. Rats were placed in a cylinder with a wire mesh floor and a smooth paintbrush stimulus was used to rub the plantar area of hind paw from the heel to the toes for a maximum of 15 s. A paw withdrawal response within 15 s was considered dynamic mechanical allodynia.

2.7.3. Thermal allodynia

We employed the paw immersion test to observe the response of rats to non-noxious heat, as described previously by Takahashi et al. (2003). Briefly, after the environmental habituation period, rats were gently handled, and their right paw was dipped into a bath containing water at 30 °C. This low-intensity stimulus yields baseline latencies (15 s) that are long enough to observe hyperalgesia or analgesia. The latency to withdraw the paw from the non-noxious bath was recorded with a stopwatch. Each rat was tested twice before the administration of drugs to obtain baseline withdrawal latencies and several times after drug treatments. If after 15 s the animals did not withdrawal their paw, the stimulus as the test was suspended. The paw withdrawal response within 15 s was considered to be a nociceptive behavior.

2.7.4. Spontaneous nociception

Inspection of rat behavior was performed during the time they stayed on the wire mesh floor, as described previously by Weissman-Fogel et al. (2008). A number of measurements were observed: gait/weight bearing disturbance, guarding, hind paw lifting and grooming. The time spent demonstrating any one of these behaviors was measured for 5 min with a chronometer and defined as spontaneous pain. Spontaneous pain was verified at several time points after the thermal injury (from 1 to 6 days) and was compared to the control group (sham). A spontaneous pain response was verified before the measurement of allodynia.

2.8. Inflammation assessment

2.8.1. Edema formation

The edema induced by a thermal burn was considered as the increase in paw thickness measured with a digital caliper (Mytutoio, Japan) as described previously by Silva et al. (2010). Paw thickness was verified at several time points (1 to 6 days) after thermal injury and compared to baseline values (before thermal injury).

2.8.2. Leukocyte infiltration markers

To estimate the inflammatory cell infiltration in the paw after a thermal injury, paw skin samples were collected to estimate the activities of myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase (NAGase) and eosinoperoxydase (EPO), markers of neutrophil, macrophage and eosinophil infiltration, respectively (Lloret and Moreno., 1995; Suzuki et al., 1983; Kang et al., 2008). Firstly, the samples were homogenized in acetate buffer (8 mM, pH 5.5) containing 0.5% HTAB and centrifuged at 16,000 g at 4 °C for 20 min, and the supernatant was collected.

For the MPO activity measurement, 10 μl of supernatant was added to 200 μl of acetate buffer (200 mM, pH 5.4) and 20 μl of 3,3′,5,5′ tetramethyl-benzidine (TMB-18.4 mM) in a 96-well plate and incubated at 37 °C for 3 min in duplicate. To stop the reaction, the microplates were incubated in an ice bath, and 30 μl of acetic acid was added. The color formed was assessed at 630 nm.

For the measurement of NAGase activity, 25 μL of the supernatant was incubated with 25 μL of 4-nitrophenyl N-acetyl-β-D-glucosaminide (2.24 nM) and 100 μL of citrate buffer (50 mM, pH 4.5) at 37 °C for 1 h. After incubation, 100 μL of glycine buffer (0.2 μM, pH 10.4) was added to stop the reaction and to allow for the development of color, and it was measured at 405 nm.

For the measurement of EPO activity, 100 μl of a substrate solution consisting of 0.1 mM of o-phenylenediamine in Tris–HCl buffer (0.05 M, pH 8.0) with 0.1% Triton X-100 and 1 mM H_2O_2 was added to 100 μl of supernatant. The reaction mixture was incubated for 30 min at 37 °C, and then the reaction was stopped by the addition of 50 μl of 4 M H_2SO_4. The enzyme activity was evaluated colorimetrically at 490 nm.

The absorbance of all reactions was measured in a Fisher Biotech Microkinetics Reader BT 2000 microplate reader. The values are expressed as optical densities, corrected for the protein content, which was measured by the method of Bradford (1976).

2.9. Histology

To confirm the leukocyte infiltration in the tissues of the right hind paws of the animals that received or did not receive a thermal injury, we carried out histological analyses. Samples were collected 6 days after the incision or sham procedure. Rats were sacrificed, and their paws were removed and fixed in alfac solution (16:2:1 mixture of ethanol 80%, formaldehyde 40% and acetic acid) and then decalcified. Each sample was embedded in paraffin wax, sectioned at 5 μm and stained with toluidine

blue. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with a 10× and 100× objective (Oliveira et al., 2011). To minimize any source of bias, the investigator analyzing the samples did not know the identity of the group that he was analyzing.

2.10. Statistical analyses

The results are expressed as the means ± S.E.M. All data were analyzed by Student's *t*-test, one-way or two-way analysis of variance (ANOVA) followed by Bonferroni's or Student–Newman–Keuls' (SNK) post hoc tests when appropriate. *P* values of less than 0.05 ($P < 0.05$) were considered significant. The I_{max} (maximal inhibition) were calculated based on the responses of the control (sham) group. To meet the ANOVA assumptions, the mechanical allodynia data were subjected to Log transformation before statistical analysis.

3. Results

3.1. HPLC analysis of the Aloe saponaria extract and physicochemical characterization of creams

The HPLC fingerprinting demonstrated that flavonoids (quercetin, rutin and kaempferol) and phenolic acids (gallic and caffeic acids) are present in the extract of *Aloe saponaria*. The quantitative analysis of *Aloe saponaria* extract revealed the presence of gallic acid (t_R=13.97 min; 1.05%; peak 1), caffeic acid (t_R=27.15 min; 0.47%; peak 2), rutin (t_R=41.54 min; 2.13%; peak 3), quercetin (t_R=50.83 min; 1.09%; peak 4) and kaempferol (t_R=69.06 min; 0.38%; peak 5) (Fig. 1 and Table 1).

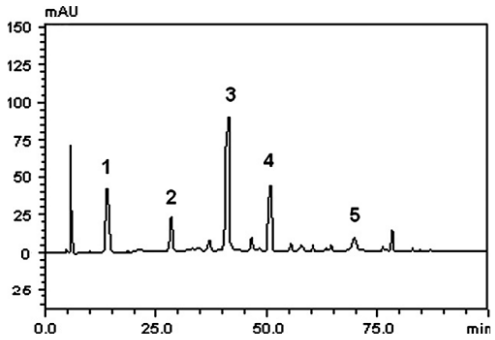

Fig. 1. Representative high performance liquid chromatography spectrum of *Aloe saponaria*, detection UV was at 325 nm. Gallic acid (peak 1), caffeic acid (peak 2), rutin (peak 3), quercetin (peak 4) and kaempferol (peak 5). Chromatographic conditions are described in the Methods section.

Table 1
Phenolics and flavonoids composition of *Aloe saponaria*.

Compounds	*Aloe saponaria*	
	mg/g	Percent
Gallic acid	10.53 ± 0.11	1.05
Caffeic acid	4.72 ± 0.02*	0.47
Rutin	21.30 ± 0.12*	2.13
Quercetin	10.94 ± 0.09	1.09
Kaempferol	3.86 ± 0.25*	0.38

Results are expressed as mean ± standard deviations (SD) of three determinations.

* $P < 0.05$ compared to the gallic acid concentration (one-way ANOVA followed by the Newman–Keuls test).

For the tests, the dry extract was incorporated into Lanette cream and some of physicochemical characterization of formulations was carried out. Regarding the spreadability, no difference ($P > 0.05$; Student's *t*-test) was observed between base cream and *Aloe saponaria* 10% cream (spreadability factor = 3.02 ± 0.47 and 2.36 ± 0.45 mm^2/g, respectively). At 0.22 s^{-1} (shear rate), the viscosity was 230,831 and 94,060 mPa s for base cream and *Aloe saponaria* 10% cream, respectively. Moreover, the formulations exhibited a non-Newtonian behavior, because the viscosity varied according to the change in the shear rate. The pH values obtained were 5.87 and 4.91 for base cream and *Aloe saponaria* 10% cream, respectively, and were compatible with topical application.

3.2. Effects of Aloe saponaria treatment on mechanical allodynia induced by a thermal injury

Compared to animals that received the sham procedure, animals that received a first-degree thermal injury and were not treated developed static mechanical allodynia, which was

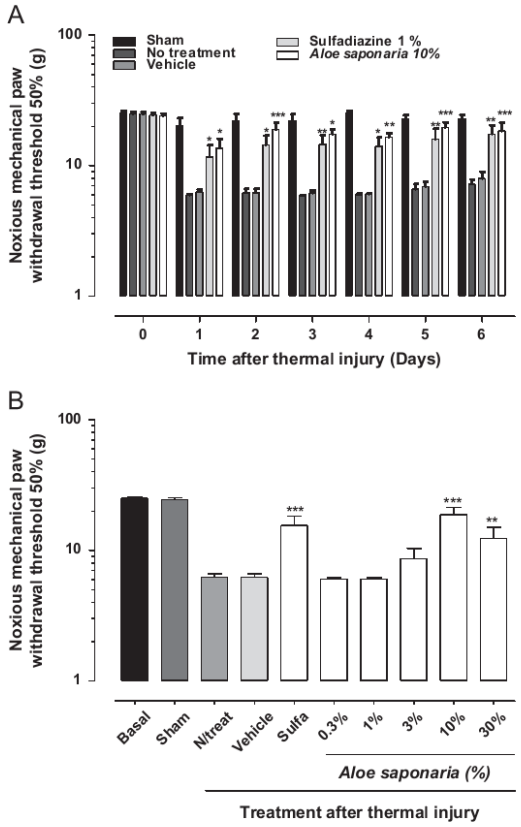

Fig. 2. Time-course (A) and dose–response curves (B) for the *Aloe saponaria* extract, no treatment (N/treat) and Sulfadiazine 1% (Sulfa) effects on static mechanical allodynia (A and B) induced by a first-degree thermal injury. The dose–response curves for allodynia were assessed at 48 h after thermal injury. Data are presented as the means ± SEM from 7 rats. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ compared to the no treatment group (Two-way ANOVA followed by Bonferroni's test).

characterized by a significant reduction in the paw withdrawal threshold in response to von Frey filaments (24.7 ± 0.9 g at baseline for the sham group vs. to 6.1 ± 0.5 g for the thermal injury group $P < 0.01$, Student's *t*-test) (Fig. 2A–B). Furthermore, compared to rats that received the sham procedure, rats that received a first-degree thermal injury also presented dynamic mechanical allodynia characterized by a significant reduction in the paw withdrawal latencies in response to a paintbrush (14 ± 3 s at baseline to 2.0 ± 0.5 s, $P < 0.01$, Student's *t*-test) (Fig. 3A–B). The topical treatment with the vehicle did not alter either static or dynamic allodynia caused by the burn. On the other hand, the treatment with *Aloe saponaria* (10%) or sulfadiazine (1%, used as a positive control) was able to reduce the static and dynamic mechanical allodynia induced by the thermal injury, an effect that started at 1 day, peaked at 2 days and was maintained up to 6 days after injury (Figs. 2 and 3A). The dose–response curve demonstrated that *Aloe saponaria* treatment at the doses of 10% and 30%, but not at 0.3% and 1%, was capable of reducing static and dynamic allodynia with a maximal inhibition of 68 ± 11% and 36 ± 5%, respectively (at the dose of 10%), demonstrating an efficacy similar to that of sulfadiazine (inhibition of 70 ± 11% and 38 ± 10%, respectively) (Figs. 2 and 3B).

Similar to the first-degree, animals submitted to second-degree thermal injury developed static and dynamic mechanical allodynia (Suppl. Fig. 1A–B). The topical treatment with *Aloe saponaria* (10%) or sulfadiazine (1%, used as a positive control), but not with vehicle, was able to reduce static (inhibitions of 57 ± 12% and 72 ± 6%, respectively) and dynamic (10 ± 5% and 15 ± 9%, respectively) mechanical allodynia with similar efficacy (Suppl. Fig. 1A–B).

3.3. Effect of Aloe saponaria treatment on heat allodynia induced by a thermal injury

Animals that received a first-degree thermal injury and were not treated developed thermal allodynia characterized by a significant reduction in the latency to non-noxious heat compared with the sham procedure (13 ± 0.6 s at baseline to 2.8 ± 0.7 s, respectively, $P < 0.01$, Student's *t*-test) (Fig. 4A–B). Topical treatment with vehicle did not alter thermal allodynia caused by the first-degree burn. On the other hand, it was observed that treatment with *Aloe saponaria* (10%) or sulfadiazine (1%, used as a positive control) was able to reduce the thermal allodynia

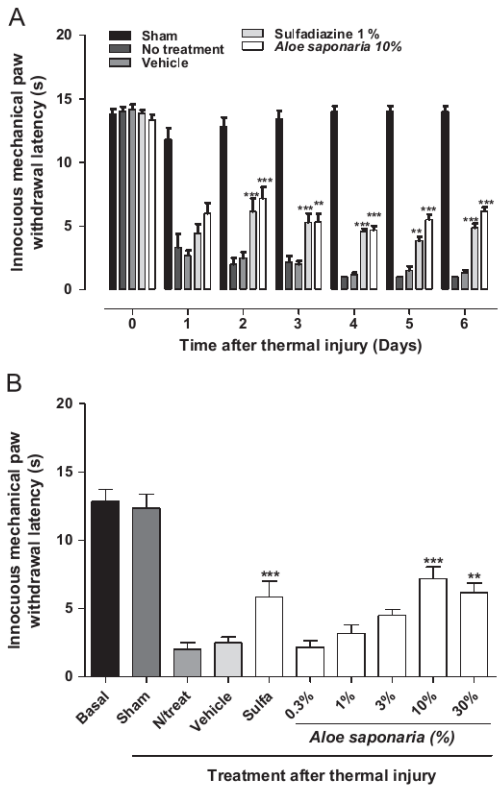

Fig. 3. Time-course (A) and dose–response curves (B) for the *Aloe saponaria* extract, no treatment (N/treat) and Sulfadiazine 1% (Sulfa) effects on dynamic mechanical allodynia (A and B) induced by a first-degree thermal injury. The dose–response curves for allodynia were assessed at 48 h after thermal injury. Data are presented as the means ± SEM from seven rats. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ compared to the no treatment group (Two-way ANOVA followed by Bonferroni's test).

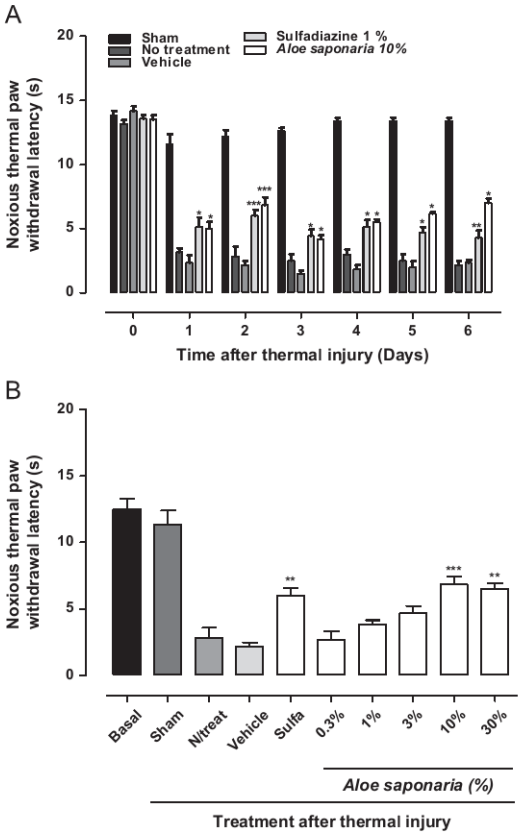

Fig. 4. Time-course (A) and dose–response curves (B) for the *Aloe saponaria* extract, no treatment (N/treat) and Sulfadiazine 1% (Sulfa) effects on thermal allodynia (A and B) induced by a first-degree thermal injury. The dose–response curves for allodynia were assessed at 48 h after thermal injury. Data are presented as the means ± SEM from 7 rats. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ compared to the no treatment group (Two-way ANOVA followed by Bonferroni's test).

induced by the thermal injury, an effect that started at 1 day, peaked at 2 days and was maintained up to 6 days after injury (Fig. 4A). The dose–response curve demonstrated that *Aloe saponaria* treatment at the doses of 10% and 30%, but not at 0.3% and 1%, was capable of reducing thermal allodynia with maximal inhibition of 29 ± 6% (at the dose of 10%) demonstrating an efficacy similar to that of 1% sulfadiazine (inhibition of 38 ± 6%) (Fig. 4B).

Similarly to the first-degree injury, animals submitted to second-degree thermal injury developed thermal allodynia (Suppl. Fig. 1C). The topical treatment with *Aloe saponaria* (10%) or sulfadiazine (1%), but not with vehicle, was capable of reducing thermal allodynia induced by the thermal injury, an effect that started at 1 day, peaked at 2 days after injury (with inhibitions of 87 ± 4% and 82 ± 4%, respectively) (Suppl. Fig. 1C)

3.4. Effect of Aloe saponaria treatment on spontaneous nociception induced by a thermal injury

Animals that received a first-degree thermal injury and were not treated presented spontaneous pain characterized by the presence of gait/weight bearing disturbance, guarding, hind paw lifting and grooming compared to the sham procedure group (58 ± 11 s, $P < 0.01$, Student's *t*-test) (Fig. 5A–B). Topical treatment with vehicle did not alter the spontaneous pain caused by the first-degree burn. On the other hand, it was observed that treatment with *Aloe saponaria* (10%) or sulfadiazine (1%, used as a positive control) was able to reduce the spontaneous pain induced by the thermal injury, an effect that started at 1 day, peaked at 2 days and was maintained up to 6 days after injury (Fig. 5A). The dose–response curve demonstrated that *Aloe saponaria* treatment at the doses of 10% and 30%, but not at 0.3% and 1%, were capable of reducing spontaneous pain with maximal inhibition of 83 ± 7% (at the dose of 10%), demonstrating an efficacy similar to that of sulfadiazine 1% (inhibition of 81 ± 8%) (Fig. 5B).

Furthermore, rats submitted to second-degree thermal injury (second-degree model) presented spontaneous pain (Suppl. Fig. 1E). The topical treatment with the vehicle did not alter spontaneous pain caused by the second-degree thermal injury, but the treatment with *Aloe saponaria* (10%) or sulfadiazine (1%, used as a positive control) was able to reduce the spontaneous pain induced by the thermal injury (71 ± 3% and 61 ± 4%,

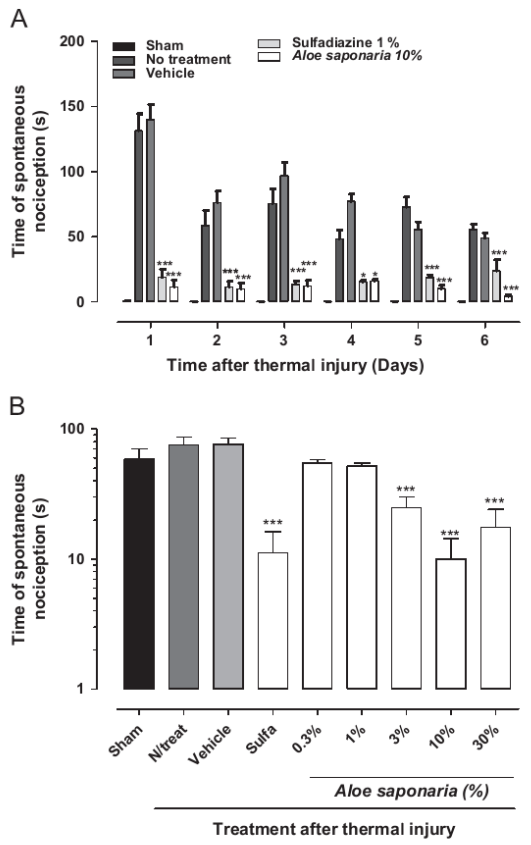

Fig. 5. Time-course (A) and dose–response curves (B) for the *Aloe saponaria* extract, no treatment (N/treat) and Sulfadiazine 1% (Sulfa) effects on spontaneous pain (A and B) induced by a first-degree thermal injury. The dose–response curves for spontaneous pain were assessed at 48 h after thermal injury. Data represented as the means ± SEM from 7 rats *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ compared to the no treatment group (Two-way ANOVA followed by Bonferroni's test).

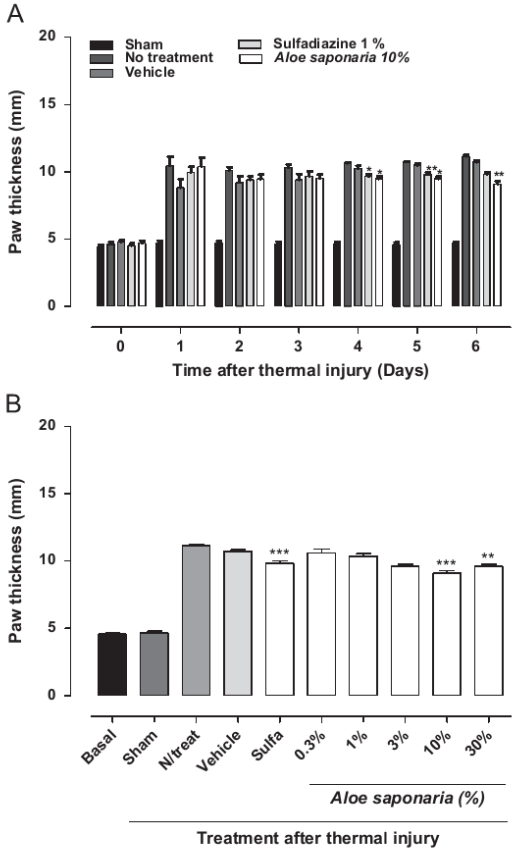

Fig. 6. Time-course (A) and dose–response curves (B) for the *Aloe saponaria* extract, no treatment (N/treat) and Sulfadiazine 1% (Sulfa) effects on paw edema (A and B) induced by a first-degree thermal injury. The dose–response curve for paw edema was assessed at 144 h after thermal injury. Data represented as the means ± SEM from seven rats. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ compared to the no treatment group (Two-way ANOVA followed by Bonferroni's test).

respectively), an effect that started at 1 day, peaked at 2 days after injury (Suppl. Fig. 1E).

3.5. Effects of Aloe saponaria treatment on paw edema induced by a thermal injury

Compared to animals that received the sham procedure, animals that received the first-degree thermal injury procedure and were not treated presented edema characterized by an increase in paw thickness (4 ± 0.1 mm at baseline compared to 11.15 ± 0.09 mm, respectively, $P < 0.01$, Student's t-test) (Fig. 6A–B). Topical treatment with the vehicle did not alter edema formation caused by the first-degree burn. On the other hand, it was observed that treatment with *Aloe saponaria* (10%) or sulfadiazine (1%, used as a positive control) was able to reduce the thermal injury-induced edema, an effect that started at day 4 and was maintained up to 6 days (peaked) after injury (Fig. 6A). The dose–response curve demonstrated that *Aloe saponaria* treatment at the doses of 10 and 30%, but not at 0.3% and 1%, was capable of reducing the edema formation with maximal inhibition of $62 \pm 2\%$ (at the dose of 10%), demonstrating an efficacy similar to that of 1% sulfadiazine (inhibition of $51 \pm 2\%$) (Fig. 6B).

Animal submitted to a second-degree thermal injury developed paw edema, which was inhibited by the topical treatment with *Aloe saponaria* (10%) or sulfadiazine (1%) (reductions of $28 \pm 5\%$ and $35 \pm 9\%$ at 2 days after injury, respectively) (Suppl. Fig. 1D).

Furthermore, we have tested the treatments in animals submitted to sham procedure (that not received thermal injury). During 6 days, none of the treatments (vehicle, sulfadiazine 1% or *Aloe saponaria* 10%) caused edema or nociception in animals (data not shown), indicating that they not produced skin irritation in animals.

3.6. Aloe saponaria Haw attenuated leukocyte infiltration induced by the thermal burn injury

To investigate whether the treatment with *Aloe saponaria* altered neutrophil, macrophage and eosinophil infiltration induced by injury, we assessed the activities of MPO, NAGase and EPO in the injured tissue (Fig. 7A – C). Six days after the first-degree thermal injury, we detected increases of 100%, $66 \pm 14\%$ and 100% in the MPO, NAGase and EPO activities, respectively, compared with the sham-injured group. The increases in the MPO, NAGase and EPO activities were inhibited by the *A. saponaria* (10%) treatment (100%, $60 \pm 3\%$ and $83 \pm 6\%$, respectively). Similarly, the silver sulfadiazine treatment (1%, used as positive control) also inhibited the increases in the MPO, NAGase and EPO activities caused by the thermal injury (inhibitory effects of $68 \pm 14\%$, $79 \pm 10\%$ and $55 \pm 4\%$, respectively).

Furthermore, we confirmed our enzymatic leukocyte infiltration detection with histological analyses. In accordance with the enzymatic results, the first-degree thermal injury-induced leukocyte infiltration observed in histological slides compared with the sham procedure submitted group (Suppl. Fig. 2A–B). The treatment with sulfadiazine 1% or *Aloe saponaria* 10%, but not with vehicle, caused a reduction in leukocytes infiltration compared with no treatment group (Suppl. Fig. 2C–E).

Finally, we also assessed the activities of MPO and EPO in the injured tissue that animals that received the second-degree thermal injury. Two days after injury, we detected an increased of MPO and EPO activities that were reduced by either *Aloe saponaria* (10%) (inhibitions of $18 \pm 3\%$ and $33 \pm 12\%$, respectively) or silver sulfadiazine (1%) treatment (inhibitions of $16 \pm 4\%$ and $37 \pm 3\%$, respectively) (Suppl. Fig. 3A–B).

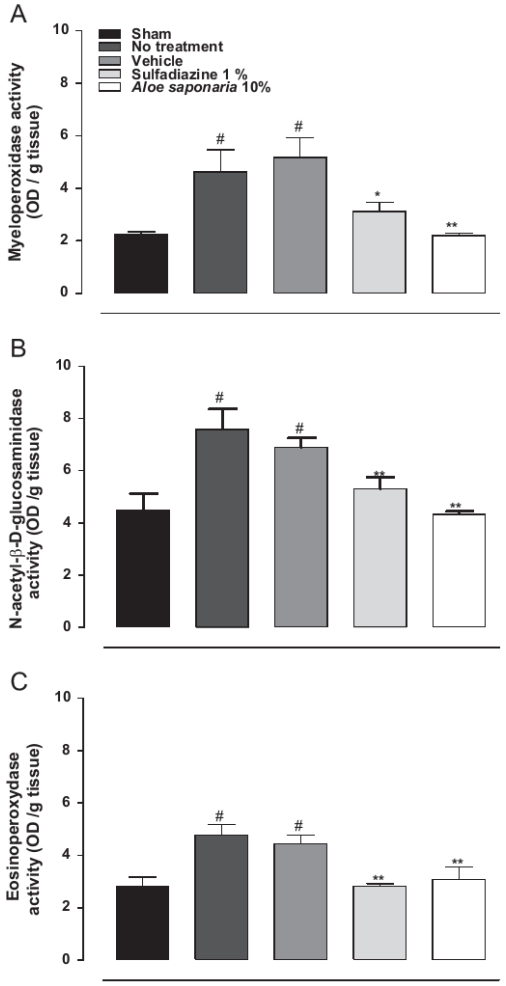

Fig. 7. Effect of the treatment with *Aloe saponaria* 10% extract or silver sulfadiazine 1% after 6 days treatment on MPO (A), NAG (B) and EPO (C) levels in the paw tissue of rats subjected to a thermal burn. Data are presented as the means ± SEM from seven rats. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the no treatment group. $\#P < 0.01$ compared to the control group. (Two-way ANOVA followed by Bonferroni's test).

4. Discussion

Aloe saponaria has been empirically used worldwide as a folk medicine for various skin disorders, including thermal injuries, and this treatment is based on anecdotal evidence or on research conducted almost exclusively with *Aloe vera* (Soares et al., 2004). Thus, we investigated the antinociceptive and anti-inflammatory effects of *Aloe saponaria* for the treatment of both first and second-degree thermal injuries, which has yet to be studied. The results of this study demonstrate that burn-induced nociception and inflammation is ameliorated by topical treatment with *Aloe saponaria*.

A preliminary HPLC analysis of *Aloe saponaria* showed several chromatographic peaks, revealing great chemical diversity. Among the substances present, flavonoids (rutin, quercetin and kaempferol) and phenolic acids (gallic and caffeic acids) were found. These

flavonoids and phenolics are widely distributed in medicinal plants. Our results are in accordance with previous findings for the same genus, which detected phenols and flavonoids in leaf extracts of *Aloe ferox*, *Aloe secundiflora* and *Aloe vera* (Rebecca et al., 2003; Wintola and Afolayan, 2011). Moreover, several studies have shown that the flavonoids and phenolics found in *Aloe saponaria* possess antinociceptive and anti-inflammatory effects in models of inflammatory pain in rodents (Lapa et al., 2009; Hajhashemi et al., 2012; Mehrotra et al., 2011). Thus, these biological activities could be responsible, at least in part, for the effects of *Aloe saponaria* observed in our study.

To evaluate the antinociceptive and anti-inflammatory activities of *Aloe saponaria*, we used a thermal injury model (Gao et al., 2010) in which the animals received a scald burn to the paw. In a scald burn, pain is the most frequent complaint of the injured patient. Patients present mechanical allodynia, thermal hyperalgesia and spontaneous pain of the skin (Summer et al., 2007). Moreover, in humans, burns cause edema and leukocyte infiltration (Kowal-Verne et al., 1997). Because we detected the same signs and symptoms in our study, this model seems to be relevant to study the anti-inflammatory and antinociceptive effects of *Aloe saponaria* after a thermal burn.

The hyperalgesia induced by mechanical stimulation of the injured site is the major source of severe pain after a burn injury (Summer et al., 2007). In accordance, our study showed that first and second-degree thermal injury model decreased the threshold of static mechanical allodynia and decreased the latency in dynamic mechanical allodynia and thermal allodynia in rats. Furthermore, burn-injured patients also describe spontaneous components of breakthrough pain. Spontaneous pain is commonly reported by patients in qualitative terms such as "stinging", "pricking", "shooting", and "pounding" (Summer et al., 2007). Spontaneous nociception after a burn could also be observed in rats subjected to the thermal injury model. Our findings showed that topical treatment with *Aloe saponaria* had an antinociceptive effect in various broad parameters of pain, such as dynamic and static mechanical allodynia, thermal allodynia and spontaneous nociception. Our findings are in agreement with a previous study that showed that systemic administration of an ethanol extract of *Aloe saponaria* presented antinociceptive effects in a model of neuropathic pain caused by successive treatment with cisplatin (Yoo et al., 2008).

A skin burn results in local tissue damage, which induces a painful inflammatory process (Sener et al., 2003). In fact, we observed that thermal injury-induced edema formation, an effect that was reduced by *Aloe saponaria* treatment. Other species from the genus *Aloe* have been previously described to have topical anti-edematogenic effects. For instance, *Aloe vera* treatment reduces edema produced by bacteria infection (Rishi et al., 2008). Similar to *Aloe saponaria*, the treatment with 1% silver sulfadiazine (positive control) had antinociceptive and anti-inflammatory effects in the thermal burn model. Silver sulfadiazine is the most commonly used topical treatment for burn injury, and several studies have shown it to be effective to treat burn injuries (Maenthaisong et al., 2007; Khorasani et al., 2009). Because *Aloe saponaria* presented efficacy similar to sulfadiazine and *Aloe vera* (Bunyapraphatsara et al., 1996 and present study), the antinociceptive and anti-inflammatory effects of this plant could be beneficial in the treatment of first and second-degree burns, apart being as effective as the reference treatments. The similar efficacy of *Aloe saponaria* and silver sulfadiazine could be due to their resemblance with regard to its properties. In fact, sulfadiazine is primarily used for burns because of its antimicrobial action (Khorasani et al., 2009) and a study indicated that *Aloe saponaria* also possesses antibacterial action (Tian et al., 2003). Furthermore, we also observed that *Aloe saponaria* extract 10% was more effective than 30% to produce antinociceptive and antiinflammatory effects. This finding is not unexpected since the relationship of the flux of a drug from ointments to skin and the drug dose follows usually an inverted-U shaped curve (Troy, 2005). In fact, the evaporation of more volatile components of a cream (such as water) may lead to the early precipitation of drugs, reducing their access to skin, which may explain why the *Aloe saponaria* dose of 30% is less effective than of 10%.

In addition to edema, burn wounds are also susceptible to infiltration by a variety of cell types including macrophages and neutrophils (Sener et al., 2003; Evers et al., 2010). The MPO, NAGase and EPO activities in injured tissue were used as a marker of neutrophil, macrophage and eosinophil infiltration, respectively (Lloret and Moreno., 1995; Kang et al., 2008). Our observation demonstrated that the MPO, NAGase and EPO activities were increased in paw tissue samples after a thermal injury, indicating neutrophil, macrophage and eosinophil infiltration into this tissue, which could contribute to injury in a thermal injury. Concomitant with its antinociceptive and anti-edematogenic effects, *Aloe saponaria* has a preventive effect in a thermal injury through inhibition of the infiltration of neutrophils, macrophages and eosinophils. In accordance with our findings, it has been found that *Aloe vera* reduced leukocyte adhesion in the endothelium of burn-wounded rats (Duansak et al., 2003). Furthermore, Yoo et al. (2008) showed that the incubation of *Aloe saponaria* extract with cultured macrophages in vitro suppressed nitric oxide production and inhibited the lipopolysaccharide (LPS)-induced mRNA increases in nitric oxide synthase, granulocyte-macrophage colony-stimulating factor and cyclooxygenase 2. Thus, the reduction in leukocyte infiltration and activation in injured tissue seems to contribute to the beneficial effects of *Aloe saponaria* on thermal injury.

Finally, changes in the physicochemical characteristics of the cream after extract incorporation could facilitate its anti-inflammatory action. In fact, the incorporation of the *Aloe saponaria* extract into Lanette cream reduced its pH and viscosity, but not its spreadability. It is important to relate that the efficacy of topical therapy depends on the patient spreading the formulation on the skin, and their distribution on the applied region, as well as the viscosity and composition (Garg et al., 2002). In this case, the formulations had similar values and the incorporation of the *Aloe* extract into the base cream did not influence its spreadability. Apart to be lower than base cream, the pH value of the *Aloe saponaria* cream was still compatible with topical application and with the slightly acidic mantle of skin pH between 4.6 and 5.8. Furthermore, the low pH of the cream *Aloe* may be favorable in the healing process, since the acid mantle has a number of functions, including antimicrobial defense and restriction of inflammation by inhibiting the release of pro-inflammatory cytokines (Prow et al., 2011).

In conclusion, the results presented in this study show that treatment with *Aloe saponaria* Haw has antinociceptive and anti-inflammatory effects in an animal that received first or second-degree model of thermal injuries. Taken together, our results support the traditional use of this plant in the treatment of burns.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2012.12.055.

References

Barros, F.M.C., Pereira, K.N., Zanetti, G.D., Heinzmann, B.M., 2007. Plantas de Uso Medicinal no Município de São Luiz Gonzaga, RS, Brasil. Latin American Journal of Pharmacy 26, 652–662.

Borghetti, G.S., Knorst, M.T., 2006. Desenvolvimento e avaliação da estabilidade física de loções O/A contendo filtros solares. Revista Brasileira de Ciências Farmacêuticas 42 (4), 531–537.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein—dye binding. Analytical Biochemistry 72, 248–254.

Bunyapraphatsara, N., Jirakulchaiwong, S., Thirawarapan, S., Manonukul, J., 1996. The efficacy of *Aloe vera* cream in the treatment of first, second and third degree burns in mice. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 2 (3), 247–251.

Capasso, F., Borrelli, F., Capasso, R., Di Carlo, G., Izzo, A.A., Pinto, L., Mascolo, N., Castaldo, S., Longo, R., 1998. Aloe and its therapeutic use. Phytotherapy Research 12, 124–127.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994. Quantitative assessment of tactile allodynia in the rat paw. Journal of Neuroscience Methods 53, 55–63.

Chithra, P., Sajithlal, G.B., Chandrakasan, G., 1998. Influence of aloe vera on the healing of dermal wounds in diabetic rats. Journal of Ethnopharmacology 59, 195–201.

Dixon, W.J., 1980. Efficient analysis of experimental observations. Annual Review of Pharmacology and Toxicology 20, 441–462.

Duansak, D., Somboonwong, J., Patumraj, S., 2003. Effects of *Aloe vera* on leukocyte adhesion and TNF-alpha and IL-6 levels in burn wounded rats. Clinical Hemorheologic Microcirculation 29, 239–246.

Evaristo, I.M., Leitão, M.C., 2001. Identificação e Quantificação por DAD-HPLC, da Fracção Fenólica Contida em Folhas de Quercussuber L. Silva Lusitana 9, 135–141.

Evers, L.H., Bhavsar, D., Mailänder, P., 2010. The biology of burn injury. Experimental Dermatology 19, 777–783.

Gao, Y., Xu, C.S., Yu, K., Li, G., Wan, F., Liu, S., Lin, J., Liu, H., Zhang, J., Li, X., Liang, S.D., 2010. Effect of tetramethylpyrazine on DRG neuron P2 × 3 receptor involved in transmitting pain after burn. Burns: Journal of the International Society for Burn Injuries 36, 127–134.

Garg, A., Aggarwal, D., Garg, S., Singela, A., 2002. Spreading of semisolid formulations. Pharmaceutical Technology 26, 84–105.

Hajhashemi, V., Zolfaghari, B., Yousefi, A., 2012. Antinociceptive and anti-inflammatory activities of *Saturejahortensis* seed essential oil, hydroalcoholic and polyphenolic extracts in animal models. Medical Principles and Practice 21, 178–182.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. Library of Congress Edition, Florida, pp. 114–117.

Jaggi, A.S., Singh, N., 2011. Exploring the potential of telmisartan in chronic constriction injury-induced neuropathic pain in rats. European Journal of Pharmacology 667, 215–221.

Kang, H.S., Lee, J.Y., Kim, C.J., 2008. Anti-inflammatory activity of arctigenin from Forsythiae Fructus. Journal of Ethnopharmacology 116, 305–312.

Khorasani, G., Hosseinimehr, S.J., Azadbakht, M., Zamani, A., Mahdavi, M.R., 2009. Aloe versus silver sulfadiazine creams for second-degree burns: a randomized controlled study. Surgery Today 39, 587–591.

Kowal-Verne, A., Walenga, J.M., Sharp–Pucci, M., Hoppensteadt, D., Gamelli, R.L, 1997. Postburn edema and related changes in interleukin-2, leukocytes, platelet activation, edothelin-1, and C1 esterase inhibitor. The Journal of Burn Care & Rehabilitation 18, 99–103.

Lapa, F.R., Gadotti, V.M., Missau, F.C., Pizzolatti, M.G., Marques, M.C.A., Dafré, A.L., Farina, M., Rodrigues, A.L.S., Santos, A.R.S., 2009. Antinociceptive properties of the hydroalcoholic extract and the flavonoid rutin obtained from *Polygala paniculata* L. in mice. Basic & Clinical Pharmacology & Toxicology 104, 306–315.

Lloret, S., Moreno, J.J., 1995. Effects of an-anti-inflammatory peptide antiflammin 2 on cell influx, eicosanoid biosynthesis and oedema formation by arachidonic acid and tetradecanoylphorbol dermal application. Biochemical Pharmacology 50, 347–353.

Loots, D.T., Van Der Westhuizen, F., Botes, L., 2007. *Aloe ferox* leaf gel phytochemical content, antioxidant capacity, and possible health benefits. Journal of Agricultural and Food Chemistry 55, 6891–6896.

Lv, R.L., Wu, B.Y., Chen, X.D., Jiang, Q., 2006. The effects of aloe extract on nitric oxide and endothelin levels in deep-partial thickness burn wound tissue in rat. Zhonghua shao shang za zhi=Zhonghua shaoshang zazhi=Chinese Journal of Burns 22, 362–365.

Maenthaisong, R., Chaiyakunapruk, N., Niruntraporn, S., Kongkaew, C., 2007. The efficacy of *Aloe vera* used for burn wound healing: a systematic review. Burns : Journal of the International Society for Burn Injuries 33, 713–718.

Mehrotra, A., Shanbhag, R., Chamallamudi, M.R., Singh, V.P., Mudgal, J., 2011. Ameliorative effect of caffeic acid against inflammatory pain in rodents. European Journal of Pharmacology 666, 80–86.

Oliveira, S.M., Drewes, C.C., Silva, C.R., Trevisan, G., Boschen, S.L., Moreira, C.G., Cabrini, D.A., Cunha, C., Ferreira, J., 2011. Involvement of mast cells in a mouse model of postoperative pain. European Journal of Pharmacology 672, 88–95.

Parihar, A., Parihar, M.S., Milner, S., Bhat, B., 2008. Oxidative stress and anti-oxidative mobilization in burn injury. Burns: Journal of the International Society for Burn Injuries 34, 6–17.

Prow, T.W., Grice, J.E., Lin, L.L., Faye, R., Butler, M., Becker, W., Wurm, E.M., Yoong, C., Robertson, T.A., Soyer, H.P., Roberts, M.S., 2011. Nanoparticles and microparticles for skin drug delivery. Advanced Drug Delivery 63, 470–491.

Rebecca, W., Kayser, O., Hagels, H., Zessin, K.H., Madundo, M., Gamba, N., 2003. Main phenolic compounds from the leaf exudate of *Aloe secundiflora* by high-performance liquid chromatography-mass spectroscopy. Phytochemical Analysis 14, 83–86.

Richardson, P., Mustard, L., 2009. The management of pain in the burns unit. Burns: Journal of the International Society for Burn Injuries 35, 921–936.

Rishi, P, Rampuria, A, Tewari, R, Koul, A., 2008. Phyromodulatory potentials of *Aloe vera* against *Salmonella* OmpR-mediated inflammation. Phytotherapy Research 22, 1075–1082.

Sener, G., Satiroglu, H., Sehirli, A.O., Kaçmaz, A., 2003. Protective effect of aqueous garlic extract against oxidative organ damage in a rat model of thermal injury. Life Sciences 73, 81–91.

Silva, M.A., Klafke, J.Z., Rossato, M.F., Gewehr, C., Guerra, G.P., Rubin, M.A., Ferreira, J., 2010. Role of peripheral polyamines in the development of inflammatory pain. Biochemical Pharmacology 82, 269–277.

Soares, E.L.C., Vendrusculo, G.S., Eisinger, S.M., Záchia, R.A., 2004. Estudo etonobotânico do uso dos recursos vegetais em São João do Polêsine, RS, Brasil, no período de outubro de 1999 a junho de 2001. I-Origem e fluxo do conhecimento. Revista Brasileira de Plantas Medicinais, 69–95.

Summer, G.J., Puntillo, K.A., Miaskowski, C., Green, P.G., Levine, J.D., 2007. Burn injury pain: the continuing challenge. The Journal of Pain 8, 533–548.

Suzuki, K., Ota, H., Sasagawa, S., Sakatani, T., Fujikura, T., 1983. Assay method formyeloperoxidase in human polymorphonuclear leukocytes. Analytical Biochemistry 132, 345–352.

Takahashi, K., Sato, J., Mizumura, K., 2003. Responses of C-fiber low threshold mechanoreceptors and nociceptors to cold were facilitated in rats persistently inflamed and hypersensitive to cold. Neuroscience Research 47, 409–419.

The Angiosperm Phylogeny Group, 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161, 105–121.

Tian, B., Hua, Y.J., Ma, X.Q., Wang, G.L., 2003. Relationship between antibacterial activity of aloe and its anthaquinone compounds. Zhongguo Zhong Yao ZaZhi 28, 1034–1037.

Troy, D.B., 2005. Remington: The Science and Practice of Pharmacy, 21st ed. Lippincott Williams and Wilkins, Baltimore, pp. 871–888.

Weissman-Fogel, I., Dashkovsky, A., Rogowski, Z., Yarnitsky, D., 2008. Vagal damage enhances polyneuropathy pain: additive effect of two algogenic mechanisms. Pain 138, 153–162.

Wintola, O.A., Afolayan, A.J., 2011. Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. Pharmacognosy Magazine 28, 325–333.

Yoo, E.A., Kim, S.D., Lee, W.M., Park, H.J., Kim, S.K., Cho, J.Y., Min, W., Rhee, M.H., 2008. Evaluation of antioxidant, antinociceptive, and anti-inflammatory activities of ethanol extracts from *Aloe saponaria* Haw. Phytotherapy Research 22, 1389–1395.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.

4.1.1. Artigo 1 - Figuras suplementares

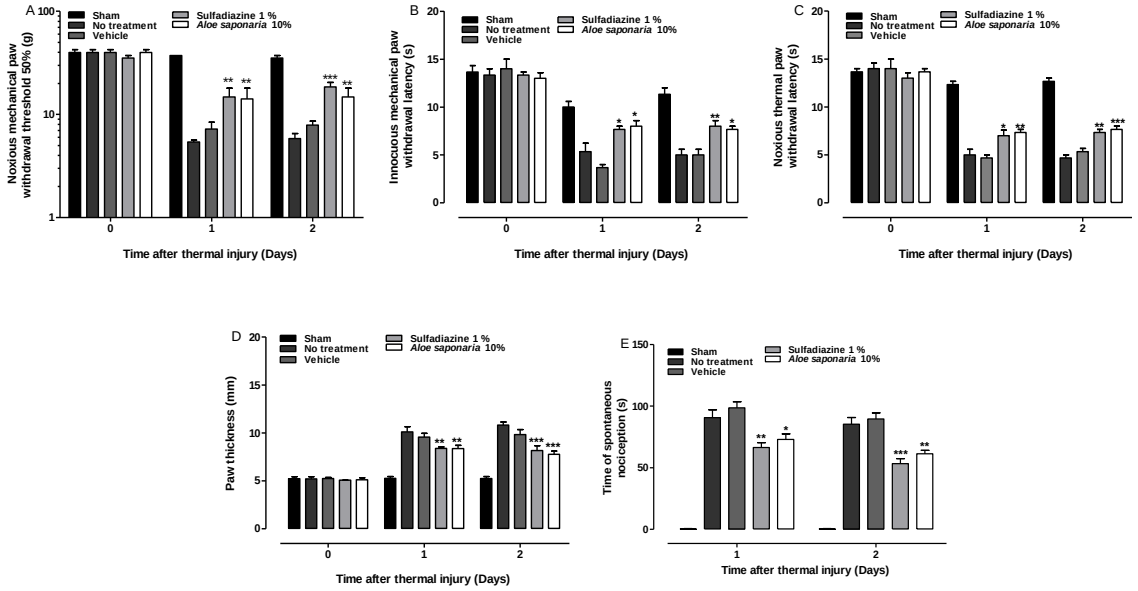


Figure 1. Time-course for the *Aloe saponaria* 10% extract, no treatment (N/treat) and Sulfadiazine 1% (Sulfa) effects on static (A) and dynamic (B) mechanical allodynia, thermal allodynia (C), paw edema (D) and spontaneous pain (E) induced by a second-degree thermal injury. The dose-response curves for allodynia were assessed at 48 h after thermal injury. Data are presented as the means ± SEM from 4 rats. *P < 0.05; ** P < 0.01 and *** P < 0.001 compared to the no-treatment group (Two-way ANOVA followed by Bonferroni's test).

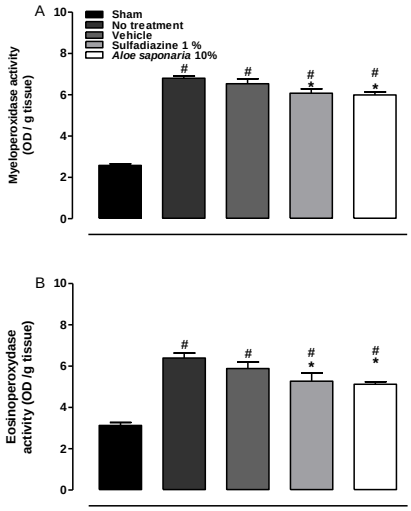


Figure 2. Effect of the treatment with *Aloe saponaria* 10% extract and sulfadiazine 1% on the MPO (A) and EPO (B) levels in the paw tissue of rats 2 days after the second-degree thermal burn. Data are presented as the means ± SEM from 4 rats. *P < 0.05 compared to the no-treatment group. # P<0.01 compared to the control group. (Two-way ANOVA followed by Bonferroni's test).

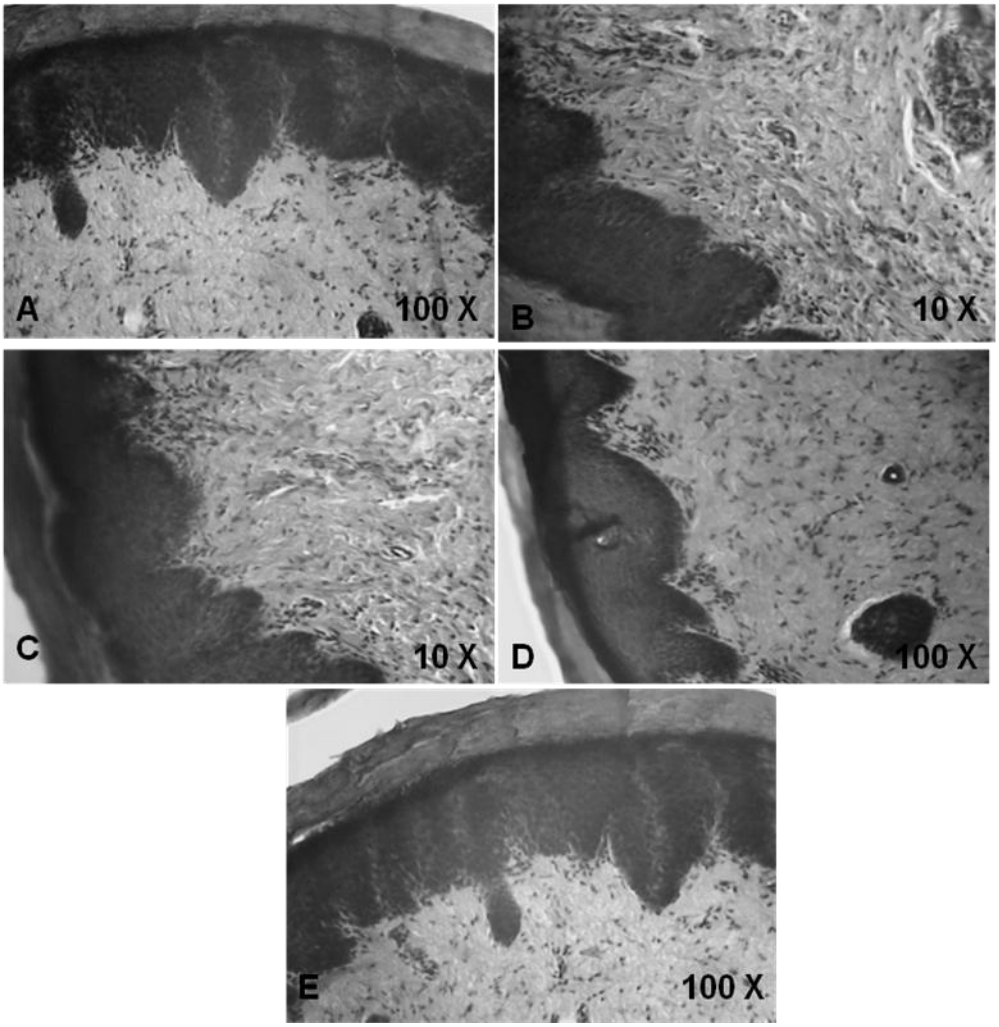
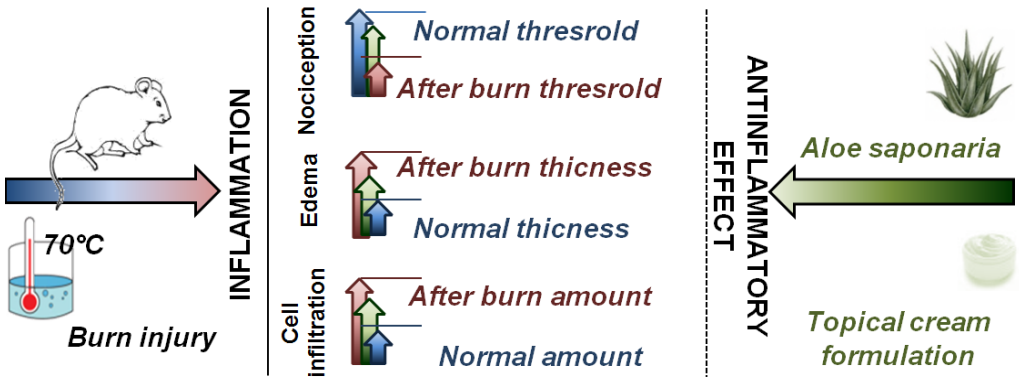
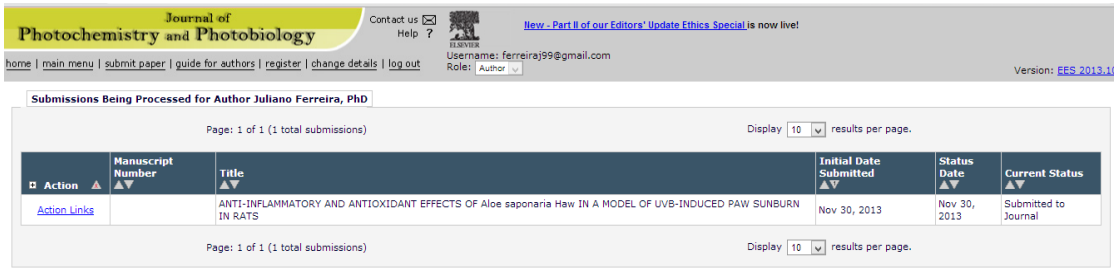


Figure 3. Representative light microphotograph showing the paw tissue of sham injured rats (A) or 6 days after first-degree thermal injury in rats with no treatment (B) and treated with vehicle (C), sulfadiazine 1% (D) or *Aloe saponaria* 10% (E).

4.1.2. Artigo 1 – Resumo gráfico



4.2. Manuscrito



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ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF *Aloe saponaria Haw* IN A MODEL OF UVB-INDUCED PAW SUNBURN IN RATS

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Highlights

- The treatment with *Aloe saponaria* reduced UVB irradiation-induced allodynia.
- Inflammation caused by UVB irradiation was decreased by *Aloe saponaria* treatment.
- *Aloe saponaria* extract reduced oxidative stress induced by UVB irradiation.

Abstract

Ultraviolet B (UVB) irradiation mainly affects biological tissues by inducing an increase in reactive oxygen species (ROS) production which leads to deleterious outcomes for the skin, including pain and inflammation. As a protective strategy, many studies have focused on the use of natural products. The aim of this study was to investigate the effects of *Aloe saponaria* on nociceptive, inflammatory, and oxidative parameters in a model of UVB-induced sunburn in adult male Wistar rats. Sunburned animals were topically treated with vehicle (base cream), 1% silver sulfadiazine (positive control) or *A. saponaria* (10%) once a day for 6 days. UVB-induced nociception (allodynia and hyperalgesia), inflammation (edema and leukocyte infiltration) and oxidative stress (increases in H_2O_2, protein carbonyl levels and lipid peroxidation and a decrease in thiol content) were reduced by both *A. saponaria* and sulfadiazine topical treatment. Furthermore, *A. saponaria* or its constituents aloin and rutin reduced the oxidative stress induced by H_2O_2 skin homogenates *in vitro.* Our results demonstrate that topical *A. saponaria* treatment displayed anti-nociceptive and anti-inflammatory effects in a UVB-induced sunburn model, and these effects seem to be related to its antioxidant components.

Keywords: Aloin; analgesic; anti-inflammatory; antioxidant; rutin; silver sulfadiazine.

Abbreviations

ANOVA, Analysis of variance; DNPH, 2,4-dinitrophenylhydrazine; DTNB, 5,5'-dithiobis-(Z-nitro-benzoic acid); EPO, eosinophil peroxidase; HPLC-DAD, high performance liquid chromatography-diode array detection; H2O2, hydrogen peroxide; LOD, limit of detection; LOQ, limit of quantification; MDA, malonyldialdehyde; Imax, maximal inhibition; MPO, myeloperoxidase; PWT, paw withdrawal threshold; PBS, phosphate buffered saline; ROS, reactive oxygen species; SS, silver sulfadiazine; TCA, trichloroacetic acid; UVR, ultraviolet radiation.

1. Introduction

Exposure to ultraviolet radiation (UVR), especially UVB (280-315 nm), can result in sunburn, which is an inflammatory process characterized by the development of pain, edema and leukocyte infiltration [1, 2]. Moreover, UVB-induced sunburn pain and inflammation is mainly mediated in affected biological tissues through impairment of the oxidant/antioxidant balance, which causes an increase in the cellular levels of reactive oxygen species (ROS) [3]. Due to the deleterious effects of ROS in the skin, many studies have focused on the establishment of a protection system to prevent and/or treat UV irradiation-induced skin damage [4]. Patients report painful responses to a non-noxious mechanical stimulus (allodynia) and a heightened response to a noxious stimulus (hyperalgesia) after sunburn, which hinders their daily activities [5] and requires treatment.

In this context, many studies have focused on the use of natural sources, especially plants that contain compounds with antioxidant and anti-inflammatory activities [4]. The *Aloe* family is composed of tropical and subtropical plants and is characterized by lanced-shaped leaves with jagged edges and, in some species, sharply tapered leaves [6]. Phytopharmacological studies of different *Aloe* species, such as *A. vera*, *A. spicata* and *A. ferox*, have shown anti-inflammatory and antioxidant effects as well as the capacity to enhance burn healing [7]. Similar to other *Aloe* species*, A. saponaria* Haw is used to treat burn injuries in southern Brazil [8], and a previous study by our group showed that it exhibits anti-nociceptive and anti-inflammatory effects in a scald burn model in rats [9]. Moreover, HPLC analysis of *A. saponaria* showed several chromatographic peaks, indicating a wide diversity of chemical compounds. These compounds include some phenolic substances, which usually exhibit antioxidant effects [9]. Thus, the goal of this study was to analyze the anti-

nociceptive, anti-inflammatory, and antioxidant effects of *A. saponaria* treatment in a model of UVB irradiation-induced sunburn in rats.

2. Material and Methods

2.1. Plant material

Plant material was collected in July 2008 at Cunhaporã (Santa Catarina), in the South of Brazil. A voucher specimen (SMDB 10.077) is deposited at the Herbarium of the Botany Department, Federal University of Santa Maria, Brazil.

2.2. Extraction

Fresh leaves were cut into small pieces and macerated with ethanol (70%) at room temperature for seven days with daily agitation. The crude hydroethanolic extracts from leaves were concentrated to dryness in a rotary evaporator at a temperature below 50ºC. For the tests, the dry extract (10%) or silver sulfadiazine (1%, used as a positive control) were incorporated into Lanett cream (Lanette® wax 12.0 g; Solid Vaseline 11 mL, propylene glycol 7.0 mL; parabens solution preservative 3.3 g; imidazolidinyl urea preservative solution 50%; distilled water for 100 g) manufactured by the Pharmacy of the Federal University of Santa Maria. Lanett cream without drugs was used as the vehicle.

2.3. Animals

Experiments were performed using adult male Wistar rats (weighing 250 - 300 g) bred in our animal house. The animals were housed at a controlled temperature (22 ± 2 ºC) with a 12 hours light/dark cycle. They were given standard lab food and water *ad libitum*. The animals were habituated to the experimental room for at least 30 minutes before the experiments were performed. The experiments were performed in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals [10]. Animals were randomly assigned to individual treatment groups, and all subsequent behavioral tests were performed blindly. Moreover, to verify the reproducibility of our data, the experiments were performed in at least two blocks. The intensities of the noxious stimuli were previously defined by pilot stimuli-response curves. The noxious stimuli and number of animals used were the minimum necessary to demonstrate the consistent and statistically significant effects of the procedures and the drug treatments. The Committee on the Use and Care of Laboratory Animals at our university approved this study (no. 119/2011).

2.4. HPLC analysis

All chemicals were analytical grade. Methanol and aloin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography-diode array detection (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector (DAD), UV-VIS detector and LC solution 1.22 SP1 software.

Isocratic phase chromatographic analyses were carried out using a C_{18} column (4.6 mm x 250 mm) packed with particles 5 µm in diameter; the mobile phase was methanol: water (60:40 v/v), the flow rate was 0.7 mL/min, and detection was performed at a wavelength of 254 nm, following a previously described method [11] with slight modifications. The presence of aloin in *A. saponaria* extract (10 mg/ml) was analyzed. A calibration curve was obtained by using aloin standard at concentrations ranging from 0.050 - 0.250 mg/ml ($y = 15472x + 1048.3$; $r = 0.9995$). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses, and the slope was determined using three independent analytical curves, as previously described [9]. The LOD and LOQ were calculated to be 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response, and S is the slope of the calibration curve.

2.5. Experimental design

To investigate the effects of *A. saponaria* on UVB-induced sunburn, adult male Wistar rats were subjected to UVB irradiation. The naive group (un-irradiated animals) was included in the experiments. Sunburned animals were topically treated with vehicle (base cream) or *A. saponaria* (10%) immediately after the UVB irradiation procedure once a day for 6 days. The doses used in our study were based in previous studies using *A. saponaria* cream to treat thermal injury and ranged from 0.3-30%; a dose of 10% achieved the best results [9]. As a positive control, we treated a separate group of sunburned animals with silver sulfadiazine (SS, 1%). Each day, we measured nociceptive (static and dynamic mechanical allodynia or thermal allodynia) and inflammatory (paw edema) parameters 30 minutes before the treatment. Moreover, enzymatic indicators of leukocyte infiltration

(MPO and EPO) and antioxidant effects (carbonyl levels, lipid peroxidation, thiol levels and neutralization of H_2O_2) in the burned tissue were also determined at 6 days and 24 hours, respectively, after the sunburn.

2.6. UVB Irradiation Model

UVB irradiation was performed as described previously by Bishop [1]. The source of UVB irradiation was a Philips TL40W/12 RS lamp (Medical-Eindhoven, Holland) mounted 20 cm above the table on which the rats were placed. The source emitted a continuous light spectrum between 270 and 400 nm with a peak emission at 313 nm. UVB output (80% of the total UV irradiation) was measured using a model IL-1700 Research Radiometer (International Light, USA; calibrated by IL service staff) with a radiometer sensor for UV (SED005) and UVB (SED240). The UVB irradiation rate was 0.27 mW/cm^2, and the dose used was 0.5 J/cm^2. The rats were first anesthetized (ketamine/xylazine) with a single intraperitoneal injection and then exposed to UVB irradiation. Only the right hind paw was exposed to UVB irradiation.

2.7. Nociceptive assessment

The measurement of the static mechanical paw withdrawal threshold was carried out using the up-and-down paradigm as described previously [9]. Briefly, rats were first acclimatized (1 hour) in individual clear Plexiglass boxes (9 x 7 x 11 cm) on an elevated wire mesh platform to allow access to the plantar surface of the hind paws. Von Frey filaments of increasing stiffness (6-100 g) were applied to the animal's hind paw plantar surface with enough pressure to bend the filament. Absence of paw lifting after 5 seconds led to the use of the next filament with increasing weight, whereas paw lifting indicated a positive response and led to the

use of a weaker filament. This paradigm continued until a total of 6 measurements were obtained or until four consecutive positive or negative responses were observed. The 50% mechanical paw withdrawal threshold (PWT) response was then calculated from the resulting scores, as described previously by Dixon [12]. The PWT was expressed in grams (g) and was evaluated for several time points after sunburn (from 1 to 6 days) and compared to the baseline values (before sunburn).

The dynamic response to a non-noxious mechanical stimulus was measured as described previously [9]. The response to a smooth paintbrush has been described as allodynia because naive rats rarely withdraw from this stimulus. Rats were placed in a cylinder with a wire mesh floor and a smooth paintbrush stimulus was used to rub the plantar area of the hind paw from the heel to the toes for a maximum of 15 seconds. A paw withdrawal response within 15 seconds was considered dynamic mechanical allodynia.

For the thermal allodynia assessment, we employed the paw immersion test as described previously [9]. The paw withdrawal response within 15 seconds was considered to be nociceptive behavior. Briefly, after the environmental habituation period, the rats were gently handled and their right paw was dipped into a water bath at 30ºC, which is considered a non-noxious heat stimulus. This low-intensity stimulus yields baseline latencies (15 seconds) that are long enough to observe hyperalgesia or analgesia. The latency to withdraw the paw from the non-noxious bath was recorded with a stopwatch. Each rat was tested twice before the administration of drugs to obtain baseline withdrawal latencies and then tested several times after drug treatments. After 15 seconds, if the animals did not withdrawal their paw, the stimulus and the test were suspended. The paw withdrawal response within 15 seconds was considered to be nociceptive behavior.

2.8. Inflammation Assessment

The increase in paw thickness induced by UVB-irradiation was considered to be edema as described previously [13]. Paw edema was verified at several time points (1 to 6 days) after UVB-induced burn and was compared to baseline values (before sunburn) expressed in millimeters (mm).

To estimate the inflammatory cell infiltration in the paw after sunburn, paw skin samples were collected to estimate the activities of myeloperoxidase (MPO) and eosinophil peroxidase (EPO), which are markers of neutrophil and eosinophil infiltration, respectively [14,15]. First, the samples were homogenized in acetate buffer (8 mM, pH 5.5) containing 0.5% HTAB and centrifuged at 16,000 x g at 4ºC for 20 minutes, and the supernatant was collected.

For the MPO activity measurement, 10 µl of supernatant was added to 200 µl of acetate buffer (200 mM, pH 5.4) and 20 µl of 3,3',5,5'tetramethyl-benzidine (TMB - 18.4 mM) in a 96-well plate and incubated at 37ºC for 3 minutes in duplicate. To stop the reaction, the microplates were incubated in an ice bath, and 30 µl of acetic acid was added to each well. The color formed was assessed at 630 nm.

For the measurement of EPO activity, 100 µl of a substrate solution consisting of 0.1 mM o-phenylenediamine in Tris–HCl buffer (0.05 M, pH 8.0) with 0.1% Triton X-100 and 1 mM H_2O_2 was added to 100 µl of supernatant. The reaction mixture was incubated for 30 minutes at 37 °C, and the reaction was stopped by the addition of 50 µL of 4 M H_2SO_4. The enzyme activity was evaluated colorimetrically at 490 nm.

The absorbance of all reactions was measured in a Fisher Biotech Microkinetcs Reader BT 2000 microplate reader. The values are expressed as

optical densities and are corrected for the protein content, which was measured using the Bradford method [16].

2.9. Oxidative stress determination

2.9.1. Antioxidant activity in vitro

For the measurement of protein carbonyl levels, lipid peroxidation, and thiol levels, the skin paw samples were collected, homogenized in phosphate buffered saline (PBS - 10 mM, pH 7,4), and centrifuged at 16,000 x g at 4ºC for 20 minutes, and the supernatant was collected. First, the 500 µL paw samples were incubated with 40 mM hydrogen peroxide (H_2O_2) or PBS (control group) for 5 minutes. Next, the samples were incubated in the absence or presence of 30 µg/mL of *A. saponaria* extract, aloin or rutin. Control experiments were carried out by exposing 500 µL of paw sample to *A. saponaria* extract, aloin or rutin in the absence of H_2O_2.

The carbonyl proteins were assayed using a method previously described Klafke [17]. Initially, 500 µL of the sample (paw) was precipitated using 250 µL of trichloroacetic acid (TCA - 10%) and centrifuged at 5.000 x g for 5 minutes. The supernatant was removed and discarded. Afterwards, 250 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) was added to the test group tube, 250 µL of HCl (2 M) was added to the white tube, and the samples were incubated at room temperature for 1 hour. At the end of the incubation time, 250 µL of 10% TCA was added, and the sample was centrifuged at 5.000 x g for 5 minutes. After centrifugation, the pellets were washed three times with 500 µL of ethanol-ethyl acetate (1:1, v/v). The precipitate was dissolved in 500 µL of protein dissolving solution (SDS - 10%) and incubated for 15 minutes. The color intensity of the

supernatant was measured using a spectrophotometer at 370 nm. The carbonyl content was calculated using the molar extinction coefficient (22 × 10−3 M/cm), and results were expressed in nmol/mg protein.

Thiol levels were determined as described by Rossato [18]. Briefly, the supernatant (50 µL) was incubated with 200 µL of Tris/HCl (200 mM, pH 8.9) and 20 µL of 5,5'-dithiobis-(Z-nitro-benzoic acid) (DTNB - 2.5 mM) at room temperature for 5 minutes. The color of the solution resulting from the reaction was measured at 405 nm with a FisherBiotech Microkinetics Reader BT 2000 (Pittsburgh, PA).

The lipid peroxidation assay was performed as described previously [18]. The supernatant (50 µL) was incubated with 150 µL of malonyldialdehyde (MDA) and incubated for 1 hour at 90ºC. The solution color resulting from the reaction was measured at 535 nm using a Hitachi U-2001 Spectrophotometer (Sataiama, Japan). Both the lipid peroxidation and thiol contents of the samples were corrected for the protein content of the samples. Protein was measured using Coomassie-Blue dye and bovine serum albumin as the standard.

We also investigated the neutralization of H_2O_2. This technique is based on monitoring the absorbance of H_2O_2 spectrophotometrically at 285 nm [19]. Different concentrations of *A. saponaria*, aloin or rutin and gallic acid (used as a positive control) were incubated with 40 mM H_2O_2 in the dark for 30 minutes. A reduction in the absorbance of H_2O_2 was considered consumption of the total H_2O_2 present in the reaction, indicating H_2O_2 neutralization. The results were expressed as % of neutralization relative to the control.

2.9.2. Antioxidant activity *ex vivo*

To evaluate lipid peroxidation and the thiol and carbonyl protein levels *ex vivo*, the paw samples were collected 24 hours after sunburn, homogenized in 500 µL of PBS (10 mM, pH 7.4) and maintained on ice for the remainder of the procedure as described above. The measurement of H_2O_2 production was performed as described previously Nakamura [20]. The paw samples were collected and homogenized in PBS (10 mM, pH 7.4) containing sodium azide (0.01 M) to inhibit catalase activity in the samples. The homogenates were then centrifuged at 10.000 x g at 4º C for 10 minutes. The supernatants and H_2O_2 standards were assayed spectrophotometrically at 560 nm.

2.10. Statistical analyses

The results are expressed as the means ± S.E.M. All data were analyzed by one-way or two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc tests when appropriate. P values of less than 0.05 ($P<0.05$) were considered significant. The I_{max} (maximal inhibition) values were calculated based on the responses of the control group. To meet the ANOVA assumptions, the mechanical allodynia data were subjected to log transformation before statistical analysis.

Results and discussion

3.1. Quantification of aloin by HPLC

In the present study, the HPLC fingerprinting of *A. saponaria* extract revealed the presence of aloin (t_R = 8.31 min; peak 2) at a concentration of 4.57 ± 0.09% (or 45.7 mg aloin/g extract) (Figure 1). The LOD and LOQ were 0.018 µg/mL and 0.053

µg/mL, respectively. Peak 1 is not a peak corresponding to important compounds from *A. saponaria;* it is characteristic of natural product extracts and is related to the dead volume of the column and is a characteristic of the mobile phase. Our study is in accordance with previous studies demonstrating that aloin is the main biologically active constituent of *Aloe* extracts [21,22]. Moreover, the concentration observed in our study was similar to that observed in previous studies; the level of this anthraquinone ranged from 0.1 to 21.5% [21].

Figure 1.

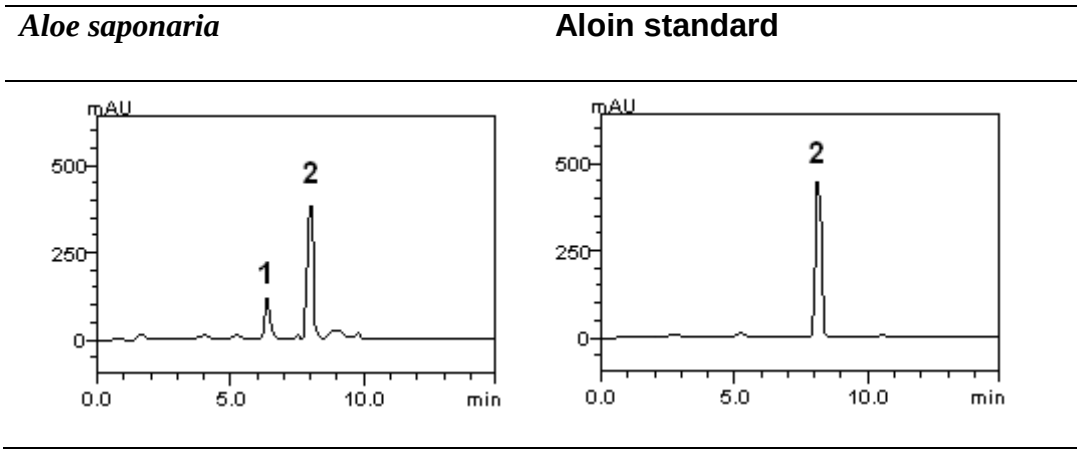


Figure 1. Representative high performance liquid chromatography spectrum of *A. saponaria* extract, showing the presence of *Aloin* detected by UV at 325 nm. Aloin (peak 2). The chromatographic conditions are described in the Methods section.

3.2. Effects of *Aloe saponaria* treatment on nociception induced by UVB irradiation

A well-characterized preclinical inflammatory pain model is the sunburn-induction model in rats. Previously, it was shown that UVB induces responses that result in hyperalgesia to mechanical and thermal stimuli [2]. To evaluate the anti-nociceptive and anti-inflammatory activities of *A. saponaria,* we used a sunburn

model in rats described previously [1], in which the animals received UVB irradiation on the hind paw. This is a good model to observe the anti-nociceptive and anti-inflammatory effects of *A. saponaria* on sunburned skin. UVB irradiation produces a concomitant reduction in the thermal and mechanical pain thresholds, referred to as allodynia [2,5]. Thus, our study showed that the untreated animals submitted to UVB irradiation developed static (34±1 g at baseline for the naïve group to 14±3 g for the sunburn group, $P<0.01$, Student's t-test) and dynamic (13.5±0.6 seconds at baseline for the naïve group to 6.3±0.6 seconds for the sunburn group, $P<0.01$, Student's t-test) mechanical allodynia. Furthermore, these animals developed thermal allodynia (12.1±0.3 seconds at baseline for the naïve group to 6.5±0.2 seconds for the sunburn group, $P<0.01$, Student's t-test) (Fig. 2A, B and C).

Moreover, these results demonstrate that topical treatment with the vehicle did not alter static and dynamic mechanical allodynia or the thermal allodynia caused by sunburn. In contrast, treatment with *A. saponaria* (10%) was able to reduce static and dynamic mechanical allodynia as well as the thermal allodynia induced by sunburn, with inhibitions of 100±14; 66±11, and 39±7%, respectively. This effect began at day 1, peaked at day 4 and was maintained up to 6 days after UVB irradiation (Fig. 2A, B, and C). Similar to the results obtained with *A. saponaria,* the treatment with silver sulfadiazine (1%, used as a positive control) was able to reduce the static and dynamic mechanical allodynia as well as the thermal allodynia induced by sunburn with inhibitions of 96±7, 73±7, and 42±3%, respectively. This effect began at day 1, peaked at day 4 and was maintained up to 6 days after UVB irradiation (Fig. 2A, B, and C). These results are in accordance with Silva, [9], who demonstrated the anti-nociceptive effects of treatment with *A. saponaria* cream in a scald burn model. In addition, 1% silver sulfadiazine is the most common topical treatment for burn injury

and several studies have shown its efficacy in treating burn injuries [23]. These results are in accordance with previous studies in which *Aloe saponaria* and silver sulfadiazine exhibited anti-nociceptive and anti-inflammatory effects of similar efficacy in a scald burn model [9].

Figure 2.

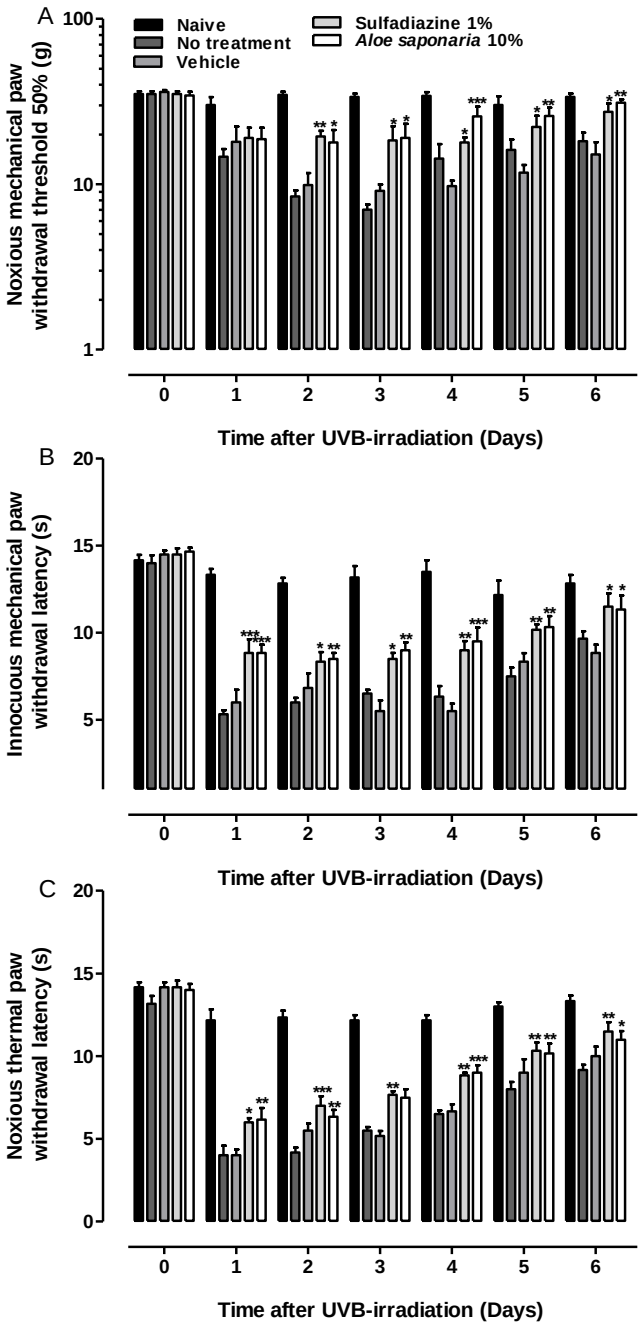


Figure 2. Time-course (A, B, and C) for the effects of *A. saponaria* extract and 1% sulfadiazine on static mechanical allodynia (A), dynamic mechanical allodynia (B) and thermal allodynia (C) induced by UVB irradiation. The data are presented as the means ± SEM from 6 rats. *P<0.05; **P<0.01 and ***P<0.001 when compared to the no-treatment or vehicle treated groups (Two-way ANOVA followed by Bonferroni's test).

3.3. Effects of *Aloe saponaria* treatment on inflammation induced by UVB irradiation

The acute exposure of skin to UVB irradiation results in inflammation associated with the formation of edema [1, 24] and leukocyte infiltration [2]. These changes are shown in our results, as untreated animals submitted to UVB irradiation developed paw edema when compared with naive animals (4.70±0.05 mm at 6 days for the naïve group to 6.39±0.08 mm at 6 days for the sunburn group, P<0.01, Student's t-test) (Fig. 3A). Treatment with the vehicle did not alter the paw edema caused by sunburn. In contrast, treatment with *A. saponaria* (10%) or SS (1%, used as the positive control) reduced (100% and 100%, respectively) the paw edema induced by sunburn, showing an effect that began at day 3, peaked at day 6 and was maintained up to 6 days after UVB irradiation (Fig. 3A). Furthermore, we determined whether the treatment with *A. saponaria* altered neutrophil and eosinophil infiltration induced by UVB irradiation by analyzing the activities of MPO and EPO enzymes in the injured tissue [9] (Fig. 3B and C). An increase of 80±8% and 63±9% in the MPO and EPO activities, respectively, was observed six days after sunburn when compared to the naïve group.

The increase in MPO and EPO activities was inhibited by *A. saponaria* (10%) treatment (59±9% and 65±14% inhibition, respectively) or SS treatment (1%, used as a positive control) (65±14% and 69±11% inhibition, respectively). In accordance with our findings, *A. vera* topical treatment has previously been shown to reduce edema produced by *Salmonella enteric* [25], and treatment with the *A. saponaria* cream also reduced the edema formation produced by a thermal burn [9]. Moreover, previous studies have demonstrated the anti-inflammatory effects of *A. vera* compounds (aloin) in the inflammatory process induced by colitis models in mice or rats [26]. Furthermore, Silva et al., [9] has shown that treatment with *A. saponaria* cream has a preventive effect in a scald burn model through the inhibition of leukocyte infiltration.

Figure 3.

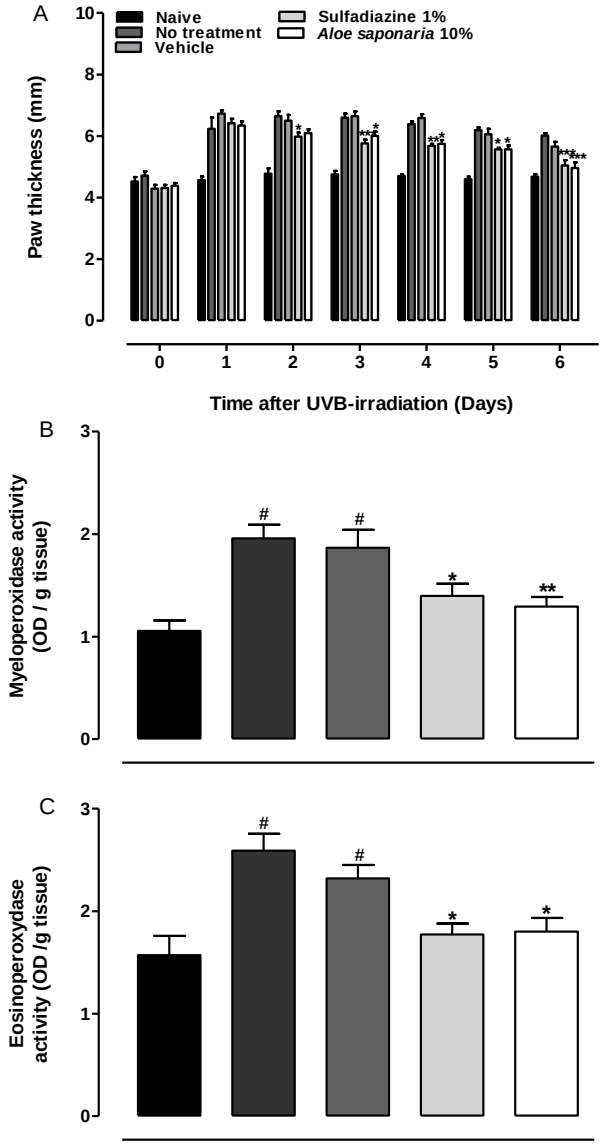


Figure 3. Time-course (A) for the effects of *A. saponaria* extract, no treatment and 1% Sulfadiazine (SS) on paw edema (A), MPO activity (B) and EPO activity (C) in the paw tissue of rats subjected to a UVB irradiation. The data are presented as the means ± SEM from 6 rats. *P< 0.05 and **P<0.01 when compared to the no treatment group. #P<0.01 when compared to the control group. (Two-way ANOVA followed by Bonferroni's test).

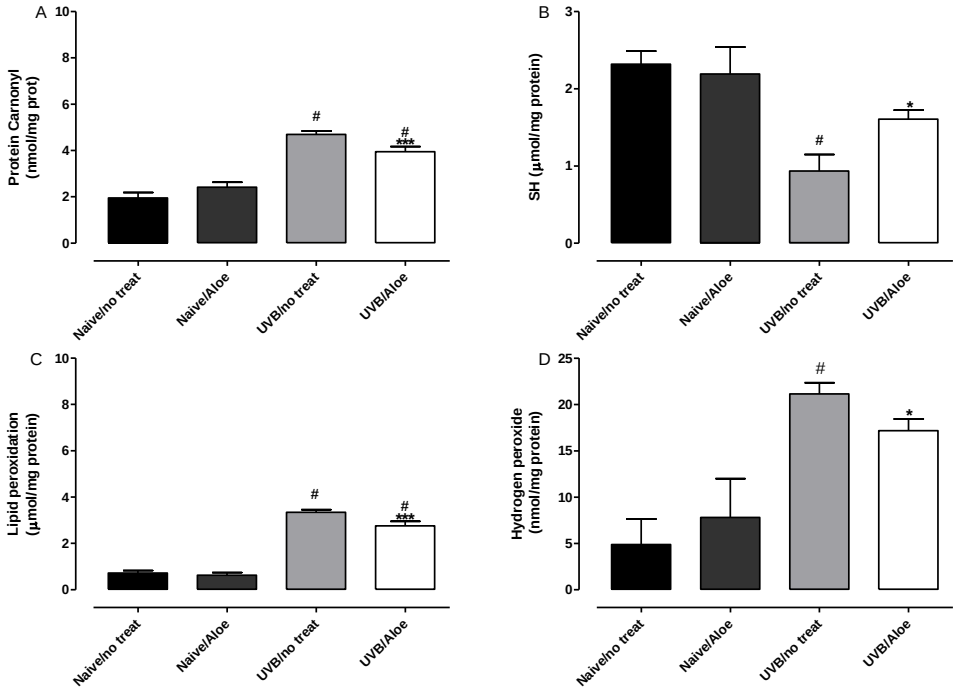
3.4. Effects of UVB irradiation on oxidative stress parameters *in vitro* and *in vivo*

UVB-induced damage is generally associated with the overproduction of ROS and free radicals and the impairment of antioxidant defense mechanisms [3,27]. To investigate the antioxidant capacity of *A. saponaria,* we measured the ability of the compound to reduce oxidative stress using protein carbonyl levels, lipid peroxidation, thiol levels and H_2O_2 content as parameters. Animals that received UVB irradiation displayed an increase in oxidative stress levels after 24 hours when compared to the naive group (2-fold increase in protein carbonyl levels, 5-fold increase in lipid peroxidation, 2-fold increase in thiol levels, and 4-fold increase H_2O_2 production) (Fig 4A, B, C, and D). These results are in accordance with other studies demonstrating that an overproduction of reactive species, such as hydrogen peroxide, and an increase in lipid peroxidation are generated during UVB light-stimulation [3,28,29].

In addition, topical treatment with *A. saponaria* did not alter the oxidative stress parameters in the naïve group. However, 24 hours after UVB irradiation and topical treatment with *A. saponaria* (10%), we observed a significant reduction in protein carbonyl levels (72±2%), lipid peroxidation (77±7%), thiol levels (51±8%) and H_2O_2 production (75±7%) when compared to the untreated group (Fig 4A, B, C, and D). These antioxidant effects *in vivo* could be due to the presence of active substances in the *A. saponaria* extract. Preliminary HPLC analysis of *A. saponaria* showed several chromatographic peaks, indicating wide chemical diversity. Among the substances present were flavonoids (rutin, quercetin and kaempferol) and phenolic acids (gallic and caffeic acid) [9]. We also observed the presence of anthraquinone (aloin). Moreover, flavonoids, phenolic acids, and aloin are widely distributed in medicinal plants and exhibit antioxidant activities [3,22,30].

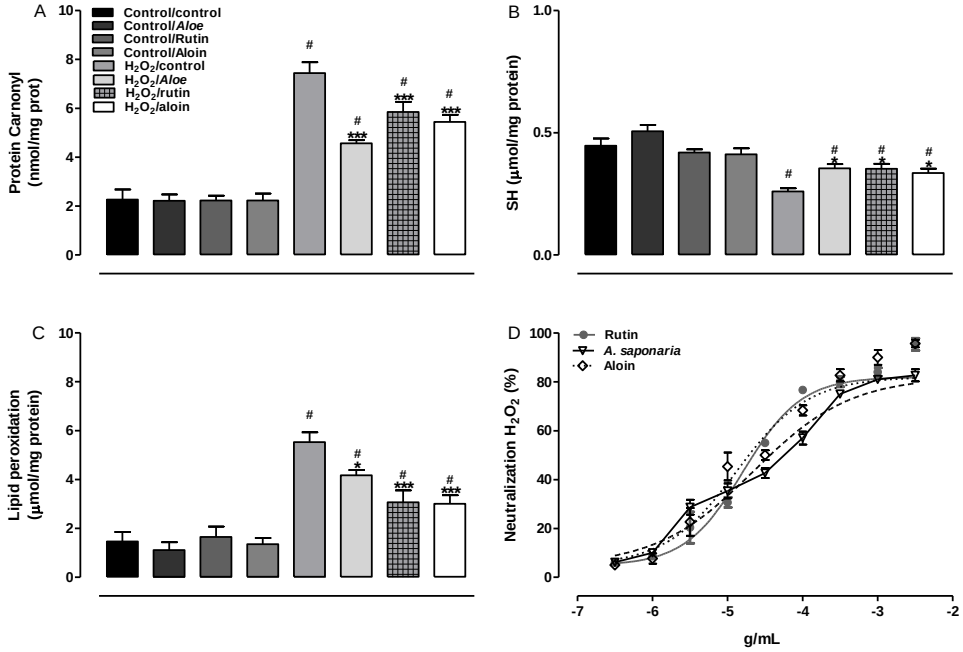
Because our *in vivo* data suggested that UVB irradiation increased the production of H_2O_2, we carried out *in vitro* experiments to determine whether *A. saponaria* could show antioxidant activity by measuring oxidative parameters induced by hydrogen peroxide. We found that *A. saponaria* extract (H_2O_2 /*Aloe*), aloin (H_2O_2/Aloin) and rutin (H_2O_2/rutin) (30 µg/mL) significantly reduced the observed increases in the protein carbonyl (Fig 5A), lipid peroxidation (Fig 5C), and thiol (Fig 5B) levels induced by hydrogen peroxide compared with the control group (H_2O_2/control) (Figure 5 A, B, C, and D). No significant difference was observed among the control groups (control/control; control/*Aloe*; control/rutin; and control/aloin). Moreover, at different concentrations (10.000 – 20 µg/mL), *A. saponaria*, aloin, and rutin were able to counteract the formation of hydrogen peroxide with ED_{50} values of 2.2 $x10^{-5}$, 1.3 $x10^{-5}$, and $1.6x10^{-5}$ µg/mL, respectively (Fig. 5D). Our results demonstrate that the two main compounds found in *A. saponaria* extract (aloin and rutin) exhibit similar antioxidant effects *in vitro.* Because the isolated compounds exhibited antioxidant potencies slightly greater than the extract, the antioxidant effects of *A. saponaria* are likely a result of the combination of several antioxidant compounds present in this plant. The antioxidant effects could be due to the combination of the two compounds present in the *A. saponaria* extract. These results are agree with those of other studies demonstrating the antioxidant effects of *A. saponaria* activity on xanthine-xanthine oxidase [22]. Moreover, studies have shown that aloin and rutin exhibit the significant antioxidant activity associated with *in vivo* injuries, such as burn trauma [31, 32].

Figure 4.



Effects of the *Aloe saponaria* cream (*Aloe*) and no treatment (no treat) on oxidative stress parameters (protein carbonyl levels, thiol levels (SH), lipid peroxidation, and hydrogen peroxide production) in the paw tissue of rats subjected to UVB irradiation (UVB). The data are presented as the means ± SEM of 4-5 rats. $^{\#}P<0.05$ when compared with the naïve and no treatment group (Naïve/no treat). *P<0.05 and ***P<0.001 when compared to the UVB irradiation and no treatment group (UVB/no treat). (Two-way ANOVA followed by Bonferroni's test).

Figure 5.



Effects of the *Aloe saponaria* extract (*Aloe*), rutin, and aloin on oxidative stress parameters (protein carbonyl levels (A), thiol levels (SH) (B), lipid peroxidation (C), and neutralization of H_2O_2 (D)) *in vitro* in the presence of hydrogen peroxide (H_2O_2) or control. $^{\#}P< 0.05$ when compared with the control (control/control) group. *P<0.05 and ***P<0.01 when compared to the hydrogen peroxide/control group (H_2O_2 /control). (One-way ANOVA followed by "Newman-Keuls" Test).

Conclusions

In conclusion, the results presented in the current study show that treatment with *A. saponaria* showed anti-nociceptive, anti-inflammatory and antioxidant effects in a sunburn model in rats. These biological activities could be due to the presence of active substances in the *A. saponaria* extract that exhibit antioxidant activity. Taken together, these results support the use of the *A. saponaria* for the treatment of burn injury

Acknowledgements

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Conflict of interest

Authors declared no conflict of interest.

References

[1] T. Bishop, D.W. Hewson, P.K. Yip, et al. Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. Pain. 131(2007), 70-82.

[2] A. Gustorff, T. Sycha, D. Lieba-Samal, et al. The pattern and time course of somatosensory changes in the human UVB sunburn model reveal the presence of peripheral and central sensitization. Pain. 154 (2013) 586-597.

[3] Y.M. Fonseca, C.D. Catini, F.T.M.C. Vicentini, et al. Protective effect of *Calendula officinalis* extract against UVB-induced oxidative stress in skin: Evauation of reduced glutathione levels and matrix metalloproteinase secretion. J Ethonopharmacol. 127 (2010) 596-601.

[4] A.K. Atoui, A. Mansouri, A. Boskou, et al. Tea and herbal infusions: their antioxidant activity and phenolic profile. Food chemistry. 89 (2005) 27-36.

[5] P.J. Zapata, D. Navarro, F. Guillén, et al. Characterization of gels from different *Aloes pp*. as a antifungal treatment: Potential crops for industrial applications. Industrial Crops and Products. 42 (2013) 223-230.

[6] M.M. Iwu, Handbook of African Medicinal Plants. Library of Congress Ed, 1993.

[7] F.M.C. Barros, K.N. Pereira, G.D. Zanetti, et al. Plantas de Uso Medicinal no Município de São Luiz Gonzaga, RS, Brasil. Latim. American. J Pharmacy. 26 (2007) 652-662.

[8] E.L.C. Soares, G.S. Vendrusculo, S.M. Eisinger, et al. Estudo etonobotânico do uso dos recursos vegetais em São João do Polêsine, RS, Brasil, no período de outubro de 1999 a junho de 2001. I-Origem e fluxo do conhecimento. Ver Bras Plantas Med. Botucatu. 6 (2004) 69-95.

[9] M.A. Silva, G. Trevisan, J.Z. Klafke, et al. Antinociceptive and anti-inflammatory effects of *Aloe saponaria* Haw on thermal injury in rats. J Ethonopharmacol. 146 (2013) 393-401.

[10] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 16 (1983) 109–110.

[11] A.T. Elamthuruthy, C.R. Shahb, T.A. Khanb, et al. Standardization of marketed *Kumariasava*-an Ayurvedic *Aloe vera* product. J Pharm Biomed Anal. 37 (2005) 937-941.

[12] W.J. Dixon, Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol. 20 (1980) 441–62.

[13] M.A. Silva, J.Z. Klafke, M.F. Rossato, et al. Role of peripheral polyamines in the development of inflammatory pain. BiochemicalPharmacology. 82 (2010) 269-277.

[14] S. Lloret, J.J. Moreno, Effects of an-anti-inflammatory peptide antiflammin 2 on cell influx, eicosanoid biosynthesis and oedema formation by arachidonic acid and tetradecanoylphorbol dermal application. Biochemical Pharmacologyl. 50 (1995) 347-53.

[15] H.S. Kang, J.Y. Lee, C.J. Kim, Anti-inflammatory activity of arctigenin from Forsythiae Fructus. Journal of Ethnopharmacology.116 (2008) 305–312.

[16] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein – dye binding. Anal Biochem. 72 (1976) 248–254.

[17] J.Z. Klafke, M.A. Silva, T.F. Panigas, et al. Effects of Campomanesia xanthocarpaon biochemical, hematological and oxidative stress parameters in hypercholesterolemic patients. J.Ethnopharmacol. 127 (2010) 299–305.

[18] M.F. Rossato, N.A. Velloso, S. de Oliveira, et al. Spinal levels of nonprotein thiols are related to nociception in mice. J Pain. 11(2010) 545-54.

[19] H. Aebi, Catalase in vitro. Methods Enzymol. 105 (1984) 121-126.

[20] Y. Nakamura, A. Murakami, Y. Ohto, et al. Suppression of tumor promoter-induced oxidative stress and inflammatory responses in mouse skin by a superoxide generation inhibitor 1'-acetoxychavicol acetate. Cancer Res. 58 (1998) 4832-4839.

[21] M.Y. Park, H.J Know, M.K. Sung, M.K. Evaluation of aloin and aloe-emodin as anti-inflammatory agents in aloe by using murine macrophages. Biosci Biotechnol Biochem. 73 (2009) 828 – 32.

[22] T. Sycha, S. Anzenhofer, S. Lehr, et al. Rofecoxib attenuates both primary and secondary inflammatory hyperalgesia: a randomized, double blinded, placebo controlled crossover trial in the UV-B pain model. Pain. 113 (2005) 316-322.

[23] G. Khorasani, S.J. Hosseinimehr, M. Azadbakht, et al. Aloe Versus Silver Sulfadiazine Creams for Second-Degree Burns: A Randomized Controlled Study. Surg Today. 39 (2009) 587–591.

[24] Y. Matsumoto-Okasaki, M. Furuse, K. Kajiya, Claudin-5 haploinsufficiency exacerbates UVB-induced oedema formation by inducing lymphatic vessel leakage. Exp Dermatol. 21 (2012) 535-561.

[25] P. Rishi, A. Rampuria, T. Rupinder, et al. Phytomodulatoty Potentials of *Aloe vera* against *Salmonella* OmpR-mediated Inflammation. Phytother Res. 22 (2008) 1075-1082.

[26] M.Y. Park, H.J Know, M.K. Sung, M.K. Dietary aloin, aloesin, or aloe-gel exerts anti-inflammatory activity in a rat colitis model. Life Sci. 88 (2011), 486 – 492.

[27] R. Aquino, S. Morelli, A. Tomaino, et al, Antioxidant and photoprotective activity of a crude extract of Culcitium reflexum H.B.K. leaves and their major flavonoids. J. Ethnopharmacol. 79 (2002) 183-191.

[28] S.C. Lee, J.W. Lee, J.E. Jung, et al. Protective role of nitric oxide-mediated inflammatory response against lipid peroxidation in ultraviolet B-irradiation skin. Br J Dermatol. 142 (2000) 653-659.

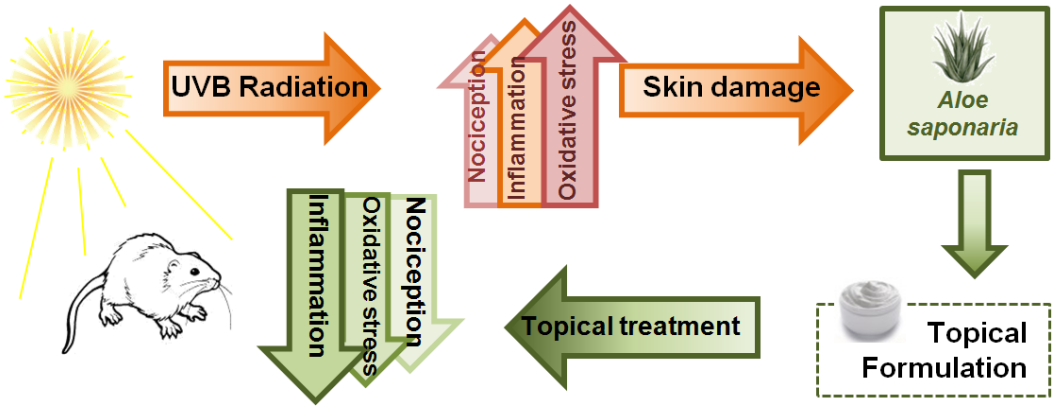
[29] V.A. Terra, F.P. Souza-Neto, R.C. Perreira, et al. Time-dependent reactive species formation and oxidatve stress damage in the skin after UVB irradiation. J Photochem Photobiol B.109 (2012) 34-41.

[30] V. Hajhashemi, B. Zolfaghari, A. Yousefi, Antinociceptive and Anti-Inflammatory Activities of Satureja hortensis Seed Essential Oil, Hydroalcoholic and Polyphenolic Extracts in Animal Models. Medi Princi Pract. 21 (2012) 178–182.

[31] A. Chaudhary, A. Bhandari, A. Pandurangan, Antioxidant potential and total phenolic content of methanolic bark extract of *Madhuca indica (koenig) Gmelin*. Anc Sci Life. 31 (2012) 132–136.

[32] E. Moretti, L. Mazzi, G. Terzuoli, et al. Effect of quercetin, rutin, naringenin and epicatechin on lipid peroxidation induced in human sperm. Reprod Toxicol. 34 (2012) 651–657.

4.2.1. Resumo gráfico



5. DISCUSSÃO

A planta *Aloe saponaria* vem sendo utilizada empiricamente no mundo inteiro (medicina popular) para várias doenças de pele, incluindo queimaduras (SOARES et al., 2004). Este tratamento é baseado na evidência anedótica ou em pesquisas realizadas quase que exclusivamente com a planta da mesma espécie, *Aloe vera* (SOARES et al., 2004). Dessa forma, foram investigados os efeitos antinociceptivos, antiinflamatórios e antioxidantes do creme contendo o extrato da *Aloe saponaria*, em lesões térmicas causadas por água quente, tanto de primeiro quanto de segundo grau. A lesão térmica induzida é de primeiro grau, sendo causada por radiação UVB. Neste estudo, pudemos observar através de dados comportamentais e bioquímicos que as lesões térmicas levam ao desenvolvimento de um processo inflamatório, que resulta em desenvolvimento de nocicepção e estresse oxidativo. Adicionalmente, também foi observado que o creme contendo o extrato da planta *A. saponaria,* conseguiu reverter estas respostas após o estabelecimento da lesão térmica.

Assim, inicialmente foi realizada uma análise preliminar dos componentes presentes na planta *A. saponaria* com HPLC. Esta análise demonstrou a presença de inúmeros picos cromatográficos, os quais revelaram uma grande diversidade de compostos químicos. Entre as substâncias presentes na *Aloe saponaria*, estão flavonoides (rutina, quercetina e canferol) ácidos fenólicos (ácido gálico e ácido caféico) e antraquinonas. Estes compostos estão distribuídos em inúmeras plantas medicinas. Tais resultados mostram-se semelhantes ao que já foi observado em resultados anteriores para o mesmo gênero, detectando fenóis, flavonoides e antraquinonas em extratos de folhas de A*. ferox, A. secundiflora e A. vera* (REBECCA et al., 2003; WINTOLA e AFOYALAN, 2011). Também foi observada a presença de um pico correspondente a presença de aloína, principal constituinte biologicamente ativo encontrado no extrato das plantas pertencentes à espécie *Aloe* (PARK et al., 2009; SYCHA et al., 2005). Ainda, de acordo com Park e colaboradores (2009), foi observada a presença de aloína (antraquinona) em uma concentração que variou de 0,1 a 21,5%. Além disso, vários estudos demonstraram que os flavonóides e compostos fenólicos encontrados na *A. saponaria* possuem efeitos antiinflamatórios e antinociceptivos em modelos de dor inflamatória em ratos (LAPA et al., 2009; HAJHASHEMI al., 2012; MEHROTRA et al., 2011).

Nestas condições, inicialmente foi investigado a possibilidade de a radiação UVB induzir um aumento dos níveis de estresse oxidativo (proteína carbonilada, níveis de tióis e conteúdo de H_2O_2). Esta avaliação foi realizada 24 horas após a

irradiação e comparada ao grupo que não recebeu nenhuma irradiação. Os resultados mostraram que a radiação aumentou 2 vezes os níveis de proteína carbonilada, 5 vezes a peroxidação lipídica, 2 vezes os níveis de tióis, e 4 vezes a produção de H_2O_2 no tecido da pata. Estes dados são semelhantes aos observados em outros estudos, os quais demonstraram que o dano induzido pela radiação UVB está geralmente associado com a superprodução de espécies reativas de oxigênios e radicais livres, como o H_2O_2, e ainda, a um aumento da peroxidação lípidica seguido de uma redução das defesas antioxidantes do organismo (FONSECA et al., 2010; AQUINO et al., 2002, TERRA et al., 2012). Em continuação, foi observado que o tratamento com o creme contendo extrato de *A. saponaria,* não alterou os parâmetros de estresse oxidativo nos animais do grupo naive. No entanto, 24 horas após a emissão da radiação UVB seguida pelo tratamento tópico com o creme, foi observado uma redução significativa dos parâmetros de estresse oxidativo analisados, quando comparados com o grupo queimado e que não foi tratado. Acredita-se, que tais efeitos antioxidantes ocorrem devido à presença de diversas substâncias antioxidantes (rutina, ácido caféico, ácido gálico, etc.) presentes na planta, que como observado em outros estudos, apresentam uma grande distribuição em plantas medicinais e exibem atividades antioxidantes (FONSECA et al., 2010; SYCHA et al., 2005; HAJHASHEMI et al., 2012).

Seguindo os resultados *in vivo*, foram realizados experimentos *in vitro* para determinar se a *A. saponaria* apresentaria atividade antioxidante, este parâmetro foi avaliado após a adição de H_2O_2. Foram observados, que os grupos tratados com o extrato da planta, ou aloína, ou rutina reduziram significativamente o aumento dos níveis de proteína carbonilada, peroxidação lipídica e níveis de tióis, os quais foram induzidos pela adição de H_2O_2 e comparados ao grupo controle (H_2O_2 /controle). Além disso, em diferentes concentrações a *A. saponaria*, aloína e rutina foram capazes de inibir a formação de H_2O_2. Além disso, foi observado que os compostos: aloína e rutina, presentes no extrato exibiram efeitos antioxidantes similares nos experimentos realizados *in vitro.* Devido aos compostos isolados terem exibido um efeito antioxidante ligeiramente maior do que o extrato, o efeito antioxidante da planta se deve, provavelmente, à uma combinação de diferentes compostos antioxidantes presentes na *A. saponaria.* Tal fato estaria de acordo, com resultados apresentados em estudos anteriores, em que foram demonstrados efeitos antioxidantes da planta sobre a enzima xantina oxidase (SYCHA et al., 2005). Além

disso, algumas pesquisas têm mostrado que a aloína e a rutina também apresentam atividade antioxidante significativa associada com lesões, tais como queimaduras (CHAUDHARY et al., 2012; MORETTI et al., 2012,

Ainda, para avaliar as atividades antinociceptiva e antiinflamatória de A. saponaria, foram realizados dois modelos de lesão térmica. No primeiro, os animais receberam uma queimadura por água quente na pata (GAO et al., 2010) mimetizando queimaduras causadas por líquidos quentes, já que, uma das queixas principais de pacientes com esse tipo de queimadura é o desenvolvimento de um processo doloroso, em que os mesmos apresentam alodínia mecânica, hiperalgesia térmica e dor espontânea no local da lesão e nas áreas adjacentes (SUMMER et al., 2007). Além disso, também foi analisado o desenvolvimento de edema e infiltração de leucócitos no sítio da lesão (KOWAL-VERNE et al., 1997). Utilizou-se também, um modelo de lesão térmica induzido por radiação UVB, mimetizando a queimadura solar (BISHOP et al., 2007). Neste modelo, trabalhos anteriores também já demonstraram a presença de respostas nociceptivas, como hiperalgesia a estímulos térmicos e mecânicos (GUSTORFF et al., 2013). Dessa maneira, acredita-se que este, seja um modelo adequado para observar os efeitos antinociceptivos, antiinflamatórios e antioxidantes do extrato da planta *A. saponaria*, já que a radiação UVB produz uma redução concomitante nos limiares de dor térmica e mecânica, conhecido como alodínia, bem como aumenta os níveis de espécies reativas (ZAPATA et al,, 2013; GUSTORFF et al,, 2013; FONSECA et al., 2010; AQUINO et al., 2002, TERRA et al., 2012). Semelhante aos estudos citados anteriormente, o presente estudo também comprovou os mesmos sinais e sintomas, o que tornou os modelos utilizados no trabalho, exemplos relevantes para avaliar os efeitos antiinflamatórios, antinociceptivos e antioxidantes da *A. saponaria* após queimaduras térmicas.

A hiperalgesia induzida por estimulação mecânica no sítio da lesão é a principal fonte de dor, após uma lesão térmica (SUMMER, et al., 2007). Além disso, pacientes com queimaduras, também apresentam desenvolvimento de dor espontânea. Este comportamento é comumente relatado pelos pacientes, como uma sensação de “picada”, “queimação”, e “choque” (SUMMER et al., 2007). Essa resposta também é observada em animais submetidos a modelos de lesão térmica por água quente. Em conformidade com dados anteriores, o nosso estudo também demonstrou que o modelo com água quente, tanto de primeiro quanto de segundo

grau, como também o modelo de queimadura solar (UVB) reduziram o limiar da alodínia mecânica estática e dinâmica e ainda da alodínia térmica. O que está de acordo com um estudo anterior, que expôs que a administração sistêmica do extrato etanólico de *A. saponaria* apresentou efeitos antinociceptivos em modelo de dor neuropática causada por tratamento sucessivo com cisplatina (YOO et al., 2008). Resultados semelhantes foram observados com o controle positivo (sulfadiazina de prata 1%), tratamento tópico comum para queimadura. Além disso, estudos demonstraram a sua eficácia no tratamento de queimaduras (KHORASANI et al., 2009).

A queimadura na pele acarreta dano tecidual local, o que leva ao desenvolvimento de um processo inflamatório doloroso (SENER et al., 2003), acompanhado da formação de edema (BISHOP et al., 2007; MATSUMOTO-OKASAKI et al., 2012) e infiltração de leucócitos (GUSTORFF et al., 2013). De fato, foi igualmente observado no nosso estudo, desenvolvimento de edema após a indução dos modelos de queimaduras. Esta resposta foi reduzida com o tratamento do creme contendo o extrato da *A. saponaria*. Em relação ao edema, outros estudos com diferentes espécies do gênero *Aloe* foram previamente descritos descrevendo efeitos anti-edematogênicos, também quando utilizadas topicamente. De acordo com nossos dados, Rishi e colaboradores (2008), também observaram que o tratamento com a *A. vera* foi capaz de reduzir o edema produzido por infecção bacteriana. Semelhante a *A. saponaria,* a sulfadiazina de prata 1% apresentou tanto efeitos antinociceptivos, quanto antiinflamatórios em modelos de queimaduras. A sulfadiazina de prata é o tratamento tópico mais comumente usado para queimaduras, e vários estudos têm mostrado que ela seja eficaz para tratar queimaduras (MAENTHAISONG et al., 2007; KHORASANI et al., 2009). Em vista os resultados apresentando, a *A. saponaria* apresentou eficácia semelhante à sulfadiazina e *A. vera* (BUNYAPRAPHATSARA et al., 1996), demonstrando que devido aos seus efeitos antinociceptivos e antiinflamatórios, seria um tratamento nas queimaduras de primeiro e segundo graus, já que é um tratamento que se mostrou tão eficaz quanto os tratamentos de referência. Essa eficácia semelhante da *A. saponaria* e sulfadiazina de prata, pode ser devido à sua semelhança com respeito às suas propriedades, já que a sulfadiazina é usada principalmente para queimaduras, devido à sua ação antimicrobiana (KHORASANI et al., 2009), da mesma forma um estudo anterior já havia indicado que *A. saponaria* também possui

ação antibacteriana (TIAN et al., 2003). Além disso, também observamos que o extrato da *A. saponaria* 10% foi mais eficaz do que o de 30% para produzir efeitos antinociceptivo e antiinflamatórios. Este resultado não é inesperado, uma vez que, a relação entre o fluxo de uma droga a partir de pomadas para a pele e a dose da droga geralmente segue uma curva em forma de U invertido (TROY, 2005). Na verdade, a evaporação dos componentes mais voláteis do creme (tal como água), pode levar à precipitação precoce de medicamentos, reduzindo o seu acesso à pele, o que pode explicar porque a dose *A. saponaria* de 30% é menos eficaz do que o de 10%.

Juntamente com a formação de edema, as lesões causadas por queimaduras, são também susceptíveis à infiltração por uma variedade de tipos de células, incluindo os macrófagos e os neutrófilos (SENER et al., 2003; Evers et al., 2010). A atividade da MPO, EPO e Nagase em tecido lesionado foram utilizadas como um marcador de infiltração de neutrófilos, macrófagos e eosinófilos, respectivamente (LLORET e MORENO, 1995; KANG et al., 2008). O presente trabalho demonstrou que tanto após a queimadura com água quente, como também após, a indução de queimadura solar, houve um aumento dos níveis de MPO, NAGase e EPO em amostras de tecidos das patas dos ratos. Estes resultados indicam infiltração de leucócitos no local da lesão, o que poderia contribuir para o desenvolvimento dos processos nociceptivos (SCHOLZ e WOOLF 2001; LOESER e TREEDE, 2008; LATREMOLIERE e WOOLF, 2009; WOOLF, 2010). Concomitante com os seus efeitos antinociceptivo e anti-edematogênico, *A. saponaria* também foi efetiva em prevenir a inibição da infiltração de neutrófilos, macrófagos e eosinófilos no sítio da lesão. O que está em concordância com estudos anteriores, nos quais foi demonstrado que *A. vera* foi capaz de reduzir a adesão dos leucócitos no endotélio de ratos submetidos à queimadura induzida com placa quente (10 segundos – 75ºC) (DUANSAK et al., 2003). Além disso, estudos anteriores demonstraram os efeitos antiinflamatórios do composto aloína presente na *A. vera* em modelos de colite em ratos (PARK et al., 2011). Yoo e colaboradores, em 2008 mostraram também que a incubação do extrato da *A. saponaria*, em cultura de macrófagos, suprimiu a produção de óxido nítrico, consequentemente inibindo o lipopolissacarídeo (LPS) capaz de aumentar a produção de óxido nítrico. Dessa maneira, a redução da infiltração de leucócitos no tecido lesionado parece contribuir para os efeitos benéficos de *A. saponaria* sobre a lesão térmica.

Outro fator analisado no trabalho foi a facilitação da ação antiinflamatória se as características físico-químicas do creme após a incorporação de extrato poderiam. Foram observados, que a incorporação do extrato da *A. saponaria* no creme Lanete reduzido o seu pH e sua viscosidade, porém não alterou seu comportamento, em relação ao espalhamento do creme. Sendo importante, discutir que a eficácia da terapia tópica, encontra-se na maneira como o paciente espalha a formulação na região afetada, e a sua distribuição na região aplicada, bem como sua viscosidade e composição (GARG et al., 2002). Neste caso, as formulações tiveram valores semelhantes, e a incorporação do extrato de *A. saponaria* na base não influenciou no seu comportamento (espalhamento). Outro fator observado foi o pH, apesar de ser menor do que o analisado no creme base, o valor do pH do creme contendo o extrato de *A. saponaria* ainda é compatível com a aplicação tópica e com o manto de pH ligeiramente ácido da pele entre 4,6 e 5,8. Além disso, o baixo pH do creme de *A. saponaria* pode ser favorável no processo de cicatrização, uma vez que o pH ácido da pele tem inúmeras funções, incluindo a defesa antimicrobiana, e à restrição da inflamação, devido a inibição da liberação de citocinas pró-inflamatórias (POW et al, 2011).

Esses resultados obtidos no trabalho demonstraram, que o tratamento com *A. saponaria Haw* apresentou efeitos antinociceptivos, antiinflamatórios e antioxidantes em modelos de lesão térmica (queimaduras). O que em conjunto, apóiam a utilização popular da planta no tratamento para queimadura, por parte da população.

6. CONCLUSÕES

Tendo em vista os resultados obtidos no presente estudo, pode-se concluir que:

6.1. O tratamento tópico com o creme contendo extrato da *Aloe saponaria* reduziu não somente a nocicepção, mas também parâmetros inflamatórios causados pelos modelos de queimadura com água quente e com radiação UVB, o que confirma a eficácia de seu uso popular no tratamento de queimaduras;

6.2. Foi observado que tanto o extrato, quanto os compostos isolados da *Aloe saponaria* (rutina e aloína) apresentaram atividade antioxidante *in vitro*, o que pode ser responsável pela redução do estresse oxidativo induzido pela radiação UVB *in vivo*, após a utilização do creme com extrato de *Aloe saponaria*.

7. REFERÊNCIAS BIBLIOGRÁFICAS

AQUINO, R., MORELLI, S., TOMAINO, A., et al., 2002. Antioxidant and photoprotective activity of a crude extract of Culcitium reflexum H.B.K. leaves and their major flavonoids. Journal of Ethnopharmacol 79, 183-191.

BEDI, M.K., PHILIP, D., SHENEFELT, M.D., 2002. Herbal Therapy in Dermatology. Archives of Dermatology 138, 232-242.

BERTIN-MAGHIT, M., GOUDABLE, J., DALMAS, E., et al., 2000. Time course of oxidative stress after major burns. Intensive Care Medicine 26, 800-803.

BILSEL, Y., BUGRA, D., YAMANER, S., 2002. Could honey have a place in colitis therapy? Effects of honey, prednisolone and disulfiram on inflammation, nitric oxide, and free radical formation. Dig Surg 19, 306-312.

BISHOP, T., HEWSON, D.W., YIP, P.K., et al., 2007. Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. Pain 131, 70-82.

BISHOP, T., HEWSON, D.W., YIP, P.K., et al., 2009. Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. Pain 131, 70-82.

BRENNAN, F., CARR, D.B., COUSINS, M., 2007. Pain management: a fundamental human right. Anesth Analg 105, 205-221.

BUNYAPRAPHATSARA, N., JIRAKULCHAIWON, S., THIRAWARAPAN, S., et al., 1996. The efficacy of *Aloe vera* cream in the treatment of first, second and third degree burns in mice. Phytomedicine 2, 247-251.

CHAUDHARY, A., BHANDARI, A., PANDURANGAN, A., 2012. Antioxidant potential and total phenolic content of methanolic bark extract of *Madhuca indica (koenig) Gmelin*. Anc Sci Life 31, 132–136.

CRAIG, K.D., 2009. The social communication model pain. Can. Psychol 50, 22-32.

CORMACK, D.H., 2008. Fundamentos de histologia. Guanabara e Koogan 2ª ed, 228-240.

COSTIGAN, M., SCHOLZ, J., WOOLF, C.J., 2009. Neuropathic Pain: A Maladaptive Response of the Nervous System. Annual Review of Neuroscience 32, 1-32.

DAS, S., MISHRA, B., GILL, K., et al., 2011. Isolation and characterization of novel protein with anti-fungal and anti-inflammatory properties from *Aloe vera* leaf gel. International Journal of Biologial Macromolecules 48, 38-41.

DAVIS, S.C., PEREZ, R., 2009. Cosmeceuticals and natural products: wound

healing. Clinics in Dermatology 27, 502-506.

De JONG, A.E., MIDDLEKOOP, E., FABER, A.W., 2007. Non-pharmacological nursing interventions for procedural pain relief in adults with burns: a systematic literature review. Burns 33, 811-827.

DUANSAK, D., SOMBOONWONG, J., PATUMRAJ, S., 2003. Effects of *Aloe vera* on leukocyte adhesion and TNF-alpha and IL-6 levels in burn wounded rats. ClinIcal Hemorheologic Microcirculation 29, 239–246.

DUPONT, L., PEREIRA, D.N., 2012. Sun exposure and Sun protection habits in high school students from city south of the country. Anais Brasileiros de Dermatologia 87, 90-95.

EVERS, L.H., BHAVSAR, D., MAILANDER, P., 2010. The biology of burn injury. Experimental Dermatology 19, 777-783.

FONSECA, Y.M., CATINI, C.D., VICENTINI, F.T.M.C., et al., 2010. Protective effect of Calendula officinalis extract against UVB-induced oxidative stress in skin: Evauation of reduced glutathione levels and matrix metalloproteinase secretion. J Ethonopharmacol 127, 596-601.

FURST, S., 1999. Transmitters involvednin antinociception in the spinal cord. Brain Res. Bull 48, 129-141.

GALLAGHER, J., GRAY, M., 2003. Is aloe vera effective for healing chronic wound? Wound Ostomy Continence Nurs 30, 68-71.

GAO, Y., XU, C.S., YU, K., et al., Liang, S.D., 2010.Effect of tetramethylpyrazine on DRG neuron P2X3 receptor involved in transmitting pain after burn. Burns 36, 127–134.

GARG, A., AGGARWAL, D., GARG, S., et al., 2002. Spreading of semisolid formulations. Pharmaceutical Technology 26, 84-105.

GRAVANTE G, et al., 2006. Apoptotic cell death in deep partial thickness burns by coexpression analysis of TUNEL and Fas. Sugery 139, 854-855.

GOLD, M.S., GEBHART, G.F., 2010. Nociceptor sensitization in pain pathogenesis. Nat. Med 16, 1248-1257.

GUSTORFF, A., SYCHA, T., LIEBA-SAMAL, D., et al., 2013. The pattern and time course of somatosensory changes in the human UVB sunburn model reveal the presence of peripheral and central sensitization. Pain 154, 586-597.

HACIMUFTUOGLU, A., HANDY, C.R., GOETTL, V.M., et al., 2006. Antioxidants attenuate multiple phases of formalin-induced nociceptive response in mice. Behav Brain Res 173, 211-216.

HAJHASHEMI, V., ZSOLFAGHARI, B., YOUSEFI, A., 2012. Antinociceptive and Anti-Inflammatory Activities of Satureja hortensis Seed Essential Oil, Hydroalcoholic and Polyphenolic Extracts in Animal Models. Medi Princi Pract 21, 178–182.

HORTON, J.W., 2003. Free radicals and lipid peroxidation mediated injury burn trauma: the role of antioxidant therapy. Toxicology 189, 75-88.

HONSNUTER, M., GUREL, A., BABUCCU, O., et al., 2004. The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burns 30, 121-125.

HUANG, H.S., PAN, H.L., STAHL, G.L., et al., 1995. Ischemia – and reperfusion-sensitive cardiac sympathetic afferents: influence of H_2O_2 and hydroxyl radicals. American Journal of Physiology 269, 888-901.

HUANG, J., ZHANG, X., MCNAUGHTON, P.A., 2006. Inflammatory pain: the cellular basis of heat hyperalgesia. Curr. Neuropharmacol 4, 197–206.

HU, Y., XU J., HU, Q., 2003. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. Journal of Agricultural and Food Chemistry 51, 7788-7791.

JULIUS, D., BASBAUM, A.I., 2001. Molecular mechanisms of nociception. Nature 413, 203-210.

KANG, H.S., LEE, J.Y., KIM, C.J., 2008. Anti-inflammatory activity of arctigenin from Forsythiae Fructus. Journal of Ethnopharmacology 116, 305–312.

KHORASANI, G., HOSSEINNIMEHR, S.J., AZADBKHT, M., ZAMANI, A., et al., 2009. Aloe Versus Silver Sulfadiazine Creams for Second-Degree Burns: A Randomized Controlled Study. *Surgery Today* 39, 587–591.

KIM H.K., PARK, S.K., ZHOU, J.L., et al., 2004. Reactive oxygen species (ROS) play an important role in a rat model of neurophatic pain. Pain 111, 116-124.

KLEIN, A.D., PENNEYS, N.S., 1988. *Aloe vera.* Journal of the American Academy of Dermatology 18, 714-120.

KOWAL-VERNE, A., WALENGA, J.M., SHARP –PUCCI, M., et al., 1997. Postburn edema and related changes in interleukin-2, leukocytes, platelet

activation, edothelin-1, and C1 esterase inhibitor.The Journal of burn care & rehabilitation 18, 99-103.

KUNER, R., 2010. Central mechanisms of pathological pain. Nat. Med 16, 1258-1266.

KRESS, M., RIEDL, B., REEH, P.W., 1995. Effects of oxygen radicals on nociceptive efferentes in the rat skin in vitro. Pain 62, 87-94.

LALONDE, C., NAYAK, U., HENNIGAN, J., et al., 1997. Excessive liver oxidant stress causes mortality in response to burn injury combined with endotoxin and is prevented with antioxidants. Journal of Burn Care and Rehabilitation.

LAPA, F.R., GADOTTI, V.M., MISSAU, F.C., et al., 2009. Antinociceptive Properties of the Hydroalcoholic extract and the Flavonoid rutin obtained from *Polygala paniculata* L. in mice. Basic & Clinical pharmacology & Toxicology 104, 306-315.

LLORET, S., MORENO, J.J., 1995. Effects of an-anti-inflammatory peptide antiflammin 2 on cell influx, eicosanoid biosynthesis and oedema formation by arachidonic acid and tetradecanoylphorbol dermal application. Biochemical *Pharmacology*l 50, 347-53.

LOESER, J.D., TREEDE, R.D., 2008. The Kyoto protocol of IASP Basic Pain Terminology. Pain 137, 473- 477.

LOOTS, D.T., VAN DER WESTHIZEN, F.H., BOTES, L., 2007. *Aloe ferox* Leaf Gel Phytochemical Content, Antioxidant Capacity, and Possible Health Benefits Journal of Agricultural and Food Chemistry 55, 6891-6896.

MACINTYRE, P.E., et al., 2010. Acute pain management: scientific evidence. Australian and New Zealand College of Anaesthetists and Faculty of Pain Medicine. 3ª Ed., pp. 1- 533.

Maenthaisong, R., Chaiyakunapruk, N., Niruntraporn, S., Kongkaew, C., 2007. The efficacy of *Aloe vera* used for burn wound healing: a systematic review. Burns 33, 713–718.

MATUSMOTO-OKASAKI, Y., FURUSE, M., KAJIYA, K., 2012. Claudin-5 haploinsufficiency exacerbates UVB0induced oedema formation by inducing lymphatic vessel leakage. Experimental Dermatology 21, 535-561.

MEHROTRA, A., SHANBHAG, R., CHAMALLAMUDI, M.R., SINGH, V.P., et al., 2011. Ameliorative effect of caffeic acid against inflammatory pain in

rodents.European Journal of Pharmacology 666, 80-86.

MERSKEY, H., BOGDUK, N., 1994. Classification of Chronic Pain. Taskforce on Taxonomy. Seattle.

MORETTI, E., MAZZI, L., TERSUOLI, G., et al., 2012. Effect of quercetin, rutin, naringenin and epicatechin on lipid peroxidation induced in human sperm. Reprod Toxicol 34, 651–657.

PARK, M.Y., KWON, H.J., SUNG, M.K., 2008. Plasma, tissue and urinary levels of aloin in rats after the administration of pure aloin. Nutrition Research and Practice 2, 17-21.

PARK, M.Y., KNOW, H.J., SUNG, M.K., 2009. Evaluation of aloin and aloe-emodin as anti-inflammatory agents in aloe by using murine macrophages. Biosci Biotechnol Biochem 73, 828 – 32.

PARK, M.Y., KNOW, H.J., SUNG, M.K., 2011. Dietary aloin, aloesin, or aloe-gel exerts anti-inflammatory activity in a rat colitis model. Life Sci 88, 486 – 492.

PARIHAR, A., PARIHAR, M.S., MILNER, S., et al., 2008. Oxidative stress and anti-oxidative mobilization in burn injury. Burn 34, 6-17.

PEREZ, R.S., ZUURMON, W.W., BEZEMER, P.D., et al., 2003. The treatment of complex regional pain syndrome type I with free radical scavengers: a randomized controlled study. Pain 102, 297-307.

PICCOLO, N.S., SERRA, M.C.V.F., LEONARDI, D.F., et al., 2008. Projeto diretrizes – Queimaduras: Diagnóstico e Tratamento Inicial. Sociedade Brasileira de Cirurgia Plástica 1-14.

PORTUGAL-COHEN, M., SOROKA, Y., MA'OR, Z., et al., 2009. Protective effects of a cream containing Dead Sea minerals against UVB-induced stress in human skin. Experimental Dermatology 18, 781-788.

POW, T.W., GRICE, J.E., LIN, L.L., et al., 2011. Nanoparticles and microparticles for skin drug delivery. Advanced Drug Delivery 63, 470-491.

REBECCA, W., KAYSER, O., HAGELS, H., et al., 2003. Main phenolic Compunds from the Leaf Exudate of *Aloe secundiflora* BY High-performance Liquid Chromatography-Mass Spectroscopy. Phytochemical Analysis 14, 83-86.

RICHARDSON, P., MUSTARD, L., 2009. The management of pain in the burns unit. Burns 35, 921-936.

RISHI, P., RAMPURIA, A. RUPINDER, T., et al., 2008. Phytomodulatoty Potentials of Aloe vera against Salmonella OmpR-mediated Inflammation. Phytother Res 22, 1075-1082.

ROBERTS, D.B., TRAVIS, E.L., 1995. Acemannan-containing wound dressing gel reduces radiation- induced skin reactions in C3H mice. International Journal of Radiant Oncology 32, 1047-1052.

ROCK, C.L., DECHERT, R.E., KHILNANI, R., et al., 1997. Caratenoids and antioxidants vitamins patients after burn injury. Journal of Burn Care Rehabilitation 18, 269-278.

SAMPEDRO, M.C., ARTOLA, R.L., MARATURE, D., 2004. Mannan from *Aloe saponaria* inhibits tumoral cell activation and proliferation. International Immunopharmacology 4, 411-418.

SASAKI, J.R., ZHANG, Q., SCHWACHA, M.G., 2011. Burn induces a Th-17 inflammatory response at the injury site. Burns 37, 641-651.

SCHOLZ, J., WOOLF, C.J., 2001. Can we conquer pain? Nat. Neurosci 5, 1062-1067.

SENER, G., SATIROGLU, H., SEHIRLI, A.O., et al., 2003.Protective effect of aqueous garlic extract against oxidative organ damage in a rat model of thermal injury.Life Sciences 73, 81 – 91.

SIMÕES, C.M.O., MENTZ, L.A., SHENKEL, E.P., et al., 1989. Plantas da Medicina Popular no Rio Grande do Sul. Editora da Universidade Federal do Rio Grande do Sul. 3ª Ed. pp. 28-29.

SOARES, E.L.C., VENDRUSCULO, G.S., EISINGER, S.M., et al., 2004. Estudo etonobotânico do uso dos recursos vegetais em São João do Polêsine, RS, Brasil, no período de outubro de 1999 a junho de 2001. I-Origem e fluxo do conhecimento. Revista Brasileira de Plantas Medicinais. Botucatu, pp 69-95.

SUMMER, G.J., PUNTILLO K.A., MIASKOWSKI, C., et al., 2007.Burn Injury Pain: The continuing Challenge. The Journal of Pain 8, 533-548.

SYCHA, T., ANZENHOFER, S., EHR, S., et al., 2005. Rofecoxib attenuates both primary and secondary inflammatory hyperalgesia: a randomized, double blinded, placebo controlled crossover trial in the UV-B pain model. Pain 113, 316-322.

SWALWELL, H., LATIMER, J., RACHEL, M.H., et al., 2012. Investigating the role

of melanin in UVA/UVB-and hydrogen peroxide-induced cellular and mitochondrial ROS production and mitochondrial DNA damage in human melanoma cells. Free Radical Biology and Medicine 52, 626-634.

TANAKA, H., LUND, T., WIIG, H., et al., 1999. High dose vitamin C counteracts the negative interstitial fluid hydrostatic pressure and early edema generation in thermally injured rats. Burns 25, 569-574.

TERRA, V.A., SOUZA-NETO, F.P., PERREIRA, R.C., et al., 2012. Time-dependent reactive species formation and oxidatve stress damage in the skin after UVB irradiation. J Photochem Photobiol B 109, 34-41.

TIAN, B., HUA, Y.J., MA, X.Q., et al., 2003. Relationship between antibacterial activityof aloe and its anthaquinone compounds. ZhongguoZhong Yao ZaZhi 28, 1034-1037.

TROY, D.B., 2005. Remington: the Science and Practice of Pharmacy, 21st ed. Lippincott Williams & Wilkins, Baltimore, pp. 871-888.

ZAPATA, P.J., NAVARRO, D., GUILLÉN, F., et al., 2013. Characterization of gels from different Aloes pp. as an antifungal treatment: Potential crops for industrial applications. Industrial Crops and Products 42, 223-230.

WINTOLA, O.A., AFOYAN, A.J., 2011. Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. Pharmacognosy Magazine 28, 325-333.

WOOLF, C.J., SALTER, M.W., 2000. Neuronal plasticity: increasing the gain in pain. Science 288, 1765-1769.

WOOLF, C.J., 2010. What is this thing called pain? J. Clin. Invest 120, 3742-3744.

YOO, E.A., KIM, S.D., LEE, W.M., et al., 2008. Evaluation of antioxidant, antinociceptive, and anti-inflammatory activities of ethanol extracts from *Aloe saponaria* Haw. Phytotherapy Research 22, 1389-1395.

YOUN, Y.K., SUH G.J., JUNG, S.E., et al., 1998. Recombinant human growth hormone decreases lung and liver tissue lipid peroxidation and increases antioxidant activity after thermal injury in rats. Journal of Burn Care Rehabilitation 19:542-548.