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Natháli Schopf Pegoraro

EFEITO ANTI-INFLAMATÓRIO DE FORMULAÇÕES PARA APLICAÇÃO TÓPICA DE ÁCIDO OLEICO E ACETATO DE DEXAMETASONA EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO DE PELE

Santa Maria, RS, Brasil
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Tese apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutor em Ciências Biológicas: Bioquímica Toxicológica**.

Orientadora: Profa. Dra. Sara Marchesan de Oliveira
Coorientadora: Profa. Dra. Letícia Cruz

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Natháli Schopf Pegoraro

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Aprovado em 12 de agosto de 2021:

Sara Marchesan Oliveira

Sara Marchesan de Oliveira, Dra. (UFSM)
(Presidente/Orientadora)

Letícia Cruz, Dra. (UFSM)
(Coorientadora)

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Dedico esta tese à minha

família, com todo o meu amor.

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RESUMO

EFEITO ANTI-INFLAMATÓRIO DE FORMULAÇÕES PARA APLICAÇÃO TÓPICA DE ÁCIDO OLEICO E ACETATO DE DEXAMETASONA EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO DE PELE

AUTORA: Natháli Schopf Pegoraro
ORIENTADORA: Sara Marchesan Oliveira
COORIENTADORA: Letícia Cruz

A pele, espaço de interface entre o ambiente e o corpo, está constantemente exposta a efeitos deletérios provenientes do meio externo, os quais são capazes de evocar uma resposta inflamatória. Uma vez que o tratamento para inflamação de pele apresenta limitações devido aos graves efeitos adversos causados, há uma necessidade constante no cenário mundial acerca do desenvolvimento de alternativas terapêuticas para tratar estes acometimentos. Neste trabalho foram desenvolvidas formulações para aplicação tópica do ácido oleico (AO), conhecido por modular mecanismos adjacentes à cicatrização de feridas. O primeiro capítulo desta tese aborda o desenvolvimento de formulações Lanette® e Pemulen® TR2 contendo AO e a avaliação do seu efeito anti-inflamatório em um modelo de queimadura de pele em camundongos *Swiss*. Ambas as formulações contendo 3% de AO inibiram o edema de orelha induzido pela radiação ultravioleta B (UVB) após tratamento único ($I_{máx}$ = 79,36 ± 7,47% e 92,58 ± 2,58%, respectivamente). Pemulen® TR2 3% AO reduziu a infiltração de células inflamatórias ($I_{máx}$ = 46,7 ± 4,0%) e o edema de orelha após tratamento repetido ($I_{máx}$ = 69,88 ± 2,31%; 60,95 ± 5,70% e 29,89 ± 6,40% em 24 h, 48 h e 72 h após UVB). No segundo capítulo, verificamos que Pemulen® TR2 3% AO foi capaz de inibir o edema de orelha ($I_{máx}$ = 76,41 ± 5,69%), a infiltração de células inflamatórias (avaliada pela atividade da enzima mieloperoxidase) ($I_{máx}$ = 71,37 ± 10,97%) e o nível da citocina inflamatória interleucina (IL)-1β ($I_{máx}$ = 94,18 ± 12,03%) induzidos por óleo de cróton em camundongos. Pemulen® TR2 3% AO inibiu o edema de orelha em um modelo de inflamação persistente causada por sucessivas administrações de óleo de cróton ($I_{máx}$ = 85,75 ± 3,08%), assim como inibiu o edema de orelha induzido por IL-1β ($I_{máx}$ = 80,58 ± 2,45%). Em ambos os modelos experimentais, observamos que o efeito antiedematogênico deve-se, pelo menos em parte, à ação do AO em receptores glicocorticoides, uma vez que este efeito foi prevenido pelo antagonista dos receptores glicocorticoides mifepristona. Este efeito anti-inflamatório do Pemulen® TR2 3% AO não esteve associado à ocorrência de efeitos adversos geralmente ocasionados pelo uso de glicocorticoides. Estes resultados sugerem que os semissólidos desenvolvidos poderiam constituir alternativas terapêuticas promissoras para tratar lesões inflamatórias de pele. Uma alternativa para melhorar a eficácia do tratamento e limitar a ocorrência de efeitos adversos é o emprego da Nanotecnologia. Por isso, temos como perspectiva para a conclusão deste trabalho, associar o AO à dexametasona em sistemas nanoestruturados, valendo-se das vantagens destes sistemas para a veiculação de ativos, a fim de propor uma terapia com potencial para tratar alterações cutâneas inflamatórias.

Palavras-chave: ácido oleico, acetato de dexametasona, corticosteroides, nanopartículas, anti-inflamatório.

ABSTRACT

ANTI-INFLAMMATORY EFFECT OF FORMULATIONS FOR TOPICAL APPLICATION OF OLEIC ACID AND DEXAMETHASONE ACETATE IN EXPERIMENTAL MODELS OF SKIN INFLAMMATION

AUTHOR: Natháli Schopf Pegoraro
ADVISOR: Sara Marchesan Oliveira
COADVISOR: Letícia Cruz

The skin, as an interface space between the environment and the body, is constantly exposed to harmful effects from the external environment, which are capable of evoking an inflammatory response. Since the treatment for skin inflammation has limitations due to the severity of the adverse effects caused, there is a constant need in the world scenario regarding the development of therapeutic alternatives to treat these affections. In this study, we developed formulations for the topical application of oleic acid (OA), a compound known to modulate mechanisms adjacent to wound healing. The first chapter of this thesis addresses the development of formulations based on Lanette® and Pemulen® TR2 containing OA and the evaluation of the anti-inflammatory effect of these formulations in a skin burn model in *Swiss* mice. Both formulations containing 3% OA inhibited the ultraviolet B (UVB) radiation-induced ear edema after single treatment (I_{max} = 79.36 ± 7.47% and 92.58 ± 2.58%, respectively). Pemulen® TR2 3% OA reduced inflammatory cell infiltration (I_{max} = 46.7 ± 4.0%) and ear edema after repeated treatment (I_{max} = 69.88 ± 2.31%; 60.95 ± 5.70% e 29.89 ± 6.40% at 24 h, 48 h and 72 h after UVB). In the second chapter, we verified that Pemulen® TR2 3% OA was able to inhibit the ear edema (I_{max} = 76.41 ± 5.69%), the infiltration of inflammatory cells (assessed by myeloperoxidase enzyme activity) (I_{max} = 71.37 ± 10.97%) and the level of the inflammatory cytokine interleukin (IL)-1β (I_{max} = 94.18 ± 12.03%) induced by croton oil in mice. Pemulen® TR2 3% OA inhibited ear edema in a model of persistent inflammation caused by successive croton oil administrations (I_{max} = 85.75 ± 3.08%), as well as inhibiting IL-1β-induced ear edema (I_{max} = 80.58 ± 2.45%). In both experimental models, we observed that the antiedematogenic effect is due, at least in part, to the action of OA on glucocorticoid receptors, since this effect was prevented by the glucocorticoid receptor antagonist mifepristone. This anti-inflammatory effect of Pemulen® TR2 3% OA was not associated with the occurrence of adverse effects usually caused by the use of glucocorticoids. These results suggest that the semisolids developed could be promising alternatives to treat inflammatory skin lesions. An alternative to improve treatment efficacy and limit the occurrence of adverse effects is the use of Nanotechnology. In this sense, a perspective for the conclusion of this study is. the association of OA and dexamethasone into nanostructured systems, benefiting from their advantages for the delivery of the active molecules, in order to propose a potential therapy to treat cutaneous inflammatory disorders.

Keywords: oleic acid, dexamethasone acetate, corticosteroids, nanoparticles, anti-inflammatory.

LISTA DE ABREVIATURAS E SIGLAS

AINEs	Anti-inflamatórios não-esteroides
AO	Ácido oleico
APC	Células apresentadoras de antígeno (do inglês *antigen-presenting cells*)
DAMPs	Padrões moleculares associados a danos (do inglês *danger-associated molecular patterns*)
DC	Célula dendrítica (do inglês *dendritic cell*)
DCI	Dermatite de contato irritante
DEX	Dexametasona
DNA	Ácido desoxirribonucleico (do inglês *deoxyribonucleic acid*)
GRs	Receptores de glicocorticoides (do inglês *glucocorticoid receptors*)
IL	Interleucina (do inglês *interleukin*)
$I_{máx}$	Inibição máxima
LC	Célula de Langerhans (do inglês *Langerhans cell*)
NF-κB	Fator Nuclear Kappa B (do inglês *nuclear factor kappa B*)
PAMPs	Padrões moleculares associados a patógenos (do inglês *pathogen-associated molecular patterns*)
PRRs	Receptores de reconhecimento de padrão (do inglês *pattern recognition receptors*)
TNF	Fator de necrose tumoral (do inglês *tumor necrosis factor*)
UVA	Radiação ultravioleta do tipo A
UVB	Radiação ultravioleta do tipo B
UVC	Radiação ultravioleta do tipo C
UVR	Radiação ultravioleta
ω-9	Ômega-9

LISTA DE ANEXOS

SUMÁRIO

1 APRESENTAÇÃO

Esta tese aborda o desenvolvimento de diferentes formulações semissólidas contendo ácido oleico destinadas ao tratamento de processos inflamatórios de pele. Ela encontra-se estruturada da seguinte forma:

INTRODUÇÃO: contém uma breve apresentação dos temas que serão abordados ao longo desta tese.

REVISÃO BIBLIOGRÁFICA: contém a revisão de literatura caracterizando, de forma mais aprofundada, os diferentes temas abordados ao longo do trabalho.

DESENVOLVIMENTO: os métodos e resultados encontram-se neste tópico apresentados sob a forma de artigos e manuscrito científico.

DISCUSSÃO: aborda as interpretações e comentários gerais acerca do desenvolvimento do trabalho e dos artigos científicos resultantes deste trabalho.

CONCLUSÃO: tópicos gerais sobre os resultados obtidos.

REFERÊNCIAS BIBLIOGRÁFICAS: contém listadas as referências utilizadas nos tópicos ‘‘Introdução’’, ‘‘Revisão bibliográfica’’ e ‘‘Discussão’’ desta tese.

ANEXOS: constam os certificados de aprovação da Comissão de Ética para o Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) e as permissões para uso dos artigos científicos no corpo da tese.

2 INTRODUÇÃO

A pele, considerada o órgão mais extenso do corpo humano, atua como interface entre o corpo e o meio ambiente. Sendo assim, está constantemente exposta à ação de agentes nocivos provenientes do meio externo, como micro-organismos, detergentes e solventes, radiação, traumas físicos e outros. Estes agentes nocivos são capazes de iniciar diferentes cascatas de sinalização, evocando respostas inflamatórias e imunológicas da pele (Pasparakis et al., 2014).

Por encontrar-se anatomicamente situada neste ambiente de interface, a pele é responsável por importantes funções que visam, em suma, manter a homeostasia do organismo e garantir sua integridade (Pasparakis et al., 2014; Romanovsky, 2014). Dentre estas funções estão o controle da perda excessiva de água e regulação da temperatura corporal, além de atuar promovendo a primeira linha de defesa do organismo (Di Meglio et al., 2011; Pasparakis et al., 2014).

A pele é organizada em três camadas principais: epiderme, derme e hipoderme. A epiderme é majoritariamente composta por queratinócitos, mas também abriga melanócitos e células do sistema imune, como as células de Langerhans e linfócitos. A derme, situada imediatamente abaixo da epiderme, é composta por fibroblastos, mastócitos, macrófagos e células dendríticas, além de elementos como fibras de colágeno e elastina, vasos sanguíneos e terminações nervosas. Devido à presença dos vasos sanguíneos, a derme é considerada a camada responsável por prover nutrientes para a pele. Estas duas camadas são sustentadas por uma terceira camada, a hipoderme, a qual constitui-se basicamente de células adiposas cuja principal função é a reserva energética (Nestle et al., 2009).

Agentes físicos, como a radiação ultravioleta (UVR), e agentes químicos, como solventes e compostos irritantes, são capazes de danificar o tecido cutâneo e desencadear neste tecido uma reação de proteção do organismo. A UVR é um componente do espectro de luz que subdivide-se em radiação ultravioleta dos tipos A (UVA), B (UVB) e C (UVC). UVA, UVB e UVC diferem de acordo com a faixa de comprimentos de onda que abrangem: 320-400 nm, 280-320 nm e 100-280 nm, respectivamente (D'Orazio et al., 2013).

O comprimento de onda impacta diretamente na capacidade da radiação em penetrar a pele humana. A radiação UVA é capaz de atingir camadas mais profundas da pele, podendo alcançar a derme; a radiação UVB exerce seus efeitos principalmente sobre a camada mais superficial da pele, a epiderme; a UVC tem seus efeitos sobre a pele humana considerados desprezíveis, uma vez que este tipo de radiação é quase que totalmente absorvido pela camada

de ozônio (Schuch et al., 2013). A capacidade da UVR em penetrar o tecido cutâneo possibilita a geração de efeitos como envelhecimento cutâneo precoce, inflamação e desenvolvimento de tumores (D'Orazio et al., 2013; Watson et al., 2016).

A incidência da UVR sobre a pele humana provoca também o aumento da produção de espécies reativas. Estas moléculas são altamente instáveis e, portanto, apresentam potencial capacidade de se ligar a ácidos nucleicos, proteínas e lipídeos, danificando-os. Os danos a estas biomoléculas estão intimamente relacionados aos processos de envelhecimento cutâneo precoce e carcinogênese (Schuch et al., 2017).

A dermatite de contato irritante (DCI), também denominada hipersensibilidade de contato, é uma doença inflamatória que se desenvolve após o contato de um agente irritante com a pele e é caracterizada pelo aparecimento de lesões eczematosas limitadas à área de contato. Na patofisiologia da DCI estão envolvidos mecanismos como a ativação do sistema imune inato, danos à barreira da pele, alterações celulares, liberação de mediadores e o recrutamento de células inflamatórias (Bains et al., 2019). Detergentes, solventes, pesticidas e moléculas de baixo peso molecular são exemplos de agentes que podem desencadear a DCI (Ale; Maibach, 2014; Bains et al., 2019). A DCI pode ser classificada como aguda ou crônica. A DCI aguda refere-se ao dano de pele induzido pela exposição a uma quantidade suficiente ou a algum irritante forte o suficiente para causar lesão em praticamente qualquer pessoa. A DCI crônica pode ser induzida pelos mesmos agentes capazes de provocar a DCI aguda que, em pequenas concentrações, provocam efeitos cumulativos na pele, levando ao desenvolvimento de lesões persistentes que se tornam crônicas (Przybilla; Rueff, 2009; Novak-Bilić et al., 2018).

O processo inflamatório é uma resposta de defesa do organismo frente à invasão de micro-organismos ou lesões teciduais que compreende inúmeros eventos, os quais têm como finalidade neutralizar agentes invasores e reparar o tecido lesionado, recuperando seu status homeostático inicial (Serhan, 2014). A inflamação é caracterizada pelo surgimento dos sinais cardinais clássicos postulados inicialmente por Celsus: eritema ou rubor, edema ou inchaço, calor ou aumento da temperatura e dor, os quais podem ocasionar a perda da função do membro afetado e prejudicar a capacidade de trabalho assim como a qualidade de vida dos pacientes (Serhan et al., 2008; Chiu et al., 2012; Nan et al., 2018).

A ocorrência de um sinal nocivo ou dano direto aos queratinócitos estimula células residentes da pele a produzirem e liberarem os mediadores inflamatórios, tais como citocinas pró-inflamatórias e quimiocinas, eicosanoides (prostaglandinas) e outros mediadores vasoativos e neuroativos. A liberação destas substâncias provoca alterações no endotélio

vascular, resultando em aumento da sua permeabilidade e extravasamento de um fluido proteico, culminando na formação de edema. Além disso, as alterações produzidas nas células do endotélio vascular induzem a expressão de moléculas de adesão e a ocorrência de uma série de eventos que possibilitam a migração de leucócitos circulantes para o local inflamado (D'Orazio et al., 2013).

A infiltração celular exacerbada e descoordenada pode levar à cronificação do processo inflamatório. Além disso, sabe-se que a inflamação está diretamente envolvida com o surgimento de inúmeras doenças. Neste sentido, conter sua progressão é de extrema importância (Kolaczkowska; Kubes, 2013). Atualmente, o tratamento de condições inflamatórias consiste principalmente na utilização de anti-inflamatórios não-esteroides (AINEs) e corticosteroides tópicos. Os fármacos corticosteroides tópicos, como a dexametasona, exercem seu efeito anti-inflamatório via receptores de glicocorticoides, por meio do controle da transcrição de genes inflamatórios, onde a transcrição de genes anti-inflamatórios é aumentada e a transcrição de genes pró-inflamatórios é reduzida (Barnes, 1998; Uva et al., 2012).

Corticosteroides são amplamente empregados devido aos seus potentes efeitos anti-inflamatórios, imunossupressores e antiproliferativos. Apesar disso, sua utilização clínica está associada ao desenvolvimento de efeitos adversos graves como atrofia cutânea, púrpura, rosácea e o efeito rebote, muitas vezes comprometendo a adesão dos pacientes ao tratamento farmacológico (Uva et al., 2012; Coondoo et al., 2014; Barnes et al., 2015). Estas limitações dos fármacos utilizados para o tratamento de inflamações cutâneas reforçam a necessidade pela busca de novas alternativas terapêuticas.

Há milhares de anos, compostos de origem natural são empregados pelo homem com finalidades terapêuticas. Muitas destas substâncias estão presentes naturalmente em extratos de plantas e óleos vegetais e têm sido estudadas pelo seu potencial em promover benefícios no tratamento de condições inflamatórias de pele (Rigo et al., 2015; Bhoir et al., 2019; Camponogara et al., 2019a, Camponogara et al., 2019b; Camponogara et al., 2019c).

O ácido oleico (AO), ou ômega-9 (ω-9), é um dos compostos facilmente encontrados em óleos vegetais e alimentos, tais como óleo de oliva, peixes, sementes oleaginosas e abacate (Viola; Viola, 2009; Roncero et al., 2016). O AO também é encontrado no organismo humano como um constituinte de membranas celulares, além de participar atuando como substrato para a síntese de hormônios (Tvrzicka et al., 2011).

Algumas evidências sugerem um importante papel do AO em modular processos inflamatórios, através da redução da expressão de citocinas pró-inflamatórias, moléculas de

adesão e da migração de células para o local inflamado. Estudos demonstram que o AO aumentou a expressão de citocinas anti-inflamatórias em modelos de inflamação induzida experimentalmente (Sales-Campos et al., 2013; Medeiros-de-Moraes et al., 2018).

Atualmente, a busca por alternativas terapêuticas eficazes para o tratamento de inflamação cutânea cuja utilização esteja associada a poucos efeitos adversos é crescente. Em vista disso, neste trabalho, desenvolvemos formulações semissólidas para aplicação tópica do AO e avaliamos seu efeito anti-inflamatório *in vivo* empregando modelos de inflamação de pele induzidas por radiação UVB e pelo agente irritante óleo de cróton em camundongos. Além disso, também desenvolvemos nanopartículas contendo AO e dexametasona, utilizando a nanotecnologia como aliada devido à sua vantagem de possibilitar a redução de dose de fármacos. A partir destas nanopartículas, preparamos semissólidos, como uma segunda alternativa ao tratamento de inflamação cutânea.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Desenvolver diferentes formulações semissólidas contendo ácido oleico e acetato de dexametasona e investigar o potencial anti-inflamatório destas formulações frente à inflamação de pele empregando dois modelos experimentais em camundongos.

3.2 OBJETIVOS ESPECÍFICOS

- Preparar e caracterizar formulações gel à base de Pemulen® TR2 ou formulações creme à base de cera Lanette® contendo diferentes concentrações de ácido oleico e avaliar o efeito anti-inflamatório destas formulações em um modelo de queimadura solar induzida por radiação UVB em camundongos *Swiss*. Investigar também se este possível efeito anti-inflamatório do ácido oleico ocorre via receptores glicocorticoides;
- Avaliar o efeito das formulações gel à base de Pemulen® TR2 em inibir a inflamação de pele do tipo dermatite tópica induzida experimentalmente em camundongos *Swiss* pela aplicação tópica do agente irritante óleo de cróton. Verificar também se o ácido oleico exerce a sua ação via receptores glicocorticoides e se sua aplicação tópica repetida induz efeitos tóxicos;
- Desenvolver formulações semissólidas de base nanotecnológica contendo ácido oleico e acetato de dexametasona, avaliar o perfil de liberação da dexametasona a partir das nanoestruturas e a permeação em pele suína da dexametasona a partir das formulações semissólidas. Investigar a *performance* anti-inflamatória *in vivo* das formulações utilizando o modelo de queimadura solar induzida por radiação UVB em camundongos *Swiss*.

4 REVISÃO BIBLIOGRÁFICA

4.1 PELE – ASPECTOS ESTRUTURAIS, FUNCIONAIS E PRINCIPAIS COMPONENTES CELULARES

A pele é um órgão complexo essencial ao corpo humano que desempenha funções vitais à sobrevivência contribuindo para a manutenção da homeostase corporal, auxiliando na manutenção da temperatura corporal e do equilíbrio hídrico, evitando a perda excessiva de água, além de conferir proteção aos tecidos mais profundos do organismo frente à insultos provenientes do ambiente externo, constituindo a primeira linha de defesa do corpo (Pasparakis et al., 2014; Romanovsky, 2014; Abdallah et al., 2017). Além destas funções, a pele participa ativamente da síntese de vitaminas e hormônios e constitui uma área sensorial-receptiva (Di Meglio et al., 2011; Bickle; Christakos, 2020).

A pele encontra-se na interface entre o corpo e o meio ambiente e, portanto, está permanentemente exposta à ação de agentes nocivos provenientes do meio externo, os quais são capazes de evocar respostas inflamatórias e imunológicas. Dentre estes agentes nocivos, pode-se citar micro-organismos patogênicos, traumas mecânicos, injúrias químicas por solventes e substâncias irritantes, e injúrias físicas por UVR, por exemplo (Pasparakis et al., 2014).

Para desempenhar tal diversidade de funções, a pele necessita de mecanismos bem coordenados entre seus constituintes celulares e não celulares. Portanto, sua estrutura está profundamente relacionada com as suas funções. A pele está estruturada em epiderme e derme, ambas estando ancoradas sobre uma terceira camada: o tecido adiposo subcutâneo (também chamado hipoderme) (Nguyen; Soulika, 2019).

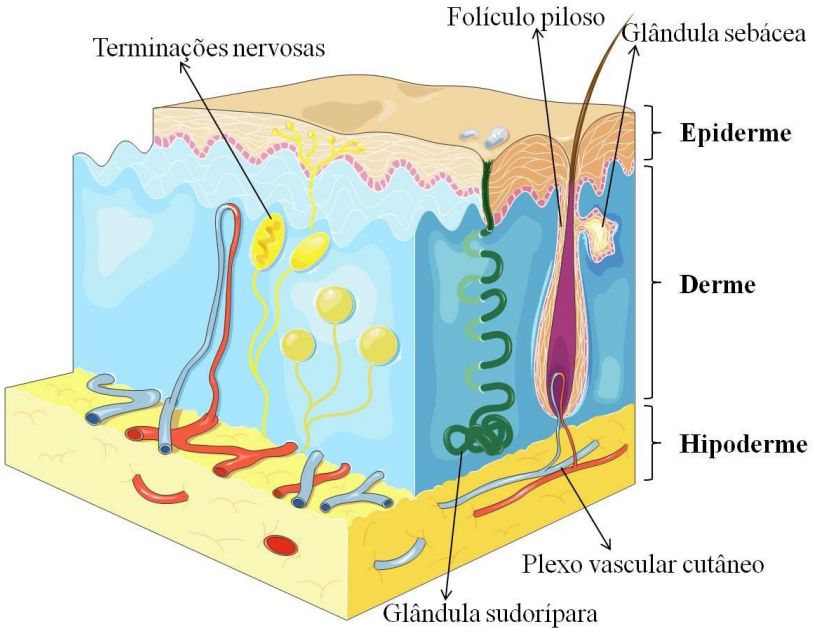

Figura 1 - Camadas da pele e seus componentes não celulares. A pele humana é composta por três camadas: epiderme, derme e hipoderme (Fonte: adaptado de smart.servier.com).

A epiderme, camada mais externa da pele, é também dividida em subcamadas: estrato córneo, estrato granuloso, estrato espinhoso e estrato germinativo (da superfície externa em direção à base) que diferem entre si quanto ao grau de diferenciação de seus queratinócitos (Pasparakis et al., 2014; Kabashima et al., 2019). De relevância dentro da epiderme destaca-se o estrato córneo, uma faixa impermeável que varia entre 10 a 30 µm de espessura e consiste de 10 a 20 camadas sobrepostas de queratinócitos mortos (também denominados como corneócitos) embebidos em uma matriz extracelular altamente hidrofóbica de três famílias de lipídeos intercelulares: ceramidas, colesterol e ácidos graxos livres (Feingold; Elias, 2014; Haftek, 2015; Matsui; Amagai, 2015). Os corneócitos são firmemente ligados por estruturas chamadas corneodesmossomos, elementos fundamentais para a coesão do estrato córneo e sua funcionalidade como barreira protetora (Haftek, 2015).

O estrato córneo, quando íntegro, participa ativamente da defesa do organismo bloqueando a entrada de agentes nocivos. Porém, quando danificado, facilita o desenvolvimento de respostas inflamatórias a estímulos danosos. Portanto, alterações na expressão destes elementos de junção e de seus processos de degradação estão intimamente relacionados com a ocorrência de algumas dermatoses (Haftek, 2015). Além disso, o estrato córneo é responsável por contribuir para a manutenção do equilíbrio hídrico da pele, regulando a perda de água transepidermal (Kabashima et al., 2019).

Os queratinócitos, as células mais abundantes na epiderme, são extremamente importantes nos processos imunológicos que ocorrem na pele, sendo responsáveis por estabelecer uma comunicação entre os sinais danosos do ambiente e as células imunes residentes da pele. Os chamados receptores de reconhecimento de padrão (PRRs, do inglês *pattern recognition receptors*) estão expressos em queratinócitos e sua ativação por estímulos danosos é responsável pela indução da produção de citocinas pró- e anti-inflamatórias, tais como IL-1, IL-10, IL-20 e fator de necrose tumoral (TNF, do inglês *tumor necrosis factor*) (Jiang et al., 2020). Além disso, a ativação de PRRs também leva à indução de outras moléculas inflamatórias como as quimiocinas, que possuem ação quimiotática atraindo células inflamatórias para o local danificado, iniciando assim a resposta imune local (Pasparakis et al., 2014).

Além de queratinócitos, a epiderme hospeda outros tipos celulares: células de Merkel (células com função sensorial), melanócitos e células do sistema imune, como as células de Langerhans (LCs, do inglês *Langerhans cells*) e linfócitos (Nestle et al., 2009; Pasparakis et al., 2014). As LCs são células apresentadoras de antígeno (APCs, do inglês *antigen-presenting cells*) que desempenham papel crucial na defesa do organismo; estão equipadas, da mesma forma que outras células imunes, com receptores específicos que funcionam como ‘‘sensores’’ em situações de perigo (Clayton et al., 2017; Rajesh et al., 2019).

Peptídeos antimicrobianos como β-defensinas e catelicidinas são moléculas anfipáticas expressas constitutivamente na pele ou após indução por estímulos inflamatórios, sendo produzidas majoritariamente por queratinócitos (Nguyen; Soulika, 2019). Os peptídeos antimicrobianos provêm um amplo espectro de proteção contra vírus, bactérias e fungos e são capazes de modular respostas imunes do hospedeiro (Clausen; Agner, 2016; Nguyen; Soulika, 2019).

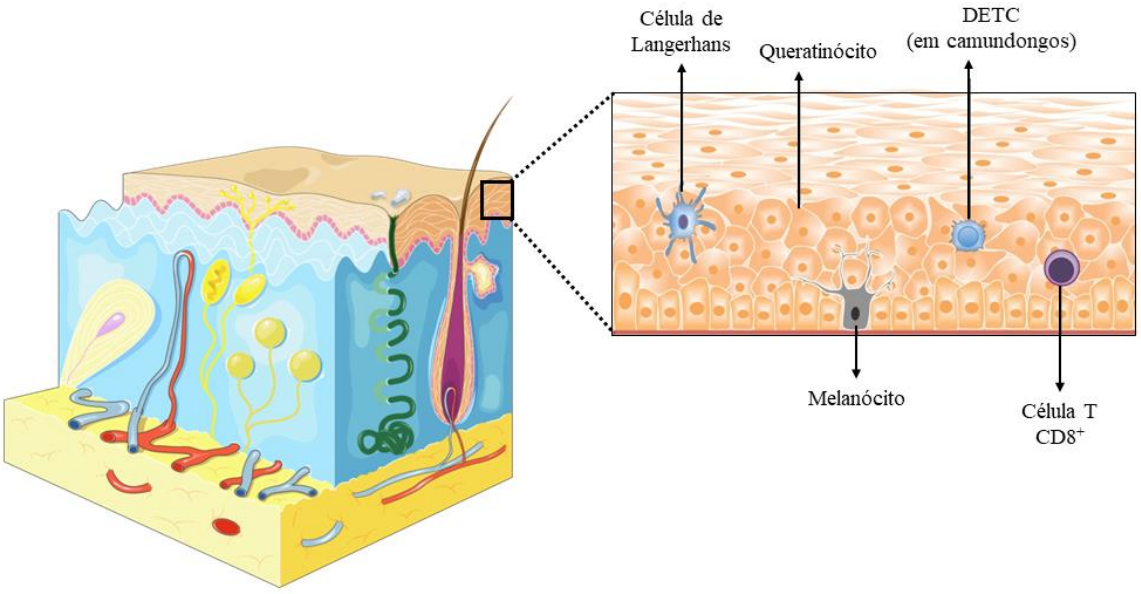

Figura 2 – Epiderme em detalhes: constituintes celulares (Fonte: adaptado de Ho e Kupper, 2019 e smart.servier.com).

Imediatamente abaixo da epiderme localiza-se a derme, a camada mais complexa da pele, que abriga células como fibroblastos, mastócitos, macrófagos, além de outras, e também elementos como fibras de colágeno e elastina, vasos sanguíneos e terminações nervosas. Devido à presença dos vasos sanguíneos, a derme é considerada a camada responsável por prover nutrientes para a pele (Jiang et al., 2020).

Fibroblastos são o principal tipo celular encontrado na camada dérmica. Sua função mais notável é a síntese de matriz extracelular, constituída de colágeno, proteoglicano e fibras elásticas, conferindo integridade estrutural à derme (Woodley, 2017). Ademais, contribuem para o desenvolvimento dos folículos pilosos e na regulação do ciclo de crescimento piloso (Driskell; Watt, 2015).

Macrófagos também residem nesta camada da pele, onde são responsáveis por remover restos teciduais e realizar a fagocitose de agentes patogênicos (Malissen et al., 2014). Macrófagos são células que apresentam plasticidade e podem ser categorizadas em dois tipos quanto às suas funções efetoras: fenótipo M1 pró-inflamatório e fenótipo M2 anti-inflamatório/pró-resolução. No entanto, cabe ressaltar que macrófagos genuínos dos fenótipos M1 e M2 podem ser gerados apenas *in vitro*. *In vivo*, estas células podem expressar marcadores M1 e M2 simultaneamente (Nguyen; Soulika, 2019).

Mastócitos também são células classicamente encontradas na derme. Sob condições normais, encontram-se próximos a vasos sanguíneos, terminações nervosas e folículos pilosos (Komi et al., 202). Em seu interior, contêm grânulos de mediadores pré-formados: histamina,

proteoglicanos sulfatados, serotonina e triptase e/ou quimase (Oliveira et al., 2018). São células conhecidas por estarem envolvidas em processos alérgicos, durante os quais produzem e liberam grande quantidade de mediadores, principalmente histamina (Siiskonen; Harvima, 2019). Os mediadores inflamatórios liberados pelos mastócitos provocam o aumento da permeabilidade vascular, formação de edema e por consequência, impactam no recrutamento de outras células imunes para o local de inflamação (Kunder et al., 2011).

Diferentemente da epiderme, onde as APCs são as LCs, as APCs encontradas na derme são conhecidas como células dendríticas dérmicas (DCs, do inglês *dendritic cells*). De maneira similar às LCs, após um estímulo de perigo, as DCs migram aos linfonodos que drenam a pele carregando o antígeno, a fim de apresentá-lo aos linfócitos T virgens (naïve) e desencadear a resposta imune adaptativa. Durante o processo de iniciação de linfócitos T, as DCs fornecem três tipos de sinais inflamatórios: a ativação de receptores de células T, a ativação de moléculas coestimulatórias e combinações específicas de citocinas. O fenótipo inflamatório adquirido por estas células após estímulo nocivo é transitório, desaparecendo logo após o estímulo ser cessado (Kashem et al., 2017).

A pele humana é também amplamente povoada por linfócitos T (ou ainda denominados células T); estimativas indicam que este órgão pode abrigar mais de 20 bilhões de células T (Clark et al., 2010). Após a apresentação de antígenos às células T naïve nos linfonodos que drenam a pele, estas células sofrem diferenciação, transformando-se em células T de memória ou células T efetoras. As células T efetoras adquirem funções como citotoxicidade (células T citotóxicas ou $CD8^+$) e capacidade de produção de citocinas (células T auxiliares ou $CD4^+$) e migram, via vasos linfáticos, até os locais da pele onde houve exposição ao antígeno. Passado o pico da resposta imunológica, estas células deixam o local lesionado via corrente sanguínea (Clark, 2010).

Residindo na pele humana são encontradas as células T γδ (1 – 10%) e células T αβ (perfazendo o restante da população), distribuídas tanto na derme quanto na epiderme. As células T epidérmicas encontram-se principalmente na proximidade de LCs, enquanto as células T dérmicas estão agrupadas em torno dos capilares, abaixo da junção dermo-epidérmica ou adjacentes aos apêndices cutâneos (Nestle et al., 2009). As células T que residem na pele são, quase que em sua totalidade, células T de memória, divididas em igual número entre células $CD4^+$ e $CD8^+$ (Nestle et al., 2009).

As células $CD4^+$ são ainda subdivididas em três tipos principais, dependendo das citocinas que produzem e liberam: Th1, Th2 e Th17. Estes três subtipos de células têm sido

identificados na pele durante diversos processos inflamatórios (Albanesi et al., 2018; Tokura et al., 2018). Além destas, também são encontradas na pele as células T regulatórias, responsáveis pela supressão da ativação e proliferação de células e da produção de citocinas (Clark, 2010). Embora as células T desempenhem importante papel em respostas imunes cutâneas contra estímulos nocivos, sabe-se que sua ativação aberrante ou inadequada pode originar desordens crônicas de pele (Ho; Kupper, 2019).

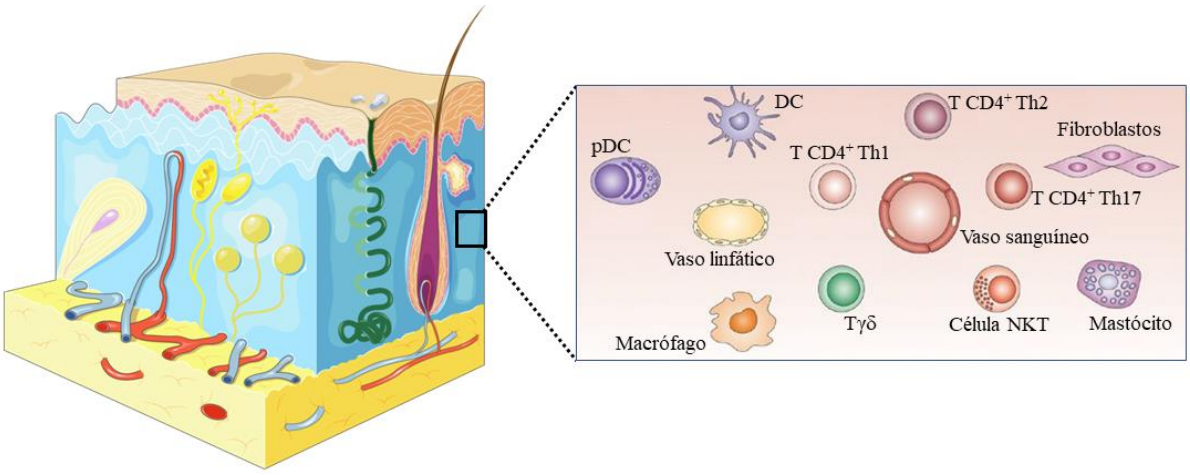

Figura 3 – Derme em detalhes: constituintes celulares e não-celulares (Fonte: adaptado de Nestle et al., 2009 e smart.servier.com).

Derme e epiderme são sustentadas por uma terceira camada, a hipoderme, a qual constitui-se basicamente de células adiposas, atuando como um tecido de reserva energética do organismo (Nestle et al., 2009; Jiang et al., 2020). Além de servir como tecido de sustentação e reserva energética, a hipoderme protege os órgãos internos do corpo absorvendo impactos e contribui para o processo de regulação térmica (Jiang et al., 2020).

Por fim, conectados à pele também estão presentes os apêndices, como os folículos pilosos e glândulas sebáceas e sudoríparas. Estes apêndices contribuem para as defesas do organismo, protegendo contra lesões mecânicas, mudanças de temperatura, UVR e contra a perda excessiva de água (Kabashima et al., 2019). Residindo nas glândulas sebáceas encontram-se os sebócitos, células responsáveis pela produção de sebo. Embora as funções do sebo na pele ainda não tenham sido completamente elucidadas, existe um consenso de que este produto atue selando os folículos pilosos, impedindo que estes funcionem como porta de entrada para micro-organismos atingirem camadas mais profundas da pele (Nguyen; Soulika, 2019). Ademais, o sebo também contribui para a manutenção do pH da pele, que em condições normais varia entre 4,1 e 5,8 (Gallo; Nakatsuji, 2011; Proksch, 2018).

Os apêndices cutâneos abrigam também fungos, bactérias e vírus que colonizam naturalmente a pele humana (tal conjunto é denominado microbiota ou microbioma), os quais a fazem por meio de relações de comensalismo, mutualismo ou parasitismo (Belkaid; Tamoutounour, 2016; Byrd et al., 2018). Recentemente, diversas evidências têm demonstrado a participação do microbioma da pele nas reações imunes cutâneas (Chen et al., 2018; Park; Lee, 2018; Kabashima et al., 2019).

Portanto, a pele é um órgão complexo que conta com estratégias tais como barreiras químicas, físicas e microbiológicas para defesa do hospedeiro contra insultos provenientes do ambiente (Nguyen; Soulika, 2019). Ademais, constitui um amplo espaço de interação dinâmica entre diversos elementos, mantendo uma rede intrincada de componentes celulares e não celulares cruciais para defesa do hospedeiro e manutenção da homeostasia tecidual (Nguyen; Soulika, 2019; Jiang et al., 2020).

4.2 INFLAMAÇÃO E MECANISMOS ADJACENTES ÀS MANIFESTAÇÕES INFLAMATÓRIAS CUTÂNEAS

O processo inflamatório é desencadeado como uma resposta biológica do sistema imune frente a um estímulo agressor. Estes estímulos que advêm do ambiente externo podem ser de natureza química, física ou biológica como, por exemplo, patógenos, compostos irritantes, UVR, células danificadas, entre outros (Chen et al., 2018). Este processo biológico é complexo, mas altamente coordenado. Envolve uma série de mediadores moleculares e componentes celulares e tem como propósito neutralizar ou remover o estímulo agressor e reparar o tecido lesionado, recuperando a homeostasia tecidual (Medzhitov, 2010; Serhan et al., 2014). O tipo e o grau da resposta inflamatória são determinados pela natureza do agente agressor e pelo tempo de persistência do estímulo danoso (Medzhitov, 2010).

A nível tecidual, a inflamação é evidenciada pelas seguintes manifestações, denominadas de sinais cardinais da inflamação, que são o edema, o eritema (ou vermelhidão), a dor e o calor (aumento da temperatura do local lesionado). Estes quatro sinais da inflamação foram postulados por Celsius há mais de dois mil anos. Muito tempo depois, em 1858, Virchow incluiu à lista um quinto sinal, a perda da função do tecido afetado, onde o indivíduo perde a habilidade ou evita usar o membro lesionado, sendo que este sinal pode ou não ocorrer, dependendo da extensão do dano tecidual (Freire; Van Dyke, 2013).

Eventos microvasculares, celulares e moleculares adjacentes ao processo inflamatório também são observados: alterações na permeabilidade vascular, extravasamento de fluido rico em proteínas, recrutamento e acúmulo de leucócitos e liberação de mediadores inflamatórios. Em resposta à lesão tecidual, são ativadas cascatas de sinalização que estimulam respostas inflamatórias objetivando o reparo do local afetado (Chen et al., 2018).

Células imunes e não imunes amplamente distribuídas pelos tecidos apresentam, em sua superfície, sensores para o reconhecimento de estímulos nocivos denominados de receptores de reconhecimento de padrão (PRRs, do inglês *pattern recognition receptors*) constituindo uma rede capaz de detectar imediatamente a invasão de agentes nocivos e/ou o dano tecidual associado a estes (Cronkite; Strutt, 2018). Os receptores Toll-like e NOD-like são exemplos de PRRs (Takeuchi; Akira, 2010). Estes PRRs estão programados para reconhecer sinais denominados padrões moleculares associados a patógenos (PAMPs, do inglês *pathogen-associated molecular patterns*) e padrões moleculares associados a dano (DAMPs, do inglês *damage-associated molecular patterns*). PAMPs são produtos derivados de patógenos, tais como o lipopolissacarídeo constituinte da parede celular de bactérias gram-negativas; DAMPs são sinais liberados de células danificadas ou necróticas após um dano tecidual. Exemplos de DAMPs incluem DNA, ATP, histonas, citocinas como IL-1α, fragmentos de matriz extracelular, dentre outros (Zindel; Kubes, 2020).

A ligação de PRRs por PAMPs ou DAMPs promove a ativação de fatores de transcrição tais como o fator nuclear kappa B (NF-κB, do inglês *nuclear factor kappa-B*) e a proteína ativadora-1, resultando na transcrição de genes inflamatórios que regulam processos celulares como migração, proliferação, apoptose e expressão de mediadores como citocinas inflamatórias e quimiocinas e de moléculas de adesão (Gong et al., 2020). A subsequente produção destes mediadores inflamatórios controla a iniciação e a manutenção do processo inflamatório e regula sua amplitude (Nedoszytko et al., 2014).

As alterações de permeabilidade vascular que ocorrem durante o processo inflamatório contribuem diretamente para aumentar a exsudação de conteúdo proteico, o qual resulta na formação de edema, e também para o acúmulo de leucócitos polimorfonucleares no tecido inflamado (Freire; Van Dyke, 2013; Schimmel et al., 2017).

Estímulos inflamatórios também são capazes de promover o processo denominado inflamação neurogênica. Neste evento, ocorre a ativação de fibras sensoriais peptidérgicas que inervam a pele, com consequente liberação de neuropeptídeos como a substância P e o peptídeo relacionado ao gene da calcitonina. A substância P liberada age sobre vênulas pós-capilares

para causar extravasamento plasmático e infiltração de leucócitos, enquanto que o peptídeo relacionado ao gene da calcitonina promove dilatação arteriolar e hiperemia (Geppetti et al., 2015).

Após a ativação de células residentes da pele (macrófagos, DCs, mastócitos, linfócitos, fibroblastos) por agentes nocivos, estas produzem e liberam mediadores inflamatórios solúveis como citocinas, quimiocinas, aminas vasoativas e prostaglandinas (Nourshargh; Alon, 2014). Estes mediadores solúveis promovem a ativação de células endoteliais da vasculatura próxima ao tecido danificado (Fullerton; Gilroy, 2016). Concomitantemente a este processo, a expressão de moléculas de adesão celular é regulada positivamente em leucócitos circulantes e em células endoteliais em uma etapa de preparação ao evento seguinte: o influxo de células inflamatórias para o local lesionado por meio de um processo conhecido como diapedese (Fullerton; Gilroy, 2016).

Os neutrófilos, que são os leucócitos mais abundantes do sangue, são as primeiras células polimorfonucleares a infiltrarem no tecido danificado (Hind; Huttenlocher, 2018). Estas células são importantes efetores durante o processo inflamatório pois apresentam mecanismos fundamentais para destruir ameaças infecciosas: como a fagocitose, liberação do conteúdo dos seus grânulos, produção de espécies reativas de oxigênio e a formação das armadilhas extracelulares dos neutrófilos (Oliveira et al., 2016).

O primeiro passo do processo de migração leucocitária é a marginação dos leucócitos próximo à parede do endotélio e a ocorrência de interações fracas entre os leucócitos e as células endoteliais de capilares nas proximidades do tecido inflamado. Este evento é possibilitado pela expressão de selectinas na superfície de células endoteliais (E-selectina e P-selectina) e de leucócitos (L-selectinas). Em seguida, a adesão firme (de alta afinidade) é mediada por receptores da família das imunoglobulinas, as integrinas leucocitárias, aos quais se ligam às moléculas de adesão intercelular e às moléculas de adesão vascular. Por fim, os leucócitos adquirem uma forma polarizada e rastejam ao longo da superfície apical endotelial, em busca de um local permissivo para sua migração em direção ao tecido danificado (Nourshargh; Alon, 2014; Filippi, 2016).

Embora a resposta inflamatória tenha caráter protetor, uma falha na remoção de materiais nocivos do tecido danificado via fagocitose, a falha na depuração de células apoptóticas e o atraso de eventos apoptóticos e do *clearance* de mediadores inflamatórios é capaz de promover a cronificação do processo. A inflamação descontrolada tem sido apontada

como a causa de muitas patologias, incluindo artrite, câncer, doenças cardiovasculares e neurodegenerativas, dentre outras (Freire; Van Dyke, 2013; Fullerton; Gilroy, 2016).

O organismo humano conta com um sistema de resolução bem orquestrado para recuperar o status homeostático após a resposta inflamatória. O período de tempo compreendido entre o pico de influxo de células inflamatórias no tecido e o *clearance* destas células e a restauração da homeostasia funcional corresponde à fase de resolução (Fullerton; Gilroy, 2016). Este processo acontece em diversas etapas. Inicialmente, o estímulo que desencadeou a inflamação é eliminado. Subsequentemente, a síntese de mediadores pró-inflamatórios é suspensa e os mediadores remanescentes no tecido são catabolizados, impedindo o recrutamento de mais células inflamatórias para o local e a formação de edema. Os leucócitos infiltrados no tecido são então removidos por três maneiras distintas: podem retornar à circulação sistêmica (processo denominado migração reversa), ser removidos via drenagem linfática ou sofrer necrose ou apoptose local com subsequente remoção por macrófagos (eferocitose). Por fim, os macrófagos também deixam o local de injúria por meio dos vasos linfáticos ou sofrem apoptose local (Serhan et al., 2007; Fullerton; Gilroy, 2016; Sugimoto et al., 2019).

A eferocitose bem sucedida também é capaz de estimular a reprogramação de macrófagos do fenótipo pró-inflamatório (M1) para o fenótipo anti-inflamatório (M2), os quais passam a liberar mediadores como as citocinas IL-10 e fator de crescimento transformante beta, além de fatores de crescimento como fator de crescimento endotelial vascular alfa e o fator de crescimento derivado de plaquetas, que estimulam a resolução e o reparo tecidual (Sugimoto et al., 2019). Outros mediadores lipídicos, chamados mediadores pró-resolução especializados, incluindo lipoxinas, resolvinas, protectinas e maresinas, também já foram reconhecidos como importantes moléculas na resolução da inflamação (Chiang; Serhan, 2017; Serhan, 2017; Sugimoto et al., 2019).

4.2.1 Resposta inflamatória induzida pela radiação ultravioleta

Além dos agentes anteriormente citados capazes de danificar o tecido cutâneo e desencadear neste tecido uma reação de defesa do organismo, figura a UVR. A UVR é um componente do espectro de luz, a qual subdivide-se em radiação UVA, UVB e UVC. Estes três subtipos diferem de acordo com a faixa de comprimentos de onda que abrangem: 320-400 nm, 280-320 nm e 100-280 nm, respectivamente (D'Orazio et al., 2013).

A faixa de comprimentos de onda que estes tipos de radiação abrangem impacta diretamente na sua capacidade de penetrar a pele humana. A radiação UVA apresenta maior faixa de comprimento de onda, portanto pode atingir camadas mais profundas da pele, podendo alcançar a derme. Por sua vez, a radiação UVB corresponde à faixa intermediária de comprimentos de onda, exercendo seus efeitos sobre a camada mais superficial da pele, a epiderme. O terceiro subtipo de UVR, a UVC, abrange os menores comprimentos de onda, porém seus efeitos sobre a pele humana são ditos desprezíveis, uma vez que este tipo de radiação é quase que totalmente absorvido pela camada de ozônio (Schuch et al., 2013).

Sabe-se que a exposição à UVR apresenta efeitos benéficos como a estimulação da síntese de vitamina D e no tratamento de doenças de pele por meio da fototerapia (Halliday et al., 2008). No entanto, a exposição excessiva à UVR também é responsável por efeitos maléficos como envelhecimento cutâneo precoce, inflamação e desenvolvimento de tumores (D'Orazio et al., 2013; Watson et al., 2016).

A exposição da pele à UVR promove a iniciação de uma cascata de citocinas e de mediadores vasoativos e neuroativos que juntos compõem a resposta inflamatória, desencadeando o surgimento dos sinais cardinais clássicos do processo inflamatório (Matsumura; Ananthaswamy, 2004; D'Orazio et al., 2013). Estes sinais são derivados de eventos como extravasamento vascular, o qual se deve ao aumento da permeabilidade endotelial e migração de células inflamatórias para o tecido inflamado (Nourshargh; Alon, 2014).

A ocorrência de um sinal nocivo estimula células residentes da pele a produzirem e liberarem os mediadores inflamatórios, tais como citocinas pró-inflamatórias e quimiocinas, eicosanoides (prostaglandinas) e outros mediadores vasoativos e neuroativos. A liberação destas substâncias provoca alterações no endotélio vascular, resultando em aumento da sua permeabilidade e extravasamento de um fluido proteico, os quais culminam na formação de edema. Além disso, as alterações produzidas nas células do endotélio vascular induzem a expressão de moléculas de adesão e a ocorrência de uma série de eventos que possibilitam a migração de leucócitos circulantes para o local inflamado (D'Orazio et al., 2013).

Alguns mediadores participam ativamente do processo inflamatório induzido por UVR. Dentre estes, pode-se citar o óxido nítrico, prostaglandinas, as moléculas de adesão intercelular e vascular, citocinas como IL-1β e TNF-α, quimiocinas, proteínas do sistema complemento, fatores de crescimento, além de cicloxigenases e outras enzimas (Clydesdale et al., 2001; Prasad; Katiyar, 2017). Outro efeito induzido por UVR na pele é o aumento da espessura da epiderme, denominada hiperqueratose (D'Orazio et al., 2013).

A ativação do NF-κB também acontece como uma consequência da exposição da pele à UVR. Este fator de transcrição é encontrado fisiologicamente na sua forma inativa no citoplasma celular ligado a proteínas inibitórias da família IκB. Após estímulos inflamatórios como a liberação de citocinas, UVR ou lesão física, ocorre a ativação deste fator, o qual desprende-se das proteínas inibitórias e transloca-se para o núcleo celular, ligando-se a regiões específicas do DNA. A ativação do NF-κB é responsável pela transcrição de genes de citocinas, quimiocinas, moléculas de adesão, enzimas inflamatórias e outros mediadores (Cooper; Bowden, 2007; López-Camarillo et al., 2012).

Queratinócitos também sofrem diretamente os efeitos deletérios da UVR. Se a dose de exposição extrapola o limiar de resposta ao dano, seu DNA é severamente danificado e estas células ativam as vias apoptóticas. Morfologicamente, são observadas como células com núcleo picnótico e citoplasma retraído, sendo denominadas *''sunburn cells''* (Laethem et al., 2005; Cezar et al., 2019).

Outro efeito relacionado à incidência da UVR sobre a pele humana é o aumento da produção de espécies reativas. Estas moléculas são altamente instáveis e, portanto, apresentam potencial capacidade de se ligar a ácidos nucleicos, proteínas e lipídeos, danificando-os. A UVR é um dos principais agentes capazes de alterar o DNA, induzindo lesões mutagênicas e citotóxicas como a formação dos dímeros de pirimidina ciclobutano e os 6-4 fotoprodutos (Rastogi et al., 2010). Os danos a estas biomoléculas estão intimamente relacionados aos processos de envelhecimento cutâneo precoce e carcinogênese (Schuch et al., 2017).

4.2.2 Inflamação na dermatite de contato irritante

As dermatites de contato são respostas inflamatórias caracterizadas por lesões eczematosas, as quais subdividem-se em DCI e dermatite de contato alérgica (DCA), sendo esta última também denominada hipersensibilidade de contato. Visto que ambas apresentam características macroscópicas muito semelhantes, sua diferenciação é difícil. Além disso, frequentemente ambos os tipos podem coexistir em um mesmo paciente. A DCI é caracterizada por lesões de forma demarcada e restritas à área de contato com o agente irritante, enquanto que na DCA as lesões apresentam-se mais distribuídas no indivíduo afetado (Eberting, 2014).

As respostas na dermatite de contato são desencadeadas pela exposição a agentes irritantes ou alérgenos que geralmente apresentam baixo peso molecular e capacidade antigênica (haptenos). Devido ao seu potencial pró-inflamatório, os haptenos desencadeiam

uma reação inflamatória de pele não-específica a antígeno (resposta imune inata), caracterizando a DCI (Bonneville et al., 2007).

Embora a DCI possa ocorrer em qualquer indivíduo, pacientes com doenças de pele, como por exemplo dermatite atópica, são mais susceptíveis devido ao dano de barreira pré-existente. Idosos e crianças também apresentam maior susceptibilidade pois a camada epidérmica da pele destes indivíduos é menos resistente, evidenciando o importante papel da barreira cutânea em conter esta patologia (Eberting, 2014).

A DCI é influenciada pelas propriedades químicas e físicas do agente irritante, sua concentração, forma de exposição, fatores ambientais e fatores relacionados à susceptibilidade do paciente (Eberting, 2014). Dependendo do potencial irritante da substância e do tempo de exposição, o processo inflamatório na DCI pode ser classificado como agudo ou crônico (Willis, 2006).

Na patogênese da DCI, o agente irritante em contato com a pele danifica as células epidérmicas e remove os lipídios desta camada. Este evento aumenta a permeabilidade da pele levando a perda de água transepidermal, evidenciando o aspecto seco da pele nas lesões de DCI. Um amplo arranjo de citocinas inflamatórias (IL-1α, IL-1β, IL-6, TNF-α), quimiocinas (CCL20, CXCL8, CCL2, CXCL1) e moléculas de adesão predominam neste processo (Eberting, 2014).

Repetidas exposições a alérgenos levam à cronificação da DCI, na qual ocorre o endurecimento da pele pela constante extração de seus lipídios. A constante perda de água transepidermal também induz a proliferação desordenada de queratinócitos e o consequente espessamento da camada epidérmica (hiperqueratose) (Eberting, 2014).

4.3 O TRATAMENTO FARMACOLÓGICO DE DOENÇAS INFLAMATÓRIAS DE PELE

Para manter a homeostasia do tecido, ambos os processos de iniciação da inflamação e sua resolução devem ser finamente coordenados e eficientes. Conter a progressão do processo inflamatório cutâneo é de extrema importância, uma vez que a infiltração celular exacerbada e descoordenada pode levar à sua cronificação (Kolaczkowska; Kubes, 2013).

Pesquisas no campo da Imunologia têm trazido opções terapêuticas para o tratamento de desordens cutâneas de caráter inflamatório. Com a maior compreensão acerca da patofisiologia das doenças de pele por meio da elucidação de eventos celulares e moleculares, terapias direcionadas com eficácia clínica sem precedentes têm sido propostas (Schlapbach;

Navarini, 2016). Estas novas terapias têm como alvo moléculas com importante contribuição nas doenças de pele, especialmente as citocinas. Como exemplos, pode-se citar os biológicos infliximab e dupilumab para o tratamento de psoríase e dermatite atópica, respectivamente (Noda et al., 2015). Apesar de promissoras, devido ao emprego de novas tecnologias e inúmeras pesquisas em seu desenvolvimento, estas opções terapêuticas trazem consigo um elevado custo agregado, inviabilizando sua ampla utilização no contexto clínico (Raval et al., 2011; Wittmann et al., 2014; Torres et al., 2021).

Em contrapartida, atualmente, devido à facilidade de acesso e baixo custo, o tratamento de condições inflamatórias consiste principalmente na utilização de AINEs e corticosteroides tópicos como a dexametasona. Os AINEs compreendem diversas moléculas que apresentam atividades anti-inflamatória, antipirética e analgésica. AINEs têm seu mecanismo de ação anti-inflamatória centrado na inibição das enzimas cicloxigenases, as quais são responsáveis pela produção de moléculas com potente ação vasodilatadora, as prostaglandinas, além de tromboxanos. A inibição da síntese de prostaglandinas vasodilatadoras resulta, como consequência, na redução do edema decorrente do processo inflamatório (Bacchi et al., 2012).

Os corticosteroides, ou glicocorticoides, desempenham sua ação farmacológica via ligação aos receptores de glicocorticoides (GRs), pertencentes à família de proteínas dos receptores hormônio-esteroides (Xavier et al., 2016). Os GRs localizam-se no citoplasma celular, onde encontram-se associados a um complexo proteico. A ligação de glicocorticoides aos GRs desencadeia a dissociação do complexo proteico e favorece a translocação do complexo glicocorticoide-GR para o núcleo celular, onde ligam-se aos elementos de resposta a glicocorticoides. Após ligação aos elementos de resposta a glicocorticoides, os fármacos pertencentes à classe dos corticosteroides tópicos exercem seu efeito anti-inflamatório por meio do controle da transcrição de genes inflamatórios, aumentando a transcrição de genes anti-inflamatórios e reduzindo a transcrição de genes pró-inflamatórios. Os GRs são capazes de induzir a expressão de diversas moléculas inibitórias, como reguladores negativos da via dos receptores Toll-like ou fatores de transcrição chave como o NF-κB e a proteína ativadora-1 (Barnes, 1998; Uva et al., 2012; Xavier et al., 2016).

Desde sua introdução na terapêutica, esta classe de fármacos vem sendo amplamente empregada, devido aos seus potentes efeitos anti-inflamatórios, imunossupressores e antiproliferativos. Entretanto, sua utilização está atrelada ao desenvolvimento de efeitos adversos graves, que compreendem atrofia cutânea, púrpura, rosácea e o efeito rebote o qual conduz a uma piora do processo inflamatório, e que muitas vezes acabam por comprometer a

aceitação dos pacientes ao tratamento farmacológico (Uva et al., 2012; Coondoo et al., 2014; Barnes et al., 2015). Similarmente, a utilização de AINEs também está associada à ocorrência de efeitos adversos: complicações gastrintestinais e cardiovasculares, toxicidade renal e retenção de fluidos são os mais relatados (Bacchi et al., 2012).

Visto que o tratamento farmacológico com ambas as alternativas de fácil acesso disponíveis atualmente, AINEs e corticosteroides, resultam em efeitos adversos graves, produtos naturais como fontes de moléculas biologicamente ativas têm chamado atenção devido à sua eficácia e menor ocorrência de efeitos adversos (Lahlou, 2013; Thomford et al., 2018).

4.4 COMPOSTOS DE ORIGEM NATURAL E O ÁCIDO OLEICO

Compostos naturais de origem mineral, vegetal e animal são utilizados pelo homem com finalidades terapêuticas há milhares de anos (Calixto, 2019). São inúmeros os exemplos de fármacos originados de produtos naturais utilizados nos dias de hoje: atorvastatina (as estatinas foram isoladas do fungo *Penicillium citrinum*), captopril (isolado do veneno de *Bothrops jararaca*), tacrolimo (isolado da actinobactéria *Streptomyces tsukubaensis*), ciclosporina (isolada do fungo *Tolypocladium inflatum*), paclitaxel [isolado de extratos de casca da árvore *Taxus brevifolia* Nutt. (Taxaceae)], penicilina (isolada de fungos do gênero *Penicillium*), entre outros (Altmann e Gertsch, 2007; Endo, 2017; Gaynes, 2017; Yang et al., 2018; Calixto, 2019; Peloso, 2020).

Medicamentos clássicos como o ácido acetilsalicílico e a morfina também foram descobertos a partir de produtos naturais. O ácido acetilsalicílico é derivado da salicilina, uma substância presente na planta *Salix alba* pertencente ao gênero *Spiraea*. A utilização terapêutica do ácido salicílico iniciou há mais de 3 mil e quinhentos anos. Registros datados de 1.500 a.C., conhecidos como papiros de Ebers, trazem recomendações do uso de infusões de folhas secas do arbusto murta para o alívio de dores reumáticas. Apesar dos registros milenares de sua utilização, foi apenas em 1897 que o químico alemão Felix Hoffmann descobriu o efeito analgésico do ácido acetilsalicílico, o qual se dá pela inibição inespecífica das enzimas cicloxigenases, inibindo a síntese de prostaglandinas. Em 1900, o ácido acetilsalicílico passou a ser comercializado como o primeiro medicamento na forma de comprimidos, com o nome comercial de Aspirina®. Nos dias de hoje, o ácido acetilsalicílico é utilizado por muitos pacientes como agente antiagregante plaquetário no tratamento e prevenção de doenças cardiovasculares (Desborough; Keeling, 2017).

O alcaloide morfina foi isolado em 1806 a partir do suco da semente da papoula (*Papaver somniferum*) pelo farmacêutico alemão Friedrich Sertürner (Krishnamurti; Chakra Rao, 2016; Calixto, 2019). Este fármaco, um potente analgésico agonista de receptores opioides é ainda amplamente utilizado e, muitas vezes, é o fármaco de escolha para o alívio da dor, especialmente em dores de alta intensidade, sendo administrado por diversas vias: oral, intravenosa, subcutânea, epidural, inalatória e outras (Krishnamurti; Chakra Rao, 2016).

No cenário nacional, um importante passo foi dado no ano de 2006 no que diz respeito à utilização terapêutica de produtos de origem natural. Por meio do Decreto nº 5.813, de 22 de junho de 2006, ficou instituída a Política Nacional de Plantas Medicinais e Fitoterápicos, com o objetivo de ‘‘garantir à população brasileira o acesso seguro e o uso racional de plantas medicinais e fitoterápicos, promovendo o uso sustentável da biodiversidade, o desenvolvimento da cadeia produtiva e da indústria nacional’’ (Brasil, 2006). Ainda neste âmbito, no presente ano foi publicada a 2ª edição do Formulário de Fitoterápicos da Farmacopeia Brasileira, um conjunto de 85 monografias, as quais contemplam 85 espécies e o total de 236 formulações à base de plantas medicinais, visando ‘‘contribuir para a expansão do desenvolvimento, produção e dispensação de produtos fitoterápicos com qualidade, tornando-os cada vez mais acessíveis para a população brasileira’’ (Brasil, 2021).

O ácido oleico, ou ômega-9, (C18:1, n-9), é um dos compostos facilmente encontrados em muitos óleos vegetais e alimentos, tais como óleo de oliva, peixes, sementes oleaginosas e frutos como o abacate (Viola; Viola, 2009; Roncero et al., 2016). O AO é o principal membro do grupo dos ácidos graxos monoinsaturados, representando cerca de 90% dos ácidos graxos monoinsaturados ingeridos pelo homem por meio da alimentação (Schwingshackl; Hoffmann, 2014). O consumo regular de AO está associado com níveis reduzidos de colesterol no sangue, menor risco de desenvolvimento de doenças cardiovasculares e redução da pressão arterial (Sales-Campos et al., 2013).

O ácido oleico também é encontrado no organismo humano como um constituinte de membranas celulares, além de participar atuando como substrato para a síntese de hormônios (Tvrzicka et al., 2011). Na pele humana, está presente como constituinte lipídico do estrato córneo, contribuindo para a funcionalidade de barreira desta camada (Sales-Campos et al., 2013).

Diversos óleos vegetais têm o ácido oleico como um de seus principais constituintes, especialmente aqueles da região Amazônica: óleo de babaçu (extraído da *Orbignya phalerata* Mart; família Arecaceae) (Souza et al., 2011), óleo de andiroba (extraído da *Carapa guianensis*

Aubl.; família Meliaceae) (Pesso, 2011), óleo de oliva (extraído da *Olea europaea* L.; família Oleaceae) (Donato-Trancoso et al., 2016) e óleo de semente de uva (extraído da *Vitis vinifera* L.; família Vitaceae) (Shivananda Nayak et al., 2011).

No Brasil, principalmente na região Amazônica, área de elevada diversidade de espécies vegetais, povos nativos empregam óleos de origem vegetal com finalidades terapêuticas (Burlando; Cornara, 2017). No entanto, a utilização destes óleos pela população geralmente ocorre de maneira empírica, sem o respaldo científico acerca de sua eficácia e segurança. Portanto, muitos estudos têm sido conduzidos atualmente com o propósito de confirmar a eficácia biológica destes compostos e garantir sua segurança para a utilização humana. Muitas destas substâncias também têm sido estudadas pelo seu potencial em promover benefícios no tratamento de condições inflamatórias de pele (Garavaglia et al., 2016; Reis et al., 2017; Alves et al., 2019).

Estudos intencionando investigar os efeitos do AO na pele já foram relatados na literatura. Algumas destas evidências sugerem um importante papel do AO em modular processos inflamatórios. Nestes estudos, os resultados mostram que este composto é capaz de promover a redução da expressão de citocinas pró-inflamatórias e de moléculas de adesão, além de reduzir a migração de células para o local inflamado, ao passo que aumenta a expressão de citocinas anti-inflamatórias, em modelos de inflamação induzida experimentalmente (Sales-Campos et al., 2013; Medeiros-de-Moraes et al., 2018). Por meio da regulação destes mecanismos, já foi demonstrado que o AO é capaz de promover benefícios na cicatrização de feridas quando aplicado diretamente sobre a pele (Cardoso et al., 2011; Sales-Campos et al., 2013; Poljšak et al., 2019; Jara et al., 2020).

4.5 SISTEMAS NANOESTRUTURADOS PARA VEICULAÇÃO DE FÁRMACOS E A ADMINISTRAÇÃO DE SUBSTÂNCIAS ATIVAS NA PELE

O termo ‘‘nano’’, de origem grega e que se refere à manipulação de materiais em escala muito diminuta (10^{-9} m), popularizou-se nas últimas décadas, quando a nanotecnologia e a nanoengenharia têm sido empregadas em muitas pesquisas científicas no campo do ‘‘*drug delivery*’’ (Lane, 2011; Vega-Vásquez et al., 2020). O advento dos sistemas nanoestruturados para liberação de fármacos trouxe consigo inúmeras vantagens para a terapêutica: o direcionamento do fármaco ao local alvo de ação no organismo, possibilitando consequentemente a redução da dose de fármaco administrada e de efeitos adversos associados

ao tratamento; e também a possibilidade de modulação da liberação do fármaco a partir destes sistemas (Chamundeeswari et al., 2019).

Dentre os sistemas de liberação de fármacos de escala nanométrica mais empregados atualmente estão as nanocápsulas e as nanoemulsões. Nanocápsulas são sistemas organizados sob a forma de vesículas, nos quais o núcleo, geralmente de caráter oleoso, é circundado por uma parede polimérica. Nestes sistemas o fármaco pode estar dissolvido no núcleo e/ou adsorvido à parede do polímero (Deng et al., 2020); nanoemulsões são emulsões nas quais o tamanho das gotículas encontra-se na faixa nanométrica, podem ser do tipo óleo em água (O/A) ou água em óleo (A/O) e são estabilizadas por tensoativos (Sutradhar; Amin, 2013). Inúmeros estudos na literatura reportam o desenvolvimento de nanocarreadores para veiculação de substâncias ativas à pele direcionadas ao tratamento de doenças que acometem este órgão (Pegoraro et al., 2017; Marchiori et al., 2017; Carter et al., 2019; Rigon et al., 2019).

A via tópica apresenta vantagens frente a outras vias de administração de fármacos, tais como a menor incidência de efeitos adversos sistêmicos e aplicação específica no local acometido pela patologia, além de ser uma via de administração não-invasiva (Zeb et al., 2018). A administração de substâncias ativas na pele normalmente intenciona: efeitos locais na epiderme, efeitos locais na derme, ou a absorção transdérmica (circulação sistêmica) (Lane, 2011). A eficácia de substâncias ativas na pele pode ser aumentada pela utilização da nanotecnologia, uma vez que partículas de tamanho diminuto podem amplificar a penetração de fármacos e cosméticos, além de permear a pele via diferentes mecanismos, permitindo um regime de dosagem simplificado e intervenções terapêuticas fáceis (Dhapte; Pokharkar, 2019).

A permeação de substâncias ativas na pele pode ocorrer por dois mecanismos distintos: via difusão passiva através da epiderme intacta (rota transepidermal) ou via apêndices cutâneos (também referida como via anexial). Visto que os apêndices cutâneos ocupam menos de 0,1% da superfície da pele humana, a permeação por esta via é considerada mínima (Lane, 2011). Entretanto, já foi demonstrado que alguns nanocarreadores podem utilizar esta via de permeação (Ghasemiyeh; Mohammadi-Samani, 2020). A rota de permeação transepidermal pode ser subdividida em dois tipos: via transcelular e via intercelular. A rota transcelular compreende a passagem do fármaco através dos corneócitos, enquanto que a via intercelular diz respeito à passagem do fármaco entre os corneócitos, e é considerada a principal via de permeação de substâncias hidrofóbicas (Ghasemiyeh; Mohammadi-Samani, 2020).

Uma vez que sistemas nanoestruturados são geralmente obtidos na forma de suspensões coloidais líquidas, sua incorporação em formulações semissólidas para facilitar a aplicação

tópica tem sido relatada (Rigo et al., 2015; Pegoraro et al., 2017; Rigon et al., 2019). Neste sentido, sabe-se que a escolha das bases semissólidas (veículos) é de fundamental importância para o ‘‘*delivery*’’ do composto ativo e seu efeito sobre o
tecido cutâneo (Silva et al., 2010; Uchechi et al., 2014). Exemplos de matérias-primas que têm sido empregadas para constituir bases semissólidas são o emulsificante polimérico Pemulen® TR2, o polímero Carbopol®, o polissacarídeo goma gelana e a cera Lanette® (Pegoraro et al., 2017; Rigon et al., 2019).

O desenvolvimento de formulações semissólidas para veiculação de ativos à pele compreende uma série de etapas de caracterização físico-química, avaliação de espalhabilidade e do comportamento reológico. Avaliações de caracterização físico-química compreendem por exemplo medida de pH, tamanho de partícula/gotícula, índice de polidispersão e potencial Zeta (Nastiti et al., 2017).

A avaliação da espalhabilidade também é uma análise fundamental para a caracterização de semissólidos. A eficácia da terapia tópica está diretamente associada à sua capacidade de espalhar formando uma camada uniforme sobre a área de aplicação. Além disso, formulações destinadas à aplicação sobre áreas lesionadas não devem necessitar de muita força para espalhar (Garg et al., 2002). Complementar à avaliação de espalhabilidade, o comportamento reológico também constitui uma importante análise de caracterização de semissólidos (Rathod e Mehta, 2015).

5 HIPÓTESE

O ácido graxo ácido oleico pode constituir uma boa alternativa terapêutica para o tratamento de doenças e condições de pele guiadas por mediadores inflamatórios uma vez que evidências da literatura sugerem que este composto é capaz de suprimir processos inflamatórios. Ademais, aliar este composto ao acetato de dexametasona associados a sistemas nanoestruturados pode proporcionar um efeito biológico ainda melhor por possibilitar a redução de dose do corticosteroide, o que pode impactar positivamente na diminuição dos seus efeitos adversos.

6 DESENVOLVIMENTO

O desenvolvimento desta tese está apresentado sob a forma de dois artigos científicos. Os tópicos ‘‘Resumo’’, ‘‘Introdução’’, ‘‘Materiais e métodos’’, ‘‘Resultados’’, ‘‘Discussão’’ e ‘‘Referências bibliográficas’’ encontram-se nos próprios artigos científicos.

Os artigos científicos foram publicados nas revistas *Inflammopharmacology (DOI: 10.1007/s10787-019-00675-5)* e *Journal of Ethnopharmacology (DOI: 10.1016/j.jep.2020.113486)*, respectivamente e, portanto, encontram-se no formato de versão publicada.

Em anexo, ao final desta tese, encontram-se as cartas de aprovação dos projetos de pesquisa pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) e as permissões para o uso dos artigos científicos nesta tese.

6.1 ARTIGO 1

Formas farmacêuticas semissólidas contendo ácido oleico exibem efeito anti-inflamatório *in vivo* via receptor de glicocorticoide em um modelo de inflamação de pele induzida por radiação UVB

Oleic acid-containing semisolid dosage forms exhibit *in vivo* anti-inflammatory effect via glucocorticoid receptor in a UVB radiation-induced skin inflammation model

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Inflammopharmacology

ORIGINAL ARTICLE

Oleic acid-containing semisolid dosage forms exhibit in vivo anti-inflammatory effect via glucocorticoid receptor in a UVB radiation-induced skin inflammation model

Natháli Schopf Pegoraro[1] · Camila Camponogara[1] · Mailine Gehrcke[2] · Laura Minussi Giuliani[2] · Dariane Trivisiol da Silva[3] · Luana Haselein Maurer[3] · Priscilla Dias[4] · Tatiana Emanuelli[3] · Letícia Cruz[2] · Sara Marchesan Oliveira[1]

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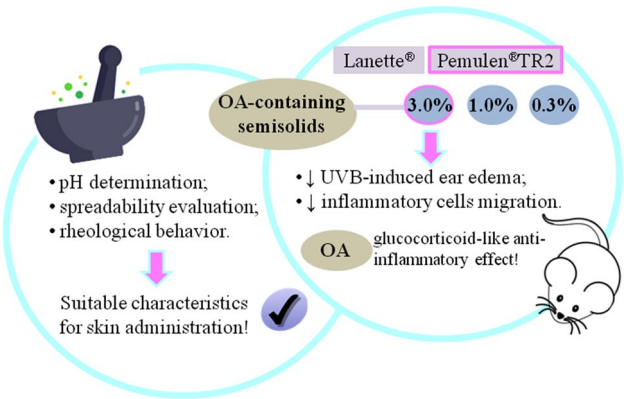
Abstract

The treatment of cutaneous inflammation with topical corticosteroids may cause adverse effects reinforcing the need for therapeutic alternatives to treat inflammatory skin disorders. We investigated the anti-inflammatory effect of oleic acid (OA), a fatty acid of the omega-9 (ω-9) family, and we point out it as an alternative to treat inflammatory skin disorders. OA was incorporated into Lanette®- or Pemulen® TR2-based semisolid preparations and the pH, spreadability, rheological behavior and in vivo anti-inflammatory performance in a UVB radiation-induced skin inflammation model in mice were assessed. The anti-inflammatory activity was verified after single or repeated treatment of the mouse ear following the UVB. The OA action on glucocorticoid receptors was investigated. Both semisolids presented pH values compatible with the deeper skin layers, appropriate spreadability factors, and non-Newtonian pseudoplastic rheological behavior. Pemulen® 3% OA inhibited ear edema with superior efficacy than Lanette® 3% OA and dexamethasone after a single treatment. Pemulen® 3% OA and dexamethasone also reduced inflammatory cell infiltration. After repeated treatments, all formulations decreased the ear edema at 24 h, 48 h and 72 h after UVB. OA in semisolids, especially Pemulen® TR2-based ones, presented suitable characteristics for cutaneous administration and its anti-inflammatory activity seems to occur via glucocorticoid receptors. OA was also capable to reduce croton oil-induced skin inflammation. Besides, the ex vivo skin permeation study indicated that OA reaches the receptor medium, which correlates with a systemic absorption in vivo. The natural compound OA could represent a promising alternative to those available to treat inflammatory skin disorders.

Graphic abstract



Keywords Omega-9 fatty acid · Skin inflammation · Sunburn · Croton oil · Skin permeation · Mifepristone

Extended author information available on the last page of the article

Abbreviations

ANOVA	Analysis of variance
BSA	Bovine serum albumin
DMSO	Dimethylsulphoxide
E_{max}	Maximal effect
I_{max}	Maximum inhibition
Lanette® 0.3% OA	Lanette®-based semisolid containing 0.3% oleic acid
Lanette® 1% OA	Lanette®-based semisolid containing 1% oleic acid
Lanette® 3% OA	Lanette®-based semisolid containing 3% oleic acid
OA	Oleic acid
Pemulen® 0.3% OA	Pemulen® TR2-based semisolid containing 0.3% oleic acid
Pemulen® 1% OA	Pemulen® TR2-based semisolid containing 1% oleic acid
Pemulen® 3% OA	Pemulen® TR2-based semisolid containing 3% oleic acid
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
TPA	12-*O*-Tetradecanoylphorbol-13-acetate
UVA	Ultraviolet radiation type A
UVB	Ultraviolet radiation type B
UVC	Ultraviolet radiation type C
UVR	Ultraviolet radiation

Introduction

Natural compounds have been employed by humans for 1000 years with therapeutic purposes. In this sense, many studies have been conducted to check and elucidate their biological activities, among these, their ability to promote benefits on skin health. Some of these compounds are present on plant extracts and vegetable oils, whose potential have been studied in the treatment of inflammatory skin conditions, including those sunburn-associated, irritant and allergic contact dermatitis, and psoriasis (Bhoir et al. 2019; Camponogara et al. 2019a, b, c; Rigo et al. 2015; Yu et al. 2015).

Oleic acid (OA) is one of the relevant fatty acids found in vegetable oils and foods, such as cod and oilseeds (Roncero et al. 2016; Viola and Viola 2009). OA is also naturally found in the human body as a constituent of cell membranes, and it participates as a substrate for hormone synthesis (Tvrzicka et al. 2011). Despite the importance of this unsaturated fatty acid in nutrition, few studies are available regarding its effects on skin diseases (Sales-Campos et al. 2013).

Evidence reports the popular use of medicinal plants containing OA on wound healing, which occurs through the topical administration of vegetable oils that present this fatty acid in their composition (Veiga-Júnior and Pinto 2002; Lordani et al. 2018). Usually, the OA is used as coadjuvant in pharmaceutical formulations to treat skin disorders but no studies reported its effect in semisolid dosage forms for cutaneous administration.

Due to its anatomical location, situated on the interface between external and internal environments, the skin is subjected to many environmental stimuli that are capable of evoking cutaneous inflammatory responses. These environmental stimuli can be physical, biological, and chemical. Besides providing the first line of defense against various insults, the skin is responsible for crucial functions to maintain the body homeostasis, such as the control of excessive water loss and thermoregulation (Nestle et al. 2009; Paspparakis et al. 2014; Serhan et al. 2008).

Among the insults that can evoke cutaneous inflammatory response is the UV radiation (UVR). UVR is a component from the electromagnetic light spectrum, which is subdivided into three types according to the wavelengths (nm) that it covers ultraviolet radiation type A (UVA; 320–400 nm), ultraviolet radiation type B (UVB; 280–320 nm), and ultraviolet radiation type C (UVC; 100–280 nm) (D'Orazio et al. 2013). Once UVC radiation is almost totally absorbed by the ozone layer of the atmosphere, the solar UVR that is relevant to human health is the UVA and UVB types (Schuch et al. 2013).

The UVA radiation can reach the deeper skin layer, i.e., dermis, while the UVB radiation is absorbed by the epidermis and it exerts its effects on this skin layer. The UVR ability to penetrate the skin contributes to inflammation, early skin aging, and cancer (D'Orazio et al. 2013; Watson et al. 2016).

UVR-induced tissue injury promotes endothelial permeability alterations, inflammatory cell migration and release of vasoactive, neuroactive, and chemical mediators, resulting in an inflammatory response characterized by erythema, heat, swelling (edema), and pain (Nourshargh and Alon 2014). UV-induced further change includes the increase in the epidermal thickness (hyperkeratosis) (D'Orazio et al. 2013). The incidence of UV radiation on human skin also results in increased production of reactive species that may damage nucleic acids, proteins, and lipids, potentially leading to carcinogenesis development and early skin aging (Schuch et al. 2017). Besides, ROS can potentially promote and contribute to the inflammatory process maintenance (Mittal et al. 2014). These effects can lead to the function loss of the injured tissue and impair the patient's work capacity and quality of life (Serhan et al. 2008; Chiu et al. 2012; Nan et al. 2018).

The treatment of cutaneous inflammation consists of the use of topical corticosteroids. However, they can cause adverse effects, such as skin atrophy, development of rosacea

and purpura, and the well-known rebound effect (Barnes et al. 2015; Coondoo et al. 2014), which limit their use. These disadvantages reinforce the need for the discovery of new effective therapeutic alternatives to treat skin diseases but with less potential to cause adverse effects.

In this sense, evidence indicates that the OA can modulate the inflammatory processes preventing their progression and reducing associated damages, without causing the severe adverse effects related to glucocorticoid therapy. Therefore, we employed an animal model of skin inflammation UV radiation-induced to provide insights into the anti-inflammatory effect of OA fatty acid incorporated into semisolid formulations and its action mechanism. To consistently demonstrate the oleic acid efficacy in treating inflamed skin, we additionally evaluated the croton oil-induced skin inflammation model. To guarantee the quality and efficacy of the formulations, we have also performed their characterization.

Methodology

Materials

Pemulen® TR2 was donated by Noveon (Cleveland, USA). Oleic acid (OA) (about 78% purity) was purchased from LabSynth (Diadema, Brazil). Croton oil, dimethylsulphoxide (DMSO) and triethanolamine were purchased from Sigma-Aldrich (São Paulo, Brazil). Imidazolidinyl urea was purchased from PharmaSpecial (São Paulo, Brazil). Dexamethasone acetate and Lanette® base were purchased from Nova Derme (Santa Maria, Brazil). Ketamine (Dopalen®) and xylazine (Anasedan®) were purchased from Ceva (Paulínia, Brazil). Formaldehyde, acetone, ethanol, and acetic acid were purchased from Vetec (Rio de Janeiro, Brazil). Hematoxylin–eosin, paraffin, and magnesium chloride were purchased from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) was purchased from Ludwig Biotecnologia (Alvorada, Brazil). All other reagents and solvents were of analytical grade and used as received.

Methods

Preparation of semisolid formulations

Two types of semisolid formulations containing OA were prepared: Pemulen® TR2-based and Lanette®-based semisolids. Pemulen® TR2 gels were prepared, with the aid of a mortar and pestle, by dispersing the polymeric emulsifier Pemulen® TR2 into distilled water, at the concentration of 0.7%. To this dispersion, 1% triethanolamine was added, immediately conducting to the gel formation, and the imidazolidinyl urea (2%) as an antimicrobial preservative agent. Lastly, the active compound OA was incorporated at three concentrations: 0.3%, 1%, and 3%. Since the obtained raw material was not 100% pure, the real content of OA was considered for its incorporation into semisolids. The semisolid containing 0.5% dexamethasone acetate was prepared by incorporating this compound solubilized in DMSO (5%) into Pemulen® TR2 vehicle.

Lanette® cream formulations were prepared by adding OA to a commercial base cream at concentrations described above. Both vehicle formulations (Pemulen® TR2 and Lanette®) were also prepared, employing the same methodology but omitting the OA in the formulation.

The real content of OA in the raw material was checked by gas chromatography analysis coupled to flame ionization detector after conversion of fatty acids into methyl esters (Hartman and Lago 1973) and this content was employed to correct the final percentage of OA into the semisolids.

Semisolid characterization

The pH, spreadability and rheological behavior of all semisolid formulations were determined immediately after preparation.

pH measurements The pH values of semisolid formulations were evaluated at room temperature (25 ± 2 °C), by immersing a calibrated potentiometer (Model Even PHS-3E, Yoke Instrument Co., China) directly in a semisolid aqueous dispersion (10%, w/v).

Spreadability evaluation The developed formulations spreadability was evaluated through the parallel plate method (Borghetti and Knorst 2006; Rigo et al. 2012). For this, an aliquot of the sample was placed in a central hole of a mold glass plate that was supported on the scanner surface (HP Officejet, model 3050 Desktop, USA). This glass plate was carefully removed to avoid the withdrawal of the formulation. So, ten glass plates with known weights were put on the formulations. Each plate was placed observing an interval of 1 min to the subsequent plate, and one image was captured at every 1 min of the interval, employing the desktop scanner.

The image registers were used to calculate the spreading area of the captured images, using the software Image J (Version 1.49q, National Institutes of Health, USA). The spreadability profiles were obtained by plotting the spreading area versus the cumulative weight of the plates. The spreadability factor was calculated for all formulations. This factor represents the formulation ability to expand on a smooth horizontal surface when a gram of weight is added to it under test conditions. The equation below was employed to calculate the spreadability factor:

$$\mathrm{Sf} = \frac{A}{W}, \quad (1)$$

in which Sf is the spreadability factor (mm^2/g), A is the maximum spread area (mm^2) after the addition of the total number of plates and W is the total weight of the plates added (g).

Rheological behavior analysis Rheological analysis of the semisolids was conducted at 25 ± 1 °C employing viscometer (RVDV-I-PRIME model, Brookfield, USA) supplied with an RV06 spindle. For this, about 30 g of the formulations was used and submitted to a range of speed between 2 and 100 rpm. The data obtained were analyzed to the best fit using Bingham, Casson, and Ostwald models (Eqs. 2–4), employing a graphical model to determine the rheological behavior:

$$\tau = \tau_0 + \eta\gamma, \quad (2)$$

$$\tau^{0.5} = \tau_0^{0.5} + \eta^{0.5}\gamma^{0.5}, \quad (3)$$

$$\tau = \kappa\gamma^n, \quad (4)$$

where τ_0 is the yield stress, η is the viscosity, n is the index of flow, κ is the index of consistency, τ is the shear stress and γ is the shear rate (Kim et al. 2003; Pegoraro et al. 2017).

In vivo experiments

Animals Male *Swiss* mice weighing about 25–30 g obtained from Biotério Central of the Federal University of Santa Maria were used in all experiments. Animals were kept on suitable cages, under controlled temperature (22 ± 2 °C), on a 12-h light–dark cycle and fed with standard laboratory chow and water ad libitum. The animals were acclimatized to the experimental room for at least 1 h before performing the experiments. All experiments were carried out between 8:00 a.m. and 5:00 p.m. The experiments were performed following national legislation (Guidelines of Brazilian Council of Animal Experimentation-CONCEA), and they followed the Animal Research: Reporting In Vivo Experiments ARRIVE guidelines (McGrath and Lilley, 2015). All procedures were approved by the Institutional Committee for Animal Care and Use of the Federal University of Santa Maria (2320290518/2018, 5582261018/2018, and 5864120819/2019). Animals were randomly assigned to different treatment groups and all the experiments were performed blindly. The number of animals and the stimuli intensity were the minimum necessary to demonstrate the consistent effects of treatments.

UVB irradiation model The UVB radiation source consisted of a Philips TL40W/12 RS lamp, which was mounted 12 cm above the surface where mice were placed. UVB lamp emits a continuous light spectrum between 270 and 400 nm, with a peak of emission at 313 nm. The UVB output (80% of the total UV radiation) was measured using a UV monitor (model MS-211-1; EKO Instruments, Tokyo, Japan). Before UVB irradiation, mice were firstly anesthetized with 90 mg/kg of ketamine + 30 mg/kg of xylazine by a single intraperitoneal injection. After the anesthetic procedure, mice were placed on the bench at a distance of 12 cm from the lamp, and only their right ear was exposed to UVB radiation for 14 min. The remaining mice corporal surface was protected from UV radiation. UVB irradiation rate was 0.27 mW/cm^2 and the dose employed was 0.5 J/cm^2 (Marchiori et al. 2017; Pegoraro et al. 2017). It is important to reinforce that mouse ear was irradiated only once.

Formulation administration and experimental design: Mice were divided into eleven groups containing six animals each, and classified as it follows: naïve (non-irradiated); irradiated untreated (UVB 0.5 J/cm^2); UVB + Lanette® vehicle; UVB + Lanette® 0.3% OA; UVB + Lanette® 1% OA; UVB + Lanette® 3% OA; UVB + Pemulen® TR2 vehicle; UVB + Pemulen® TR2 0.3% OA; UVB + Pemulen® TR2 1% OA; UVB + Pemulen® TR2 3% OA; UVB + 0.5% dexamethasone acetate (positive control). Mouse ear was topically treated after UVB irradiation with semisolid formulations (15 mg/ear), with the aid of a spatula, according to the experimental groups. Two types of treatment were conducted: (1) single treatment immediately after UVB irradiation (single exposure); (2) repeated treatments that began immediately, 24 h, and 48 h after UVB irradiation (single exposure).

Ear edema measurement: The ear edema was assessed through the measurement of the ear thickness, before (basal measure) and after the UVB radiation. An increase in ear thickness after UVB irradiation when compared to basal values was considered as ear edema. For the single treatment protocol, the ear thickness measurement was performed before and at 24 h after UVB irradiation and/or plus treatments; for the repeated treatments protocol, the ear thickness was measured before and at 24 h, 48 h, and 72 h after UVB irradiation and/or plus treatments. The ear thickness was evaluated using a digital micrometer (Digimess, São Paulo, Brazil) in animals previously anesthetized.

The micrometer was applied near the tip of the ear, just distal to the cartilaginous ridges (Pegoraro et al. 2017; Silva et al. 2011). Ear thickness was expressed in μm, as the difference between basal thickness and ear thickness at every time point. To minimize the variation, a single investigator performed the measurements throughout each experiment.

Assessment of inflammatory cell infiltration The inflammatory cell infiltration to the irradiated ear tissue was assessed by histological analysis. Separate groups of mice were used to evaluate histological changes in ear tissue at 24 h after receiving UVB irradiation or UVB irradiation plus treatments with semisolid formulations. After ear edema assessment, mice were euthanized, the right ear was removed and fixed in Alfac solution (16:2:1 mixture of ethanol 80%, formaldehyde 40%, and acetic acid). Each sample was then embedded in paraffin, sectioned at 5 µm and stained with hematoxylin–eosin. A representative area was selected, and a quantitative analysis of the number of inflammatory cells was performed using 10 × objectives (Piana et al. 2016). To minimize the source of bias, this analysis was performed blindly. The inflammatory cells quantification was performed by counting the cells per field using the Image J software, and three fields from six distinct histological slides of each group were analyzed.

Oleic acid anti-inflammatory activity via glucocorticoid receptors To verify if the OA anti-inflammatory activity is dependent on the glucocorticoid receptors, animals received a pre-treatment with a glucocorticoid receptor antagonist, mifepristone (50 mg/kg; s.c.; saline containing 10% ethanol) 15 min before the ear irradiation (UVB, 0.5 J/cm^2) plus topical treatments. Ear thickness was evaluated before and at 24 h after UVB irradiation and the ear edema was expressed as described above (Camponogara et al. 2019a; Mendes et al. 2016).

Croton oil-induced acute skin inflammation model Mice were previously anesthetized with 90 mg/kg of ketamine + 30 mg/kg of xylazine by a single intraperitoneal injection and the acute ear edema was induced by a croton oil single topical application (1 mg/ear dissolved in acetone; 20 µL/ear) given in the right mouse ear. After croton oil application, mice were treated with the semisolid developed formulations or dexamethasone (0.5%; employed as a positive control). Six hours after the croton oil or croton oil plus treatment application, the ear thickness of the animals was verified, and then they were euthanized, and ear biopsies were collected for further analysis (Brum et al 2016; Piana et al 2016; Rigon et al 2019).

Formulation administration and experimental design: Mice were divided in seven groups containing six animals: Naïve; Croton oil (1 mg/ear); Croton oil + Pemulen® TR2 vehicle; Croton oil + Pemulen® TR2 0.3% OA; Croton oil + Pemulen® TR2 1% OA; Croton oil + Pemulen® TR2 3% OA; Croton oil + 0.5% dexamethasone acetate (positive control). Mouse ear was topically treated, immediately after croton oil application with semisolid formulations (15 mg/ear).

Ear edema measurement: The ear edema was assessed as described above. The increase in ear thickness 6 h after croton oil administration when compared to basal values was considered as ear edema (Pegoraro et al. 2017; Silva et al. 2011).

Ex vivo experiments

Porcine skin permeation study The permeation study was performed on Franz-type vertical diffusion cells, using porcine skin as the membrane. The pig ear tissue was obtained from a slaughterhouse (Santa Maria, Brazil). An infinite dose of the semisolid containing OA (0.5 g) was spread on the top of the skin. The receptor medium, phosphate buffer, pH 5.5, was maintained at 37 °C under constant magnetic stirring for 8 h. At the end of 8 h of experiment, the excess of the formulation was removed from the skin, and tape stripping was performed to quantify the OA in the *stratum corneum* (18 rounds of strip tapes; Masterfix®). For epidermis and dermis separation, the skin tissue was maintained for 45 s in a water bath at 60 °C, and after that, the epidermis was removed using a spatula (Rigon et al 2019). The receptor medium was also collected for OA quantification. The quantification of OA in the skin layers and receptor medium from samples without the semisolid containing OA was also performed since OA is naturally present in the skin. The samples were extracted using chloroform and methanol (Bligh and Dyer 1959) and converted into methyl esters (Hartman and Lago 1973). The percentage of OA of total fatty acids in the different skin layers was quantified by gas chromatography.

Statistical analysis

The semisolid characterization results are presented as mean ± standard deviation (SD). Results of in vivo evaluations are presented as the mean + standard error of the mean (SEM), and they are reported as geometric means plus its respective 95% confidence limits. The maximum inhibitory effect (I_{max}) was calculated based on the response of the control groups. Statistical significance between groups was assessed by one-way or two-way analysis of variance (ANOVA) followed by post hoc Newman–Keuls test or Tukey's test, when appropriate. *P* values less than 0.05 ($p < 0.05$) were considered as indicative of significance. All statistical tests were carried out using GraphPad Prism 6.00 Software (San Diego, USA).

Results

Semisolid characterization

The pH values and spreadability factors of the semisolid formulations developed are presented in Table 1. The pH values

were situated next to the neutral range (6.0–7.2). The pH values obtained for dexamethasone acetate and Pemulen® TR2 semisolids were higher than their related Lanette® ones ($p \leq 0.001$). No significant difference was observed between the three different concentrations of OA Lanette®-based semisolids pH neither between the three different concentrations of OA Pemulen® TR2-based semisolids pH ($p > 0.05$).

The spreadability factor was also calculated as a parameter of semisolids characterization. This factor presented values ranging close to 2.0 for all formulations, regardless of the vehicle employed. No statistical difference was observed in spreadability factor between all semisolids developed ($p > 0.05$).

In respect to the rheological evaluation, all the semisolids developed, regardless of the vehicle employed, presented non-Newtonian flow behavior since an increase in the shear rate conducted to the viscosity decrease. Concerning the mathematical modeling of the rheograms, it indicated that the data fitted better to the Ostwald model, presenting pseudoplastic behavior, as it can be seen in Table 2.

Table 1 pH values and spreadability factor for the developed semisolids

Formulation	pH	Spreadability factor ($mm^2 g^{-1}$)
Lanette® vehicle	6.01 ± 0.07	2.12 ± 0.15
Lanette® 0.3% AO	6.13 ± 0.04	1.89 ± 0.19
Lanette® 1% AO	6.27 ± 0.19	2.38 ± 0.48
Lanette® 3% AO	6.11 ± 0.07	2.23 ± 0.68
Pemulen® TR2 vehicle	7.05 ± 0.13	1.81 ± 0.22
Pemulen® TR2 0.3% OA	7.06 ± 0.06	1.83 ± 0.38
Pemulen® TR2 1% OA	7.11 ± 0.08	1.85 ± 0.56
Pemulen® TR2 3% AO	7.16 ± 0.02	1.87 ± 0.47
Dexamethasone acetate	7.17 ± 0.07	1.89 ± 0.30

The results are expressed as SEM of three independent experiments. One-way ANOVA followed by post hoc Tukey's test

In vivo experiments

Oleic acid single application reduces the UVB radiation-induced ear edema and inflammatory cell infiltration

The UVB radiation-induced ear edema model was employed to investigate the effects of OA on two different types of semisolid formulations on inflammatory parameters induced by UVB radiation. UVB radiation increased the ear thickness, characterizing the ear edema formation, with a maximal effect (E_{max}) = 74 ± 4 µm, at 24 h after UVB exposure. OA 0.3%, 1% and 3% incorporated in Lanette®, but not the vehicle, decreased the mouse ear edema, with maximal inhibitions (I_{max}) of 42.26 ± 3.62%, 57.10 ± 1.21%, and 79.36 ± 7.47%, respectively. Importantly, Lanette® 3% OA reduced the ear edema similar to 0.5% dexamethasone acetate, used as a positive control, which presented an I_{max} of 77.74 ± 2.69% (Fig. 1a).

OA 0.3%, 1% and 3% incorporated in Pemulen® TR2 also effectively reduced the UVB irradiation-induced ear edema with inhibitions of 48.52 ± 2.66%, 72.41 ± 0.84%, and 92.58 ± 2.58%, respectively. Pemulen® vehicle caused a minimum effect of 13.51 ± 4.56%. It is worth pointing out that the Pemulen® 1% OA produced an antiedematogenic effect similar to the 0.5% dexamethasone acetate (Fig. 1b), while the Pemulen® 3% OA was more effective than dexamethasone (I_{max} of 77.46 ± 3.27%).

The histological analysis of the mice ears at 24 h after UVB irradiation or UVB irradiation plus treatments with semisolids was also performed to investigate the inflammatory cells infiltration to the damaged tissue. This analysis revealed that UVB radiation increased the inflammatory cell infiltration (107 ± 3 inflammatory cells per field) when compared to the naïve group (50 ± 4 inflammatory cells per field) (Figs. 2, 3).

Topical treatment with semisolid Lanette®-based formulations was not capable of significantly decreasing the tissue

Table 2 Rheological behavior [(regression coefficients) (r^2)] for mathematical modeling in shear rate–shear stress curves to models of Bingham, Casson, and Ostwald

Formulation	Mathematical model		
	Bingham	Casson	Ostwald
Lanette® vehicle	0.9628 ± 0.0104	0.9904 ± 0.0039	0.9835 ± 0.0105
Lanette® 0.3% AO	0.9484 ± 0.0100	0.9903 ± 0.0034	0.9887 ± 0.0038
Lanette® 1% AO	0.9343 ± 0.0125	0.9760 ± 0.0108	0.9879 ± 0.0102
Lanette® 3% AO	0.9185 ± 0.0079	0.9759 ± 0.0045	0.9969 ± 0.0026
Pemulen® vehicle	0.9667 ± 0.0110	0.9921 ± 0.0030	0.9771 ± 0.0156
Pemulen® 0.3% AO	0.9409 ± 0.0600	0.9808 ± 0.0256	0.9657 ± 0.0069
Pemulen® 1% AO	0.9511 ± 0.0158	0.9878 ± 0.0073	0.9923 ± 0.0051
Pemulen® 3% AO	0.9440 ± 0.0023	0.9849 ± 0.0034	0.9957 ± 0.0021
Dexamethasone acetate	0.9694 ± 0.0023	0.9963 ± 0.0008	0.9906 ± 0.0015

The results are expressed as SEM of three independent experiments

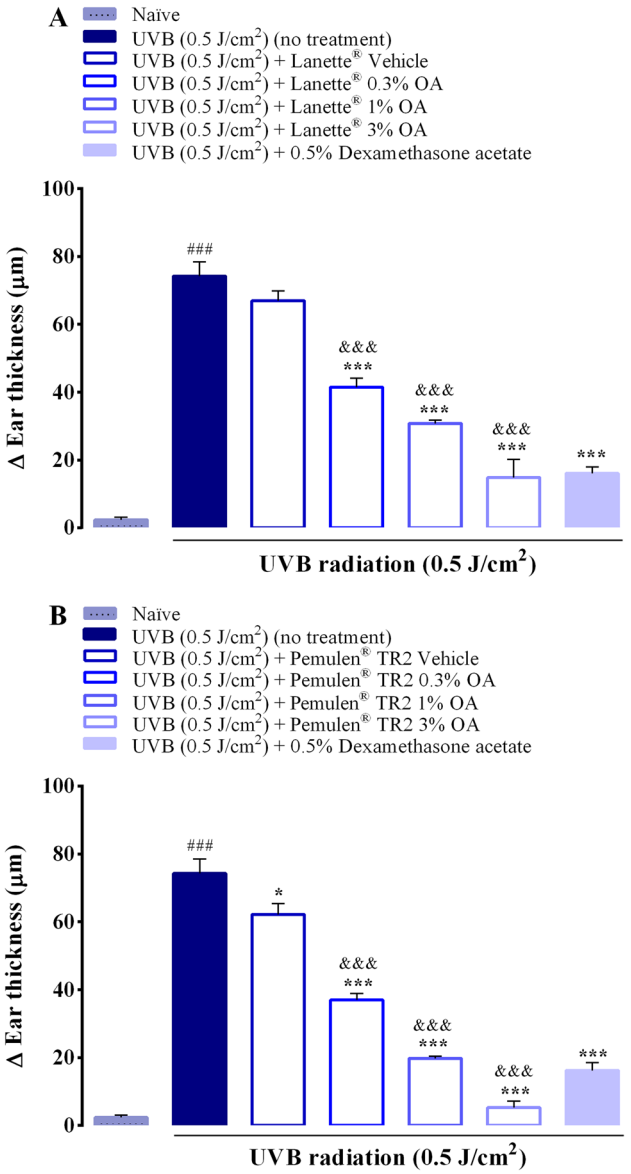


Fig. 1 Antiedematogenic effect of semisolid formulations on the ear edema induced by UVB irradiation in mice. All formulations (15 mg/ear) were applied immediately after UVB irradiation. Ear thickness was measured at 24 h after ear irradiation and UVB irradiation plus treatments using Lanette® (**a**) and Pemulen® TR2 (**b**) semisolid formulations as a base. Each bar represents the mean + SEM ($n = 6$); $^{\#\#\#}p < 0.001$ shows a significant difference when compared to the naïve group; $^{*}p < 0.05$ and $^{***}p < 0.001$ show a significant difference when compared to the UVB (0.5 J/cm^2) (no treatment) group; $^{\&\&\&}p < 0.001$ shows a significant difference when compared to the their respective vehicle formulations (UVB + Lanette® or UVB + Pemulen® vehicle group). One-way ANOVA followed by post hoc Newman–Keuls test

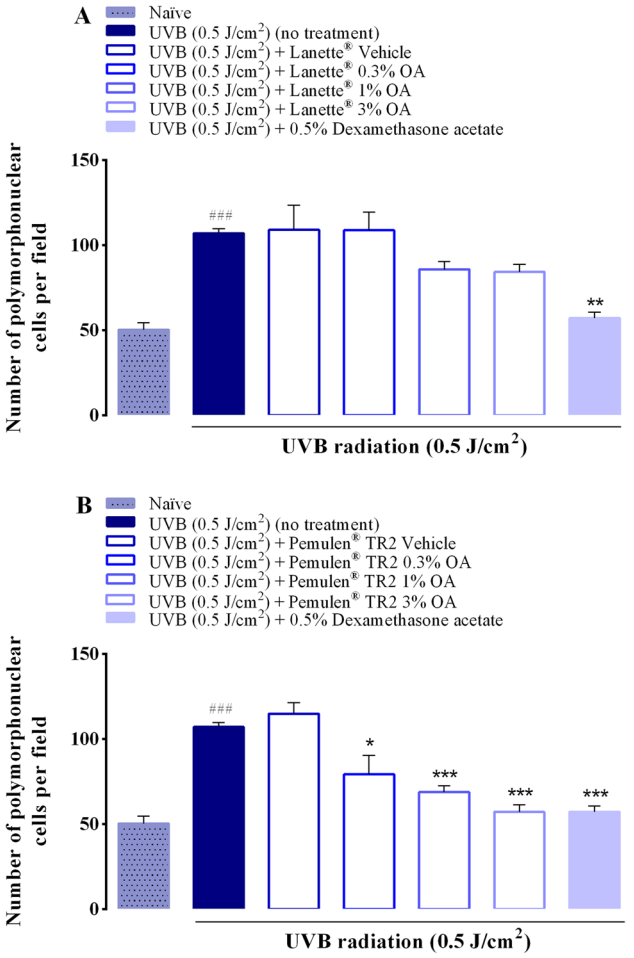


Fig. 2 Quantification of polymorphonuclear cells per field of the mice' ear tissue at 24 h after the UVB radiation or UVB radiation plus treatments using Lanette® (**a**) and Pemulen® TR2 (**b**) semisolid formulations as a base. Each bar represents the mean + SEM ($n = 6$). $^{\#\#\#}p < 0.001$ shows a significant difference when compared to the naïve group; $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ show a significant difference when compared to the UVB (0.5 J/cm^2) (no treatment) group. One-way ANOVA followed by post hoc Tukey's test

cell infiltration when compared to the UVB group although Lanette® 1% and 3% OA reduced the UVB-induced inflammatory cell infiltration in 19.78 ± 4.23% and 21.18 ± 4.08%, respectively. On the other hand, Pemulen®-based semisolids containing 0.3%, 1%, and 3% OA reduced this parameter with I_{max} of 25.93 ± 10.47%, 35.83 ± 3.65%, and 46.73 ± 4.07%, respectively. Similarly, the positive control dexamethasone acetate presented an I_{max} of 46.54 ± 3.12% (Figs. 2, 3).

Oleic acid repeated application reduces the UVB radiation-induced ear edema

The UVB radiation increased the ear thickness of the mice in 65 ± 3 µm, 73 ± 4 µm, and 67 ± 4 µm at 24 h, 48 h, and 72 h after UVB exposure, respectively. Lanette® 3% OA and Pemulen® TR2 3% OA decreased the ear edema with I_{max} of 56.78 ± 3.11% and 69.88 ± 2.31%, respectively, when compared to the UVB-irradiated group, at 24 h after

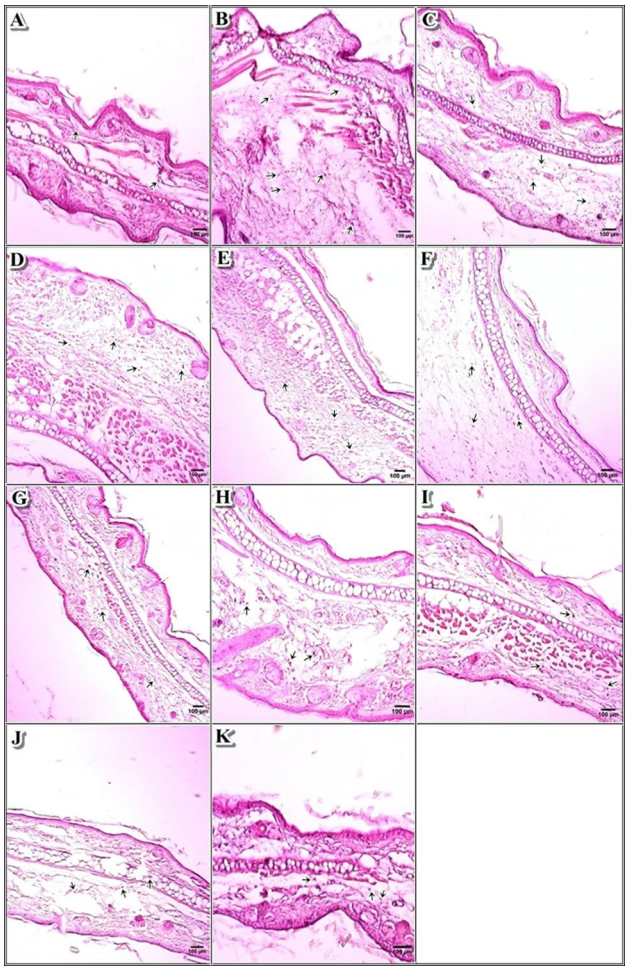


Fig. 3 Effect of the semisolid formulations topically applied to UVB-induced inflammatory cell infiltration. Histological changes (**a**–**k**; hematoxylin–eosin 10× objectives) of the ear tissue of mice at 24 h after UVB irradiation or UVB irradiation plus treatments. **a** Naïve; **b** UVB 0.5 J/cm^2 (no treatment); **c** UVB 0.5 J/cm^2 + Lanette® vehicle; **d** UVB 0.5 J/cm^2 + Lanette® 0.3% OA; **e** UVB 0.5 J/cm^2 + Lanette® 1% OA; **f** UVB 0.5 J/cm^2 + Lanette® 3% OA; **g** UVB 0.5 J/cm^2 + Pemulen® vehicle; **h** UVB 0.5 J/cm^2 + Pemulen® 0.3% OA; **i** UVB 0.5 J/cm^2 + Pemulen® 1% OA; **j** UVB 0.5 J/cm^2 + Pemulen® 3% OA; **k** UVB 0.5 J/cm^2 + 0.5% dexamethasone acetate. The arrows indicate the presence of inflammatory cells in the ear tissue. Scale bar of 100 µm

UVB exposure. This antiedematogenic effect was similar to that caused by dexamethasone acetate (75.11 ± 2.41%). Similarly, Lanette® 3% OA and Pemulen® 3% OA also were capable of reducing the ear edema effectively, with I_{max} of 31.37 ± 3.37% and 60.95 ± 5.70%, respectively, at 48 h after UVB exposure. The antiedematogenic effect of Pemulen® 3% OA was similar to that exerted by dexamethasone acetate (76.89 ± 2.42%).

At 72 h after UVB radiation, both formulations Lanette® and Pemulen® containing 3% OA reduced the ear thickness with I_{max} of 32.41 ± 5.27% and 29.89 ± 6.40%, respectively,

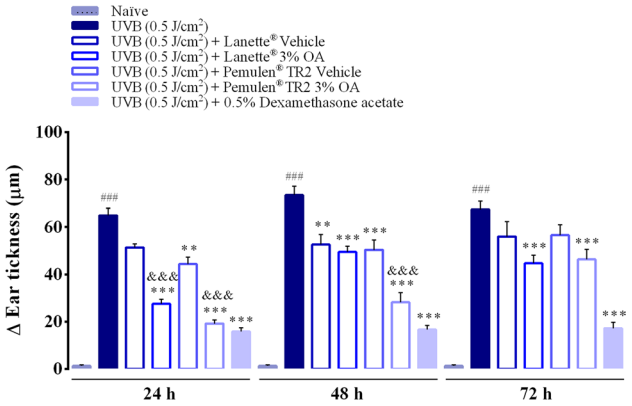


Fig. 4 Antiedematogenic effect of OA formulations on the ear edema induced by UVB irradiation in mice. All formulations (15 mg/ear) were applied immediately after UVB irradiation and reapplied at 24 h and 48 h after UVB radiation. Ear thickness was measured at 24 h, 48 h, and 72 h after ear irradiation and UVB irradiation plus treatments using the semisolid formulations. Each bar represents the mean + SEM ($n = 6$); $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{**}p < 0.01$ and $^{***}p < 0.001$ show a significant difference when compared to the UVB (0.5 J/cm^2) (no treatment) group; $^{\&\&\&}p < 0.001$ shows a significant difference when compared to their respective vehicle formulations (UVB + Lanette® or UVB + Pemulen® vehicle groups). One-way ANOVA followed by post hoc Newman–Keuls test

while dexamethasone acetate decreased the ear edema in 74.02 ± 3.97% (Fig. 4).

Oleic acid presents anti-inflammatory activity via glucocorticoid receptors

Pre-treatment with mifepristone did not change the UVB-induced ear edema. As expected, semisolids containing OA and dexamethasone acetate reduced the ear thickness with I_{max} of 87.45 ± 2.76 and 83.26 ± 4.13, respectively. However, mifepristone pre-treatment was able to prevent the antiedematogenic effect presented by both these formulations by 92.25 ± 6.03 and 87.36 ± 4.78, respectively, when compared to the group treated only with Pemulen® 3% OA and dexamethasone acetate (Fig. 5).

Oleic acid reduces the croton oil-induced ear edema

We also employed the croton oil as an irritant agent to induce skin inflammation in mice ears and assess the OA ability to act as an anti-inflammatory agent in another inflammation model. Croton oil increased the mouse ear thickness with a maximum effect (E_{max}) of 87 ± 6 µm when compared to the naïve group. Pemulen® TR2-based semisolids containing OA at 0.3% and 1% reduced the ear edema with I_{max} of 36.69 ± 5.94 and 49.72 ± 5.99, respectively. The inhibitory effect showed by Pemulen® TR2 3% OA (I_{max} of 75.28 ± 5.62%) was similar to that presented by the positive

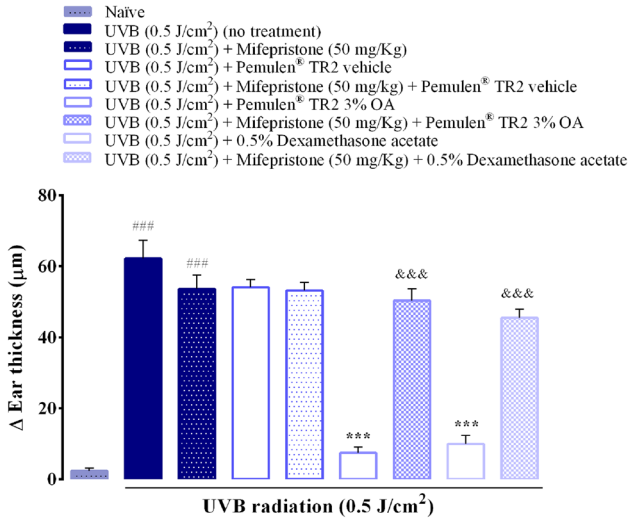


Fig. 5 Reversal of the antiedematogenic activity of OA and dexamethasone by mifepristone. Mifepristone (50 mg/kg, s.c.) was administered 15 min before ear irradiation with UVB, while semisolids containing OA and dexamethasone acetate were applied immediately after irradiation. Each bar represents the mean + SEM ($n = 6$); $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{***}p < 0.001$ shows a significant difference when compared to the UVB (0.5 J/cm^2) (no treatment) group; $^{\&\&\&}p < 0.001$ shows a significant difference when compared to the UVB + Pemulen® 3.0% OA or UVB + 0.5% dexamethasone acetate groups. One-way ANOVA followed by Tukey's test

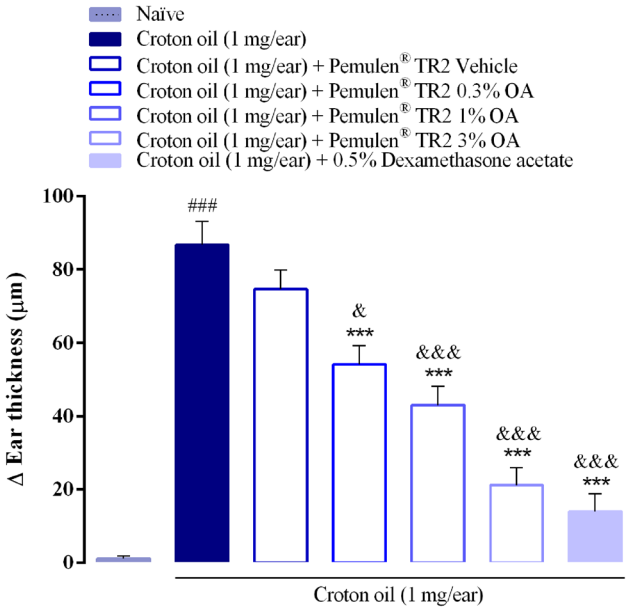


Fig. 6 Antiedematogenic effect of semisolid formulations on the ear edema induced by croton oil topical application in mice. All formulations (15 mg/ear) were applied immediately after mice received croton oil. Ear thickness was measured at 6 h after croton oil or croton oil plus treatments using Pemulen® TR2 semisolid formulations containing oleic acid or dexamethasone acetate. Each bar represents the mean + SEM ($n = 7$); $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{***}p < 0.001$ shows a significant difference when compared to the croton oil group. $^{\&}p < 0.05$ and $^{\&\&\&}p < 0.001$ show a significant difference when compared to the Pemulen® TR2 Vehicle group. One-way ANOVA followed by post hoc Tukey's test

control 0.5% dexamethasone acetate (I_{max} of 83.63 ± 2.12%) (Fig. 6).

Ex vivo experiments

Porcine skin permeation study

The OA distribution in skin layers and receptor medium is shown in Fig. 7. The result was expressed as the content of OA of the total amount of fatty acids identified in the samples. The results showed that a significant difference in OA content between skins treated with OA semisolid and non-treated was only observed at receptor medium. A 235.23 ± 6.56% (2.4-fold) increase was observed in the content of OA in the receptor medium collected from the permeation of skin treated with the semisolid containing OA when compared to the non-treated skin ($p < 0.001$).

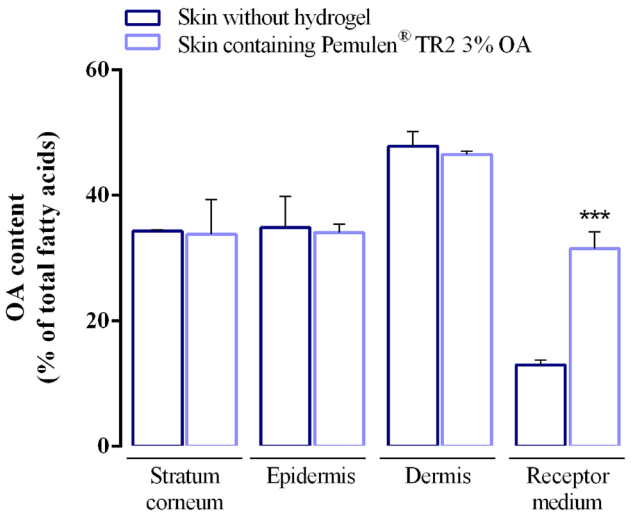


Fig. 7 OA content into skin layers from porcine skin after 8 h incubation or not with semisolid containing 3% OA. The results were expressed as the mean + SD of three independent experiments. $^{***}p < 0.001$ shows a significant difference when compared to the skin non-treated with OA-containing semisolid. One-way ANOVA followed by post hoc Newman–Keuls' test

Discussion

Worldwide, there is a constant interest in the development of new topical products, like gels and creams, intended for the treatment of dermatological disorders. This information is supported by a large number of published studies comprising this topic (Ourique et al. 2011; Santos et al. 2013).

This constant interest is due to the advantages conferred by the semisolid dosage forms, e.g., the ease to deliver a wide variety of hydrophilic and lipophilic drugs to the skin and mucosa (Nabi et al. 2016). We employed semisolid dosage forms to deliver the OA to the skin due to their ability to promote the retention of the active compound incorporated over the skin, prolonging its absorption, besides being easily administered, it is a non-invasive way to deliver drugs (Chang et al. 2013).

In the context of pharmaceutical semisolid development, it has been recognized that the choice for the vehicle to deliver the active compound to the skin impacts directly on its effects on cutaneous tissue. Thus, due to their fundamental importance to semisolid development, the raw materials should be carefully chosen (Nwoko et al. 2014; Otto et al. 2009). In this sense, we selected two types of formulation bases as vehicles to test the active compound in the skin: Lanette® and Pemulen® TR2. Consequently, two types of semisolid dosage forms were formed: emulsion and gel.

Pemulen® TR2 is a polymeric emulsifier, which is part of the copolymers from acrylic acid and methacrylate groups, which presents hydrophilic and lipophilic regions, with similar chemical structure and properties of Carbopol® (Ravenel 2010). This gel-former presents many advantages that justify its choice to develop the present study. Among them, it is relevant to point out the capability to form highly stable semisolids with very low concentrations (normally less than 1%), low irritancy and mucoadhesive properties that facilitate their adherence to the skin (Shahin et al. 2011; Szucks et al. 2008).

The first step on the development of semisolid formulations comprises the evaluation of their physicochemical, spreadability and flow characteristics. In this context, we measured the pH, and we performed the evaluations of spreadability, and rheological behavior of the developed semisolids intended to skin application.

As expected, the pH values obtained for the Lanette®-based formulations were around 6.0, according to similar studies (Mazzarino and Knorst 2007; Silva et al. 2013). Concerning the Pemulen® semisolids, the pH values were close to 7.0. It is important to reinforce that these pH values are close to the skin physiological pH range and in the range of the body internal environment pH, therefore, suitable for the intended administration route (Ali and Yosipovitch 2013).

The efficacy of topical therapy is conditioned to the spreading of the medicine on a uniform layer on the damaged skin to guarantee a standard dose of the active compound. Furthermore, a formulation developed to treat injuries cannot require too much force to spread once these damaged areas are often also painful and sensitive (Garg et al. 2002). Therefore, the determination of spreadability factor is an important parameter for the semisolid development intended to skin application once it is performed to verify if the semisolids would present ease of application (Garg et al. 2002). Our results showed that the spreadability factors for all semisolids were around 2.0, according to that obtained in other studies employing the use of gel-forming agents (Marchiori et al. 2017; Pegoraro et al. 2017; Rigo et al. 2015). Moreover, no statistical difference in spreadability factor values was observed between the bases of the formulations employed.

Besides the spreadability evaluation, rheological behavior is another fundamental assessment in the scope of semisolid characterization. This analysis showed that all semisolids developed presented non-Newtonian pseudoplastic flow, which is characteristic of solutions of gelling agents and semisolids (Aulton 2005; Rathod and Mehta 2015). Furthermore, the data were applied to three mathematical models to confirm flow behavior. From these models, it was possible to obtain the linear regression coefficient (r), which allowed concluding that the obtained data fitted better to the Ostwald model, frequently used to describe the non-Newtonian pseudoplastic flow. This flow behavior presented by the semisolids is desirable, as it means that they can easily flow and spread in the applied area. If the formulation spreads easily, not much force is required to its application, an important favorable characteristic in the development of formulations intended to treat injured areas. This result is clinically relevant once the pain is a symptom frequently associated with burned areas (Rigo et al. 2015).

Since the semisolids presented good flow behavior and spreadability, we investigated the anti-inflammatory potential of the OA in both semisolid types in a skin inflammation model UVB radiation-induced in mice. It has been well known that UVB is capable of inducing an inflammatory process in mice, which is characterized by erythema, edema, and inflammatory cells infiltration (Kripke 1994; Marchiori et al. 2017; Nan et al. 2018; Pegoraro et al. 2017).

An important marker of skin inflammation is edema, which is the result of increased vascular permeability (vasodilation) and consequent leakage of exudate into inflamed tissue (Fullerton and Gilroy 2016; Medzhitov 2008, 2010). All semisolids developed containing OA were capable of reducing the ear edema after its application. Besides, 3% OA exhibited a higher efficiency to that shown by the positive control, dexamethasone acetate, a drug clinically used to treat skin inflammatory disorders. This antiedematogenic effect agrees with that presented by Morais et al. (2017), which demonstrated the anti-inflammatory effect of many seed oils topically applied to an ear edema model induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA). In this study, the ear edema inhibition conferred by cashew nut and pequi oils was attributed to the presence of OA, the main constituent of these vegetable oils. Moreover, the OA and other monounsaturated fatty acids promote the wound healing

in inflammatory process, especially in skin lesions experimentally induced, like burns, diabetic wounds and pressure ulcers (Cardoso et al. 2011; De Caterina et al. 1994; Lima-Salgado et al. 2011; Oh et al. 2009; Rodrigues et al. 2012; Rowan et al. 2015).

Another signal that characterizes the skin inflammation is the inflammatory cells infiltration to the damaged tissue. These cells are attracted by the release of several chemoattractants on the inflammatory site (Ortega-Gómez et al. 2013; Sadik et al. 2011). Pemulen®-based semisolids containing OA, but not Lanette®-based semisolids, were capable of reducing the number of polymorphonuclear cells on the damaged tissue. Our results were similar to the results found by Favacho et al. (2010) that demonstrated in vivo anti-inflammatory activity (reduction of edema and inflammatory cells infiltration) of *Euterpe oleracea* Mart. oil in inflammation models in experimental animals. This anti-inflammatory activity was attributed to OA, the major component of the studied oil.

Once the reduction of inflammatory cells infiltration is crucial to prevent the occurrence of a chronic inflammatory process (Nestle et al. 2009), it is important to note that OA just reduced the inflammatory cell infiltration when it was delivered by Pemulen® TR2. This result reinforces the best performance of Pemulen® TR2 compared to Lanette® as a base to vehicle OA in the inflamed skin, and these data suggest that Pemulen® TR2 OA could avoid the development of a chronic inflammatory process, due to its in vivo efficacy in the acute inflammation.

This discrepancy observed between both vehicles employed could be attributed to the OA partition coefficient (log $P_{o/w} = 7.421$ at 25 °C). The partition coefficient of a compound indicates its lipophilic or hydrophilic character; the OA partition from the base to the skin is favored when semisolid vehicle presents hydrophilic characteristic (as in the case of Pemulen®), once this is a lipophilic substance. In other words, this means that in this case, OA exhibits more affinity to the skin lipid layer stratum corneum than with the gel network and this fact could enable a greater output of this compound from the semisolid to the skin, impacting in a better in vivo performance. Instead, OA tends to be more retained in the semisolid when incorporated into Lanette® vehicle, once this base presents a more lipophilic character and, therefore, smaller is its output to the skin layers (Jankowski et al. 2017; Leo et al. 1971; Zhu et al. 2016). Moreover, the use of hydrogels presents important properties for topical application of medicines: non-oily aspect, cooling effect, and the ability to be simply removed using water. The cooling effect is an important feature to their use in skin burns once the heat is one sign of inflammatory processes (Peppas et al. 2000).

Another factor that could have contributed to the better effect of OA into Pemulen® semisolids are the pH values of the formulations. Since oleic acid is an acidic compound and its *p*Ka is around 5.02, at pH values higher than this, there is the prevalence of the ionized forms, which could favor its skin absorption. Once the pH values from Pemulen®-based semisolids are higher than that for the Lanette® based, the prevalence of ionized forms is possible higher for oleic acid into Pemulen®-based semisolids. In this sense, the increase in oleic acid solubility promoted for this ionization could be higher when this compound is incorporated into Pemulen®, increasing its delivery from the semisolid to the skin and consequently improving its biological effect.

It is important to mention that several available products, like cosmetics and medicines, employ lipids as formulation excipients. Among these medicines, there are those intended for the treatment of cutaneous disorders. Even though these lipids are used as formulation excipients, many of them present biological activities, for example, the compounds present in vegetable oils, as the OA (Cosmetic Ingredient Review 2019). It is already known that skin care products containing high lipid content are advantageous to counter skin dryness and in the treatment of inflammatory conditions (Reuter et al 2008). In this sense, compounds as the OA could be beneficial to treat inflammatory processes.

Despite the availability of anti-inflammatory drugs and topical glucocorticoids intended to treat skin inflammatory disorders, the uncontrolled, abuse or the misuse of them is associated with the occurrence of several severe adverse effects, like the development of rosacea, skin atrophy, papules and pustules and the rebound effect (Roth 2012; Xiao et al. 2015). Systemic adverse effects of topical glucocorticoids include hyperglycemia, glaucoma, cataract, hypertension, among others (Hengge et al. 2006). In this sense, the use of products of natural origin as a treatment or adjuvant to treat skin inflammatory conditions could soften the adverse effects of the medicines available nowadays. According to toxicological guidelines covering the OA actions to its cosmetic use, no photosensitization effect was produced in human skin with the maximum OA concentration of 13% (Cosmetic Ingredient Review 1987, 2019). Based on this, at the concentration purposed by us, OA skin administration may not produce adverse effects.

In summary, OA effectively reduced the UVB-induced ear edema at 24 h after irradiation and maintained this effect at 48 h and 72 h. Moreover, this compound was also able to reduce the inflammatory cell infiltration to the injured tissue.

We have further carried out a croton oil-induced skin inflammation model to consistently demonstrate the OA anti-inflammatory effect. Croton oil, by its main constituent 12-*O*-tetradecanoylphorbol-13-acetate (TPA), has been recognized as a compound able to experimentally induce skin inflammation in rodents. This inflammatory process promoted by croton oil is well marked, constituted by the classical signs of inflammation, as erythema, edema and

polymorphonuclear leukocyte infiltration (Stanley et al 1991; Bald et al 2016; Piana et al 2016). Employing this model, we once again demonstrated the prospect of this natural compound in treating inflammatory skin disorders.

Based on our results, considering both skin inflammation models employed, Pemulen®-based containing 3% OA showed to be the most promising semisolid dosage form. We also hypothesized that OA anti-inflammatory activity might be due to the action on glucocorticoid receptors, once this compound presented a similar effect to that showed by dexamethasone acetate. To confirm this hypothesis, we demonstrated that the OA anti-inflammatory activity was prevented by the glucocorticoid receptor antagonist mifepristone. This result suggests that the activity of this compound depends on the glucocorticoid receptors and, therefore, that it exerts glucocorticoid-like effects. However, further studies are needed to elucidate other molecular mechanisms involved in the OA biological activity and understand the reasons why there are no reports concerning the OA adverse effects similar to that presented by glucocorticoids even when present at high concentration into semisolids.

Finally, we investigated the permeation of OA into skin layers, to understand its location and explain its biological effect. We observed that OA does not deposit in skin layers, but it reaches the receptor medium, which indicates a systemic absorption of this compound. Since OA reached the systemic absorption (observed by its presence at receptor medium), our semisolid dosage form can be considered a system to the transdermal delivery of OA.

Conclusion

We have demonstrated the development of two types of semisolid dosage forms and the assessment of their biological activity. The semisolids presented suitable spreadability and flow behavior. Besides, these semisolids containing OA presented great in vivo anti-inflammatory efficacy employing two distinct skin inflammation models. Among the bases evaluated, Pemulen® TR2 showed to be the most promising one as a vehicle to the active compound OA. Therefore, Pemulen® TR2 containing OA could represent an interesting therapeutic alternative to that commercially available nowadays to the treatment of cutaneous inflammatory disorders.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Affiliations

Nathália Schopf Pegoraro[1] · Camila Camponogara[1] · Mailine Gehrcke[2] · Laura Minussi Giuliani[2] · Dariane Trivisiol da Silva[3] · Luana Haselein Maurer[3] · Priscilla Dias[4] · Tatiana Emanuelli[3] · Letícia Cruz[2] · Sara Marchesan Oliveira[1]

✉ Sara Marchesan Oliveira
saramarchesan@hotmail.com; saramarchesan@ufsm.br

[1] Graduate Program in Biological Sciences: Biochemistry Toxicology, Center of Natural and Exact Sciences, Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Av. Roraima 1000, Camobi, Santa Maria, RS 97105-900, Brazil

[2] Graduate Program in Pharmaceutical Sciences, Center of Health Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil

[3] Graduate Program in Food Science and Technology, Center of Rural Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil

[4] Graduate Program in Genetics and Biochemistry, Biotechnology Institute, Federal University of Uberlândia, Uberlândia, MG, Brazil

6.2 ARTIGO 2

Ácido oleico exibe efeito anti-inflamatório expressivo em um modelo de dermatite de contato irritante induzida por óleo de cróton sem ocorrência de efeitos tóxicos em camundongos

Oleic acid exhibits an expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis without the occurrence of toxicological effects in mice

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Oleic acid exhibits an expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis without the occurrence of toxicological effects in mice

Natháli Schopf Pegoraro [a], Camila Camponogara [a], Letícia Cruz [b], Sara Marchesan Oliveira [a,*]

[a] Graduate Program in Biological Sciences: Biochemistry Toxicology, Center of Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil
[b] Graduate Program in Pharmaceutical Sciences, Center of Health Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil

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ABSTRACT

Ethnopharmacological relevance: Cutaneous inflammatory diseases, such as irritant contact dermatitis, are usually treated with topical corticosteroids, which cause systemic and local adverse effects limiting their use. Thus, the discovery of new therapeutic alternatives able to effectively treat skin inflammatory disorders, without causing adverse effects, is urgently needed.

Aim of the study: To investigate the topical anti-inflammatory effect of oleic acid (OA), a monounsaturated fatty acid, into Pemulen® TR2-based semisolid dosage forms, employing a croton oil-induced irritant contact dermatitis model in mice.

Materials and methods: Male *Swiss* mice were submitted to skin inflammation protocols by acute and repeated applications of croton oil. The anti-inflammatory activity of Pemulen® TR2 hydrogels containing OA was evaluated by assessing oedema, inflammatory cell infiltration, and pro-inflammatory cytokine IL-1β levels. The mechanisms of action of OA were evaluated using cytokine IL-1β application or pretreatment with the glucocorticoid antagonist mifepristone. Possible toxic effects of OA were also assessed.

Results: Pemulen® TR2 3% OA inhibited the acute ear oedema [maximal inhibition (I_{max}) = 76.41 ± 5.69%], similarly to dexamethasone (I_{max} = 84.94 ± 2.16%), and also inhibited ear oedema after repeated croton oil application with I_{max} = 85.75 ± 3.08%, similar to dexamethasone (I_{max} = 81.03 ± 4.66%) on the day 7 of the experiment. Croton oil increased myeloperoxidase activity, which was inhibited by Pemulen® TR2 3% OA (I_{max} = 71.37 ± 10.97%) and by 0.5% dexamethasone (I_{max} = 96.31 ± 3.73%). Pemulen® TR2 3% OA also prevented the increase in pro-inflammatory cytokine IL-1β levels induced by croton oil (I_{max} = 94.18 ± 12.03%), similar to 0.5% dexamethasone (I_{max} = 87.21 ± 10.58%). Besides, both Pemulen® TR2 3% OA and 0.5% dexamethasone inhibited IL-1β-induced ear oedema with an I_{max} of 80.58 ± 2.45% and 77.46 ± 1.92%, respectively. OA and dexamethasone anti-inflammatory effects were prevented by 100% and 91.43 ± 5.43%, respectively, after pretreatment with mifepristone. No adverse effects were related to Pemulen® TR2 3% OA administration.

Conclusions: OA demonstrated anti-inflammatory efficacy similar to dexamethasone, clinically used to treat skin inflammatory conditions, without presenting adverse effects.

Abbreviations: ALT, glutamic pyruvic transaminase; ANOVA, analysis of variance; AST, glutamic oxaloacetic transaminase; CONCEA, Brazilian Council of Animal Experimentation; E_{max}, maximal effect; HTAB, hexadecyltrimethylammonium bromide; ICD, irritant contact dermatitis; I_{max}, maximum inhibition; IL-1β, interleukin 1 beta; MPO, myeloperoxidase; NF-κB, nuclear factor-kappa B; OA, oleic acid; OD, optical density; Pemulen® 0.3% OA, Pemulen® TR2-based semisolid containing 0.3% oleic acid; Pemulen® 1% OA, Pemulen® TR2-based semisolid containing 1% oleic acid; Pemulen® 3% OA, Pemulen® TR2-based semisolid containing 3% oleic acid; s.c., subcutaneous injection; SEM, standard error of the mean; TMB, tetramethylbenzidine; TNF-α, tumour necrosis factor-alpha; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; ω-9, omega 9.

* Corresponding author. Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Av. Roraima 1000, Camobi, Zipcode 97105-900, Santa Maria, RS, Brazil.
E-mail address: saramarchesan@ufsm.br (S.M. Oliveira).

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1. Introduction

The skin tissue consists of the interface between the external and internal environments of the human body. It is composed of a complex structure with multiple cellular and non-cellular components that play the first line of body defence. Due to its role as the organism's interface, the skin is exposed to and affected by different challenges, including chemical, physical, and biological agents that are capable of damaging it. The damage provoked by these agents could evoke inflammatory skin diseases such as irritant contact dermatitis (ICD) (Serhan et al., 2008; Nestle et al., 2009; Eberting et al., 2014; Pasparakis et al., 2014).

ICD is a skin inflammatory response, associated with innate immune system activation in response to contact with an external stimulus that occurs without a prior sensitisation by the external agent (Eberting et al., 2014). This agent can be a caustic or irritating substance or a physical agent whose contact with the skin results in a direct cytotoxic effect, associated with skin barrier disruption, cellular alterations, and the release of pro-inflammatory substances (Eberting et al., 2014).

Nowadays, cutaneous inflammatory disorders as ICD are treated with topical corticosteroids (Frosch and John, 2010; Brasch et al., 2014). However, these drugs can cause severe adverse effects like skin atrophy, rosacea, and purpura development, increased blood glucose levels, and the rebound effect (Coondoo et al., 2014; Barnes et al., 2015), directly compromising their clinical use. These disadvantages reinforce the need for the discovery of new effective therapeutic alternatives to treat these inflammatory skin diseases with less potential to cause adverse effects.

Natural compounds, *e.g.* plant derivatives, have been employed by humans for thousands of years to treat a variety of diseases, including cutaneous disorders. Nowadays, they remain excellent options for medicine development due to the variety of biologically active compounds they contain and the possibility of treatment with fewer adverse effects (Yuan et al., 2016).

Several natural compounds also present effectiveness in treating skin inflammation (Dawid-Pać, 2013;Camponogara et al., 2019a,c). This pharmacological effect is possible due to the ability of these compounds to act through inflammation-associated pathways, reducing the inflammatory process (Calixto et al., 2004; Maione et al., 2015; Arulselvan et al., 2016).

Many essential and widely clinically used drugs were discovered in natural products and based on their popular use, such as morphine, codeine, and acetylsalicylic acid (Calixto, 2019). An example of a medicinal plant is *Cordia verbenacea* A. DC. (Boraginaceae family) (known as 'erva baleeira' or 'maria-milagrosa' or 'whaling herb'), popularly used in Brazil to treat tumours and inflammation. The anti-inflammatory effects of *C. verbenacea* were observed in pre-clinical and clinical studies, attracting the interest of a pharmaceutical company. A semisolid preparation containing *C. verbenacea* essential oil resulted in the development of a topical anti-inflammatory medicine called Acheflan® (Calixto, 2005; Balbani et al., 2009).

A natural compound that attracted our interest was a compound of the omega-9 (ω-9) family. Oleic acid (OA) is a fatty acid naturally found in vegetable oils and foods, such as cod and oilseeds (Roncero et al., 2016; Viola and Viola, 2009). OA also occurs naturally in the human body, as it is part of the cell membrane and participates in hormone synthesis (Tvrzicka et al., 2011).

In Brazil, more specifically, in the rich plant diversity Amazonian region, natives employ several vegetable oils with therapeutic purposes (Burlando and Cornara, 2017). It is important to highlight that literature data report the ethnopharmacological use of vegetable oils containing OA as one of their major components to treat skin diseases as well as for skincare (Burlando and Cornara, 2017). Among these oils, we highlight babassu oil (from *Orbignya phalerata* Mart; Arecaceae family) (Souza et al., 2011), andiroba oil (from *Carapa guianensis* Aubl.; Meliaceae family) (Pesso, 2011), olive oil (from *Olea europaea* L.; Oleaceae family) (Donato-Trancoso et al., 2016), and grape seed oil (from *Vitis vinifera* L.; Vitaceae family) (Shivananda Nayak et al., 2011).

Recently, our research group showed the effective anti-inflammatory action of OA to treat UVB radiation-induced sunburn (Pegoraro et al., 2019). However, neither study demonstrated its effectiveness as a topical anti-inflammatory agent against ICD. In skin inflammatory disorders, such as ICD, oedema is considered the first sign and a classical marker of skin inflammation, resulting in increased vascular permeability and the proliferation of epidermal keratinocytes (Medzhitov, 2008; Xu et al., 2016). Another event that occurs during skin inflammation and that is also a result of increased vascular permeability is leukocyte infiltration to damaged sites (Stanley et al., 1991; Ortega-Gómez et al., 2013; Xu et al., 2016). These processes are the result of the release of inflammatory mediators at the inflamed site after contact with a harmful stimulus, including proinflammatory cytokines [like interleukin 1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α)], and chemokines (Turner et al., 2014). These mediators are responsible for attracting inflammatory cells to inflamed sites, amplifying and sustaining inflammation (Kolaczkowska and Kubes, 2013).

In this sense, we aimed now to evaluate the anti-inflammatory efficacy of OA by employing an ICD model induced by croton oil. Croton oil, by its main constituent 12-*O*-tetradecanoylphorbol-13-acetate (TPA), has been recognised as a compound able to experimentally induce ICD in rodents, promoting erythema, oedema, and polymorphonuclear leukocyte infiltration (Stanley et al., 1991; Bald et al., 2016; Piana et al., 2016; Camponogara et al., 2019a).

We employed this irritant agent to induce an inflammatory process in the mouse ear, and we evaluated inflammatory markers to assess the efficacy of the natural compound OA in treating cutaneous inflammatory disorders, such as ICD. Moreover, we investigated possible mechanisms of action and the adverse effects of the OA-containing semisolid dosage form.

2. Material and methods

2.1. Materials

Pemulen® TR2 was donated by Noveon (Cleveland, USA). Oleic acid (OA) (about 78% purity) was obtained from LabSynth (Diadema, Brazil). Croton oil, hexadecyltrimethylammonium bromide (HTAB), tetramethylbenzidine (TMB), and mifepristone were purchased from Sigma Aldrich (São Paulo, Brazil). Dexamethasone acetate was purchased from Nova Derme (Santa Maria, Brazil). Ketamine (Dopalen®) and xylazine (Anasedan®) were purchased from Ceva (Paulínia, Brazil). Formaldehyde, ethanol, sodium citrate, acetone, and acetic acid were purchased from Vetec (Rio de Janeiro, Brazil). Hematoxylin-eosin and paraffin were obtained from Merck (Darmstadt, Germany). Enzyme-linked immunoassay for IL-1β measurement was purchased from Peprotech (São Paulo, Brazil). Laboratory kits for biochemical tests were obtained from Labtest Diagnóstica (Lagoa Santa, Brazil). All other reagents and solvents were of analytical grade and used as received.

3. Methods

3.1. Preparation of semisolid formulations

Semisolid dosage forms containing OA or dexamethasone acetate, as well as the vehicle, were prepared as previously described by Pegoraro et al. (2019).

3.2. Animals

Male *Swiss* mice (25–30 g; 4–5 weeks of age) were produced and provided by the Federal University of Santa Maria and used in all experiments. Animals were kept in suitable cages, under controlled temperature (22 ± 2 °C) and a 12 h light-dark cycle, and fed with standard laboratory food and water *ad libitum*. The animals were acclimatised to the experimental room for at least 1 h before performing the

experiments. All experiments were carried out between 8:00 a.m. and 5:00 p.m., and they were performed following national legislation (Guidelines of Brazilian Council of Animal Experimentation – CONCEA) and followed the Animal Research: Reporting *In Vivo* Experiments ARRIVE guidelines (McGrath and Lilley, 2015) and the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals – PHS Policy). All conducted procedures were approved by the Institutional Committee for Animal Care and Use of the Federal University of Santa Maria (protocol numbers 7412190319/2019 and 5582261018/2018). The number of animals and the intensity of stimuli were the minimum necessary to demonstrate the consistent effects of treatments.

3.2.1. Acute croton oil application-induced irritant contact dermatitis

Acute ear oedema was induced by a croton oil single topical application (1 mg/ear dissolved in acetone; 20 μL/ear) given in the right mouse ear. After croton oil application, mice's ears were topically treated with the semisolid developed formulations or dexamethasone (0.5%; employed as positive control). Ear thickness was measured before and 6 h after the croton oil or croton oil plus treatment application. Next, mice were euthanised to collect ear biopsies for further analysis (Brum et al., 2016; Piana et al., 2016; Rigon et al., 2019).

3.2.2. Repeated croton oil application-induced irritant contact dermatitis

Skin inflammation by croton oil multiple topical applications (0.4 mg/ear) in the mouse right ear, on days 1, 3, and 5 of the experimental protocol was also induced. Topical treatments with semisolid dosage forms were applied to the same ear twice a day from the day 5 until the day 9 of the experimental protocol. The ear thickness was measured once a day, during the experimental period. On the last day of the experiment (day 9), the animals were euthanised, and ear biopsies were collected for further analysis (Horinouchi et al., 2013; Camponogara et al., 2019a,b).

3.2.3. Formulation administration and experimental design

Mice were divided in eight groups containing seven animals each and classified as it follows: naïve; croton oil (1 mg/ear); croton oil + Pemulen® TR2 vehicle; croton oil + Pemulen® TR2 0.3% OA; croton oil + Pemulen® TR2 1% OA; croton oil + Pemulen® TR2 3% OA; croton oil + 0.5% dexamethasone acetate (positive control). Topical treatments (15 mg/ear) were applied in the mouse ear after croton oil application, according to the experimental groups described above.

3.2.4. Ear oedema measurement

Mouse ear oedema was evaluated through the measurement of the ear thickness, before (basal measure) and after the croton oil application. An increase in ear thickness after croton oil application when compared to the basal value was considered as indicative of ear oedema. The ear thickness was evaluated using a digital micrometer (Digimess, São Paulo, Brazil) in animals previously anaesthetised, as described previously (Silva et al., 2011; Pegoraro et al., 2017, 2019). Ear thickness was expressed in μm, as the difference between basal thickness and ear thickness at every time point. A single investigator performed all the measurements to minimize the variation.

3.2.5. Assessment of inflammatory cells infiltration

3.2.5.1. Myeloperoxidase (MPO) activity measurement. The inflammatory cells infiltration to the inflamed tissue was evaluated from MPO activity determination since its measure is directly related to the number of neutrophils in the tissue. Six hours after the irritant agent and treatment application, ear samples were collected, homogenised in acetate buffer (80 mM, pH 5.4) containing 0.5% HTAB and centrifuged at 16.000×*g* at 4 °C for 30 min, as previously described (Oliveira et al., 2014). Supernatants were incubated with acetate buffer and TMB solution (18.4 nM) at 37 °C for 10 min. Samples were spectrophotometrically analysed at 630 nm and the results were expressed as optical density (OD)/mL of the sample.

3.2.5.2. Histopathological analyses. Complementary to the MPO activity measurement, the leukocytes infiltration to the inflamed tissue was assessed by histopathological analyses. Separate groups of mice were used to evaluate histopathological changes into ear tissue at 6 h after receiving the croton oil or croton oil plus topical treatment application. Six hours after ear oedema assessment, mice were euthanised, and the right ear was collected and fixed in Alfac solution (16:2:1 mixture of ethanol 80%, formaldehyde 40%, and acetic acid). The samples were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin. A representative area was selected and the quantitative analysis of the number of leukocytes was performed using 20x objectives (Piana et al., 2016) and qualitative histopathological changes (hydropic swelling, exocytosis, spongiosis, and spongiotic vesicles) were assessed from 40x objectives (Willis, 2006; Park et al., 2011). These analyses were performed blindly to minimize the source of bias. The leukocyte quantification was performed by counting the cells per field using the Image J software, and 3 fields from 6 distinct histological slides of each group were analysed (Camponogara et al., 2019a,b).

3.2.6. Interleukin (IL)-1β pro-inflammatory cytokine levels

Six hours after the croton oil application, animals were euthanised, and the right ear was collected and prepared according to Walker et al. (2013). After centrifugation, the supernatant obtained was used to determine the IL-1β levels using an enzyme-linked immunoassay kit (Peprotech®, Brazil). The results were expressed as ng cytokine/mL of sample.

3.2.7. Skin inflammation model induced by IL-1β application

IL-1β (10 ng/ear; 20 μL acetone) was topically applied in the mice's right ear to cause acute ear oedema. The topical treatments were administrated immediately after the IL-1β application. The ear oedema was measured as described above, before and at 1 h after ear oedema induction and topical treatment application (Camponogara et al., 2019a, b).

3.2.8. Oleic acid anti-inflammatory activity via glucocorticoid receptors

We also verified if the OA anti-inflammatory activity is dependent on the glucocorticoid receptors. Animals were pre-treated with a glucocorticoid receptor antagonist, mifepristone (50 mg/kg; s. c.; dissolved in saline containing 10% ethanol) 15 min before the croton oil administration and topical treatments. Ear thickness was measured before and at 6 h after receiving croton oil. The ear oedema was expressed in μm, as described above (Mendes et al., 2016; Camponogara et al., 2019b; Pegoraro et al., 2019).

3.2.9. Biochemical markers of toxicity

Alanine transaminase (ALT) and aspartate transaminase (AST) activities, glucose, urea, and creatinine levels were employed as indicators of hepatic, pancreatic, and renal alterations, respectively (Badalov et al., 2007; Ozer et al., 2008; Van Meer et al., 2014; McGill, 2016; Chu et al., 2016; Sharma et al., 2020). On the day 9 after the repeated application of croton oil or croton oil plus topical treatments, the animals were euthanised, and blood samples were collected by the cardiac punch. Blood samples were centrifuged at 3.000 rpm for 10 min to obtain the serum. AST and ALT activities and the glucose, urea, and creatinine serum levels were assessed by spectrophotometry using Labtest® kits according to the manufacturer's specifications (Labtest Diagnostica, Brazil) (Camponogara et al., 2019a).

3.3. Statistical analysis

The results are presented as the mean + standard error of the mean (SEM) and are reported as geometric means plus its respective 95% confidence limits. The maximum inhibitory effect (I_{max}) was calculated based on the response of the control groups, considered as 100% of the effect. Statistical significance between groups was assessed by one-way or two-way (repeated measures) analysis of variance (ANOVA) followed by Tukey's and Dunnett's post hoc tests. P-values less than 0.05 ($p < 0.05$) were considered as indicative of significance. All statistical tests were carried out using GraphPad Prism 6.00 Software (San Diego, USA).

4. Results

4.1. Oleic acid reduces the croton oil-induced acute ear oedema and inflammatory cells infiltration

Croton oil increased the mice's ear thickness with a maximum effect (E_{max}) of 87 ± 6 μm when compared to the naïve group. Pemulen® TR2-based semisolids containing OA at 0.3% and 1% reduced acute ear oedema with an I_{max} of 36.59 ± 5.81% and 50.64 ± 5.83%, respectively. Dexamethasone acetate (0.5%), inhibited the acute ear oedema with an I_{max} of 84.94 ± 2.16%. The inhibitory effect showed by Pemulen® TR2 3% OA (I_{max} = 76.41 ± 5.69%) was similar to that presented by the positive control (Fig. 1).

Additionally, we verified if the topical treatments can reduce the inflammatory cells infiltration to the injured tissue through the MPO enzyme activity measurement. The croton oil increased the neutrophils infiltration to the ear tissue evaluated at 6 h after its administration when compared to the naïve group. Topical treatments with Pemulen® TR2-based semisolids containing OA at 0.3%, 1%, and 3% reduced the MPO activity with an I_{max} of 42.89 ± 12.33%, 78.57 ± 4.50%, and 71.37 ± 10.97%, respectively, while 0.5% dexamethasone acetate reduced this parameter by 96.30 ± 3.73% (Fig. 2). No statistical difference was verified when comparing topical treatment with semisolids containing the three oleic acid concentrations with dexamethasone acetate.

We also demonstrated the reduction of leukocytes infiltration into the damaged tissue employing histological counting evaluated at 6 h after the croton oil administration (Fig. 3). We confirmed that the topical treatments with oleic acid-containing semisolid dosage forms reduced the leukocytes infiltration to the inflammatory site. Croton oil increased the number of leukocytes (70 ± 6 cells per field) when compared to the naïve group (34 ± 6 cells per field). Pemulen® TR2 3% OA inhibited this inflammatory signal with higher I_{max} (100%) than 0.5% dexamethasone acetate (I_{max} = 95.82 ± 9.37%); however, there was no statistical significance between both experimental groups. Also, no statistical difference was observed when comparing the lower oleic acid concentrations (Pemulen® TR2 0.3% OA and Pemulen® TR2 1% OA) with the dexamethasone acetate group.

The histopathological features were also assessed by histological analysis in mouse ear at 6 h after croton oil application or croton oil plus treatments. Qualitative tissue changes croton oil-induced are presented in Fig. 4. Hydropic swelling, exocytosis, spongiosis, and spongiotic vesicles were observed in mouse ear tissue after croton oil application.

4.2. Oleic acid reduces ear oedema induced by repeated application of croton oil

Besides the acute ear oedema model induced by croton oil, we evaluated the anti-oedematogenic effect of OA in reducing the oedema induced by multiple croton oil applications. Multiple topical

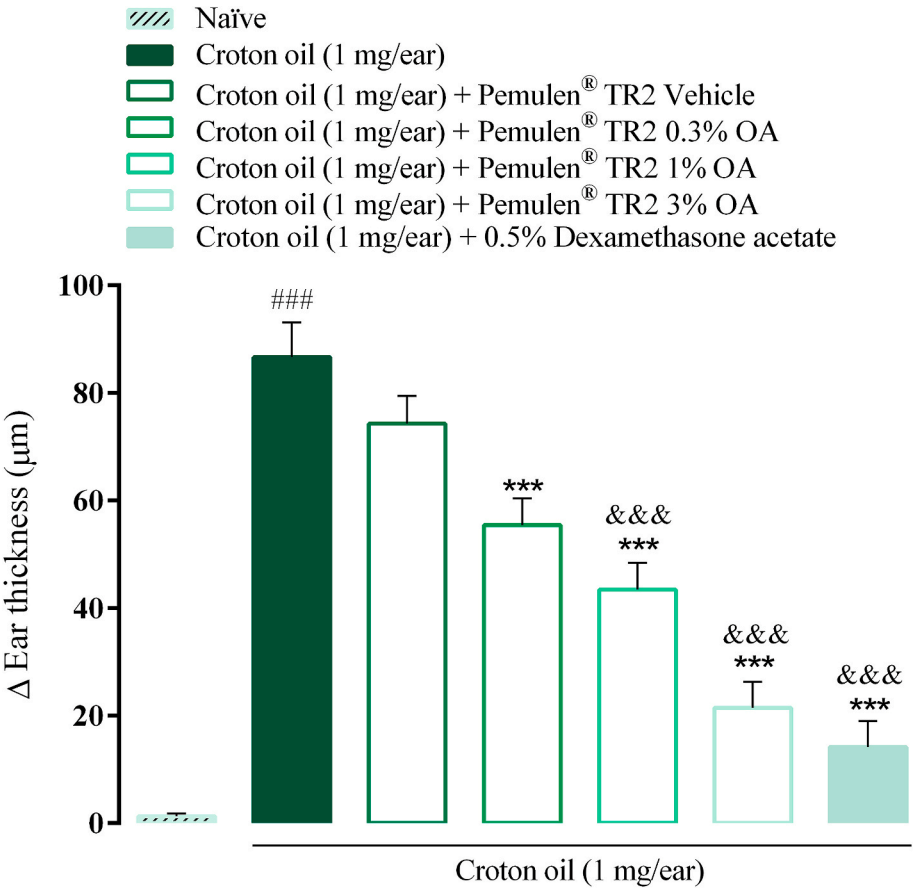

Fig. 1. Anti-oedematogenic effect of semisolid dosage forms containing oleic acid (0.3–3%) and dexamethasone acetate (0.5%) topically applied (15 mg/ear) on the acute croton oil application-induced irritant contact dermatitis in mice. All formulations were topically applied (15 mg/ear) immediately after mouse received croton oil. Ear thickness was measured at 6 h after croton oil or croton oil plus topical treatment application. Each bar represents the mean + SEM ($n = 7$); $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{***}p < 0.001$ shows a significant difference when compared to the croton oil group; $^{\&\&\&}p < 0.001$ shows a significant difference when compared to the Pemulen® TR2 vehicle group. One-way ANOVA followed by post hoc Tukey's test.

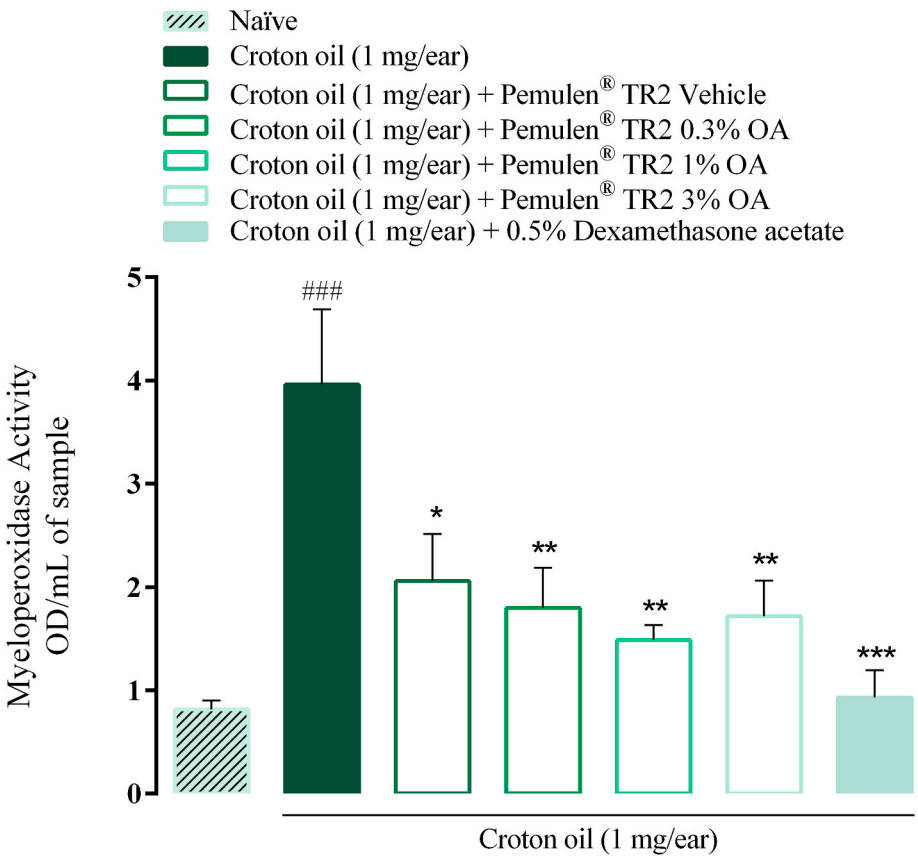

Fig. 2. Effect of semisolid dosage forms containing oleic acid (0.3–3%) and dexamethasone acetate (0.5%) topically applied (15 mg/ear) on the MPO enzyme activity after acute croton oil application-induced irritant contact dermatitis in mice. MPO activity was measured at 6 h after croton oil and the croton oil plus treatment application. Each bar represents the mean + SEM ($n = 7$). $^{###}p < 0.001$ indicates a significant difference when compared to the naïve group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ denotes significant difference when compared with the croton oil group. One-way ANOVA followed by post hoc Tukey's test.

applications of croton oil increased the ear thickness with E_{max} of 227 ± 24 μm on the day 6 of the experiment when compared to the naïve group. Pemulen® TR2 3% OA inhibited the croton oil-induced ear oedema from the day 6 until day 9 (last day) of the experiment, with an I_{max} of 85.75 ± 3.08% on the day 7. Similarly, 0.5% dexamethasone acetate inhibited the ear oedema from the day 6 up to the day 9 of the experiment with an I_{max} of 81.03 ± 4.66% on the day 7 (Fig. 5).

4.3. Oleic acid effectively reduces the croton oil-induced increase on IL-1β cytokine levels and the ear oedema IL-1β-induced

The croton oil (1 mg/ear) increased the cytokine IL-1β levels in mouse inflamed ear when compared to the naïve group. Effectively, Pemulen® TR2 3% OA reduced the IL-1β levels with an I_{max} of 94.18 ± 12.03%, which was similar to that showed by 0.5% dexamethasone acetate (I_{max} = 87.21 ± 10.58%) (Fig. 6).

Since IL-1β tissue levels was reduced by OA topical treatment, this inflammatory cytokine was employed to induce an acute inflammatory process in mouse skin. Topical IL-1β application promoted a skin inflammation evidenced by local oedema. IL-1β increased mice's ear thickness with an E_{max} of 73 ± 4 μm at 1 h after its application. Pemulen® TR2 3% OA reduced the IL-1β-induced ear oedema with an I_{max} of 80.58 ± 2.45%, which was similar to that presented by the positive control 0.5% dexamethasone acetate (I_{max} = 77.46 ± 1.92%) (Fig. 7).

4.4. Oleic acid anti-inflammatory effect is glucocorticoid receptor-dependent

Croton oil caused ear oedema, which did not alter by the pretreatment with the glucocorticoid antagonist mifepristone. Pemulen® TR2 3% OA and 0.5% dexamethasone acetate reduced the croton oil-induced ear oedema with an I_{max} of 84.72 ± 4.93% and 83.51 ± 5.69%, respectively. Mifepristone prevented both Pemulen® TR2 3% OA and 0.5% dexamethasone anti-oedematogenic effect by 100% and 91.43 ± 5.43%, respectively (Fig. 8).

4.5. Oleic acid into semisolid dosage forms does not cause adverse effects in vivo

We also investigated if OA leads to the occurrence of some adverse effects after nine days of its repeated application. Initially, we observed that none of the treatments caused mice body weight loss (data not shown) neither behavioural alterations as immobility nor in the locomotion pattern.

Neither Pemulen® TR2 3% OA nor 0.5% dexamethasone altered AST and ALT enzymes activities and urea and creatinine levels, used as toxicity indicators. On the other hand, Pemulen® TR2 vehicle and 0.5% dexamethasone acetate increased the blood glucose levels when compared to the naïve group. Importantly, increased blood glucose levels were not observed in animals topically treated with Pemulen® TR2 3% AO (Table 1).

5. Discussion

The human skin provides defence against the environment due to its anatomical location at the interface between the human body and the outside environment (Pasparakis et al., 2014). The human skin has a well-developed immune system that acts with coordinated mechanisms to respond to harmful stimuli and restore skin homeostasis after an injury (Nestle et al., 2009; Kabashima et al., 2019).

Skin exposure to irritant agents leads to an inflammatory state, which is a body protective reaction aiming to eliminate the inciting stimulus, resulting in tissue repair/healing (Fullerton and Gilroy, 2016). This inflammatory state is characterised by the development of inflammatory signs like erythema, oedema, heat, and pain; another sign that can occur is the loss of function of the affected tissue/limb

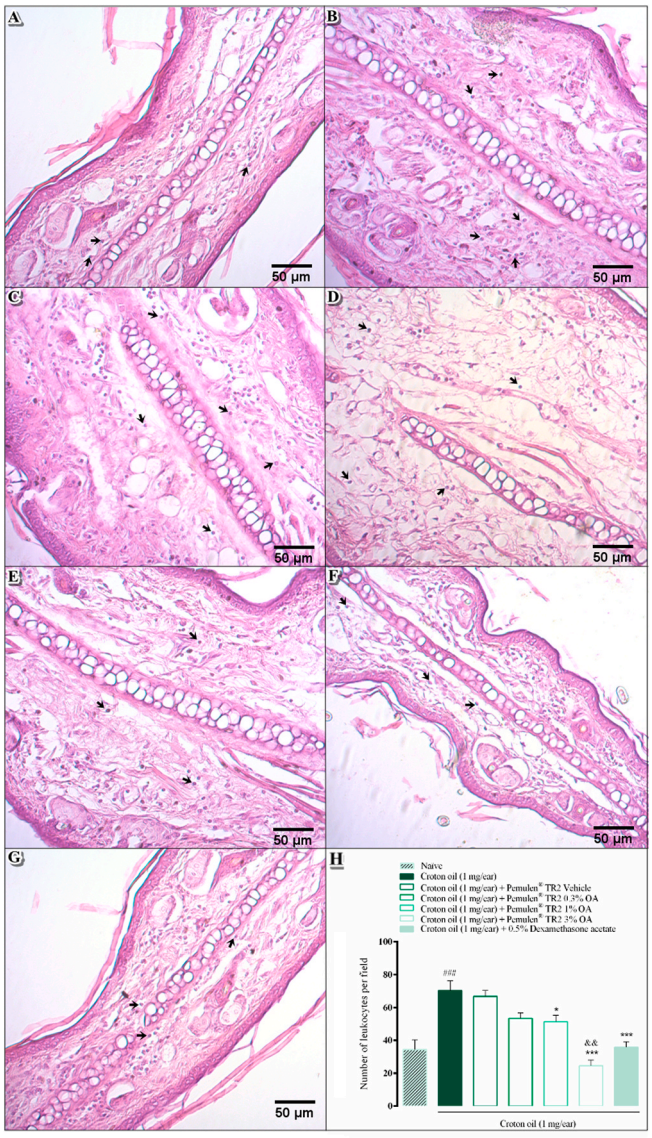

Fig. 3. Effect of the semisolid dosage forms containing oleic acid (0.3–3%) and dexamethasone acetate (0.5%) (15 mg/ear) on acute croton oil application-induced irritant contact dermatitis in mice. Histological changes (A-H; hematoxylin-eosin 10x objectives) of the ear tissue of mice at 6 h after croton oil or croton oil plus treatments. A: naïve; B: croton oil (1 mg/ear) (no treatment); C: croton oil (1 mg/ear) + Pemulen® TR2 vehicle; D: croton oil (1 mg/ear) + Pemulen® TR2 0.3% OA; E: croton oil (1 mg/ear) + Pemulen® TR2 1% OA; F: croton oil (1 mg/ear) + Pemulen® TR2 3% OA; G: croton oil (1 mg/ear) + 0.5% Dexamethasone acetate; H: number of leukocytes per field. The arrows indicate the presence of leukocytes in the ear tissue. Scale bar of 50 μm. Each bar represents the mean + SEM ($n = 7$). $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{*}p < 0.05$ and $^{***}p < 0.001$ show a significant difference when compared to the croton oil group; $^{\&\&}p < 0.01$ indicates significant difference when compared to the Pemulen® TR2 1% OA group. One-way ANOVA followed by post hoc Tukey's test.

(Fullerton and Gilroy, 2016). These inflammatory signals result in the release of several soluble mediators by skin resident cells, *e.g.* cytokines, chemokines, vasoactive amines, and complement system proteins, among others. These mediators evoke alterations to the local vasculature and lead to an increase in blood flow, fluid leakage, and circulating cell infiltration into adjacent tissue, amplifying the inflammatory process (Fullerton and Gilroy, 2016; Schwager and Detmar, 2019).

The therapies available to treat skin inflammatory disorders such as ICD are limited and promote several local and systemic adverse effects, compromising their clinical use. One example is the topical glucocorticoid dexamethasone, an effective drug to treat skin inflammation whose therapeutic use is associated with increased blood pressure and glucose levels, delayed wound healing, decreased bone density, and water retention, among others (Poetker and Reh, 2010). In this sense, the search for effective therapeutic alternatives with fewer adverse effects to treat skin inflammation is of fundamental relevance (Camponogara et al., 2019a,c; Pegoraro et al., 2019).

Our research group recently developed Pemulen® TR2-based hydrogels containing oleic acid with efficacy in treating sunburn-induced skin inflammation (Pegoraro et al., 2019). Here, we reinforced this anti-inflammatory effect of oleic acid employing a croton oil-induced ICD model. Croton oil is an irritant agent that has been employed to experimentally induce skin inflammation in rodents. This biological effect is due to its main constituent, TPA, which causes erythema, oedema, and inflammatory cell infiltration, characterising an inflammatory process (Stanley et al., 1991; Bald et al., 2016; Piana et al., 2016).

Oleic acid presented topical anti-inflammatory activity via glucocorticoid receptors, similar to dexamethasone acetate, on croton oil-induced ICD without increasing blood glucose levels, an important adverse effect related to glucocorticoids like dexamethasone (Badalov et al., 2007; Sharma et al., 2020). This discrepancy between OA and dexamethasone is very important especially in the treatment of inflammatory processes in diabetic patients who present increased glucose levels (Tamez-Pérez et al., 2015). Oleic acid could be employed not only to treat sunburn-induced inflammation but also for the treatment of ICD.

Regarding the toxicity markers, both urea and creatinine are renal toxicity markers (Van Meer et al., 2014). Creatinine is an endogenous cation formed in the liver and muscle by a multistep process and eliminated via the kidney by combining glomerular filtration and active transport; it is the most widely used marker to assess renal injury (Van Meer et al., 2014). Elevated serum creatinine levels are associated with renal injury (Chu et al., 2016). However, there is no evidence in the literature that reduced creatinine levels indicate any toxicity parameter. We verified that the creatinine levels in Pemulen® TR2 3% OA-treated animal group was significantly lower than in the naïve group ($p < 0.05$). Our results are in accordance with that obtained in previous studies by Camponogara and co-workers (2019a,b) that also obtained lower creatinine levels in the vehicle group than in the naïve group.

AST and ALT enzymes are liver toxicity markers, and elevated serum AST and ALT levels are associated with liver injury (Ozer et al., 2008; McGill, 2016). Here, we demonstrated that AST and ALT levels did not differ between the naïve and Pemulen® TR2 3% OA groups. Like creatinine levels, lower values of these biomarkers are not considered indicatives of toxicity. Our results are similar to those found by Camponogara and co-workers (2019), which was observed lower ALT serum activity in dexamethasone-treated topically animals (29 ± 9 U/L) than in naïve animals (36 ± 3 U/L). Considering the above, the topical treatment employing the semisolid dosage forms containing OA can be considered safe according to these preliminary toxicological tests. No indication of hepatic or renal toxicity was observed after topical treatment with OA at the employed doses, enabling its repeated use in the clinic.

Our study showed that OA inhibited ear oedema, the first inflammatory sign, after acute and repeated applications of croton oil. These results ensure that this proposed treatment could be employed to treat short and long-term inflammatory processes. Our results are in agreement with literature data, which demonstrate that the anti-oedematogenic effect of vegetable oils may be associated with OA. Lescano et al. (2015) showed that the anti-inflammatory effect of bocaiuva oil [extracted from *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart.; Arecaceae family; known as 'coco-de-espinho'], employing a model of carrageenan-induced paw oedema in *Wistar* rats, may be related to OA, the main constituent of this oil. Further, açaí oil (from *Euterpe oleracea* Mart.; Arecaceae family) presents anti-inflammatory activity in croton

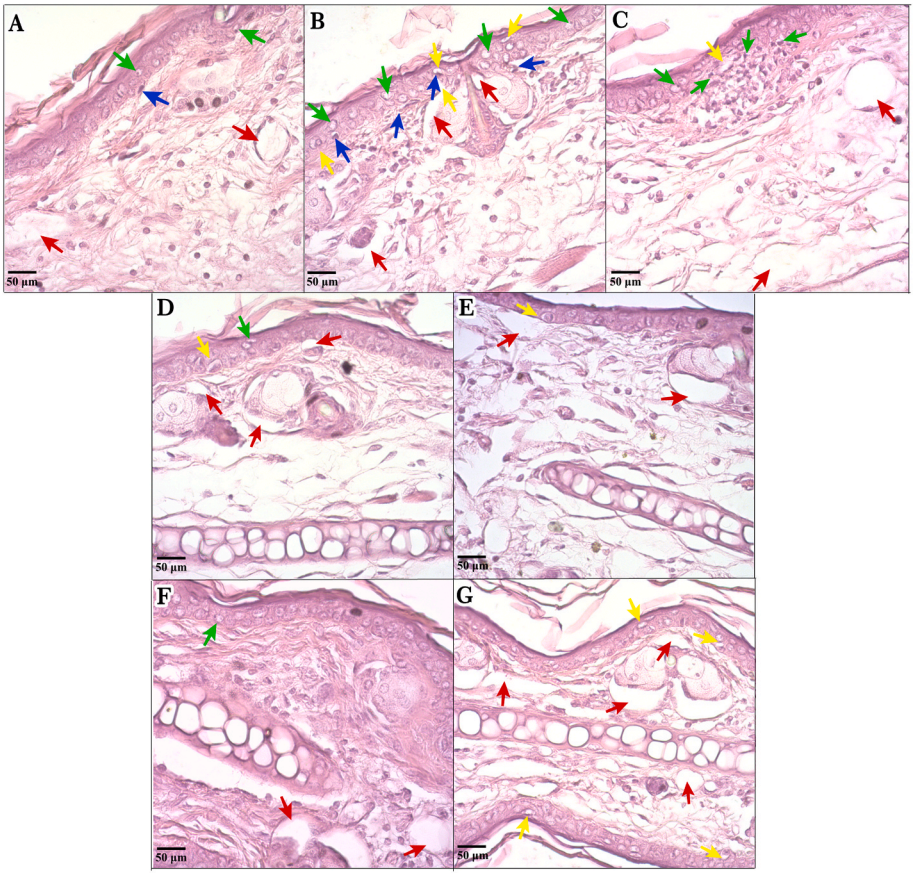

Fig. 4. Qualitative histopathological analyses (A-G; hematoxylin-eosin; 40x objectives) into mice's ears tissue observed in the acute croton oil application-induced irritant contact dermatitis model. Arrows indicate the histopathological changes as follow: green arrows – hydropic swelling (epidermis); blue arrows – exocytosis (epidermis and dermal-epidermal junction); yellow arrows – spongiosis (epidermis); red arrows – spongiotic vesicles (dermis). A: naïve; B: croton oil (1 mg/ear) (no treatment); C: croton oil (1 mg/ear) + Pemulen® TR2 vehicle; D: croton oil (1 mg/ear) + Pemulen® TR2 0.3% OA; E: croton oil (1 mg/ear) + Pemulen® TR2 1% OA; F: croton oil (1 mg/ear) + Pemulen® TR2 3% OA; G: croton oil (1 mg/ear) + 0.5% Dexamethasone acetate. Scale bar, 50 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

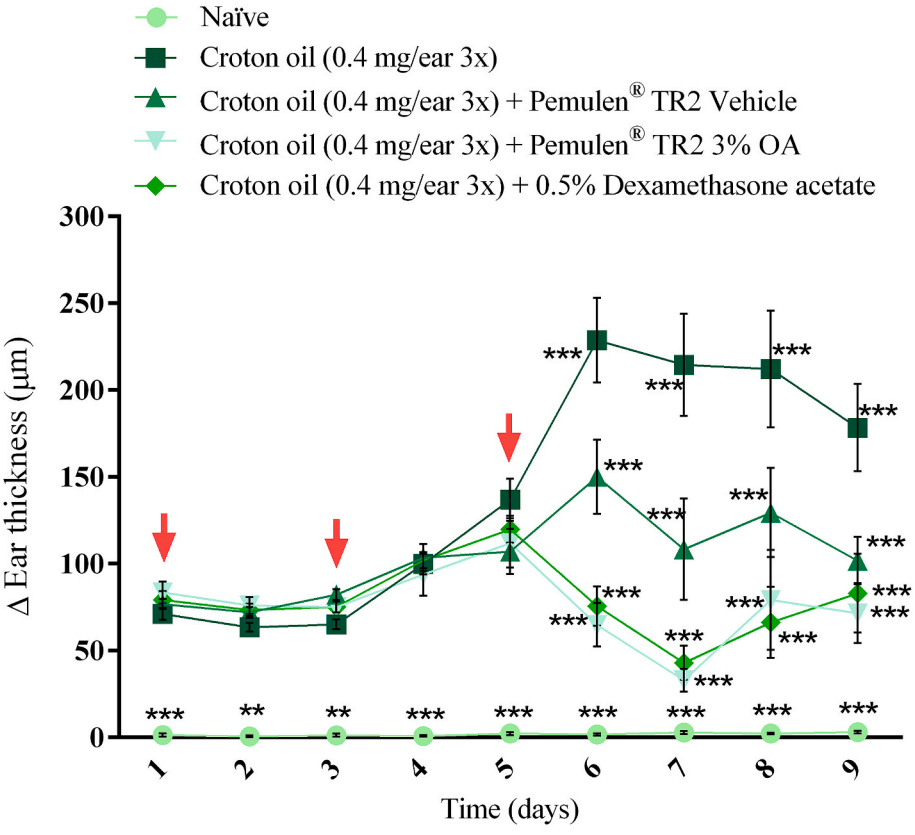

Fig. 5. Anti-oedematogenic effect of semisolid dosage forms containing oleic acid (3%) and dexamethasone acetate (0.5%) topically applied (15 mg/ear) on the ear oedema induced by croton oil repeated application-induced irritant contact dermatitis in mice. Ear oedema was measured once a day for 9 days. The red arrows indicate the days when animals received croton oil administration (0.4 mg/ear). Treatments were topically applied twice a day, starting on day 5 of the experiment. Each line represents the mean + SEM for 7 animals. ***$p < 0.001$ indicates a significant difference when compared with the croton oil group. Two-way (repeated measures) ANOVA followed Dunnett's post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

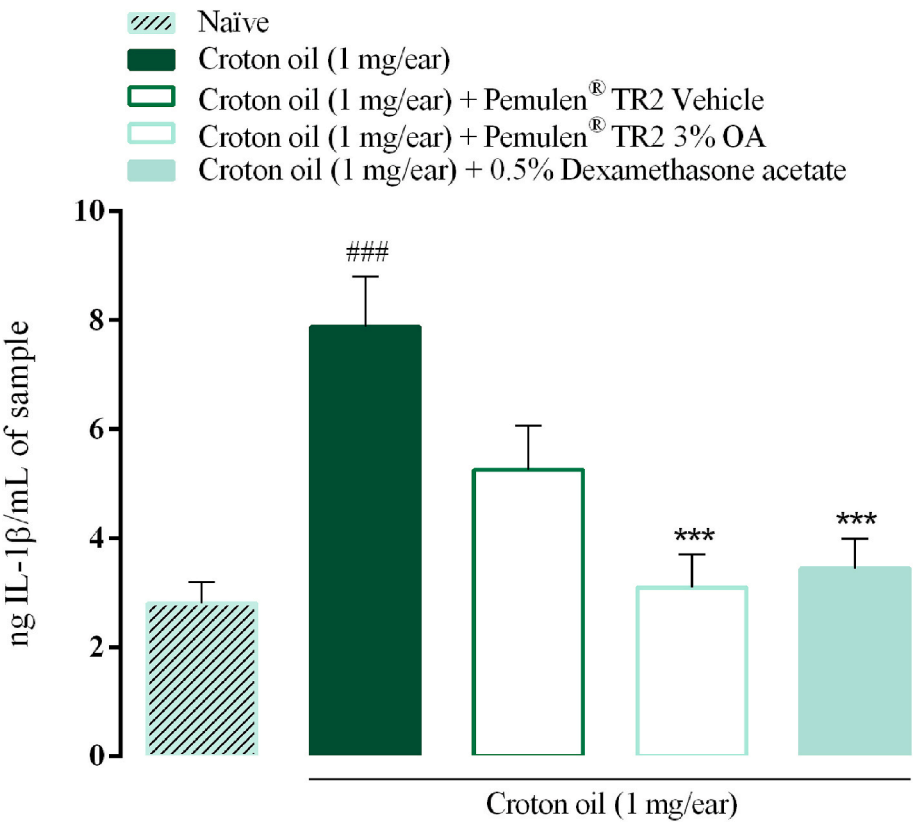

Fig. 6. Effect of the semisolid dosage forms containing oleic acid (3%) and dexamethasone acetate (0.5%) topically applied (15 mg/ear) on the levels of the proinflammatory cytokine IL-1β in the croton oil application-induced irritant contact dermatitis in mice. Each value represents the mean + SEM ($n = 7$). $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{***}p < 0.001$ shows a significant difference when compared to the croton oil group. One-way ANOVA followed by post hoc Tukey's test.

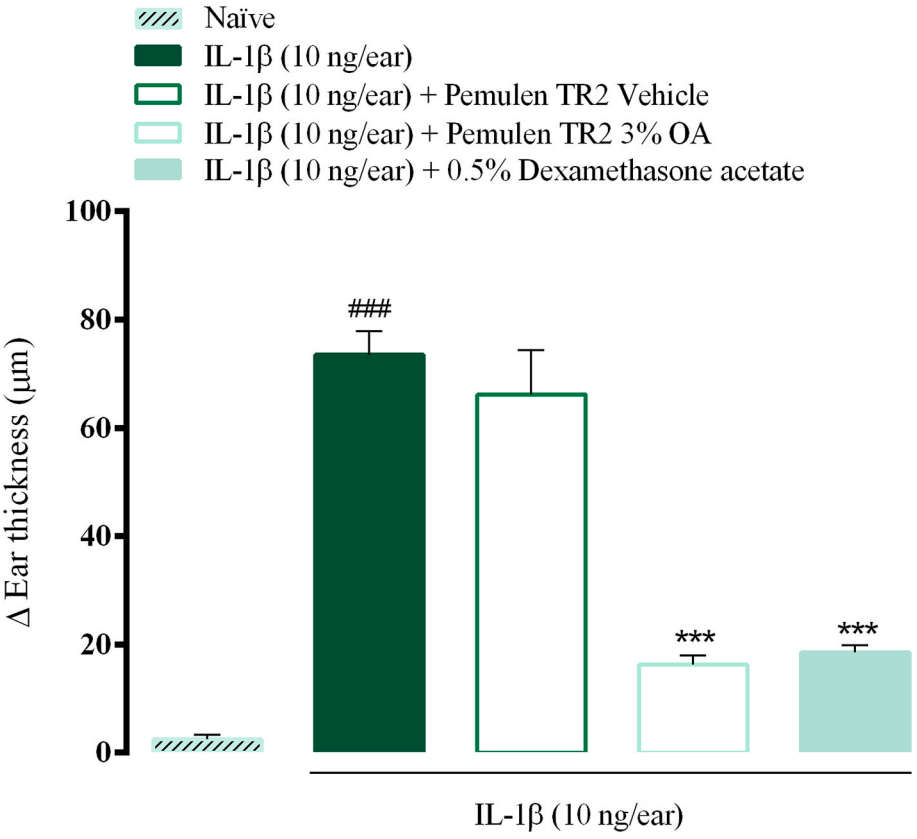

Fig. 7. Anti-oedematogenic effect of the semisolid dosage forms containing oleic acid (3%) and dexamethasone acetate (0.5%) topically applied (15 mg/ear) on the acute ear oedema induced by the IL-1β proinflammatory cytokine. All formulations (15 mg/ear) were topically applied immediately after mice received IL-1β. Ear thickness was measured at 1 h after IL-1β or IL-1β plus treatment application. Each bar represents the mean + SEM ($n = 7$); $^{###}p < 0.001$ shows significant difference when compared to the naïve group; $^{***}p < 0.001$ shows significant difference when compared to the IL-1β group. One-way ANOVA followed by post hoc Tukey's test.

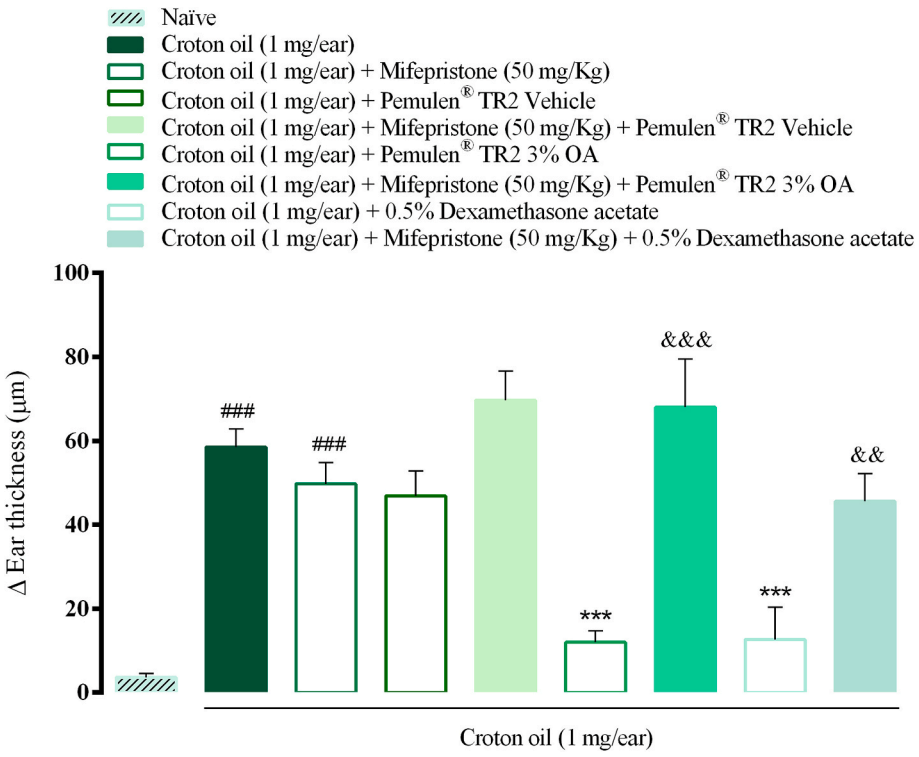

Fig. 8. Reversal of the anti-oedematogenic effect of oleic acid and dexamethasone by the glucocorticoid antagonist mifepristone. Mifepristone (50 mg/kg, s.c.) was administered 15 min before the treatments. Treatments occurred immediately after the topical administration of croton oil. Each bar represents the mean + SEM ($n = 7$); $^{###}p < 0.001$ shows a significant difference when compared to the naïve group; $^{***}p < 0.001$ shows a significant difference when compared to the croton oil group; $^{\&\&}p < 0.01$ and $^{\&\&\&}p < 0.001$ indicate significant difference when compared to the related group that not received the mifepristone. One-way ANOVA followed by post hoc Tukey's test.

Table 1
Effects of oleic acid and dexamethasone-containing semisolid dosage forms on toxicity biochemical parameters.

Experimental groups	ALT (U/L)	AST (U/L)	Urea (mg/mL)	Creatinine (mg/dL)	Glucose (mg/dL)
Naïve	36.88 ± 3.35	22.86 ± 7.16	15.72 ± 4.26	1.56 ± 0.44	72.67 ± 2.80
Croton oil	26.94 ± 2.24	16.59 ± 3.45	14.01 ± 2.22	0.59 ± 0.10	76.97 ± 2.88
Pemulen® TR2 Vehicle	62.76 ± 12.25	13.27 ± 2.08	18.63 ± 0.32	0.86 ± 0.14	103.71 ± 9.94**
Pemulen® TR2 3% AO	28.72 ± 5.67	14.77 ± 1.14	17.00 ± 0.90	0.37 ± 0.13$^{\#}$	81.75 ± 4.89$^{\&\&\&}$
0.5% Dexamethasone acetate	22.01 ± 11.67	13.98 ± 0.90	13.83 ± 2.36	0.53 ± 0.18	138.40 ± 6.09***

Each value represents the mean ± SEM (n = 7). **p < 0.01 and ***p < 0.001 denote significant difference when compared with the naïve group; $^{\&\&\&}$p < 0.001 indicates significant difference when compared to the 0.5% dexamethasone acetate group; $^{\#}$p < 0.05 indicates significant difference when compared to the naïve group. One-way ANOVA followed by post hoc Tukey's test.

oil-induced irritant dermatitis, which may be attributed to the presence of OA, the major constituent in this vegetable oil (Favacho et al., 2011). Oliveira et al. (2010) and Saraiva et al. (2008, 2009) showed the anti-inflammatory effect of pequi oil (extracted from *Caryocar coriaceum* Wittm.; Caryocaraceae family) in several inflammation models, including those induced by croton oil, and attributed this activity to unsaturated fatty acids present in the oil, among them OA.

Besides reducing inflammation-associated oedema, effective treatment to restrain inflammatory cell infiltration to injured tissue is needed since excessive infiltration and activation may lead to an increase in mediator release, contributing to cell chemotaxis and allowing the establishment of chronic inflammation (Nestle et al., 2009; Kolaczkowska and Kubes, 2013). The new therapy to treat skin inflammatory diseases proposed in this study, OA, proved to be effective in also reducing this parameter. Oleic acid reduced myeloperoxidase activity, an indirect measure of neutrophil leukocytes influx (Winterbourn et al., 2000; Kato, 2016), which is the first cellular subtype to infiltrate into damaged sites (Mortaz et al., 2018), confirming the topical anti-inflammatory activity of OA. In addition to that reported by Lescano et al. (2015), our results are also in accordance with those described by Cardoso et al. (2011) who demonstrated reduced inflammatory infiltration in the skin wounds of mice treated with OA. A reduction in the number of leukocytes infiltrating the injured tissue was also confirmed by histology.

Increased cell infiltration occurred after croton oil application. Topical treatment with Pemulen® TR2 OA and dexamethasone reduced this inflammatory parameter, demonstrated by both histological analysis and myeloperoxidase activity. The histological analysis did not enable an exact differentiation between leukocyte types in the tissue. However, since myeloperoxidase activity is an indirect marker of neutrophil infiltration (Winterbourn et al., 2000; Van der Veen et al., 2009; Huang et al., 2016), we showed that most infiltrated cells in the analysed tissue were neutrophils.

Pro-inflammatory cytokines are also important mediators in skin inflammatory processes. They are released by cells activated after inflammatory stimuli and contribute to skin resident cell activation and to the recruitment of leukocytes and other cell types to the skin, besides acting as signalling molecules to mediate and regulate immune and inflammatory processes by altering the expression of inflammation-related genes (Jensen, 2010; Turner et al., 2014; Bou-Dargham et al., 2016). Activated skin resident and recruited cells sustain the inflammatory environment by releasing cytokines (Sokol and Luster, 2015). Important cytokines involved in skin inflammation are members of the IL-1 family, like IL-1β (Jensen, 2011; Fields et al., 2019).

Besides the functions IL-1 family members in pro-inflammatory processes, these mediators also have effects on cell proliferation, differentiation, and apoptosis (Garlanda et al., 2013; Turner et al., 2014). It is already known that IL-1 family members contribute to adaptive immunity by inducing T lymphocyte differentiation into Th17 cells (Louten et al., 2009; Sandquist and Kolls, 2018). Moreover, IL-17 and other Th17

cell-derived cytokines induce granulopoiesis and consequent neutrophil proliferation and accumulation (Cua and Tato, 2010).

Since the cytokine IL-1β is crucial to the establishment of inflammation, a therapeutic alternative capable of reducing its tissue levels is of high relevance. We demonstrated that topically applied croton oil induced an increase in the IL-1β levels in mouse ear tissue. Besides reducing the IL-1β levels in the damaged ear, OA inhibited the ear oedema induced by the topical application of IL-1β, corroborating the effects on ear oedema and inflammatory cell infiltration.

We can hypothesise that the irritant agent croton oil leads to keratinocyte activation, stimulating them to the release cytokines, among then IL-1β, supporting the increased levels observed in mouse ear tissue. Additionally, it could also be hypothesised that this IL-1β induction by croton oil led to increased vascular permeability and consequent leukocyte infiltration into the injured tissue, as observed by histological analysis and myeloperoxidase activity. Damaged tissue inflammatory cells can also be involved in sustaining the number of leukocytes accumulated in the tissue, since skin infiltrating cells also release cytokines, attracting more cells (Oliveira et al., 2016). Furthermore, we speculated that IL-1-induced Th17 responses could contribute to the increased number of inflammatory cells in the damaged tissue by inducing neutrophil proliferation.

It is also known that the dysregulation of pro-inflammatory cytokine signalling leads to aberrant immunity and the development of several diseases (Dinarello, 2011; Hahn et al., 2017). For this reason, skin disease treatments are directed to the discovery of an effective therapy capable of restraining these effects by inhibiting key cytokines (Schlapbach and Navarini, 2016). Cytokine release and binding to their receptors triggers the activation of pro-inflammatory transcription pathways, which can control inflammation-related gene expression (Kataoka, 2009). Thus, inhibiting pro-inflammatory transcription pathway activation is also an important mechanism to suppress inflammation (Rodrigues et al., 2012). Interestingly, the topical therapy Pemulen® TR2 3% OA proposed by us effectively inhibited the increase in IL-1β, indicating that this anti-inflammatory effect could be interesting in clinical practice.

Glucocorticoids can play their biological effects by acting in two different ways at the cellular level: by genomic or non-genomic pathways. In the genomic pathway, the activation of the glucocorticoid receptor results in the transcription of genes with anti-inflammatory functions; the activation of this receptor also negatively regulates the expression of pro-inflammatory players by the transrepression mechanism. The non-genomic pathway involves the modulation of cell activation and responsiveness (Uva et al., 2012). Since our compound was found to activate glucocorticoid receptors, we believe that these mechanisms could play a role in its anti-inflammatory activity.

OA presented anti-inflammatory potential to treat skin inflammatory disorders such as ICD without causing adverse effects, supporting its clinical use. However, further studies evaluating the expression of chemokine and nuclear factor-kappa B (NF-κB) transcription pathway activation could be of great relevance to better understand the mechanisms involved in the anti-inflammatory action of OA.

6. Conclusion

Oleic acid presents *in vivo* anti-inflammatory efficacy in skin inflammation models induced by croton oil in mice, which seems to be glucocorticoid receptor-dependent. OA demonstrated anti-inflammatory efficacy similar to dexamethasone acetate, a clinical medicine widely used to treat skin inflammatory conditions, without causing adverse effects in the preliminary tests evaluated. Therefore, OA represents an attractive therapeutic alternative to treat cutaneous inflammatory disorders such as ICD.

Author contributions

Participated in research design: N.S.P., S.M.O.
Conducted experiments: N.S.P, C.C.
Performed data analysis: N.S.P., S.M.O.
Wrote or contributed to the writing of the manuscript: N.S.P, C.C., L. C., S.M.O.
All the authors reviewed the manuscript.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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7 DISCUSSÃO GERAL

Investigamos a eficácia do AO em modelos *in vivo* de doenças inflamatórias de pele uma vez que diversos relatos da literatura já demonstram o efeito benéfico do ácido graxo AO nos processos de cicatrização de feridas de pele (Cardoso et al., 2011; Jara et al., 2020; Poljŝak et al., 2020).

Cardoso e colaboradores (2011) testaram ácidos graxos na busca por alternativas terapêuticas capazes de acelerar o processo de cicatrização de feridas. Para tal, utilizaram como modelo animal camundongos da linhagem BALB/c e demonstraram que, ao longo deste processo de cicatrização, diversos mediadores inflamatórios têm seus níveis aumentados. Ainda, demonstraram que o tratamento com o ácido graxo AO foi capaz de reduzir o nível tecidual destes mediadores pró-inflamatórios, sugerindo a ação deste composto como um modulador de respostas inflamatórias durante a cicatrização, acelerando o processo de reparação de feridas.

Uma vez que a inflamação constitui uma das etapas do processo de cicatrização e, visto que o AO é tão eficaz na cicatrização de feridas, testamos a eficácia de uma formulação tópica contendo AO em modelos de desordens inflamatórias de pele em camundongos. Além de investigar o efeito anti-inflamatório do AO em sua forma livre, objetivamos associar os benefícios de sistemas nanoestruturados na veiculação de fármacos (Rajendran et al., 2018; Carter et al., 2019), utilizando-se destes para realizar a entrega do AO no tecido cutâneo.

Em nossos primeiros achados, referentes aos artigos científicos 1 e 2, demonstramos a habilidade do AO em inibir desordens inflamatórias cutâneas induzidas pela radiação UVB e pelo agente irritante óleo de cróton em camundongos, respectivamente. Nestes primeiros estudos, demonstramos que o AO regulou negativamente importantes parâmetros inflamatórios, como o edema, a infiltração leucocitária e a inibição de uma das mais importantes citocinas pró-inflamatórias: a interleucina 1 beta (IL-1β). Ademais, nós também induzimos o processo inflamatório de pele com a citocina IL-1β em camundongos e mostramos a inibição deste processo após o tratamento dos animais com o AO.

Em ambos os artigos científicos, os efeitos anti-inflamatórios demonstrados pelo AO foram similares àqueles obtidos pelo tratamento dos animais com a dexametasona (DEX; grupo controle), um corticosteroide classicamente utilizado no tratamento destas patologias (Fathallah et al., 2015). Além disso, demonstramos em ambos os modelos de inflamação de pele que o AO pode estar desempenhando sua ação farmacológica por meio da ativação de GRs, de maneira

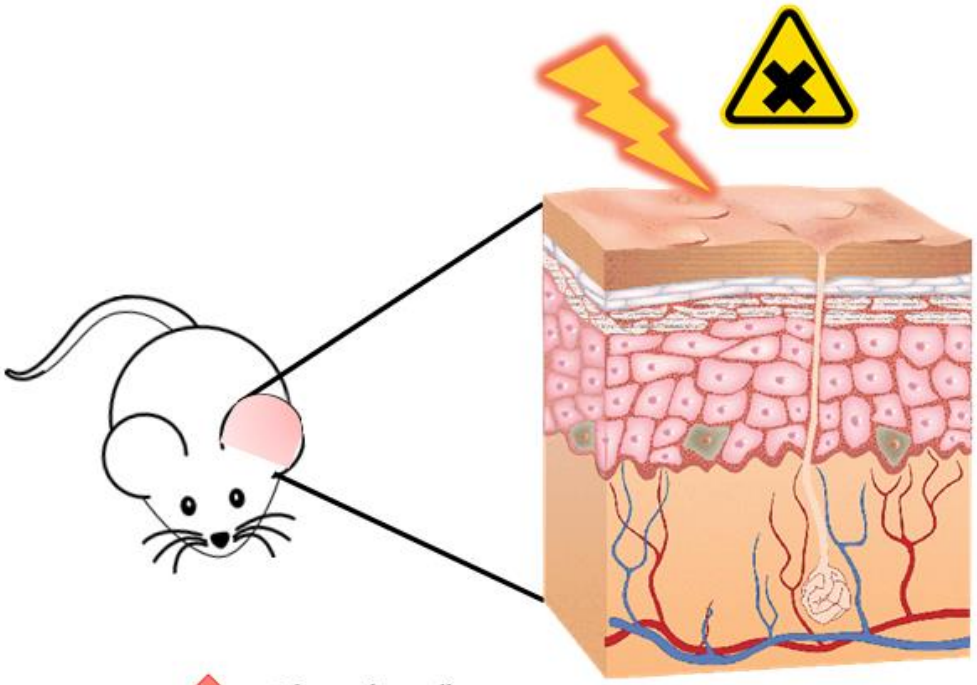
similar aos fármacos pertencentes à classe dos corticosteroides. Embora tenhamos demonstrado que estes efeitos são, pelo menos em parte, mediados por GRs (Xavier et al., 2016), observamos que os tratamentos tópicos com AO e DEX não compartilham um dos principais efeitos indesejados relacionados ao uso de corticosteroides: o aumento dos níveis de glicose sanguínea.

Outro tópico a ser ponderado diz respeito às diferenças estruturais entre a pele de camundongos, modelo amplamente utilizado no *screening* de novos fármacos, e a pele humana. A espessura da epiderme murina é menor que a humana; em contrapartida, a densidade de pêlos é maior. A derme de murinos não apresenta glândulas sudoríparas, ao contrário da pele humana. Além disso, a pele de camundongos apresenta ainda uma camada muscular dérmica, denominada panículo carnoso, que é ausente na derme humana. Apesar do exposto, estes modelos animais são excelentes em mimetizar aspectos patológicos observados em doenças cutâneas humanas e, portanto, de grande valia para a pesquisa de alternativas terapêuticas na área da Dermatologia (Zomer; Trentin, 2018; Nguyen; Soulika, 2019).

8 CONCLUSÃO

Com base nos resultados obtidos ao longo deste trabalho, verificamos que o AO possui atividade anti-inflamatória tópica, observada quando testamos diferentes formulações semissólidas contendo este ácido graxo em dois modelos animais de inflamação de pele: um modelo de dermatite de contato irritante e um modelo de queimadura solar. Ademais, demonstramos, em ambos os modelos, que o AO pode estar desempenhando seu efeito via ativação de receptores glicocorticoides e, mais importante, sem compartilhar um dos principais efeitos adversos associados ao uso de fármacos desta classe: o aumento do nível de glicose sanguínea. Neste contexto, estes achados fornecem evidências que as formulações desenvolvidas podem constituir relevantes estratégicas terapêuticas para tratar doenças inflamatórias de pele.

GRAPHICAL ABSTRACT



Edema de orelha;
Infiltração de células inflamatórias;
Mediadores inflamatórios – IL-1, IL-6, TNF

1
Pemulen® TR2
3% AO

Edema de orelha;
Infiltração de leucócitos.

Via receptor de glicocorticoides

2
Pemulen® TR2 3% AO

Edema de orelha;
Infiltração de leucócitos;
Nível de IL-1β

Inibição do edema de orelha induzido por IL-1β

Via receptor de glicocorticoides

Ausência de efeitos tóxicos

9 PERSPECTIVAS

- Desenvolver formulações semissólidas de base nanotecnológica contendo ácido oleico e acetato de dexametasona;

- Avaliar o perfil de liberação da dexametasona a partir das nanoestruturas e a permeação em pele suína da dexametasona a partir das formulações semissólidas;

- Investigar a *performance* anti-inflamatória *in vivo* das formulações utilizando o modelo de queimadura solar induzida por radiação UVB em camundongos *Swiss*;

- Investigar se o efeito anti-inflamatório das formulações semissólidas contendo as nanopartículas de DEX + AO é sustentado por até 72 horas após o tratamento único dos animais, empregando o modelo de inflamação de pele induzida por UVB;

- Avaliar a atividade das enzimas mieloperoxidase e N-acetil-β-D-glicosaminidase (NAGase), marcadores de infiltração celular, no tecido da orelha dos camundongos 72 horas após UVB + tratamento com as formulações semissólidas contendo as nanopartículas de DEX + AO;

- Verificar os níveis das citocinas pró-inflamatórias IL-6 e MIP-2 no tecido da orelha dos camundongos 72 horas após UVB + tratamento com as formulações semissólidas contendo as nanopartículas de DEX + AO;

- Avaliar a ocorrência de efeitos adversos após o tratamento tópico da orelha dos camundongos durante 14 dias consecutivos com as formulações semissólidas contendo as nanopartículas de DEX + AO.

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11 ANEXOS

11.1 CERTIFICADOS DE APROVAÇÃO DOS PROJETOS REALIZADOS COM ANIMAIS EXPERIMENTAIS PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DA ATIVIDADE ANTI-INFLAMATÓRIA DE FORMULAÇÕES SEMISSÓLIDAS CONTENDO ÁCIDO OLEICO EM UM MODELO DE INFLAMAÇÃO DE PELE", protocolada sob o CEUA nº 2320290518 (ID 002087), sob a responsabilidade de **Sara Marchesan de Oliveira** *e equipe*; *Natháli Schopf Pegoraro*; *Camila Camponogara Dalla Pozza*; *Letícia Cruz* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 09/08/2018.

We certify that the proposal "EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SEMISOLID FORMULATIONS CONTAINING OLEIC ACID IN A SKIN INFLAMMATION MODEL", utilizing 156 Heterogenics mice (156 males), protocol number CEUA 2320290518 (ID 002087), under the responsibility of **Sara Marchesan de Oliveira** *and team*; *Natháli Schopf Pegoraro*; *Camila Camponogara Dalla Pozza*; *Letícia Cruz* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 08/09/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 07/2018 a 07/2019 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	4 a 5 semanas	N:	156
Linhagem:	Swiss			Peso:	25 a 30 g		

Local do experimento: Laboratório de Neurotoxicidade e Psicofarmacologia do Prédio 18.

Santa Maria, 07 de julho de 2020

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DA ATIVIDADE ANTI-INFLAMATÓRIA DO ÁCIDO OLEICO EMPREGANDO UM MODELO DE INFLAMAÇÃO DE PELE INDUZIDA POR ÓLEO DE CRÓTON", protocolada sob o CEUA nº 5582261018 (ID 002285), sob a responsabilidade de **Sara Marchesan de Oliveira** *e equipe; Natháli Schopf Pegoraro; Camila Camponogara Dalla Pozza; Letícia Cruz* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 29/01/2019.

We certify that the proposal "EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF OLEIC ACID EMPLOYING A SKIN INFLAMMATION CROTON OIL-INDUCED MODEL", utilizing 49 Heterogenics mice (49 males), protocol number CEUA 5582261018 (ID 002285), under the responsibility of **Sara Marchesan de Oliveira** *and team; Natháli Schopf Pegoraro; Camila Camponogara Dalla Pozza; Letícia Cruz* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 01/29/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 01/2019 a 07/2020 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	4 a 5 semanas	N:	49
Linhagem:	Swiss			Peso:	25 a 30 g		

Local do experimento: Laboratório de Neurotoxicidade e Psicofarmacologia do Prédio 18 - UFSM

Santa Maria, 07 de julho de 2020

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DO EFEITO ANTI-INFLAMATÓRIO DO ÁCIDO OLEICO EMPREGANDO UM MODELO DE INFLAMAÇÃO DE PELE INDUZIDA POR ÓLEO DE CRÓTON", protocolada sob o CEUA nº 7412190319 (ID 002503), sob a responsabilidade de **Sara Marchesan de Oliveira** *e equipe; Natháli Schopf Pegoraro; Camila Camponogara Dalla Pozza; Letícia Cruz* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 21/05/2019.

We certify that the proposal "EVALUATION OF ANTI-INFLAMMATORY EFFECT OF OLEIC ACID EMPLOYING A SKIN INFLAMMATION CROTON OIL-INDUCED MODEL", utilizing 168 Heterogenics mice (168 males), protocol number CEUA 7412190319 (ID 002503), under the responsibility of **Sara Marchesan de Oliveira** *and team; Natháli Schopf Pegoraro; Camila Camponogara Dalla Pozza; Letícia Cruz* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 05/21/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 06/2019 a 07/2020 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	4 a 5 semanas	N:	168
Linhagem:	Swiss			Peso:	25 a 30 g		

Local do experimento: Laboratório de Neurotoxicidade e Psicofarmacologia do Prédio 18.

Santa Maria, 07 de julho de 2020

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Coordenadora da Comissão de Ética no Uso de Animais
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Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

Santa Maria, 20 de agosto de 2019
CEUA N 7412190319

IIlmo(a). Sr(a).
Responsável: Sara Marchesan De Oliveira
Área: Bioquímica E Biologia Molecular

Título da proposta: "AVALIAÇÃO DO EFEITO ANTI-INFLAMATÓRIO DO ÁCIDO OLEICO EMPREGANDO UM MODELO DE INFLAMAÇÃO DE PELE INDUZIDA POR ÓLEO DE CRÓTON".

Parecer Consubstanciado da Comissão de Ética no Uso de Animais UFSM (ID 001695)

A Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria, no cumprimento das suas atribuições, analisou e **APROVOU** a Emenda (versão de 06/agosto/2019) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "Solicitamos a aprovação da CEUA para realizar um experimento adicional: a indução de processo inflamatório pela administração de IL-1?. Por meio deste experimento, gostaríamos de avaliar a capacidade do ácido oleico incorporado à formulação semissólida em reduzir o processo inflamatório induzido pela administração desta citocina pró-inflamatória. Gostaríamos de realizar este experimento pois, com base em resultados dos experimentos anteriores, observamos que esta citocina está aumentada na pele de animais submetidos ao modelo de inflamação induzida por óleo de cróton. Para a realização de tal experimento e conclusão deste projeto, pedimos a autorização da CEUA e liberação de mais 35 animais (5 grupos x 7 animais por grupo). ".

Comentário da CEUA: "Emenda aprovada em seus aspectos éticos".

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DO MECANISMO DE AÇÃO DO ÁCIDO OLEICO", protocolada sob o CEUA nº 5864120819 (ID 002715), sob a responsabilidade de **Sara Marchesan de Oliveira** *e equipe*; *Natháli Schopf Pegoraro*; *Camila Camponogara Dalla Pozza*; *Letícia Cruz* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 15/10/2019.

We certify that the proposal "EVALUATION OF OLEIC ACID MECHANISM OF ACTION", utilizing 54 Heterogenics mice (54 males), protocol number CEUA 5864120819 (ID 002715), under the responsibility of **Sara Marchesan de Oliveira** *and team*; *Natháli Schopf Pegoraro*; *Camila Camponogara Dalla Pozza*; *Leticia Cruz* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 10/15/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 12/2019 a 12/2020 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	4 a 5 semanas	N:	54
Linhagem:	Swiss			Peso:	25 a 30 g		

Local do experimento: Laboratório de Neurotoxicidade e Psicofarmacologia do Prédio 18.

Santa Maria, 07 de julho de 2020

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "EFEITO DE FORMULAÇÕES NANOESTRUTURADAS CONTENDO ÁCIDO OLEICO E ACETATO DE DEXAMETASONA EM UM MODELO DE INFLAMAÇÃO DE PELE EM CAMUNDONGOS", protocolada sob o CEUA nº 4369251019 (ID 002911), sob a responsabilidade de **Sara Marchesan de Oliveira** *e equipe; Natháli Schopf Pegoraro; Camila Camponogara Dalla Pozza; Letícia Cruz; Mailine Gehrcke* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 31/03/2020.

We certify that the proposal "EFFECT OF NANOSTRUCTURED FORMULATIONS CONTAINING OLEIC ACID AND DEXAMETHASONE ACETATE IN A SKIN INFLAMMATION MODEL IN MICE", utilizing 72 Heterogenics mice (72 males), protocol number CEUA 4369251019 (ID 002911), under the responsibility of **Sara Marchesan de Oliveira** *and team; Natháli Schopf Pegoraro; Camila Camponogara Dalla Pozza; Letícia Cruz; Mailine Gehrcke* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 03/31/2020.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 04/2020 a 04/2021 Área: Departamento de Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	4 a 5 semanas	N:	72
Linhagem:	Swiss			Peso:	25 a 30 g		

Local do experimento: Laboratório de Neurotoxicidade e Psicofarmacologia do Prédio 18.

Santa Maria, 07 de julho de 2020

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

Santa Maria, 20 de outubro de 2020
CEUA N 4369251019

Ilmo(a). Sr(a).
Responsável: Sara Marchesan De Oliveira
Área: Departamento De Bioquímica E Biologia Molecular

Título da proposta: "EFEITO DE FORMULAÇÕES NANOESTRUTURADAS CONTENDO ÁCIDO OLEICO E ACETATO DE DEXAMETASONA EM UM MODELO DE INFLAMAÇÃO DE PELE EM CAMUNDONGOS".

Parecer Consubstanciado da Comissão de Ética no Uso de Animais UFSM (ID 002380)

A Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria, no cumprimento das suas atribuições, analisou e **APROVOU** a Emenda (versão de 21/setembro/2020) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "A presente emenda objetiva a substituição dos grupos experimentais inicialmente delineados neste projeto. Isto se deve ao fato de termos necessitado realizar alterações nos grupos experimentais de testes preliminares de desenvolvimento das formulações para uso tópico. Visto que todos os dados farão parte de um mesmo manuscrito científico, optamos por alterar os grupos experimentais dos testes in vivo, para que fiquem alinhados com os grupos experimentais das demais avaliações. ".

Comentário da CEUA: "Emenda aprovada.".

Profa. Dra. Patrícia Severo do Nascimento
Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

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Expected presentation date	Jul 2021

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Oleic acid exhibits an expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis without the occurrence of toxicological effects in mice

Author: Natháli Schopf Pegoraro,Camila Camponogara,Letícia Cruz,Sara Marchesan Oliveira
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