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# AVALIAÇÃO DO PROCESSO INFLAMATÓRIO APÓS USO DO GLICIRRIZINATO DIPOTÁSSIO NA CICATRIZAÇÃO DE FERIDAS POR SEGUNDA INTENÇÃO EM MODELO ANIMAL

Bragança Paulista

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*"Cada vez mais, sobressaem-se aqueles que reconhecem a importância de um sorriso, uma dose extra de paciência, uma amizade desinteressada, enfim, aqueles que sabem levar a vida com leveza, tornando o ambiente mais agradável a sua volta"*

*"Não serão nossos gritos a fazer a diferença e sim a força contida em nossas mais delicadas e íntegras ações"*

***Rosana Braga***

# RESUMO

A cicatrização de feridas é caracterizada por um processo sistêmico e complexo de atividades celulares e moleculares. O Glicirrizinato Dipotássico (DPG), um produto secundário derivado do ácido glicirrízico, possui vários efeitos biológicos, como antialérgico, antioxidante, antibacteriano, antiviral, gastroprotetor, antitumoral e antiinflamatório. Este estudo teve como objetivo avaliar o efeito anti-inflamatório do DPG tópico na cicatrização de feridas cutâneas por segunda intenção, em modelo experimental in vivo. Para tanto, foram usados 24 ratos Wistar machos, divididos aleatoriamente em 6 grupos (n=4, cada). Excisões circulares foram realizadas em ratos e tratadas topicamente por 14 dias após a indução da ferida. Foram realizadas análises macroscópicas e histopatológicas. A expressão gênica foi avaliada por qPCR em tempo real. Nossos resultados mostraram que o tratamento com DPG causou diminuição do exsudato inflamatório, bem como levou à ausência de hiperemia ativa. Aumentos no tecido de granulação, reepitelização tecidual e colágeno total também foram observados. Além disso, o tratamento com DPG reduziu a expressão de citocinas pró-inflamatórias (*Tnf-α*, *Cox-2*, *Il-8*, *Irak-2*, *Nf-kB* e *Il-1*) e up-regulou *Il-10*, demostrando efeitos anti-inflamatórios em todos os três períodos de tratamento. Com base em nossos resultados, concluímos que o DPG atenua o processo inflamatório ao promover a cicatrização de feridas cutâneas por meio da modulação de diversos mecanismos e vias de sinalização, incluindo as anti-inflamatórias, por meio da modulação da expressão de citocinas pró e anti-inflamatórias. Isso envolve a modulação da expressão de citocinas pró e anti-inflamatórias; promoção de novo tecido de granulação; angiogênese; e reepitelização tecidual, todos os quais contribuem para a remodelação tecidual.

**Palavras-chave:** Cicatrização de pele. Inflamação. DPG. Modelo animal. Ratos.

# ABSTRACT

Wound healing is characterized by a systemic and complex process of cellular and molecular activities. Dipotassium Glycyrrhizinate (DPG), a side product derived from glycyrrhizic acid, has several biological effects, such as being antiallergic, antioxidant, antibacterial, antiviral, gastroprotective, antitumoral, and anti-inflammatory. This study aimed to evaluate the anti-inflammatory effect of topical DPG on the healing of cutaneous wounds by secondary intention in an in vivo experimental model. Twenty-four male Wistar rats were used in the experiment, and were randomly divided into six groups of four. Circular excisions were performed and topically treated for 14 days after wound induction. Macroscopic and histopathological analyses were performed. Gene expression was evaluated by real-time qPCR. Our results showed that treatment with DPG caused a decrease in the inflammatory exudate as well as an absence of active hyperemia. Increases in granulation tissue, tissue reepithelization, and total collagen were also observed. Furthermore, DPG treatment reduced the expression of pro-inflammatory cytokines (*Tnf-α*, *Cox-2*, *Il-8*, *Irak-2*, *Nf-kB*, and *Il-1*) while increasing the expression of *Il-10*, demonstrating anti-inflammatory effects across all three treatment periods. Based on our results, we conclude that DPG attenuates the inflammatory process by promoting skin wound healing through the modulation of distinct mechanisms and signaling pathways, including anti-inflammatory ones. This involves modulation of the expression of pro- and anti-inflammatory cytokine expression; promotion of new granulation tissue; angiogenesis; and tissue re-epithelialization, all of which contribute to tissue remodeling.

***Keywords:*** *Skin Wound Healing. Inflammation. DPG. Animal model. Rats.*

# LISTA DE SÍMBOLOS E ABREVIAÇÕES

AG – Ácido glicirrízico

COL-1 – Colágeno do tipo I

COL-3 – Colágeno do tipo II

COX-2 – Ciclooxigenase-2

CXCL1 – do inglês Chemokine (C-X-C motif) ligand 1

CXCL8 – do inglês Chemokine (C-X-C motif) ligand 8

DPG – Glicirrizinato Dipotássio

EEPQ – Epitélio estratificado pavimentoso queratinizado

EGF – do inglês Epidermal Growth Factor

FGF – do inglês Fibroblast Growth Factor

G – Glicirrizina

GM-CSF – do inglês Granulocyte and Monocyte Colony Stimulating Factor

HaCaT – do inglês Human Keratinocytes

HMGB1 – do inglês High Mobility Group Box 1 Protein

IFN-γ – Interferon gama

ILs – interleucinas

IL-1 – Interleucina-1

IL-1α – Interleucina-1 alfa

IL-6 – Interleucina-6

IL-8 – Interleucina-8

iNOS – do inglês Inducible Nitric Oxide Synthase

KGF – do inglês Keratinocyte Growth Factor

LOX-5 – do inglês 5-Lipoxygenase

M1 – Macrófagos do tipo 1

M2 – Macrófagos do tipo 2

MAPK– do inglês Mitogen-Activated Protein Kinase

M-CSF – do inglês Monocyte Colony Stimulating Factor

MEC – Matriz extracelular

MIP-1α – do inglês Macrophage Inflamatory Protein-1

MMPs – Metaloproteinases de matriz

MyD88 – do inglês Myeloid Differentiation Factor 88

MYOD – do inglês Transcription factor – basic helix-loop-helix protein family

MYOG – do inglês Transcription factor – basic helix-loop-helix protein family

NF-κB – do inglês Nuclear Factor kappa B

PDGF – do inglês Platelet-Derived Growth Factor

PGE2 – Prostaglandina E2

TCD – Tecido conjuntivo denso

TCF – Tecido conjuntivo frouxo

TGF-α – do inglês Alpha-Transforming Growth Factor

TGF-β – do inglês Beta-Transforming Growth Factor

TIMPs – do inglês Tissue Inhibitors of Metalloproteinases

TNF-α – do inglês Alpha-Tumor Necrosis Factor

VEGF – do inglês Vascular Endothelial Growth Factor

11β-HSD – do inglês 11β-Hydroxyesteroid Dehydrogenase

α-SMA – do inglês Alpha-Smooth Muscle Actin

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# SUMÁRIO

# 1. INTRODUÇÃO

## 1.1 Estrutura da pele

A pele, ou tegumento, é o órgão que reveste todo o corpo correspondendo à proporção de 15-20% do peso corporal. Constituída pelos tecidos epitelial e conjuntivo, apresenta-se composta por duas camadas principais, a epiderme e a derme. Sob a derme, há uma camada de tecido adiposo, denominada hipoderme ou tecido subcutâneo (YOUSEF; ALHAJJ; SHARMA, 2022; ROSS; PAWLINA, 2016), possuindo várias funções essenciais ao organismo, tais como: proteção contra a entrada de agentes microbianos para o meio interno; proteção contra impactos mecânicos; controle da perda líquida corporal (água, eletrólitos) e termorregulação (conservação e dissipação do calor) (YOUSEF; ALHAJJ; SHARMA, 2022; SAHLE et al., 2015; KIERSZENBAUM, 2008; NITZ et al., 2006) **(FIGURA 1).**

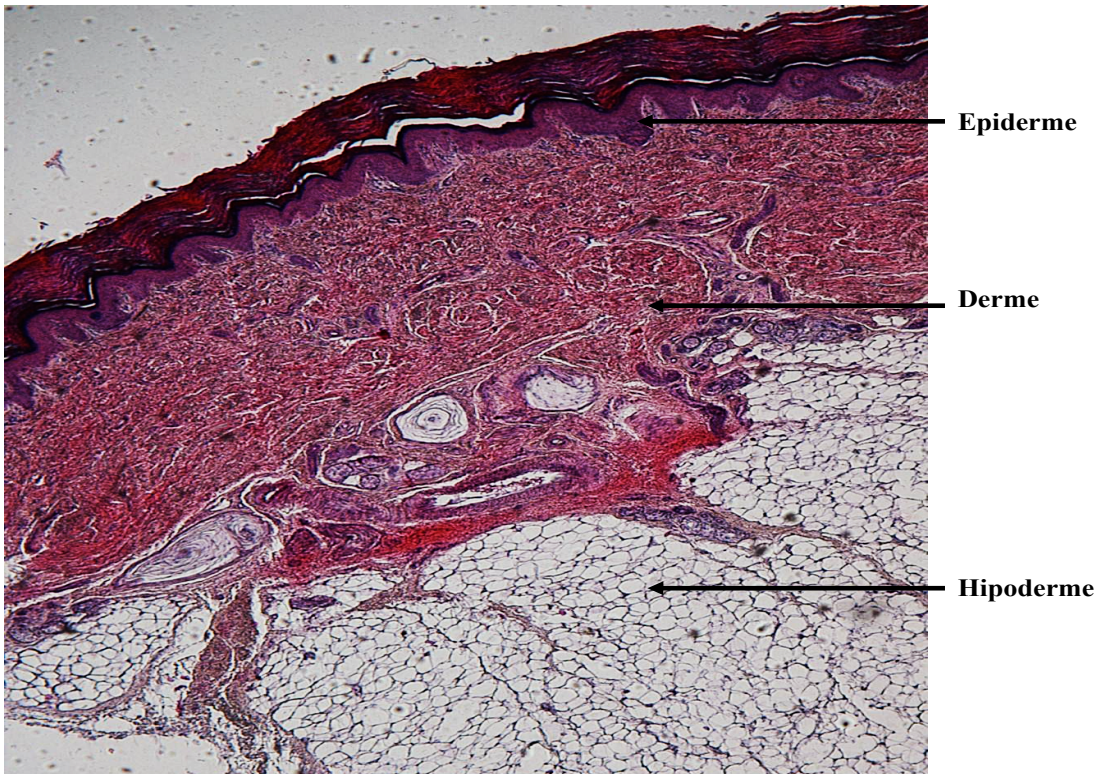


**FIGURA 1. Organização esquemática da pele.** Epiderme, derme e hipoderme. Fonte: Acervo pessoal.

A epiderme, camada mais superficial da pele, tem sua origem a partir da ectoderme e é constituída por epitélio estratificado pavimentoso queratinizado (EEPQ) associado à lâmina própria. O EEPQ é subdividido em cinco camadas e/ou estratos celulares, e partindo do meio interno para a superfície encontramos as camadas e/ou estratos: Camada basal ou germinativa (composta por células basais, altamente proliferativas, de formas cúbicas ou prismáticas, sobre a lâmina basal); Camada espinhosa (composta por queratinócitos poligonais, contendo queratina e desmossomos); Camada granulosa (composta por células alongadas, com núcleos ovais, dispostas próximas à superfície cutânea, contendo grânulos de queratina e substâncias extracelulares como lipídios e colágeno); Camada lúcida, geralmente identificada na pele espessa (YOUSEF; ALHAJJ; SHARMA, 2022; ROSS; PAWLINA, 2016) composta por células anucleadas, claras e semitransparentes, sendo visível apenas nas regiões palmares e plantares) e Camada córnea (composta por células mortas, anucleadas, contendo queratina e lipídios) (YOUSEF; ALHAJJ; SHARMA, 2022; ALMEIDA, 2019; KIERSZENBAUM, 2008).

Distribuídos nestas 5 camadas celulares, além dos queratinócitos produtores de queratina, encontram-se: os melanócitos – células de origem neural encontradas na camada basal e responsáveis pela produção de melanina, pigmento que confere coloração à pele, pelos e cabelos, e que também atua na proteção contra os raios solares (SCHIAFFINO, 2010; KIERSZENBAUM, 2008); as células de Langherans – células dendríticas (células apresentadoras de antígenos) (STOITZNER, 2010; KIERSZENBAUM, 2008); e as células de Merkel – células que possuem terminações nervosas livres e que atuam na percepção ao tato (YOUSEF; ALHAJJ; SHARMA, 2022; KIERSZENBAUM, 2008; DÂNGELO; FATTINI, 1998).

A derme, camada intermediária da pele, de origem mesodérmica, é constituída por tecido conjuntivo e garante a sustentação da epiderme, estando dividida em camada papilar – mais superficial, composta por tecido conjuntivo frouxo (TCF) (fibroblastos, fibras elásticas finas e fibras colágenas), na qual são encontradas papilas dérmicas formando a junção dermoepidérmica e camada reticular – mais profunda, composta por tecido conjuntivo denso (TCD), na qual são encontrados feixes espessos de fibras colágenas e fibras elásticas, vasos sanguíneos e linfáticos, terminações nervosas, glândulas sebáceas e sudoríparas e elementos celulares tácteis (YOUSEF; ALHAJJ; SHARMA, 2022; ALMEIDA, 2019; KIERSZENBAUM, 2008).

Vários tipos celulares se encontram na derme, por exemplo: fibroblastos e fibrócitos. Os fibroblastos, células fusiformes com núcleos ovalados, participam da síntese dos elementos da matriz extracelular, o que permite e promove a formação do tecido de granulação, levando a reepitelização e mantendo o tecido conjuntivo íntegro, sendo células essenciais na cicatrização (PAGNANO et al., 2009; CARVALHO, 2002). Os fibrócitos também contribuem para a síntese de colágeno e matriz extracelular, participando na fase de remodelagem do processo cicatricial da pele e de processos inflamatórios através da produção de fatores de crescimento e angiogênicos (QUAN et al., 2004).

A hipoderme, ou tecido subcutâneo, é constituída por TCF e, principalmente, por células adiposas. Os feixes de TCF se entrelaçam com fibras elásticas, formando alvéolos com as células adiposas, juntamente com folículos pilosos, neurônios sensoriais e vasos sanguíneos. Entretanto, esta camada não faz parte da pele (YOUSEF; ALHAJJ; SHARMA, 2022; KIERSZENBAUM, 2008).

### 1.2 Respostas celulares durante a cicatrização de feridas

A cicatrização de feridas se caracteriza por um processo sistêmico e complexo de atividades celulares e moleculares, que ocorre naturalmente, objetivando o reparo anatômico e funcional da região afetada, independente do agente que a causou; sejam estes, agentes mecânico, físico, químico ou patológico. Este evento compõe-se de quatro fases básicas: hemostática, inflamatória, proliferativa e remodeladora ou de maturação, as quais envolvem; divisão celular, proliferação, migração, neovascularização, síntese de elementos protéicos, contração, reepitelização e remodelamento estrutural do tecido lesado (SHEDOEVA et al., 2020; RODRIGUES et al., 2019; LINDLEY et al., 2016; MENDONÇA; COUTINHO, 2009) **(FIGURA 2)**.

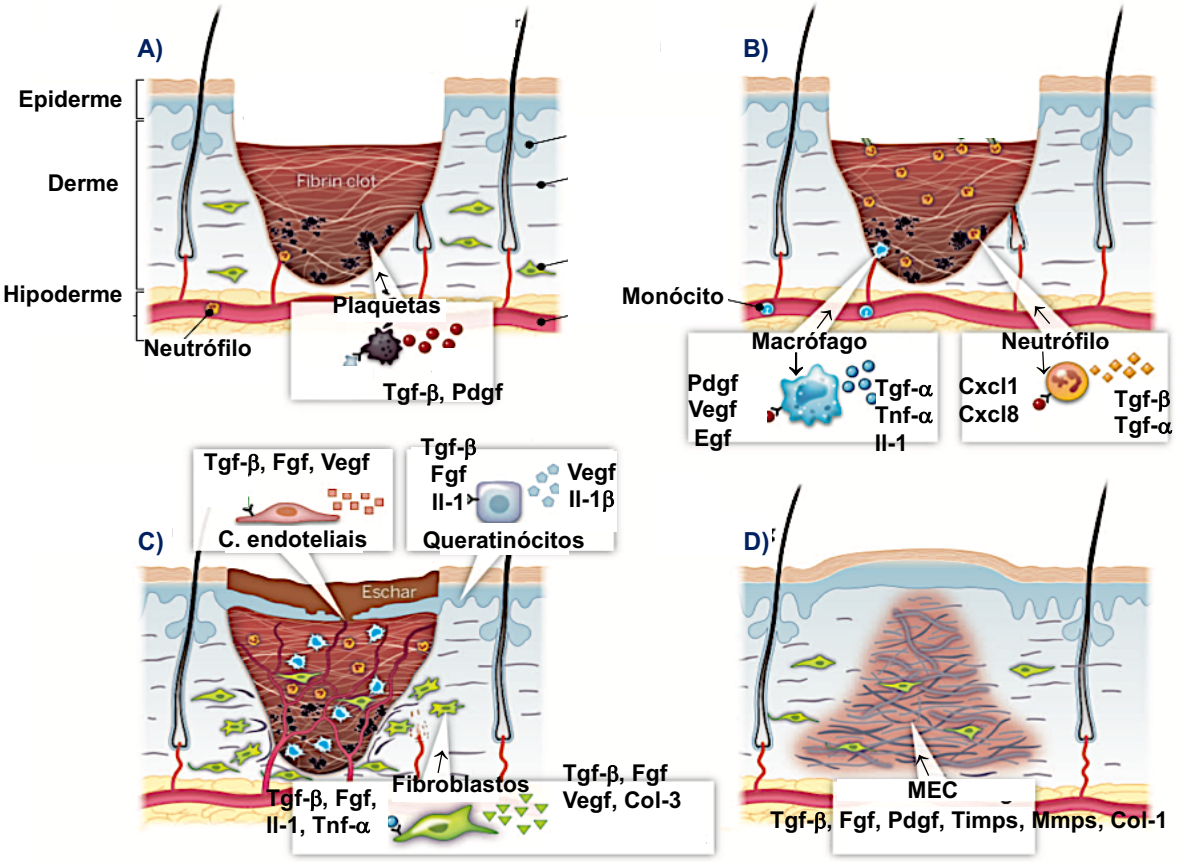


**FIGURA 2. Principais tipos celulares e fatores de crescimento envolvidos em cada uma das fases da cicatrização. A) Fase hemostática (após lesão):** Recrutamento de polimorfonucleares (neutrófilos) e plaquetas. Liberação do fator de crescimento de plaquetas (PDGF) e fator de crescimento transformante beta (TGF-β). Coágulo de fibrina. **B) Fase inflamatória (24 – 96 horas):** Recrutamento de mais neutrófilos, monócitos e macrófagos. Liberação de TGF-β e TGF-α, PDGF, fator de crescimento endotelial vascular (VEGF), interleucina-1 (IL-1), fator de necrose tumoral alfa (TNF-α), fator de crescimento epidermal (EGF) e das quimiocinas 1 e 8 (CXCL1 e 8). **C) Fase proliferativa (4º dia – 2ª/3ª semana):** Proliferação e migração de células endoteliais, fibroblastos e queratinócitos. Deposição da MEC provisória e colágeno 3 (COL-3). Liberação de VEGF, PDGF, IL-1, TNF-α, interferons (IFNs), fatores de crescimento derivados de fibroblastos (FGFs) e fator de crescimento derivado dos queratinócitos (KGF). **D) Fase remodeladora (3ª semana – meses e/ou anos):** Maturação e remodelação da MEC. Síntese de colágeno 1 (COL-1). Liberação de TGF-β, PDGF, FGF, metaloproteinases de matriz (MMPs) e inibidores das metaloproteinases (TIMPs).

Fonte: figura adaptada de Sun; Siprashvili; Khavari, 2014.

A cicatrização pode ocorrer por união primária, secundária ou terciária. Entretanto, a efetivação desse processo dependerá de fatores, como: agente causador, quantidade e qualidade de tecido que se perdeu no momento da lesão e da presença ou não de infecção no sítio lesionado (TAZIMA; VICENTE; MORIYA, 2008).

A cicatrização por união primária, ou primeira intenção, ocorre após incisões cirúrgicas nas quais, há perda mínima de tecidos, ausências de edema e infecção local, e suas bordas aproximadas por suturas cirúrgicas.

A cicatrização por união secundária, ou segunda intenção, ocorre em feridas traumáticas com bordas separadas, que se caracterizam por perda mais extensa de células e tecidos, presença ou não de infecção local, e alta produção de tecido de granulação, importante para o processo de reparação (ROSS; PAWLINA, 2016). Neste caso o leito da ferida é mantido exposto e se fecha por contração e reepitelização, com ou sem o uso de tratamentos local ou sistêmico.

Na ferida por terceira intenção, na qual há perda maciça de tecidos e infecção, as bordas do sítio lesionado são aproximadas por síntese cirúrgica, posteriormente a um tratamento inicial (TAZIMA; VICENTE; MORIYA, 2008).

Conforme mencionado, após a lesão, distintos tipos de células e moléculas são ativados e permeiam os processos de reparo em cada fase da cicatrização. Esses processos são comandados em parte pela sinalização entre células hematopoiéticas, imunológicas e células residentes do tegumento.

**Fase hemostática:** Imediatamente após a lesão, múltiplas respostas (vasoconstrição, formação de tampão plaquetário e coagulação) são desencadeadas afim de interromper a perda sanguínea e reestabelecer a hemostasia local (RODRIGUES et al., 2019; GOLEBIEWSKA; POOLE, 2015). Primeiramente, as células musculares lisas vasculares levam a vasoconstrição, a qual é desencadeada por vasoconstritores (endotelinas, catecolaminas e prostaglandinas) liberados pelo endotélio e células danificadas (GODO; SHIMOKAWA, 2017; GOLEBIEWSKA; POOLE, 2015). A seguir, ocorre a agregação das plaquetas e formação do tampão plaquetário em resposta a presença de colágeno no interior da matriz subendotelial (SCULLY et al., 2020; GOLEBIEWSKA; POOLE, 2015).

Posteriormente, fatores da coagulação são ativados e convertem o fibrinogênio em uma malha de fibrina. O tampão plaquetário junto a malha de fibrina formam um coágulo, que detém

a perda sanguínea e dá suporte provisório a migração de células (leucócitos, queratinócitos e fibroblastos) necessárias para o reparo (RODRIGUES et al., 2019; ZAIDI; GREEN, 2019). Nesta fase, o fator de crescimento derivado das plaquetas (PDGF) e fator de crescimento transformador beta (TGF-β) são os principais mediadores liberados pelas plaquetas, os quais são importantes para as fases subsequentes da cicatrização (SCULLY et al., 2020; RODRIGUES et al., 2019).

**Fase inflamatória:** Horas após a lesão, inicia-se a inflamação que é mediada principalmente por leucócitos (neutrófilos, monócitos, linfócitos, macrófagos); bem como, por interleucinas (ILs), fator de necrose tumoral alfa (TNF-α), interferon gama (IFN-γ), quimiocinas (GERMOLEC et al., 2018; WEISS, 2008; GUYTON; HALL, 2008) e por moléculas de adesão (L-selectina, P-selectina e E-selectina), liberadas pelos leucócitos, células endoteliais e plaquetas, respectivamente (GERMOLEC et al., 2018; WEISS, 2008).

A inflamação caracteriza-se por vasodilatação, aumento da permeabilidade vascular, extravasamento de líquido para o interstício, edema tecidual, migração de neutrófilos, ativação de macrófagos e, liberação de citocinas e quimiocinas (GERMOLEC et al., 2018; WEISS, 2008; GUYTON; HALL, 2008). Tem como objetivo, a remoção do agente danoso, isolamento da área e prevenção contra os microrganismos.

As plaquetas presentes sintetizam PDGF que, associado a outros fatores, como as quimiocinas CXCL1 e CXCL8, atuam no recrutamento de polimorfonucleares (neutrófilos) (ELLIS et al., 2018; SU; RICHMOND, 2015; JONHNSON; WILGUS, 2014; BAO et al., 2009). As células danificadas liberam peróxido de hidrogênio, mediadores lipídicos e quimiocinas (TNF-α, IL-1, IFN-γ) (NOSENKO; AMBARYAN; DRUTSKAYA, 2019; SPIEKSTRA et al., 2007), que desencadeiam sinais para o recrutamento de células inflamatórias (VAN DER VLIET; JANSSEN-HEININGER, 2014; KOLACZKOWSKA; KUBES, 2013), dos neutrófilos em particular, a partir da primeira hora após a lesão (SU; RICHMOND, 2015; GUYTON; HALL, 2008).

O TNF-α, constitutivo no tecido cutâneo (YUZHAKOVA et al., 2016), é capaz de induzir, bem como, de controlar a inflamação e resposta imune (FELDMANN, 2002). A IL-1 em particular, estimula a síntese de citocinas pró-inflamatórias, a expressão de moléculas de adesão e de células inflamatórias pela medula óssea (MACLEOD et al., 2021; TAN et al., 2018), a liberação de prostaglandinas e a proliferação celular, aumentando a inflamação (MARTIN et al.,

2021). A IL-1β regula positivamente a expressão gênica e a secreção da sintase óxido nítrico induzível (iNOS) e ciclooxigenase-2 (COX-2), ambas moduladoras da síntese dos mediadores inflamatórios; prostaglandinas E2 (PGE2) (SOBOLEWSKI et al., 2010; WADA, et al., 2007; ABD-EL-ALEEM et al. 2001), fator ativador de plaquetas (PAF) e óxido nítrico (NO) (GERMOLEC et al., 2018).

Os neutrófilos ativados agem contra microorganismos e degradam a matriz danificada nas primeiras 24h após a lesão (BERMAN; MADERAL; RAPHAEL, 2017; SU; RICHMOND, 2015; GILLITZER; GOEBELER, 2001). Uma neutrofilia é estabelecida após uma inflamação aguda/grave devido ao transporte das substâncias inflamatórias para a medula óssea via corrente sanguínea (SU; RICHMOND, 2015; GUYTON; HALL, 2008). Além disso, nesse caso, as substâncias inflamatórias liberadas podem estimular os capilares medulares à mobilização e síntese de mais neutrófilos, amplificando a infiltração de neutrófilos (SU; RICHMOND, 2015).

Macrófagos teciduais fixos são ativados a partir da primeira meia hora e, aumentam de volume, proliferando rapidamente. A seguir, monócitos provenientes da circulação se infiltram no tecido e lentamente se diferenciam em macrófagos maduros, tornando-se ativos para realizarem fagocitose após alguns dias (BERMAN; MADERAL; RAPHAEL, 2017; RODERO et al., 2014). Os fatores estimuladores de colônia de monócitos (M-CSF) e de colônias de granulócitos-monócitos (GM-CSF) estão implicados na regulação da resposta dos monócitos-macrófagos, na inflamação (GERMOLEC et al., 2018; GUYTON; HALL, 2008).

Os macrófagos pró-inflamatórios (M1) são induzidos nas primeiras 24 a 48h no local da ferida e inicialmente expressam as citocinas TNF-α, IL-1, IFN-γ (NOSENKO; AMBARYAN; DRUTSKAYA, 2019; HE; MARNEROS, 2013; DELAVARY et al., 2011), IL-8 e IL-6) (MATSUSHIMA; YANG; OPPENHEIM, 2022; AVAZI et al., 2019; JIANG et al., 2012).

Após 48 a 96h, os macrófagos anti-inflamatórios (M2) atuam na limpeza do tecido (HE; MARNEROS, 2013; DELAVARY et al., 2011; GUYTON; HALL, 2008) e na degradação dos neutrófilos restantes, afim de eliminar a inflamação (GALLI et al., 2011; GUYTON; HALL, 2008). Além disso, auxiliam na transição para a fase proliferativa (YANEZ et al., 2017; RUH, 2013), com liberação dos fatores de crescimento endotelial vascular (VEGF), PDGF (WILLENBORG et al., 2012; DELAVARY et al., 2011), de crescimento epidérmico (EGF) e TGF-α e β (WILLENBORG et al., 2012), preparando o tecido local para a angiogênese e

granulação subsequente (RUH, 2013; ISAAC et al., 2010; TAZIMA; VICENTE; MORIYA, 2008; CAMPOS; BRANCO; GROTH, 2007). O TGF-β, liberado pelas plaquetas e outras células, auxilia na regulação da resposta inflamatória por inibição da síntese de IL-2, IFN-γ e TNF-α e indução da angiogênese e deposição de MEC (AKDIS et al., 2016).

Fisiologicamente a inflamação é mediada inicialmente por intensa ativação de fatores pró-inflamatórios e, posteriormente, esse evento é inibido por alterações na resposta imune, cedendo lugar a ativação de fatores anti-inflamatórios e consequentemente ao início da reparação estrutural/funcional do tecido (EL AYADI; JAY; PRASAI, 2020; GERMOLEC et al., 2018; WEISS, 2008; GUYTON; HALL, 2008). No entanto, a resolução de um evento inflamatório se condiciona diretamente à gravidade e intensidade da lesão, ao equilíbrio regulatório entre as vias pró-inflamatórias e anti-inflamatórias e a capacidade de resposta e adaptação imune do organismo afetado (EL AYADI; JAY; PRASAI, 2020; GERMOLEC et al., 2018; WEISS, 2008; GUYTON; HALL, 2008). Em casos de condições e/ou doenças com ativação contínua/crônica de mediadores inflamatórios, esse evento se prolonga culminando em inflamação persistente, podendo levar a destruição dos tecidos e fibrose (WULLAERT; BONNET; PASPARAKIS 2011; GERMOLEC et al., 2018; WEISS, 2008).

**Fase proliferativa:** Aproximadamente, ao 3º e/ou 4º dia após a lesão, um novo tecido conjuntivo/granulação começa a se formar simultaneamente com a neovascularização (angiogênese) e a reepitelização tecidual (SHEDOEVA et al., 2020; RODRIGUES et al., 2019; SHAW; MARTIN, 2016; KASUYA; TOKURA, 2014). Esta fase pode perdurar até a 2ª ou 3ª semana após o dano tecidual.

A angiogênese (migração de células endoteliais e formação de novos vasos e capilares) mediada pela ação do fator de crescimento endotelial vascular (VEGF) (MELINCOVICI et al., 2018; JONHNSON; WILGUS, 2014), do fator de crescimento de fibroblastos (FGF), PDGF, TGF-β e angiopoietinas se intensifica, permitindo a nutrição dos tecidos neoformados, a manutenção da homeostase e auxiliando na proliferação e regeneração do tecido (RODRIGUES et al., 2019; EILKEN; ADAMS, 2010). VEGF e seus receptores são expressos predominantemente em células endoteliais vasculares e linfáticas, mas também por monócitos, macrófagos, queratinócitos e fibroblastos (MELINCOVICI et al., 2018).

Uma mistura composta por vasos sanguíneos recém-formados com fibroblastos, macrófagos, colágeno do tipo III (COL-3) e outras proteínas da MEC (tecido de granulação) se estabelece no leito da ferida (BALBINO; PEREIRA; CURI, 2005). Quando o tecido de granulação alcança o nível da epiderme, completando-a, a circulação vai se restabelecendo (BALBINO; PEREIRA; CURI, 2005).

Ainda nessa fase, o número de macrófagos M2 e a remoção das fibras colágenas danificadas são aumentados; assim como a proliferação, migração e diferenciação dos fibroblastos em miofibroblastos, o depósito de matriz proteica (fibronectina, COL-3) e de outros elementos da MEC (proteoglicanos, elastina e laminina), levando a contração da ferida e posterior reparação da derme (ROSS; PAWLINA, 2016; TAZIMA; VICENTE; MORIYA, 2008; KIERSZENBAUM, 2008; CAMPOS; BRANCO; GROTH, 2007).

Durante a contração da ferida, o colágeno é organizado de forma perpendicular às bordas da ferida, aumentando a resistência mecânica do tecido. Nesse processo, a área a ser reepitelizada é reduzida. Os fibroblastos são diferenciados em miofibroblastos ricos em actina do músculo liso alfa (α-SMA) (TOMASEK et al., 2002), evento mediado principalmente através de regulação das interações célula-MEC e dos fatores de crescimento FGF e TGF-β (HINZ, 2015). É através desse processo, conhecido como fibroplasia, que a matriz extracelular vai sendo substituída por um tecido mais flexível e resistente (ROSS; PAWLINA, 2016; TAZIMA; VICENTE; MORIYA, 2008; KIERSZENBAUM, 2008; CAMPOS; BRANCO; GROTH, 2007).

Além disso, ocorre também a proliferação, diferenciação e migração dos queratinócitos e, das células epidermais e subjacentes a partir do estrato basal. Estas migram das bordas em direção ao centro da ferida, objetivando reepitelizar a ferida e restaurar as múltiplas camadas da epiderme (ROUSSELLE et al., 2019; ROSS; PAWLINA, 2016; KASUYA; TOKURA, 2014).

As citocinas IL-1 e TNF-α estimulam os fibroblastos (BARRIENTOS et al., 2008) a produzirem os fatores de crescimento dos queratinócitos (KGF) e de fibroblastos (FGF), que promovem a proliferação e migração dos queratinócitos (RUH, 2013; ISAAC et al., 2010; CAMPOS; BRANCO; GROTH, 2007). As metaloproteinases da matriz (MMPs), principalmente MMP-1 e MMP-9, sintetizadas por fibroblastos e macrófagos são cruciais para a atividade migratória dos queratinócitos (WAGER; LEAVESLEY, 2015), assim como a plasmina, visto que

esta facilita a migração e a degradação da matriz provisória rica em fibrina (ROUSSELLE et al., 2019).

Ao final desta fase e posteriormente, os queratinócitos ativados vão sendo revertidos para o fenótipo presente nas células da camada basal, através de mediação do TGF-β e a proliferação celular vai sendo reduzida, dando lugar a remodelação do tecido (PASTAR et al., 2014).

**Fase de remodelação:** Por volta da 2ª ou 3ª semana a ferida tende a regredir, passando por remodelação tecidual e reparo final. Porém, esses eventos podem perdurar por meses ou até anos, a depender dano inicial e estímulos presentes (SHEDOEVA et al., 2020; RODRIGUES et al., 2019).

A angiogênese regride, bem como a deposição dos elementos da MEC e o tecido de granulação vai sendo eventualmente substituído por tecido conjuntivo normal (GURTNER et al., 2008). Os fibroblastos são os principais responsáveis pela remodelação da MEC. Inicialmente substituem a fibrina por hialuronano, fibronectina e proteoglicanos e, posteriormente atuam sobre a formação das fibras de colágeno (DARBY et al., 2014).

Nesse momento, ocorre um aumento na resistência do tecido neoformado, devido a diminuição da deposição do COL-3 formado inicialmente e da parcial substituição deste por colágeno do tipo I (COL-1) (PLIKUS et al., 2017; DARBY et al., 2014), o que determinará a qualidade final da cicatriz e integridade cutânea (REILLY; LOZANO, 2021; SAN ANTONIO et al., 2020; RICARD-BLUM, 2011). Concomitante a substituição nos tipos de colágenos, ocorre também mudanças na organização destes na derme; de uma disposição de fibras paralelas/aleatórias para fibras entrelaçadas/organizadas ao longo das margens da ferida (DARBY et al., 2014; TAZIMA; VICENTE; MORIYA, 2008; GURTNER et al., 2008; CAMPOS; BRANCO; GROTH, 2007).

O colágeno é encontrado em grandes quantidades no organismo (SAN ANTONIO et al., 2020; RICARD-BLUM, 2011). Colágenos fibrilares (I, III e V), associado a fibrilas (XII, XIV, XVI e VI) e não fibrilares (IV e XVIII) estão presentes no tecido cutâneo (REILLY; LOZANO, 2021; XUE; JACKSON, 2015). Os tipos (I e III) comumente participam da cicatrização. Vias moleculares extracelulares e intracelulares complexas, envolvendo enzimas proteolíticas de membrana e a internalização de fragmentos e colágeno intacto seguido de clivagem enzimática, respectivamente, participam da degradação do colágeno (SAN ANTONIO et al., 2020), processo

estreitamente relacionado a inflamação, angiogênese e a reepitelização da ferida (MATHEW-STEINER; ROY; SEN, 2021; SAN ANTONIO et al., 2020).

Esse evento é equilibrado em parte pela ação dos fatores de crescimento PDGF e TGF-β (ISAAC et al., 2010), bem como, pelo aumento na expressão dos inibidores de metaloproteinases (TIMPs) e diminuição na expressão das metaloproteinases de matriz (MMPs) (CALEY; MARTINS; O'TOOLE, 2015), objetivando controlar a contração da ferida e a remodelação da derme. O PDGF estimula a degradação do COL-1 e aumenta a síntese do COL-3, enquanto que o TGF-β estimula a síntese de COL-1 (ISAAC et al., 2010).

As MMPs degradam colágeno e outros componentes da MEC (CALEY; MARTINS; O'TOOLE, 2015) e têm suas atividades controladas por citocinas liberadas pelas células inflamatórias, endoteliais, fibroblastos e queratinócitos (ISAAC et al., 2010).

O equilíbrio entre a síntese dos elementos proteicos da nova matriz e lise da MEC danificada constitui fator de grande importância no processo da remodelação tecidual e cicatrização final; visto que uma síntese excessiva de colágeno e de fatores de crescimento, bem como disfunções dos miofibroblastos pode levar a cicatriz hipertrófica ou queloides (PLIKUS et al., 2017; ELDER et al., 2011). Ambas as situações são distúrbios da cicatrização decorrentes principalmente de resposta inflamatória excessiva, descontrole na remodelação da MEC e excesso de colágeno (ELDER et al., 2011).

Por fim, as respostas do reparo tecidual vão regredindo e quando macrófagos, células endoteliais, fibroblastos e miofibroblastos anteriormente ativados se esvaem do local da ferida, a cicatrização pode então ser completada (LAROUCHE et al., 2018).

## 1.3 *Glycyrrhiza glabra* (alcaçuz)

### 1.3.1 Características botânicas do alcaçuz

A *Glycyrhiza glabra* é uma planta herbácea perene pertencente à família Fabaceae **(FIGURA 3A)**, originária da Ásia e sul da Europa e, conhecida popularmente como alcaçuz (WAHAB et al.; 2021; BARONE et al., 2020; AZEVEDO et al., 2018; DASTAGIR; RIZVI,

2016). O gênero *Glycyrhiza* Fabaceae agrupa cerca de 30 espécies, como por exemplo: *G. glabra, G. korshinskyi, G. eurycarpa, G. inflata,* entre outras (WAHAB et al.; 2021; FIORE et al., 2005).

A *G. glabra* apresenta folhas pinadas com comprimento de 7 a 15 cm, flores de colorações roxas esbranquiçadas à azuladas pálidas dispostas em inflorescência solta e frutos oblongos de 2 a 3 cm com muitas sementes. Cresce aproximadamente até 1 m de altura, sendo típica de solos arenosos, argilosos e/ou úmidos e, suas raízes são capazes de fixar nitrogênio devido à simbiose com bactérias *Rhizobium* (PASTORINO et al, 2018; FIORE et al., 2005).

Geralmente, as raízes são mais utilizadas para fins fitoterápicos e farmacológicos, entretanto, mais recentemente, alguns compostos presentes nas raízes também foram encontrados nas folhagens, em menores quantidades (SIRACUSA et al. 2011; HAYASHI; SUDO, 2009; DASTAGIR; RIZVI, 2016).

### 1.3.2 Fitoquímica e Compostos bioativos

Desde os tempos remotos, a *G. glabra* vem sendo estudada, buscando-se maior entendimento das propriedades nutritivas e farmacológicas presentes principalmente em suas raízes (BARONE et al., 2020; AZEVEDO et al., 2018). O alcaçuz possui proteínas, aminoácidos, polissacarídeos, sais minerais, pectinas e esteróis (WANG Q et al., 2015). Além disso, suas raízes são ricas principalmente em diversos compostos biológicos, como: triterpenos, saponinas (glicirrizina, ácidos liquirítico, botulínico e 18-β-glicérico) (BARONE et al., 2020; AZEVEDO et al., 2018; RIZZATO et al., 2017), estrógenos, fitoesteróis (sitosterol e estigmasterol), cumarinas, vitaminas (B1, B2, B3, B5, E e C) (WANG Q et al., 2015; SMMLER; PAULI; CHEN, 2013), chalconas e flavonoides (liquiritina, isoliquiritina e licurasídeo) (BARONE et al., 2020; AZEVEDO et al., 2018; RIZZATO et al., 2017; DASTAGIR; RIZVI, 2016).

O principal produto derivado do alcaçuz, a glicirrizina (G), uma saponina triterpenóide foi identificada e isolada em pesquisas fitoquímicas por volta de 1920 (YU et al., 2015; AZEVEDO et al., 2018). A G hidrolisada dá origem ao ácido glicirrízico (AG), um composto utilizado pela indústria farmacêutica e cosmética, por apresentar propriedades antitumoral, antialérgica, antiviral, antibiótica e anti-inflamatória (LEITE et al., 2022; BARONE et al., 2020; PASTORINO et al., 2018; SUN Z et al., 2019; SALEEM et al., 2011; CHERNG et al., 2011; SHIBATA, 2000).

Outros compostos isolados do alcaçuz, como o extrato etanólico e a glabridina (BARONE et al., 2020; AZEVEDO et al., 2018), bem como, o Glicirrizinato Dipotássio (DPG – $C_{42}H_{60}K_2O_{16}$) **(FIGURA 3B)**, produto secundário derivado do AG, também apresentam propriedades anti-inflamatória, antialérgica, antioxidante, antibacteriana, antiviral, antitumoral e gastroprotetora (VITALI et al., 2013; SHIM et al., 2012; ANDERSEN, 2007).

Desde então, o interesse pelo estudo das propriedades farmacológicas dos compostos do alcaçuz tem crescido bastante, buscando-se o desenvolvimento de produtos para auxiliar principalmente no tratamento de doenças/alterações inflamatórias.

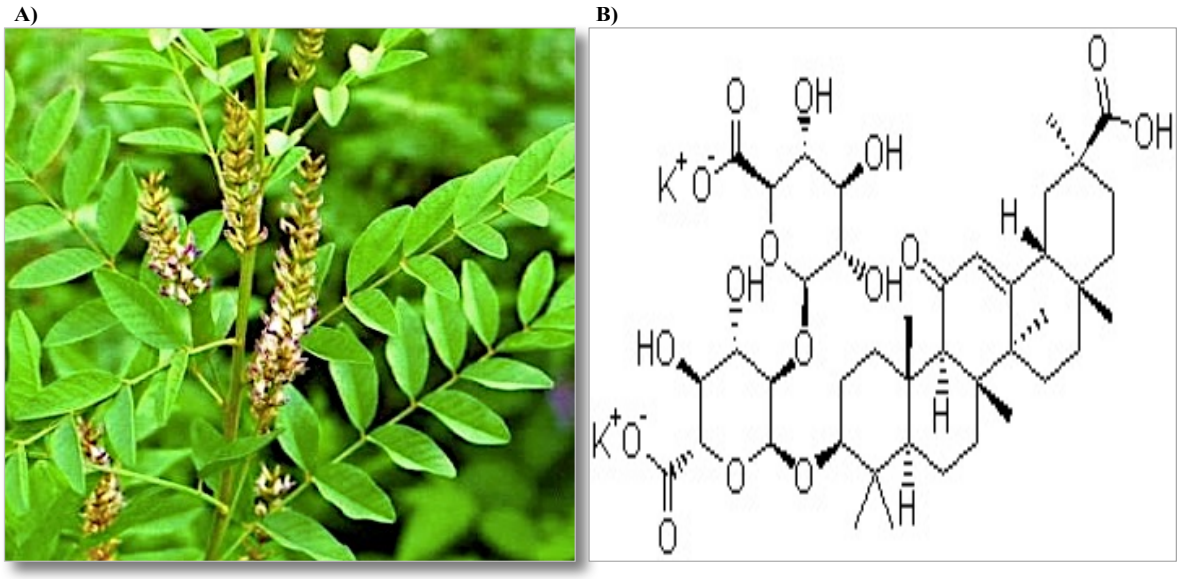


**FIGURA 3. A)** Imagem de espécime adulta da planta *Glycyrhiza glabra* (alcaçuz) apresentando flores. **B)** Estrutura molecular do Glicirrizinato Dipotássio (DPG – $C_{42}H_{60}K_2O_{16}$). Fonte: http://shalomnature.com/pt/chas/628-alcacuz-glycyrrhiza-glabra-100gr.html. Acessado em 10 set. 2019.

### 1.3.3 Propriedades farmacológicas do alcaçuz

O alcaçuz é um dos fitoterápicos mais antigos e populares do planeta. Conforme citado, seus principais derivados (glicirrizina, glabridina, ácidos 18β-glicirretínico e glicirrízico, hispaglabridina, liquiritina, glicirrizinato dipotássio, entre outros) possuem diversas propriedades farmacológicas: antioxidante, antitussígena, antiulcerativa, antimicrobiana, antiviral, antitumoral, anti-inflamatória, entre outras.

Os flavonoides, principalmente as isoflavonas (glabridina e hispaglabridina) desencadeiam atividade antioxidante (RACKOVA et al., 2007; SHARMA; PANDEY, 2013). A G auxilia na descongestão do trato respiratório acelerando a secreção de muco (SHARMA et al., 2017), assim como a liquiritina, bloqueia a tosse por inibição da capsaicina, desencadeando atividade antitussígena (KAMEI et al., 2003).

Promove efeito antiulcerativo, através de aumento da concentração de prostaglandinas no tubo digestivo e consequentemente da secreção de muco e aumento da proliferação celular (DAMLE 2014; JAFARIAN; GHAZVINI, 2007), assim como, prolonga a vida útil das células da mucosa estomacal (RAM et al., 2010).

As saponinas, alcaloides e flavonoides derivados do alcaçuz promovem atividade antimicrobiana sobre bactérias Gram-positivas e Gram-negativas (CHAKOTIKA et al, 2017; AJAGANNANAYAR et al., 2014; WANG L et al., 2015; GUPTA et al., 2008), assim como os triterpenóides (glicirrizina e ácido 18β-glicirretínico), promovem atividade antiviral com inibição da replicação viral (MICHAELIS et al., 2011), através de redução da ligação da proteína do grupo de alta mobilidade (HMGB1) ao DNA (WANG L et al., 2015) e, através de mediação das vias de sinalização das proteínas quinase C, caseína II e dos fatores de transcrição; proteína ativadora 1 (AP-1) e NF-kB (CINATI et al., 2003).

Além disso, os ácidos 18β-glicirretínico e glicirrízico (HUANG et al,. 2016; HASAN et al., 2016; LEE et al., 2008), bem como a glabridina (HSIEH et al., 2016; JIANG et al., 2016) induzem a transição da permeabilidade mitocondrial, levando a apoptose de células tumorais, sugerindo potencial terapêutico para o tratamento de tumores.

Atualmente, a G, o AG e o ácido glicirrizínico, vem sendo utilizados como coadjuvantes no tratamento de doenças reumáticas (AZEVEDO et al., 2018) e úlceras gástricas e duodenais (ANVISA-RDC n°26, 2014), respectivamente; afim de potencializar a ação terapêutica e reduzir os efeitos colaterais dos corticosteróides (AZEVEDO et al., 2018; ANVISA-RDC n°26, 2014; ANDERSEN, 2007).

Por fim, o DPG, apresenta ações; antitumoral (RICHARD, 2021; REHMAN 2020; BONAFÉ et al., 2019), regenerativa (BUENO, 2019), cicatrizante (LEITE et al., 2021), antibacteriana, antialérgica e anti-inflamatória similar aos corticosteróides, porém, não induzindo

aos efeitos colaterais (eritema, hiperidrose ou reações alérgicas cutâneas) observados no uso deste (VITALI et al., 2013; SHIM et al., 2012; ANDERSEN 2007).

Estudos demostraram que o AG assim como o DPG são capazes de inibir a proliferação celular, e induzir a apoptose em linhagens de células tumorais GBM (U251) (LI et al., 2014) e (U87MG e T98G) (BONAFÉ et al., 2019), respectivamente. De modo complementar verificou-se que o mecanismo de ação envolve a redução da proteína p65 (LI et al., 2014), responsável pela ativação da via NF-kB, a qual responde pela regulação de genes antiapoptóticos e fatores de invasão celular (BRASSESCO et al., 2013).

Mais recentemente, outros estudos sugerem um potencial efeito terapêutico do DPG na regeneração muscular (BUENO, 2017) e regeneração/cicatrização cutânea (LEITE et al., 2021) de camundongos e ratos, respectivamente. Relatou-se expressão precoce das proteínas 1 (MYOD) e miogenina (MYOG) após 3-24 horas e 3 dias, respectivamente, com formação de fibras musculares diferenciadas após tratamento da mionecrose causada por envenenamento botrópico (BUENO, 2017). Na cicatrização cutânea, houve proliferação epidérmica e reepitelização mais efetiva, bem como maior presença/distribuição de colágeno do tipo I nos animais tratados com DPG (LEITE et al., 2021).

### 1.3.4 Efeitos anti-inflamatórios

A ação anti-inflamatória do alcaçuz tem sido bem descrita desde os tempos mais remotos. Muitos estudos relatam a atividade anti-inflamatória, principalmente da glicirrizina e do ácido glicirrízico sobre condições inflamatórias dos sistemas respiratório, digestivo, hepático, renal, entre outros. Entretanto, estudos relativos a atividade anti-inflamatória do DPG são mais escassos.

A G desencadeia atividade anti-inflamatória através de inibição da isoenzima 11β-hidroxiesteroide desidrogenase (11β-HSD), o que impede a conversão do cortisol em cortisona levando a um consequente aumento do cortisol plasmático (AZEVEDO et al., 2018). Em edemas das vias respiratórias, inibe seletivamente a enzima pró-inflamatória 5-lipoxigenase (LOX-5) diminuindo a síntese de leucotrienos e a inflamação (AZEVEDO et al., 2018).

A G (WANG Y et al., 2018; SHEN et al., 2015), bem como, o AG (KIM et al., 2017) inibem a sinalização de HMGB-1 e consequentemente as respostas imune/inflamatória e proliferativa (WANG Y et al., 2018; KIM et al., 2017; SHEN et al., 2015) melhorando os sintomas inflamatórios da dermatite atópica (WANG Y et al., 2018) e reduzindo a proliferação de fibroblastos dérmicos (KIM et al., 2017), em camundongos. Ademais, a G também inibiu a ativação de mastócitos induzidos por rmHMGB-1, regulando a proteína tirosina quinase (CD117) e a via de sinalização NF-κB, em uma linhagem celular murina derivada de mastócitos (P815) (WANG Y et al., 2018), bem como, reduziu a expressão de mRNA de *Hmgb-1* e os níveis séricos de TNF-α e IL-1β em lesões cutâneas de ratos (SHEN et al., 2015) resultando em uma redução da inflamação cutânea.

No sistema hepático, o ácido 18β-glicirretínico inibe a formação de granulomas e a produção de citocinas inflamatórias através de redução da proteína inflamatória de macrófagos (MIP-1α), MyD88 e TNF-α (XIAO et al., 2010), assim como protege contra a inflamação hepática grave através de inibição de MAPK/NF-kB (YIN et al., 2017), em camundongos.

Sabe-se que ativação da via de sinalização do NF-κB pode promover a produção de citocinas pró-inflamatórias sustentando o processo inflamatório (WANG Y et al., 2018). Foi demonstrado que o AG é capaz de bloquear a via de sinalização do NF-κB dependente de ativação da proteína quinase ativada por mitógeno p38 (MAPK) e da fosfatidilinositol 3-quinase (PI3K/Akt), levando a inibição das citocinas iNOS e COX-2 e da expressão dos genes *IkBα* e *p65* (LIU W et al., 2018) e, consequentemente da inflamação cutânea, em camundongos. O AG também reduz a resposta inflamatória e é capaz de proteger as células contra a apoptose através de mediação da via MAPK/NF-κB e consequente translocação nuclear do NF-κB e do gene regulador de apoptose (*Bax*), resultando em diminuição da liberação das citocinas pró-inflamatórias (IL-1α e β; IL-1; TNF-α, COX-2, PGE2) em uma linhagem de queratinócitos humanos (HaCaT) (AFNAN et al., 2016).

Por fim, a ação anti-inflamatória do DPG refere-se ao seu efeito inibitório sobre a enzima hialuronidase, compreendida no dano à matriz extracelular e responsável pela liberação da amina vasoativa (histamina – $C_5H_9N_3$) a partir de grânulos presentes nos mastócitos e, sobre a liberação de mediadores inflamatórios, como os leucotrienos e as prostaglandinas (HUANG; ZHOU, 2022; VITALI et al., 2013; SHIM et al., 2012; ANDERSEN, 2007).

# 2. OBJETIVOS

## 2.1 Objetivo geral

Avaliar o efeito anti-inflamatório do DPG tópico em feridas cutâneas por segunda intenção, em modelo experimental *in vivo*.

## 2.2 Objetivos específicos

– Verificar o efeito do DPG na reepitelização.

– Identificar a presença de exsudato inflamatório e hiperemia ativa, de tecido de granulação e reepitelização.

– Quantificar o conteúdo de colágeno total.

– Avaliar a expressão de genes pró-inflamatórios (*Cox-2, Tnf-α, Il-8, Irak2, Nf-kβ e Il-1α*).

– Avaliar a expressão do gene anti-inflamatório (*Il-10*).

– Avaliar a expressão de genes proliferativos (*Vegf e Col-1*).

# 3. ARTIGOS PUBLICADOS

## 3.1. Capítulo I

LEITE, C. S.; BONAFÉ, G. A.; CARVALHO, S. J.; MARTINEZ, C. A. R.; ORTEGA, M. M.; RIBEIRO, M. L. The Anti-Inflammatory Properties of Licorice (*Glycyrrhiza glabra*)-Derived Compounds in Intestinal Disorders. **Int. J. Mol. Sci.**, v. 23, n. 8, p. 4121, 2022.

O propósito desta revisão foi examinar estudos recentes que abordam sobre as propriedades farmacológicas de alguns compostos bioativos derivados do alcaçuz e relatar suas propriedades anti-inflamatórias e antioxidantes, bem como seus efeitos terapêuticos em distúrbios do trato gastrointestinal. Apresentamos o amplo envolvimento dos compostos: Glicirrizina (GA), Ácido glicirretínico (GA) e Glicirrizinato dipotássio (DPG) em distúrbios gastrointestinais, bem como o potencial desses compostos para superar esses distúrbios.

Esses compostos possuem propriedades anti-inflamatórias e antioxidantes que afetam os distúrbios gastrointestinais por meio de diferentes mecanismos de ação. Isso fornece um histórico interessante para entender como os compostos G, GA e DPG agem e contribuem para o desenvolvimento de estratégias terapêuticas naturais e para o estabelecimento de modelos de pesquisa.

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*Review*

# The Anti-Inflammatory Properties of Licorice (*Glycyrrhiza glabra*)-Derived Compounds in Intestinal Disorders

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**Abstract:** Intestinal diseases, such as inflammatory bowel diseases (IBDs) and colorectal cancer (CRC), are a significant source of morbidity and mortality worldwide. Epidemiological data have shown that IBD patients are at an increased risk for the development of CRC. IBD-associated cancer develops against a background of chronic inflammation and oxidative stress, and their products contribute to cancer development and progression. Therefore, the discovery of novel drugs for the treatment of intestinal diseases is urgently needed. Licorice (*Glycyrrhiza glabra*) has been largely used for thousands of years in traditional Chinese medicine. Licorice and its derived compounds possess antiallergic, antibacterial, antiviral, anti-inflammatory, and antitumor effects. These pharmacological properties aid in the treatment of inflammatory diseases. In this review, we discuss the pharmacological potential of bioactive compounds derived from Licorice and addresses their anti-inflammatory and antioxidant properties. We also discuss how the mechanisms of action in these compounds can influence their effectiveness and lead to therapeutic effects on intestinal disorders.

**Keywords:** *Glycyrrhiza glabra*-derived compounds; glycyrrhizin (G); glycyrrhetinic acid (GA); dipotassium glycyrrhizinate (DPG); inflammation; oxidative stress; intestinal disorders



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## 1. Introduction

Licorice (*Glycyrrhiza glabra*) has been used in traditional Chinese medicine for thousands of years. Clinically, it is used widely to treat immune systems, respiratory, and digestive diseases [1–6], and no severe side effects have been reported so far [7]. In addition, Licorice-derived compounds possesses antiallergic, antibacterial, antiviral, anti-inflammatory, and anticarcinogenic effects [8–10]. These pharmacological properties aid in inflammatory disease treatment [11–13] (Figure 1).

The main bioactive compounds isolated from Licorice are glycyrrhizin (G) and glycyrrhetinic acid (GA) [14]. G is a triterpene glycoside complex and has been shown to possess cytotoxic effects against several cancer cell lines such as colon, lung, leukemia, melanoma, and glioblastoma (GBM) [9,15–21]. Additionally, the incidence of liver carcinogenesis in patients with hepatitis C was clinically reduced after G administration [22]. GA, an aglycone of G, has been demonstrated to have pro-apoptotic effects on human hepatoma, promyelocytic leukemia, stomach cancer, Kaposi sarcoma-associated herpesvirus-infected cells, and prostate cancer cells in vitro by inducing DNA fragmentation and oxidative stress [23–25]. In addition, several genotoxic studies have indicated that G is neither

teratogenic nor mutagenic and may possess anti-genotoxic properties under certain conditions [26,27]. As a result, there is a high level of use of Licorice and GZ in the US with an estimated consumption of 0.027–3.6 mg/kg/day [27].

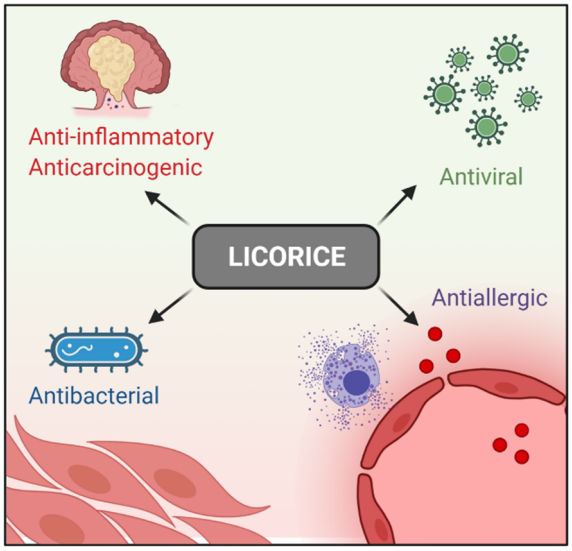


**Figure 1.** Licorice pharmacological properties.

However, GA oral efficacy is impaired due to its low solubility and permeability through the gastrointestinal mucosa [28]. It has been shown that GA administered through nanocarriers (GA-F127/TPGS-MMs) [29], micellar carrier based on polyethylene glycol-derivatized GA (PEG-Fmoc-GA) [30], and microparticles [31] increase absorption significantly [28–31]. Both G and GA have been prescribed for several therapeutic purposes, such as cancer and inflammation; however, side effects have pointed out the problem of their toxicity [32].

Dipotassium glycyrrhizinate (DPG), a dipotassium salt of GA, has been recently used as a flavoring and skin conditioning agent with demonstrated anti-allergic and anti-inflammatory properties [32]. It can inhibit leukotriene and reduce histamine levels with an apparent lack of adverse side effects [32–34]. In addition, it has been demonstrated that DPG has anti-inflammatory, antioxidant, immunomodulatory, anti-ulcerative, and antitumoral properties [11,13,35].

In this context, this review examines recent studies on the pharmacological properties of some bioactive compounds derived from Licorice and addresses their anti-inflammatory and antioxidant properties, as well as their therapeutic effects on gastrointestinal disorders.

## 2. G, GA, and DPG-Mediated Anti-Inflammation Regulation

As stated previously, Licorice compounds such as G, GA, and DPG have anti-inflammatory, antioxidant, antiviral, immunomodulatory, and antitumor properties [11–13]. Inflammation is an evolutionarily conserved, tightly regulated protective mechanism that comprehends immune, vascular, and cellular biochemical reactions. The normal inflammatory response is temporally restricted and, in general, beneficial to the host. Chronic inflammatory response, on the other hand, is a risk factor for the development of several diseases such as ischemic heart disease, stroke, cancer, and diabetes mellitus, among others [36,37]. Taking this into account, natural compounds have been widely used to treat all sorts of inflammatory conditions.

The anti-inflammatory effects of G and GA have long been reported. G has exerted anti-inflammatory actions by inhibiting the generation of reactive oxygen species (ROS) by neutrophils, the most potent inflammatory mediator at the site of inflammation [38]. Moreover, G has enhanced interleukin (IL)-10 production by liver dendritic cells in mice with hepatitis [39]. GA has presented anti-inflammatory and anticarcinogen effects on

several tumor cell lines such as human hepatoma (HLE), promyelocytic leukemia (HL-60), stomach cancer (KATO III), and prostate cancer (LNCaP e DU-145) by both DNA fragmentation and gene deregulation required for oxidative stress control [23–25].

More recently, one study exposed the U251 GBM cell line to different concentrations of GA (1 mM, 2 mM, and 4 mM), and the authors observed that the inhibition of cell proliferation and colony formation, apoptosis stimulation, and significant decrease in p65 protein, which is responsible for the activation of the nuclear factor kappa B (NF-κB) pathway [40]. The NF-κB pathway is constantly activated in GBM and is responsible for the aggressiveness of the disease and regulation of the expression of anti-apoptotic genes, and cell adhesion and invasion factors [41]. Thus, some studies have suggested that the inhibition of the NF-κB pathway could decrease the resistance of tumor cells to chemotherapy and contribute to increasing the survival of patients with GBM [42–45].

Recently, a study has shown that DPG exposure has anti-tumoral effects on GBM cell lines (U87MG and T98G) through cell proliferation decrease and apoptosis stimulation. Furthermore, DPG anti-tumoral effect was related to NF-κB pathway suppression by *IRAK2* and *TRAF6* mediating miR-16 and miR-146a, respectively. Finally, the authors have also shown that DPG was able to inhibit the subpopulation of stem cells essential for tumor formation, survival, and recurrence [46].

## 3. G, GA, and DPG-Mediated Crosstalk between Inflammation and Oxidative Stress Pathways

Oxidative stress consists of an imbalance of endogenous pro-oxidant and antioxidant activities, characterized by excessive formation of high ROS and reactive nitrogen species (RNS) [47]. Small amounts of ROS are synthesized physiologically and act on cell homeostasis; however, in the disease context, the excessive synthesis of ROS disrupts the antioxidant defense system, causing cellular apoptosis [47]. This condition is commonly associated with oxidative changes such as lipid peroxidation, protein carbonylation, carbonyl adduct, nitration, and DNA impairment as well as the induction of inflammatory processes, leading to several diseases [48,49]. Cyclooxygenase type 2 (Cox-2) and inducible nitric oxide synthase (iNOS) enzymes, responsible for the release of pro-inflammatory mediators, prostaglandin E2 (Pge-2), and nitric oxide (NO), play relevant roles in oxidative and acute inflammatory processes [49].

The high mobility group box 1 (Hmgb1) cytokine plays an important role in the pathologic process of endothelial permeability under oxidative stress [49]. DPG and G have presented antioxidant effects due to their negative modulation of Hmgb1 in the DSS-induced colitis mice model [49]. It has been shown that G inhibits Hmgb1-cytokine secretion by blocking the Cytochrome C release and caspase-3 activity, consequently inhibiting apoptosis in inflammation-related stroke rat models [50,51]. In addition, the G compound decreases the iNOS, TNF-α, IL-1β, and IL-6 expression levels by the modulation of p38 mitogen-activated protein kinases (p38-MAPK) and c-Jun *N*-terminal kinase (p-JNK) signaling pathways in brain vascular cells [51] and by preventing oxidative stress and apoptosis through the inhibition of p38-MAPK, p-JNK, and NF-κB signaling pathways in lung cells [52].

Accordingly, the G compound can inhibit oxidative stress and inflammatory response by attenuating the activity of the Hmgb1 and NF-κB signaling pathways, with decreased levels of malondialdehyde (MDA) and cytokines (TNF-α, IL-1β and IL-6) in lung cells [53]. Moreover, G increases glutathione-S-transferase (GSTs) levels, decreases MDA, and negatively regulates the expression of TNF-α, IL-6, iNOS, and monocyte chemotactic protein-1 (MCP-1) in liver cells [54]. G compound has been shown to suppress NF-κB pathway through inhibiting the toll-like receptor 4 (TLR4) in renal cells [55] and reducing the formation of intracellular ROS. Moreover, an activation of the AMP/nuclear factor erythroid-2-related factor-2 (NRF2) pathways in vitro was observed, positively regulating the antioxidant enzymes, HO-1, NQO-1, and GCLC and negatively regulating TNF-α, IL-1β, and IL-6 [56].

According to descriptions, GA also suppresses oxidative stress and neuroinflammation induced by A1C13 through TLR4/NF-κB signaling pathway inhibition [57]. In accordance, one study has observed that GA was able to attenuate oxidative stress and neuroinflammation induced by rotenone reducing the activation of the ionized calcium-binding adapter molecule-1 (Iba-1), preventing glutathione depletion, lipid peroxidation inhibition, and attenuation of the induction of COX-2 and iNOS [58]. In addition, a restored mitochondrial complex I and IV, a reduction in the generation of ROS, the release of Cytochrome C, and ultimately cell apoptosis inhibition after exposure to GA in brain tissue of adult Sprague Dawley Rats were observed [59].

GA can suppresses lipopolysaccharide (LPS)-induced oxidative stress, inflammation, and apoptosis through activation of the extracellular signal-regulated kinase (ERK) pathway, and inhibition of the NF-κB in renal cells [60]. GA also suppresses oxidative stress and inflammation through activation of the NRF-2 and HO-1 pathways and IκB and NF-κB p65 signaling inhibition in cardiac cells [61].

In the liver tissue of rats, GA inhibits NTiO2-induced apoptosis by superoxide dismutase (SOD) and glutathione peroxidase (GPx) activation [61]. Moreover, it has been shown that GA can inhibit caspase-3 and -9 at mitochondria in HepG2 cells, positively and negatively regulating Bcl-2 and Bax proteins expression, respectively [62]. Table 1 summarizes the studies used in this review.

**Table 1.** Summary of studies showing the autoinflammatory and anti-tumoral effects of G, GA, and DPG.

| Model | Compound (Dose) | Mechanism | Reference |
|---|---|---|---|
| In vitro (KATO III and HL-60) | G (1 to 10 mg/mL) | Antitumor activity ↑ apoptosis | [23] |
| In vitro (HLE, KATO III, and HL-60) | G (0.1 to 1 mg/mL) | Antitumor activity ↑ apoptosis | [24] |
| In vitro (DU-145 and LNCaP) | G (1 to 20 mM) | Antitumor activity ↑ apoptosis | [25] |
| In vitro (Caco3, HT29, and RAW 264.7)<br>In vivo (Acute lung injury mice model) | DPG (300 µM)<br>DPG (3 and 8 mg/kg/day) | ↓ TNF-α, IL-1β, and IL-6, as well as HMGB1 receptors, RAGE and TLR4 | [34] |
| In vitro (neutrophils) | G (0.05, 0.5, and 5.0 µg/mL) | ↓ ROS | [38] |
| In vivo (Con A-induced hepatitis)<br>Ex vivo (liver dendritic cells) | G (2 mg/mouse)<br>G (0.1 mg/mL) | ↑ IL-10 and ↓ liver inflammation | [39] |
| In vitro (U251) | GA (1, 2, 4 mM) | Anticancer effect ↓ proliferation and ↑ apoptosis possibly related to the NF-κB mediated pathway | [40] |
| In vitro (U87MG and T98G) | DPG (0.1 to 2 mM) | Anticancer effect ↓ proliferation and ↑ apoptosis. ↓ NF-κB pathway | [46] |
| In vivo (DSS-induced colitis mice model) | DPG (8 mg/kg/day) | ↓ colitis, at the earlier stages, ↓ inflammation though AMPK-COX-2-PGE. At later times ↓ iNOS and COX-2 in HMGB1-dependent manner | [49] |
| In vivo (mechanical thrombectomy rat model) | G (2, 4 and 10 mg/kg/day) | ↓ HMGB1 and its downstream inflammatory factors, and ↓ oxidative stress | [50] |
| In vivo (Focal cerebral I/R injury rat model) | G (4 mg/kg/day) | ↓ HMGB1 and ↑ apoptosis through the blockage of the JNK and p38 | [51] |
| In vivo (Sepsis-induced acute lung injury rat model) | G (25 and 50 mg/kg/day) | ↓ inflammatory responses, oxidative stress damage, and apoptosis though ↓ NF-κB, JNK, and p38 MAPK | [52] |
| In vivo (Acute lung injury mice model) | G (20 and 40 mg/kg/day) | ↓ LPS-induced lung injury via blocking HMGB1/TLRs/NF-κB pathway | [53] |

**Table 1.** *Cont.*

| Model | Compound (Dose) | Mechanism | Reference |
| --- | --- | --- | --- |
| In vitro (RAW 264.7 and bone marrow monocytes) | G (25 to 100 μM) | ↓ RANKL-induced osteoclastogenesis and oxidative stress through ↑ AMPK/Nrf2 and ↓ NF-κB and MAPK | [56] |
| In vivo (Parkinson rat model) | GA (50 mg/kg/day) | ↓ dopamine neuron loss and ↓ Iba-1 and GFAP ↑ antioxidant enzyme activity, ↓ lipid peroxidation, ↓ pro-inflammatory cytokines | [58] |
| In vivo (Vascular dementia rat model) | GA (20 mg/kg/day) | ↓ release of cytochrome-c and ↑ Bcl2, and ↑ the endogenous antioxidants | [59] |
| In vitro (HBZY-1)<br>In vivo (sepsis-induced acute kidney injury mice model) | GA (50 and 100 μM)<br>GA (25 and 50 mg/kg/day) | ↓ oxidative stress via ↑ ERK signaling pathway. ↓ NF-κB | [60] |
| In vivo (myocardial ischemic injury-rat model) | GA (10 and 20 mg/kg/day) | ↓ oxidative stress and inflammatory cytokines. ↑ Nrf2 antioxidant response ↓ NF-κB activation | [61] |
| In vitro (HEPG2) | G (5, 25 and 125 μg/mL) | ↓ $H_2O_2$-induced oxidative stress, ↑ apoptosis | [62] |
| In vitro (HT29) | GA (1, 5 and 10 μM) | ↓ TNF-α-mediated IL-8 through ↓ MAPK and the IKB/NF-κB pathway | [63] |
| In vivo (DSS-induced colitis mice model) | GA (10 and 50 mg/kg/day) | ↓ colitis, ↓ inflammation by regulating COX-2 and NF-κB | [64] |
| In vivo (rat model of ulcerative colitis) | G (40 mg/kg/day) | ↓ colitis, ↓ inflammatory injury via suppression of NF-κB, TNF-α, and ICAM-1 | [65] |
| In vivo (TNBS-induced experimental colitis mice model) | G (10, 30 and 90 mg/kg/day) | ↓ colitis, ↓ IFN-γ, IL-12, TNF-α, and IL-17 and ↑ IL-10 | [66] |
| In vivo (DSS-induced colitis rat model) | G (2 mg rectally) | ↓ colitis, ↓ IL-1β, IL-6, TNF-α, Cxcl-2, Mcp1, and MPO | [67] |
| In vivo (TNBS-induced experimental colitis rat model) | GA (2, 10 and 50 mg/kg, rectally and 10 mg/kg/day) | ↓ colitis, ↓ serum levels of TNF-α and IL-1β, ↓ colon MPO and MDA, and ↑ SOD | [68] |
| In vivo (rat model of ulcerative colitis) | G (100 mg/kg/day) | ↓ colitis, when combined with emu synergistically ↓ of PPARγ and TNF-α | [69] |
| In vivo (TNBS-induced experimental colitis mice model) | G (50 mg/kg/day) | ↓ colitis, ↓ HMGB1 on DC/macrophage mediated Th17 proliferation | [70] |
| In vivo (indomethacin-induced small intestinal injury mice model) | GA (100 mg/kg/day) | ↓ TNF-α, IL-1β, and IL-6, ↑ indomethacin-induced small intestinal damage | [71] |
| In vivo (DSS-induced colitis mice model) | G (100 mg/kg/day) | ↓ colitis, regulated the phosphorylation of transcription factors such as NF-κB p65 and Iκ B α | [72] |
| In vivo (DSS-induced colitis mice model) | DPG (8 mg/kg/day) | ↑ mucosal healing by ↓ CXCL1, CXCL3, CXCL5, PTGS2, IL-1β, IL-6, CCL12, CCL7; ↑ wound healing genes COL3A1, MMP9, VTN, PLAUR, SERPINE, CSF3, FGF2, FGF7, PLAT, TIMP1 and ↑ extracellular matrix remodeling genes, VTN, and PLAUR | [73] |

## 4. Therapeutic Effect of G, GA, and DPG for Intestinal Disorders

Crohn's Disease (CD) and Ulcerative Colitis (UC) are the main inflammatory bowel diseases (IBD) that affect the gastrointestinal tract. These diseases are characterized by chronic and progressive inflammation of the gastrointestinal tract, associated with extraintestinal manifestations such as arthritis, uveitis, erythema nodosum, gangrenous pyoderma,

and cholangitis [74,75]. It has been shown that inflammatory reaction and the increased production of ROS are commonly associated with the pathogenesis of IBD [49,76]. ROS are toxic to cells and their overproduction causes breakage of the various lines of defense that make up the mucosal barrier [76]. The dysregulation of the immune response and the exaggerated release of pro-inflammatory/interleukin cytokines (IL-1, IL-6, IL-8, and TNF-α) culminate in the exacerbation of intestinal inflammation [63,64]. Thus, oxidative stress is considered an initial step to the colonic epithelium inflammation of patients with IBD.

Patients with IBD are at increased risk of developing colorectal cancer (CRC). Epidemiological data from patients with UC estimate that the risk of CRC is approximately 2- to 3-fold more than the general population, and patients with CD appear to have a similar increased risk [77]. Chronic inflammation is the most important aspect of neoplastic progression, resulting in dysplastic precursor lesions that may arise from different areas of the colon [78]. The overproduction of ROS can damage the DNA of the chronically inflamed colonic mucosa cells, increasing the mutation rate in genes related to development of CRC [76,79]. Oxidative reactions are an integral part of the inflammatory response and are generally associated with CRC development [80]. The potential mechanisms for the natural alkaloids in the treatment of UC has been recently described, showing that its positive effects are closely related to the modulation of oxidative stress, immune response, intestinal microbiota, and improvement of the gut barrier function [81]. Considering that oxidative stress is one of the main factors related to the development of IBD and IBD-related CRC, it is possible that the use of active principles found in *Glycyrrhiza glabra* extract may be effective for the treatment and prevention of both diseases [82] (Table 1).

Several studies have evaluated the effectiveness of G, GA, and GL in experimental models of induced colitis [65–68,83]. The oral administration or application of enemas containing these drugs, alone or associated with other substances with anti-inflammatory activity, can reduce the inflammatory process of the colonic mucosa, and oxidative tissue damage as well as improve epithelial healing of the colonic mucosa [68,83].

Yuan et al. were the first authors to evaluate the effects of Glycyrrhizinate extract in an experimental model of acetic acid-induced colitis [65]. The authors described that Glycyrrhizinate extract has a potent anti-inflammatory effect that is mediated by the suppression of NF-κB, TNF-α, and ICAM-1 in colonic mucosa. Three years later, Sun et al. investigated the therapeutic potential of G in trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis in mice [66]. After colitis induction by TNBS, G was administered by gavage (10 mg/kg, 20 mg/kg, and 30 mg/kg) for 10 days. G significantly ameliorated TNBS-induced colitis and dose-dependently decreased macroscopic and microscopic inflammation scores, and MPO activity. Mechanistically, G downregulated the colonic levels of the pro-inflammatory cytokines IFN-α, IL-12, TNF-α, and IL-17 and increased the anti-inflammatory cytokine IL-10. The efficacy of G topical application in the treatment of a rat model of UC was also evaluated in experimental colitis models induced by dextran sodium sulfate (DSS). G significantly ameliorated the extent of colitis, which was associated with a decrease in the expression levels of pro-inflammatory cytokines and chemokines, including interleukin IL-1β, IL-6, TNF-α, CXCL2, and CCL2 in the inflamed mucosa. G also inhibited MPO activity in the inflamed mucosa and had a therapeutic effect on experimental colitis in rats [67]. A synergistic effect was observed when G was combined with emu oil in a colitis rat model induced by acid acetic. The authors observed that the treatment combination significantly improved their ability to reduce macroscopic and microscopic lesions as well as to decrease MPO levels and enhanced downmodulation on PPARγ and TNF-α expression [69]. More recently, Chen et al. reported that G ameliorated colitis and decreased the production of inflammatory mediators such as HMGB1, IFN-γ, IL-6, TNF-α, and IL-17. Furthermore, G suppressed the proliferation of Th17 cells in colitis and inhibited the ability of dendritic cells and macrophages to induce the differentiation of Th17 cells that was enhanced in the presence of HMGB1 [70].

To find the best route of administration, Liu et al. compared rectal and oral treatments with GA in TNBS-induced colitis in rats. Both rectally and orally administered treatment

effectively attenuated colitis at different dosages. Furthermore, administration by both routes decreased serum levels of TNF-α and IL-1β, colon MPO activity and MDA concentration, and elevated SOD activity [68]. It has been demonstrated that GA is capable of blocking prostaglandin-E2 synthesis via blockade of COX-2 resulting in concurrent augmentation of nitric oxide production on indomethacin-induced small intestinal injury in mice [71]. In addition, using an ulcerative colitis mice model, GA reduced IL-6 and IL-1β, regulating the phosphorylation of NF-κB and IkB-α, and the expression of COX-2 and PGE2 in an ulcerative colitis model [72]. The anti-inflammatory mechanisms of both G and GA were described as mediated by IFN-y, TNF-alpha, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, and IL-17. Moreover, G and GA mediated intercellular adhesion molecules 1, P-selectin, iNOS, and the NF-κB pathway through the nuclear translocation of NF-κB and activation of STATs 3 and 6 [11]. In human colonic epithelial cell line HT-29, GA exhibits the inhibitory activity on TNF-α and IL-8 production and the blockade of the MAPK and the IKB/NF-κB pathways [63].

The use of DPG as a therapeutic strategy to overcome intestinal inflammation was also evaluated. Vitali et al. studied the DPG effects on HMGB1, an early pro-inflammatory cytokine that is released from injured cells during inflammation. In vitro assays show that DPG significantly reduces the release of HMGB1 as well as expression levels of pro-inflammatory cytokines, TNF-α, IL-1β and IL-6. In vivo, DPG decreases the severity of DSS-induced colitis as well as intestinal inflammation reduction mediated by a downregulation of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, as well as HMGB1 receptors, RAGE, and TLR4 [34]. Posteriorly, the same research group showed that DPG has a protective effect on colitis and inflammation through the inhibition of oxidative tissue stress [49]. It was observed that DPG can decrease oxidative stress through the inhibition of iNOS and COX-2 ameliorating DSS-induced colitis in mice. It was demonstrated in vitro that DPG decreases inflammation-related oxidative stress, through (i) an earlier ability to promote AMP-activated kinase (AMPK)-phosphorylation and (ii) a later HMGB1-dependent mechanism. Moreover, DPG can also improve colonic inflammation in DSS-induced colitis mice model through downregulation of the pro-inflammatory genes (CXCL1, CXCL3, CXCL5, PTGS2, IL-1β, IL-6, CCL12, and CCL7), and upregulation of genes involved in healing (COL3A1, MMP9, VTN, PLAUR, SERPINE, CSF3, FGF2, FGF7, PLAT, and TIMP1), which contribute to accelerating intestinal mucosa repair in induced colitis [73]. It has recently been shown that DPG increases the expression of the receptors farnesoid-X-receptor (FXR), pregnane-X-receptor (PXR), and G-protein-coupled-receptor (GPCR; TGR5), decreasing the oxidative stress and consequently intestinal/hepatic inflammation in DSS colitis animal model, with a decrease in IL-8 [84]. Figure 2 summarizes the main mechanism of action of G, GA, and DPG.

The evidence from all these experimental studies suggests that the bioactive compounds from Licorice (*Glycyrrhiza glabra*) have anti-inflammatory and antioxidant effects in intestinal disorders through different mechanisms of action. Considering that these molecular features are also important in human intestinal disorders, it is reasonable to assume that Licorice might have similar activity in humans. Therefore, several clinical studies have focused on the pharmacological effects of Licorice on intestinal diseases (Table 2). However, to date, there is no clinical evidence showing the effect of Licorice in patients with IBD.

It has also been shown that Licorice has potentially serious side effects for humans [27]. Clinical studies have shown that the most important side effects of Licorice and *glycyrrhizin* are hypertension and hypokalemic-induced secondary disorders [85]. Biochemical studies indicate that G inhibits 11beta-hydroxysteroid dehydrogenase, the enzyme responsible for inactivating cortisol. As a result, continuous, high-level exposure to GZ compounds can produce hypermineralocorticoid-like effects in both animals and humans [7,27]. Chronic use of Licorice can lead to hypokalemia and hypertension. Some people are more sensitive to licorice exposure [7]. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased 11-beta-hydroxysteroid dehydrogenase activities, hypertension, and anorexia nervosa [85]. These side-effects are reversible upon withdrawal

of Licorice or Glycyrrhizin [27]. It can be assumed from these data that the consumption of Licorice extract products presents no concern for safe use as a supporting drug in patients with IBD. However, multicenter, randomized clinical studies that include a larger number of patients are still necessary to verify the benefits of using Licorice extracts in the treatment of IBD, and as a natural therapeutic strategy to prevent CRC in patients with extensive and active forms of long-term IBD.

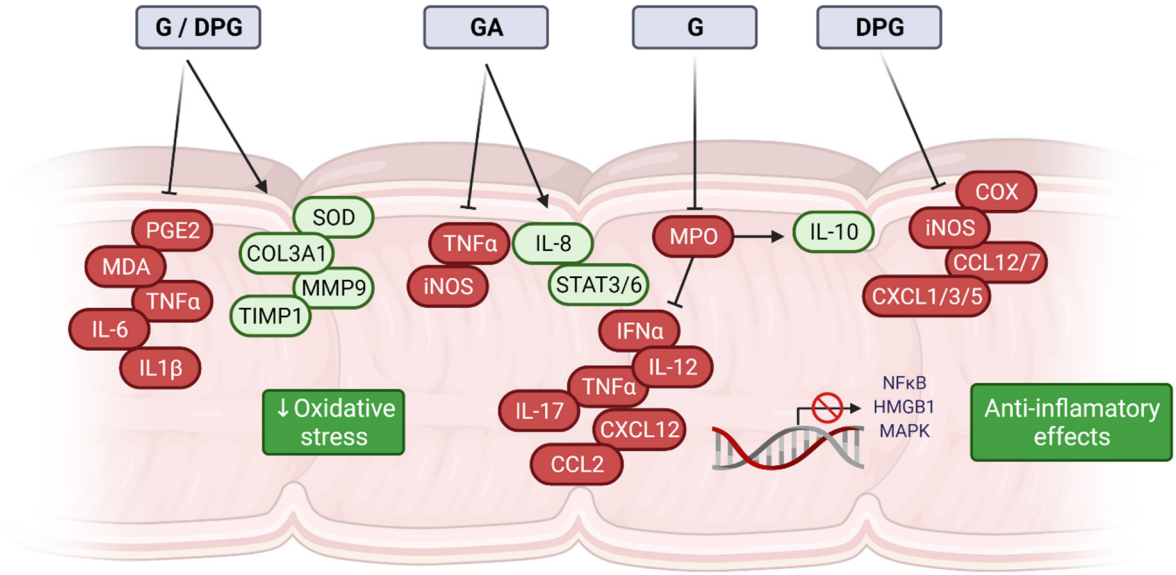


**Figure 2.** Molecular mechanisms of *Glycyrrhiza glabra*-derived compounds in intestinal disorders. Compounds derived from *Glycyrrhiza glabra* have anti-inflammatory potential. G, GA, and DPG act through the inhibition of HMGB1, TLR4, and RAGE receptors and significantly regulate important cytokines, interleukins, and genes involved in the inflammatory process. These effects are related to the capacity of regulating important inflammatory signaling pathways such as HMGB1, NF-κB, and MAPK. Oxidative stress is significantly reduced because of cellular and molecular changes, and consequently, the inflammatory process is attenuated as a result of treatment with these compounds.

**Table 2.** Clinical trials with Licorice in intestinal disorders.

| Drug | Clinical Trial | Phase | N of Pts | Status | Diseases | Results |
|---|---|---|---|---|---|---|
| Traditional Chinese Medication (containing 3 g of Licorice) | NCT03135821 | 2, 3 | 104 | Unknown | Irritable bowel syndrome | NA |
| Traditional Chinese Medicine (17 g herbal extract containing G) | NCT00676975 | 2 | 104 | Complete | Irritable bowel syndrome | NA |
| Modified Gegen Qinlian Decoction (containing 6 g of Licorice) | NCT04057547 | 1 | 60 | Recruiting | Ulcerative colitis | NA |
| Modified Gegen Qinlian Decoction (containing 6 g of Licorice) | NCT04312477 | 1 | 60 | Recruiting | Irritable bowel syndrome | NA |
| Traditional Chinese Medicine (17 g herbal extract containing 2 g of G) | NCT04368663 | NA | 100 | Recruiting | Pneumatosis cystoides intestinalis | NA |

Abbreviation: NA, not available.

## 5. Conclusions and Future Perspectives

The broad involvement of Licorice-derived compounds in intestinal disorders and their potential to overcome these disorders and the mechanism of action is presented in this review. In summary, the evidence from all these experimental studies suggests that the bioactive compounds obtained from Licorice have anti-inflammatory and antioxidant properties that affect anti-intestinal disorders through different mechanisms of action.

This provides an interesting background for understanding how G, GA, and DPG compounds act and contributes to the development of natural therapeutic strategies and to the establishment of research models. In addition, more research is needed to determine the mechanism of action in different biological activities. Clinical trials on G, GA, and DPG are also required to validate these pharmacological effects, to establish these compounds as promising pharmaceuticals, and to fill some gaps regarding their safety and toxicological characteristics.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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## 3.2 Capítulo II

LEITE, C. S.; BONAFÉ, G. A.; PIRES, O. C.; SANTOS, T. W.; PEREIRA, G. P., PEREIRA, J. A.; ROCHA, T.; MARTINEZ, C. A. R.; ORTEGA, M. M.; RIBEIRO, M. L. Dipotassium glycyrrhizininate improves skin wound healing by modulating inflammatory process. **Int. J. Mol. Sci.**, v. 24, n.4, p. 3839, 2023.

O propósito deste estudo foi avaliar os efeitos do DPG na cicatrização de feridas em ratos após 3, 7 e 14 dias de tratamento, abordando o envolvimento de fatores pró e anti-inflamatórios.

Evidenciamos o potencial do Glicirrizinato dipotássio (DPG) sobre a atenuação do processo inflamatório e promoção da reepitelização tecidual, bem como sua modulação sobre a expressão de genes pró e anti-inflamatórios, proliferativos e remodeladores.

Nossos achados nos levaram à conclusão adicional de que o DPG reduz a inflamação ao promover a cicatrização de feridas na pele por meio da modulação de vários mecanismos e vias de sinalização, tais como: anti-inflamatório, por meio da modulação da expressão de citocinas pró e anti-inflamatórias; promoção de novo tecido de granulação, angiogênese e reepitelização tecidual, indicada pela modulação do VEGF e estimulação da síntese de colágeno; e contribuindo para a reepitelização tecidual.

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# Dipotassium Glycyrrhizininate Improves Skin Wound Healing by Modulating Inflammatory Process

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**Abstract:** Wound healing is characterized by a systemic and complex process of cellular and molecular activities. Dipotassium Glycyrrhizinate (DPG), a side product derived from glycyrrhizic acid, has several biological effects, such as being antiallergic, antioxidant, antibacterial, antiviral, gastroprotective, antitumoral, and anti-inflammatory. This study aimed to evaluate the anti-inflammatory effect of topical DPG on the healing of cutaneous wounds by secondary intention in an in vivo experimental model. Twenty-four male Wistar rats were used in the experiment, and were randomly divided into six groups of four. Circular excisions were performed and topically treated for 14 days after wound induction. Macroscopic and histopathological analyses were performed. Gene expression was evaluated by real-time qPCR. Our results showed that treatment with DPG caused a decrease in the inflammatory exudate as well as an absence of active hyperemia. Increases in granulation tissue, tissue reepithelization, and total collagen were also observed. Furthermore, DPG treatment reduced the expression of pro-inflammatory cytokines (*Tnf-α*, *Cox-2*, *Il-8*, *Irak-2*, *Nf-kB*, and *Il-1*) while increasing the expression of *Il-10*, demonstrating anti-inflammatory effects across all three treatment periods. Based on our results, we conclude that DPG attenuates the inflammatory process by promoting skin wound healing through the modulation of distinct mechanisms and signaling pathways, including anti-inflammatory ones. This involves modulation of the expression of pro- and anti-inflammatory cytokine expression; promotion of new granulation tissue; angiogenesis; and tissue re-epithelialization, all of which contribute to tissue remodeling.

**Keywords:** skin wound healing; inflammation; DPG; animal model; rats

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## 1. Introduction

Wound healing is characterized by a systemic and complex process of cellular and molecular activities that occur naturally, aiming to repair the affected region anatomically and functionally. This event is composed of four basic phases: hemostatic, inflammatory, proliferative, and remodeling or maturation, which involve cell division, proliferation, migration, neovascularization, synthesis of protein elements, contraction, re-epithelialization, and structural remodeling of the injured tissue [1–3]. After tissue injury, multiple responses (vasoconstriction, platelet buffering, and coagulation) are triggered to restore local hemostasis [2,4]. Within 24 h, an inflammatory state characterized by vasodilation,

increased vascular permeability, tissue edema, neutrophil migration, macrophage activation, and the release of pro-inflammatory cytokines sets in at the site [5–7]. In this period, cytokines, interleukins, and chemokines [8] stimulate the synthesis of other pro-inflammatory mediators [9,10], as well as the release of prostaglandins, increasing the inflammatory condition [11]. Subsequently, starting on the third or fourth day, a new tissue (granulation) begins to form simultaneously with neovascularization and tissue re-epithelialization [1,2,12], an event that can last for up to 2 or 3 weeks. Finally, after the second or third week, the wound tends to regress, undergoing tissue remodeling and final repair. However, this event can last for months or years, depending on the initial damage and the persistence of stimuli [1,2,13].

Natural compounds, especially those derived from various plant species, have been used successfully in studies on the treatment of cutaneous wounds and inflammatory changes in animal models [14,15]. Licorice is the common name for the perennial herbaceous plant *Glycyrhiza glabra*, which is a member of the Fabaceae family and is native to Asia and southern Europe. It has been studied since ancient times for a better understanding of the nutritional and pharmacological properties present mainly in its roots. These roots are rich in several biological compounds, such as saponins [16,17], estrogens, phytosterols, coumarins, vitamins [18,19], chalcones, and flavonoids [16,17,20]. Glycyrrhizin (G), the main triterpenoid saponin derived from licorice, found in about 41.84-114.33 mg/g [21], is known to give rise to glycyrrhizic acid (GA), a compound that has antitumor, antiallergic, antiviral, antibiotic, and anti-inflammatory properties [16,22–25]. In addition, dipotassium glycyrrhizinate (DPG), a side product derived from GA, has antiallergic, antioxidant, antibacterial, antiviral, anti-inflammatory [26–28], and antitumor [29,30] effects. More recently, it has been described as having gastroprotective [22] and healing [31] properties related to DPG.

It has been described that the anti-inflammatory action of DPG refers to its inhibitory effect on the enzyme hyaluronidase, which is involved in damage to the extracellular matrix and is responsible for the release of histamine from granules present in mast cells, as well as that of inflammatory mediators such as leukotrienes and prostaglandins (PGs) [26–28,32]. This effect is similar to those of corticosteroids, but does not induce the side effects (i.e., erythema, hyperhidrosis, or allergic skin reactions) observed with the use of these drugs [26–28].

Although a recent study found that rats treated with DPG experienced effective epidermal proliferation and re-epithelialization, as well as an increase in type I collagen levels [31], the mechanism of action of this chemical on other crucial stages of wound healing has hardly been investigated. Thus, this work aimed to evaluate the effects of DPG on wound healing in rats after 3, 7, and 14 days of treatment, addressing the involvement of pro- and anti-inflammatory factors.

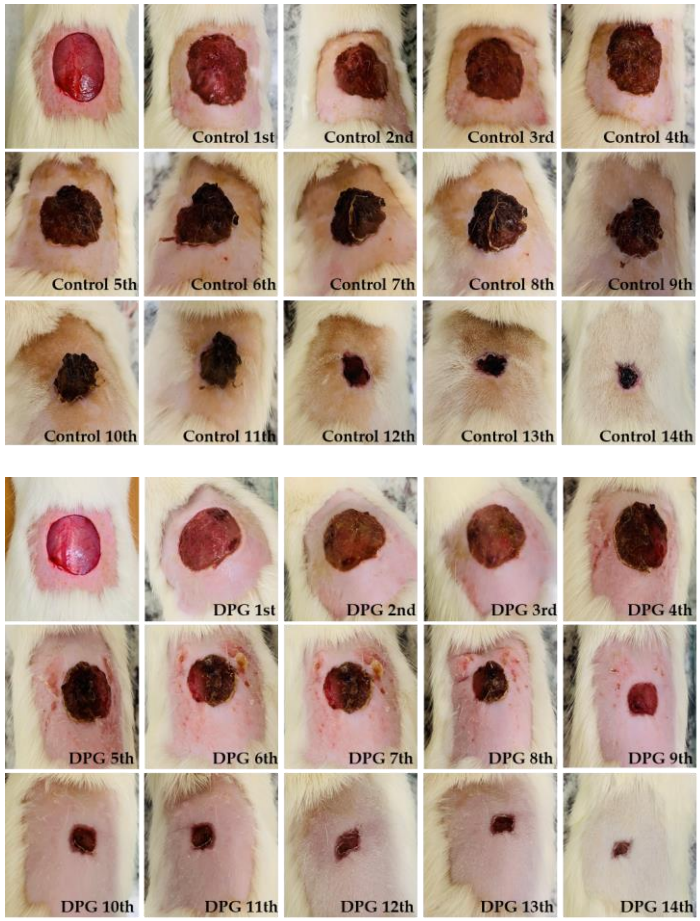
## 2. Results and Discussion

### *2.1. DPG Attenuates Inflammation, Promoting Formation of New Granulation Tissue and Tissue Re-Epithelialization*

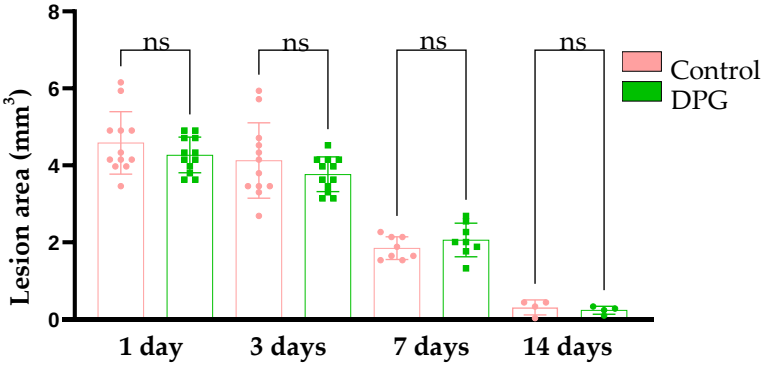
Wound healing simultaneously involves distinct cellular types in its hemostatic, inflammatory, proliferative, and tissue remodeling phases [2]. Thus, it is important to emphasize that the speed and efficiency of wound repair, as well as scar formation, are closely related to a balance between inflammatory and proliferative responses [33,34].

According to the macroscopic observations, it was noted that both the treated groups (DPG) and the untreated groups (Control) showed tissue re-epithelialization at the end of the 14th day of experimentation. Although the wounds in the DPG-treated groups were apparently smaller, there were no significant differences between the groups when compared at days 1, 3, 7, and 14 (day 1, DPG vs. Control ($p$-value = 0.2630); day 3, DPG vs. Control ($p$-value = 0.1924); day 7, DPG vs. Control ($p$-value = 0.4871) and; day 14, DPG vs. Control ($p$ = 0.8460)) of experimentation, respectively (Figure 1A,B). These results support

a prior study in which the authors noted progressive healing of the excisional incision following 7, 14, and 21 days of DPG treatment [31].




(**A**)




(**B**)

**Figure 1.** (**A**) Representative macroscopic images of wound healing areas of untreated adult animals (Control—left panel) and those treated with DPG (DPG—right panel) throughout the experimental period (0 to 14 days); (**B**) Quantitative analysis of the measurements of wound healing areas on days 1, 3, 7, and 14, ns—non significant.

Physiologically, the inflammatory process manifests singular characteristics, such as inflammatory exudate (vasodilation, increased permeability, and recruitment of leukocytes) and active hyperemia (congested vessels and reddish tissue coloration) [6]. The data

obtained in this study indicate that there was a trend towards reduction of the inflammatory exudate after treatment with DPG, suggesting a potential anti-inflammatory effect of DPG (Figure 2A,E). At the third and seventh days of treatment, there was a trend toward decreased active hyperemia in the DPG-treated groups when compared to the control group. However, on day 14, hyperemia was significantly absent in the DPG group ($p$-value 0.05). Although no significant differences were initially observed between the groups, the total absence of active hyperemia in the animals treated on day 14 may suggest that the DPG promoted an anti-inflammatory effect (Figure 2B,E). These findings agree with other studies and suggest that the DPG may have promoted a more accentuated reduction in vasodilation, vascular permeability, leukocyte infiltration, release of pro-inflammatory mediators, and, consequently, inflammation, thereby inducing an improvement in subsequent cell proliferation [6,7]. Additionally, glycyrrhizin, a compound similar to DPG, was demonstrated to be able to inhibit NO synthesis and decrease inflammation, increase cell proliferation, and speed up wound healing in vitro by a subsequent study evaluating anti-inflammatory, antioxidant, and proliferative activities [35].

On the third day, we noticed the absence of granulation tissue in both groups, which is physiologically normal during this period. On the seventh day, it was significantly higher in the DPG group ($p$-value < 0.05). On the 14th day, the granulation tissue was similar in both groups (Figure 2C,E). Once the inflammatory process is over, the path towards reconstruction of the damaged tissue continues, and intense cell proliferation and migration take place. During the proliferative phase, new tissue (granulation) begins to form simultaneously with angiogenesis and tissue re-epithelialization [1,2,12]. In general, the formation of granulation tissue composed of fibroblasts, COL-III, new vessels, scattered macrophages, and VEGF-permeated connective tissue begins between the fourth and fifth day after the initiation of tissue healing [36,37]. In our study, we observed that there was a greater amount of granulation tissue in the treated animals around the seventh day, a phase of intense cell proliferation. The use of collagen-containing microcapsules containing glycyrrhizin has also been shown to increase hydroxyproline content, capillary, and fibroblast proliferation in granulation tissue, as well as to increase and uniform collagen fiber formation, promoting neovascularization and wound healing in rats [38].

On day 3, there was an absence of re-epithelialization in both groups, which is physiologically normal during this period. This tissue begins to form between the sixth and fourteenth day after the start of tissue repair. On the seventh day, this characteristic showed a tendency to increase in the DPG group. However, on day 14, the increase in re-epithelialization was significantly greater in the DPG group ($p$-value 0.0001) (Figure 2D,E). From the seventh day of healing, depending on the characteristics of the wound, re-epithelialization tends to occur. It is characterized by the migration of keratinocytes and epithelial cells from the wound margins toward the center and the subsequent formation of skin appendages [37,39]. Keratinocytes stimulated by keratinocyte growth factor (KGF) [40,41] repair the epithelial layer [36,42]. Taken together, our findings suggest that DPG may positively influence cell proliferation and migration in the dermis and epidermis, and, thus, the scar. Consistent with previous studies, more effective epidermal proliferation and re-epithelialization were observed during the proliferative phase of skin healing in rats treated with DPG for 7 days [31]. Additionally, rats given glycyrrhizin showed decreased inflammation, accelerated re-epithelialization at day 8, better tissue remodeling, and enhanced collagen deposition at day 16 [43].

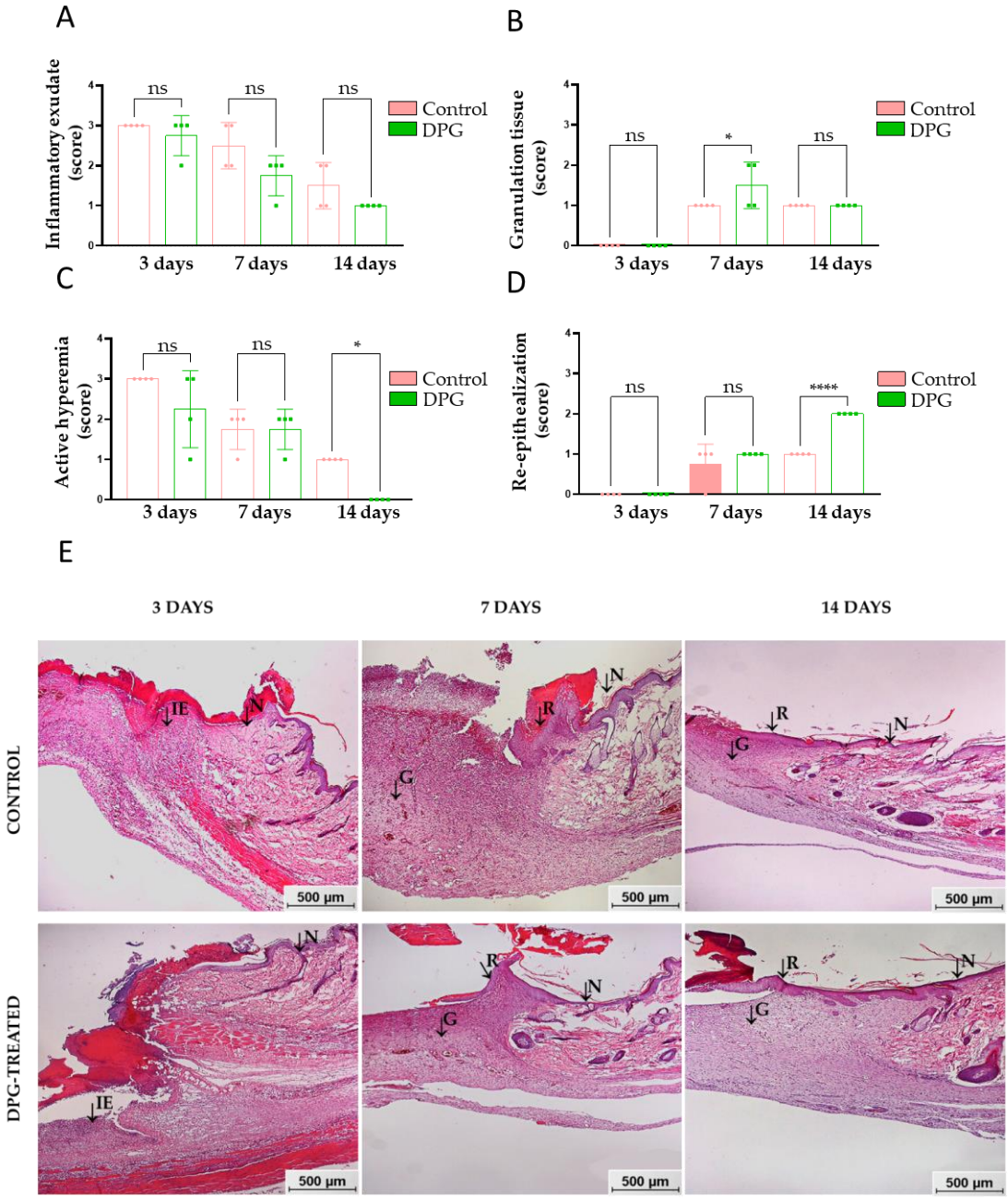



**Figure 2.** Semi-quantitative characteristics of wound healing areas at days 3, 7, and 14 of experimentation. (**A**) Inflammatory exudate; (**B**) active hyperemia, DPG vs. Control, day 14 (* $p < 0.05$); (**C**) granulation, DPG vs. Control, day 7 (* $p < 0.05$); (**D**) re-epithelialization, DPG vs. Control, day 14 (**** $p < 0.0001$); (**E**) representative histological images of the wound healing areas of the animals. Note the presence of normal tissue (N), inflammatory exudate (IE), granulation tissue (G), and re-epithelialization tissue (R). HE staining: 50× magnification (bar = 500 µm).

Concerning total collagen, at days 3 and 7, although there was no significant difference, a relatively higher density of total collagen content was observed in the DPG group. On day 14, it was observed that treatment with DPG promoted a significant increase in total collagen levels ($p$-value < 0.05) (Figure 3A,B). Similar improvements in healing were seen during skin wound remodeling in DPG-treated rats, as was an increase in the presence/distribution of type I and III collagens [31]. Furthermore, histological examinations of the wound sites in rats treated with licorice revealed an increase in collagen synthesis/deposition as well as re-epithelialization. [44].

During proliferation, the deposition and organization of a provisional matrix rich in collagen, mainly type 3, also occur [45]. Fibroblasts initially synthesize greater amounts of COL-3 and, later, COL-1 [45], with 80–90% of the collagen present in the normal reticular dermis generally being type 1, arranged in intertwined and organized fibers [46]. Subsequently, during wound contraction, the collagen is arranged perpendicular to the wound edges, while the area to be re-epithelialized is reduced. In the course of proliferation and remodeling of the tissue, the myofibroblasts contract, reapproximating the edges of the wound, and the provisional ECM is replaced by a more resistant tissue, which determines features of the skin's integrity and the final quality of the scar [45,47]. In agreement, we saw greater type I collagen expression during the 14-day tissue remodeling phase, primarily in the DPG-treated group, indicating a more robust recovery.

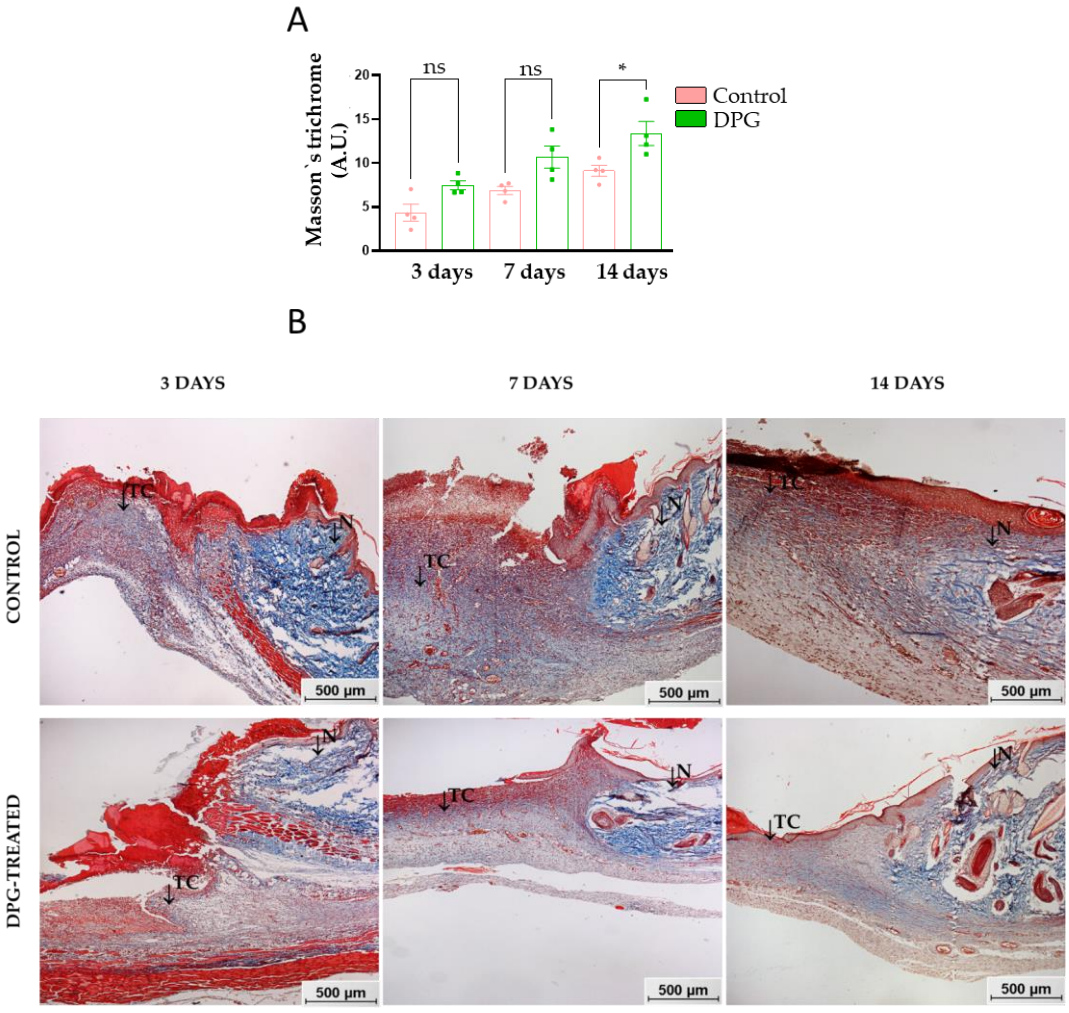



**Figure 3.** (**A**) Quantitative pixel density evaluation of total collagen content in wound scar areas over days 3, 7, and 14 of experimentation, DGP vs. Control, day 14 (* $p < 0.05$). (**B**) Representative histological images of the wound scar areas of the animals, both untreated and treated with DPG, throughout the experimentation period. Note that the presence of normal tissue (N) and the presence of total collagen (TC) is highlighted. TM staining: 50× magnification (bar = 500 µm).

### *2.2. DPG Modulates the Expression of Pro- and Anti-Inflammatory, Proliferative, and Remodeling Genes*

In the present work, we also evaluated the effects of DPG ($C_{42}H_{60}K_2O_{16}$) in pro-inflammatory (*Cox-2*, *Tnf-α*, *Nf-kb*, *Il-1α*, *Il-6*, *Il-8*, *Irak-2* e *Hmgb-1*), anti-inflammatory (*Il-10*), angiogenic (*Vegf*), and structural remodeling (*Col-1*) genes, mainly to understand its effect on the wound repair of rat skin.

In response to tissue injury, several pro-inflammatory factors are synthesized and secreted by neutrophils, macrophages, and other cells, as well as anti-inflammatory mediators and growth and structural factors, which act in the different phases of wound healing [45]. At first, platelets, leucocytes, and endothelial cells release inflammatory mediators, mainly COX-2 [48,49], IL-1 e IL-8 [11,50,51], TNF-$\alpha$, IFN-$\gamma$, CXCL1, CXCL8 [52,53], and adhesion molecules [6,7]. These mediators and others recruit large numbers of inflammatory cells [54–56], mainly neutrophils [52,53], to the wound site, which aim to degrade the damaged matrix and remove the damaging agent, preventing infection [53,57].

Following the above, in the present study, the gene expression levels of several pro-inflammatory markers were investigated. We discovered that DPG treatment had an immediate effect, significantly lowering *Tnf-α* expression (Figure 4A). Similarly, we also observed that on the third and seventh days, there was a significant decrease in the expression levels of *Cox-2*, *Il-8,* and *Irak2* in the DPG groups (Figure 4B–D). In the case of *Nf-kb* and *Il-1*, we observed mRNA repression after 7 days, as well as after 7 and 14 days of DPG treatment (Figure 4E,F, respectively). Finally, DPG significantly increased the expression of *Il-10* after the third, seventh, and fourteenth days (Figure 4G). Il-10 has been demonstrated to diminish neutrophil and macrophage infiltration at the site of injury, as well as the release of pro-inflammatory cytokines (Il-1b, Il-6, and TNF-$\alpha$), when it is present in infiltrating epidermal and mononuclear cells [58]. A murine full-thickness wound model has shown that the use of IL-10 (ovIL-10) is effective in reducing macrophage infiltration and suppressing pro-inflammatory mediators, which accelerate granulation tissue formation and re-epithelialization and enhance wound revascularization, positively regulating skin repair [59]. As a result, we saw that DPG administration raises Il-10 levels, lowers the expression of pro-inflammatory cytokines, and reduces inflammatory infiltration. Our data show that DPG helps to improve re-epithelialization by lessening the inflammatory process overall.

Specifically, TNF-$\alpha$, which is constitutive in cutaneous tissue [8,60], is released mainly by neutrophils, lymphocytes, mast cells, macrophages, and keratinocytes during the inflammatory response [6]. It is also able to induce and control the inflammatory process through binding to TNFR1-p55 and TNFR2-p75, triggering pro-inflammatory signaling cascades [61]. TNF-$\alpha$ activates and regulates in-loop NF-kB, a factor that regulates the transcription of genes encoding pro-inflammatory cytokines and also mediates keratinocyte survival and proliferation [62]. Its signaling rapidly and transiently activates the transcription of NF-kB-dependent target genes, such as IL-1β, IL-6, and IL-8 [63,64]. In fact, we observed accentuated *Tnf-α* and *Nf-kb* reductions on the third and seventh days, respectively (Figure 4A–E). DPG also promoted significant increases in *Il-10* expression (Figure 4G) and in the total collagen content during the three evaluated periods (Figure 3A), suggesting an apparent anti-inflammatory and proliferative mediator's modulation. Our findings also suggest that DPG decreased *Tnf-α* expression on the third day (Figure 4A), which may contribute to the significant reductions in *Nf-kb* and *Il-8* on the seventh day of treatment (Figure 4C–E). TNF-$\alpha$, IL-1, IL-6, and NF-kB levels were significantly reduced in rats treated for 3, 7, and 14 days with lupeol, a natural triterpene found in olive, fig, mango, carrot, soybean, melon seed, and grapes. In contrast, increased IL-10 levels were verified. As a result, the NF-kb pathway was disrupted, resulting in decreased inflammation, angiogenesis, and improved collagen proliferation and deposition in all three exposure periods [65]. It has been demonstrated that DPG was able to drastically lower the expression levels in a distinct biological setting. *Tnf-α, Il-1β,* and *Il-6,* through *Hmgb1* inhibition in vitro, reduced intestinal epithelial inflammation and the severity of colitis in animals [66]. Additionally, DPG can reduce the renal inflammatory process by blocking *Tnf-α* and *Il-1β*, in vitro and in vivo, through the NF-kB and MAPK inhibitory pathway [67].

Furthermore, it is known that moderate levels of TNF-$\alpha$ favor skin recovery by recruiting immune/inflammatory cells, contributing to the normal transition between the inflammation and cell proliferation phases [33,68]. On the other hand, a drastic reduction

in leukocyte infiltration and expression of pro-inflammatory cytokines, in addition to excessive angiogenesis, less re-epithelialization, and fibrous tissue formation, was reported in mice with TNFR1-p55 inactivation [68]. In contrast, excess TNF-$\alpha$ retards the proliferation of keratinocytes and fibroblasts, leading to persistent inflammation and delayed skin healing [33,68]. We observed a repression, but not a suppression, of *Tnf-α* during the inflammatory phase, which remained stable during proliferation in the treated groups (Figure 4A). It is suggested that DPG might possibly balance the expression of *Tnf-α* and dependent cytokines, promoting anti-inflammatory effects without impairing the physiological transition between the inflammatory and proliferative phases. In a psoriasis-like murine inflammatory model and in epidermal keratinocytes (HaCaT), it has been demonstrated that glycyrrhizin can reduce inflammation and enhance healing through regulation of Tnf-$\alpha$-induced ICAM-1 expression via NF-kB/MAPK signaling [69].

NF-kB also has a role in the regulation of homeostasis in response to inflammatory stimuli, expressing cytokines [62], cell adhesion molecules, and growth factors during healing [70]. NF-kB acts primarily through activation of the canonical IkB/NF-kB signaling pathway [71,72]. Similar to TNF-regulation, the persistence of NF-kB activation can induce chronic inflammation on the one hand, but on the other, its suppression also causes negative changes and, in some cases, inflammation [73]. Thus, it is clear that a careful balance between TNF-$\alpha$ and NF-kB activation and inhibition is required for the maintenance of cellular homeostasis as well as the induction and resolution of skin inflammation during healing, as observed in our study. The repression, but not suppression, of *Nf-kb* seen in treated animals (Figure 4E) suggests that DPG may possibly promote an anti-inflammatory effect by balancing the expression of Nf-kb and dependent cytokines. Accordingly, it has been demonstrated in a study utilizing glycyrrhizic acid, another licorice derivative, that this substance can quicken wound healing in a mouse skin model. Mechanistically, it has been discovered that the NF-kB signaling pathway mediates increased cell migratory activity and inhibition of the inflammatory process [74].

COX-2 can modulate PGE2 synthesis during the inflammatory phase by mediating TNF-$\alpha$/IL-8 signaling pathways [6,75], such as MAPKp38/NF-kB/AP-1 [76–78], among others, thus increasing the inflammatory status. It was reported that in rat wounds, COX-2 was expressed mainly in the basal layer of the epidermis, peripheral hair follicle cells, and fibroblast-like cells and capillaries around the wound 12 h after injury, reaching a peak at day 3 [79]. Subsequently, a gradual decrease in COX-2 was reported between days 3, 5, 7, and 14, as well as high levels on day 3 and a gradual decrease on days 7 and 14 in curcumin-treated rats and controls, respectively [80]. Moreover, in a murine model of a pressure ulcer, it was reported that the selective COX-2 inhibitor celecoxib can repress iNOS, Cox-2, and PGE2 and, consequently, the inflammatory process, promoting cell differentiation and re-epithelialization with improved healing [81]. Similarly, in our study, we observed significantly decreased expression of *Cox-2* mRNA on days 3 and 7, which remained lower on day 14 in the animals treated with DPG. In control animals, expression gradually increased between days 3 and 7 and decreased on day 14 (Figure 4B). These findings suggest that the repression of *Cox-2* by DPG in the initial phase of the repair allows the tissue to recover more rapidly, since it diminishes the synthesis of PGE, thus reducing inflammation.

The inhibition of Cox expression using Cox-1 and 2 siRNA or ibuprofen, as well as by diclofenac, caused a reduction in PGE2 and VEGF release in HaCaT cells and mouse excisional wounds, respectively, leading to a consequent impairment of neovascularization, both in vivo and in vitro [82]. On the other hand, we found a likelihood of a balance in *Vegf* expression in the treated groups (Figure 4H), suggesting that DPG, in addition to remarkably reducing the inflammatory process, may have also promoted positive and early effects on vascular proliferation (neoangiogenesis). In another study, COX-2 inhibition by topical hesperidin hydrogels accelerated dermal regeneration in mice, resulting in early wound contraction and reducing mean healing time by 5–7 days, with increased collagen [83]. Conversely, COX-2 overexpression can stimulate aberrant inflammatory

and fibrogenic responses, leading to severe inflammation [84], and can exacerbate fibroblast proliferation and collagen synthesis, leading to abnormal healing [85], events that could be suppressed by DPG.

Other cytokines, such as IL-8, also act mainly in the acute phase of the inflammatory process [6,86,87]. IL-8 is expressed by leukocytes, monocytes, and macrophages, and to a lesser extent by fibroblasts, endothelial cells, and keratinocytes [87]. It is secreted in response to inflammatory stimulation, primarily through the induction of IL-1, TNF-, and IFN-, and it functions by binding to CXCR1, CXCR2, IL-8R, and IL-8RA on inflammatory and endothelial cells [51,88]. High levels of IL-8 and TNF-$\alpha$ are known to increase the secretion of other pro-inflammatory cytokines, exacerbating inflammation [89–91] and decreasing the proliferative and migratory capacities of epithelial cells and fibroblasts, thus directly contributing to delayed healing and the induction of chronic wounds [92]. In our study, we found that DPG significantly reduced *Il-8* expression during the inflammatory and proliferative phases (Figure 4E), as well as Tnf-$\alpha$ expression during the inflammatory phase (Figure 4A). It is suggested that DPG suppresses the expression of *Il-8* and, consequently, of other cytokines induced by these, mainly in inflammatory cells.

In immune and inflammatory cells, IRAK-2-mediated IL-1/TRL signaling controls inflammation [93,94]. IRAK-2 activity is critical for the TRL and/or IL-1R signaling pathways [95], promoting early activation of NF-kB and induction of inflammatory mediators [96]. In skin inflammation, IRAK-2 acts mainly on keratinocytes, triggering the regulation of an alternative epidermal differentiation pathway through effects on the epidermal differentiation-associated transcription factor (TF-ZNF750) [90], which enhances the immune response, thus elevating the pro-inflammatory condition [97,98]. In the present study, we observed a suppression in the expression of Irak-2, mainly during the inflammatory and proliferative phases, in treated animals (Figure 4D), suggesting a possible modulation of the DPG on some of the signaling pathways mentioned above. IRAK-2-deficient mice have been shown to synthesize reduced levels of pro-inflammatory cytokines [99], and they are more resistant to inflammatory processes and septic shock [100,101]. There are few studies on IRAK-2 in skin healing, and little is known about the function of its signaling in the epidermis under healthy and/or inflammatory conditions. However, the relationship of IRAK-2 activation to the severity of chronic inflammatory skin diseases (psoriasis and dermatitis cutanea) [97] and tumor progression [93] is clear. Therefore, as previously mentioned, the *Irak-2* suppression observed in the treated animals suggests that relevant activity of DPG is involved in the attenuation of cutaneous inflammation through negative modulation of *Irak-2* (Figure 4D).

Furthermore, during the inflammatory phase, inactive IL-1 is induced by TLR/TNF activation or IL-1 receptor activation by active IL-1 or IL-1 [101,102]. IL-1 can stimulate the secretion of other acute phase cytokines, COX-2, and PGEs through activation of the ERK1/2, MAPKp38 and JNK signaling pathways [103–105]. In addition, it can stimulate inflammation via the MAPK/AP-1 pathways [106] and IL-1/NF-kB [105] in fibroblasts and the Myd88/TRAF6/NF-kB pathway in epidermal stem cells [107]. In our study, there was similar low expression of *Il-1* in treated and control animals at day 3, but at days 7 and 14, this expression was remarkably repressed by DPG.

At the end of the inflammatory phase and during the transition to the proliferative phase, the inflammatory mediators are important in promoting the synthesis and activity of the inflammatory cells that previously predominated, gradually providing more room for the anti-inflammatory factors that regulate or re-establish physiological balance and the subsequent intense proliferation [6,7,108]. In the meantime, macrophages (M1) give way to macrophages (M2) [109], which degrade the remaining neutrophils, eliminating inflammation, and act in the transition to proliferation [110,111]. Monocytes, macrophages (M2), lymphocytes, and epidermal cells release growth factors [6,112,113] and anti-inflammatory cytokines [114]. These cytokines, mainly IL-10, attenuate skin inflammation by directing subsequent angiogenesis, granulation, re-epithelialization, and regeneration [114,115], in addition to helping to prevent fibroproliferative disorders [114,116]. Skin IL-

10 expression has been shown to reduce TNF- and IL-1 mRNA expression in vitro [112] and in vivo [117], as well as to attenuate the inflammatory response via the IL-10R/STAT3 signaling pathway, regulating TLR4/NF-kB in dermal fibroblasts and reducing fibroblast differentiation, ECM deposition, collagen network contraction, and hypertrophic scar formation [112,117]. In our study, we verified an accentuated and significant increase in the expression of *Il-10* in all periods (3, 7, and 14 days) (Figure 4G), mainly during the inflammatory phase, as well as a reduction in *Tnf-α* and *Nf-kb* in treated animals (Figure 4A-E). It is suggested that a positive modulation of the DPG leads to the induction of anti-inflammatory genes, which, consequently, may help to achieve a balance between inflammatory response and resolution and tissue proliferation and reorganization, leading to the formation of a more uniform scar without signs of cutaneous fibrosis.

We also evaluated the effect of treatment on the expression levels of the marker *Vegf*. The data obtained in this work indicate that initially, at 3 days, DPG induces the expression of this gene. At 7 days, however, repression by treatment was observed. On day 14, no significant differences were observed between the two groups (Figure 4H). VEGF is one of the most important pro-angiogenic factors. It is secreted by endothelial cells, macrophages, keratinocytes, and fibroblasts in this period [42,118], and removes pro-angiogenic effects through binding to FLT-1/VGFR1, FLK-1/KDR/VEGFR2, and FLT-4/VEGFR3 receptors [118,119]. Studies on the expression pattern of VEGF in skin healing in mice have reported that mRNA *Vegf* and VEGF levels increase in the first 24 h [120,121], rising gradually on days 3 and 5 and returning to normalization between the 7th [121,122] and 14th days [121]. In our study, we observed a significant increase in *Vegf* expression on day 3, with a tendency to gradually decrease between days 7 and 14 in treated animals. These findings suggest that initially, the DPG may have stimulated early angiogenesis by inducing a greater release of VEGF during the inflammatory phase (Figure 4H). Subsequently, it may have contributed to maintaining the balance of Vegf synthesis by fibroblasts and keratinocytes during cell proliferation, resulting in better tissue recovery and scar formation without evidence of fibrosis. In rat wounds, collagen and glycyrrhizin microcapsules have been demonstrated to up-regulate Vegf and miRNA-21, encouraging neovascularization [38]. In rat wounds treated with licorice extract, improved healing was also seen, along with increased angiogenesis and collagen deposition, which was mediated by the upregulation of *Vegf*, *Fgf*, and *Tgf* expression levels [44].

Ultimately, we evaluated the effects of treatment on the expression of *Col-1*. The data presented in this work indicate that after only 14 days of treatment with DPG, an increase in the expression of this gene was observed (Figure 4I). As already mentioned, during the wound remodeling phase, the COL-3-rich transitional ECM is replaced by a fibrillar, interwoven, and organized COL-1-based scar [45,122–124]. It has been reported that increased Col-1 expression and hydroxyproline levels were found during proliferation and remodeling (7 and 14 days) and inflammation and remodeling (3 and 14 days), respectively, in the healing of diabetic rats treated with calcium alginate [125]. Additionally, it was shown that the application of amitriptyline-based nanoparticles (Amitrip) to diabetic rat wounds could quicken tissue remodeling by increasing hydroxyproline levels and collagen deposition due to up-regulation of Vegf and Col-1 [126]. In our study, we observed a higher level of synthesis of total collagen in animals treated during proliferation and remodeling (7 and 14 days) (Figure 3A), as well as a higher level of expression of *Col-1* in animals treated during remodeling (14 days) (Figure 4I). These data suggest that the DPG, in addition to inducing an increase in total collagen, may also have more precisely balanced the transition from COL-3 produced initially to COL-1, which is predominant in the mature scar, without inducing fibrosis. In agreement, it has been demonstrated that using injectable hydrogels loaded with DPG (HP-3/DPG10) promotes efficient tissue remodeling in mice by decreasing inflammation, promoting quick wound healing, and increasing collagen deposition. Additionally, although the literature suggests a link between elevated collagen synthesis and the development of tissue fibrosis, this fact is more

closely linked to an escalation of the inflammatory process [127–129] [128–130] and collagen production, particularly type III collagen [130,131]. We observed rapid inflammatory process resolution, improved levels of total collagen, and significant expression of type I collagen during wound remodeling, indicating the maturity and resilience of the new tissue. However, collagen III levels have been found to increase in the deep dermis of hypertrophic scars and keloids [130,131].

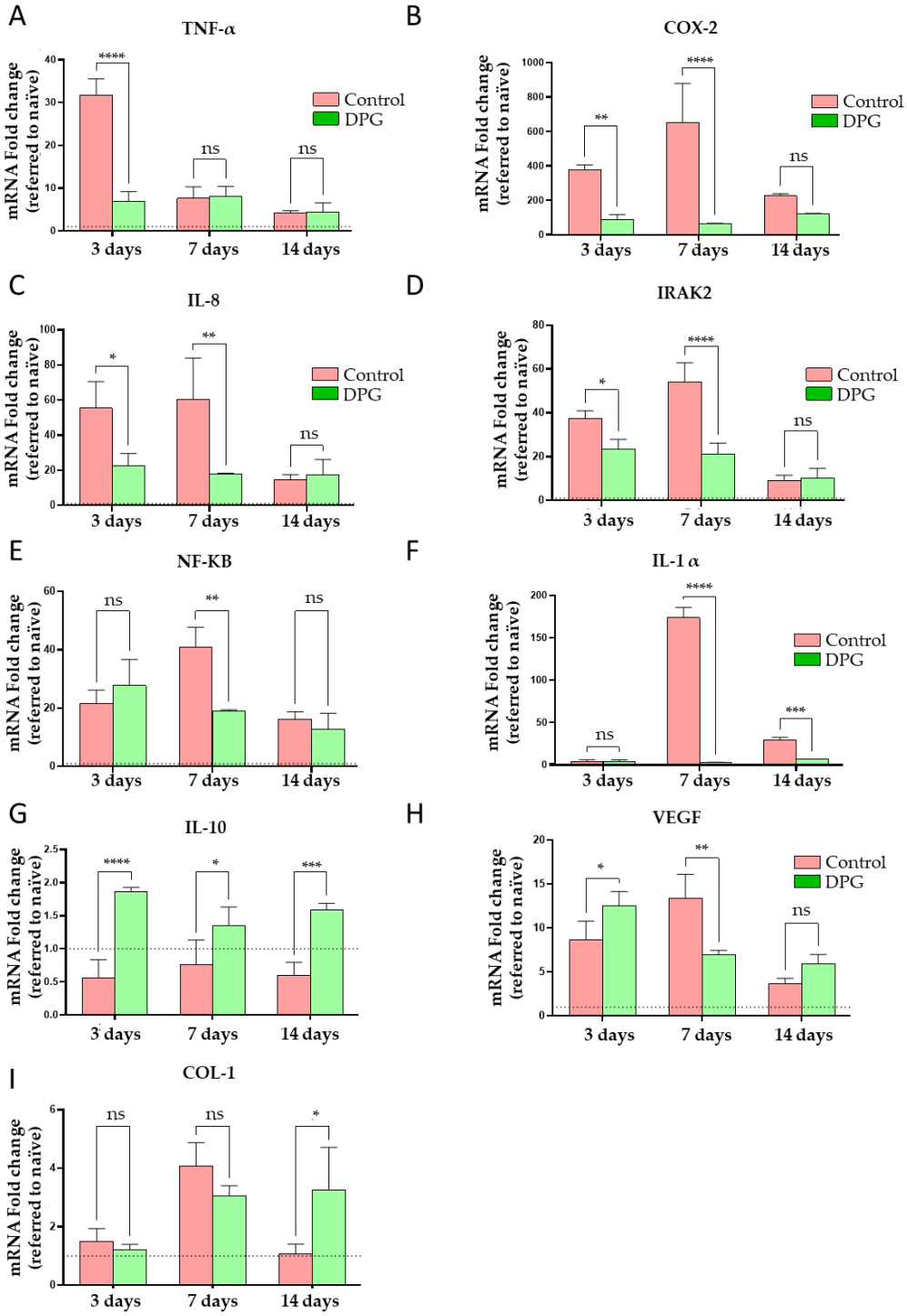



**Figure 4.** Quantitative analysis of the expression of mRNAs in the wound healing samples throughout the days of experimentation in the Control and DPG groups. (**A**) *Tnf-α* (**** $p < 0.0001$); (**B**) *Cox-2* (** $p < 0.01$, **** $p < 0.0001$); (**C**) *Il-8* (* $p < 0.05$, ** $p < 0.01$); (**D**) *Irak-2* (* $p < 0.05$, **** $p < 0.0001$); (**E**) *Nf-kb* (** $p < 0.01$); (**F**) *Il-1α* (**** $p < 0.0001$, *** $p < 0.001$); (**G**) *Il-10* (**** $p < 0.0001$, *** $p < 0.001$); (**H**)

*Vegf* (* $p < 0.05$, ** $p < 0.01$); and (**I**) *Col-1* (* $p < 0.05$). Ns—not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ when compared to the control group.

## 3. Materials and Methods

### *3.1. DPG Gel Cream*

Dipotassium glycyrrhizinate ($C_{42}H_{60}K_2O_{16}$) was manipulated as a cream at a concentration of 2%. It was supplied by Verdi Cosmetics (register number: 64.786.031/0001-00, Joanópolis, Brazil) (Table 1).

A cream gel was prepared using water, Sepigel 305, Dipotassium Glycyrrhizinate, and Euxyl PE 9010. Procedure: Dipotassium Glycyrrhizinate (2 g) was dissolved in water (93.6 mL). Next, Sepigel 305 (4 g) was added, aiming at controlling the viscosity of the formula. Finally, the preservative Euxyl PE 9010 (0.4 mL) was added. All ingredients were constantly mixed until complete homogenization. The prepared cream gel was stored in a clean, dry container and kept in a common refrigerator until use throughout the experimental period. The cream gel was used for topical application onto the wounds for 14 consecutive days during the experiment. We emphasize that the compound was used only for experimentation in this study, and is not currently marketed for the same purpose [31,132].

**Table 1.** Composition of the dipotassium glycyrrhizinate (DPG) formulation—2% gel cream.

| Ingredient | % | Function | INCI Name | CAS Number |
|---|---|---|---|---|
| Aqua | 93.600 | Solvent | Aqua | 7732-18-5 |
| Sepigel 305 | 4.000 | Viscosity controlling | Polyacrylamide, C13-14 Isoparaffin (and) Laureth 7 | 9003-05-8/246538-79-4/68439-50-9/9002-92-0/7732-18-5 |
| Dipotassium Glycyrrhizinate | 2.000 | Active | Dipotassium Glycyrrhizinate | 68797-35-3 |
| Euxyl PE 9010 | 0.400 | Preservative | Phenoxyethanol (and) Ethylhexylglycerin | 122-99-6/70445-33-9 |
| Total | 100.000 | | | |

### *3.2. Animals and Experimental Groups*

The research was previously approved by the Ethical Committee for Animal Use (CEUA) of São Francisco University (protocol n° 007.06.2020), following the guidelines of the Brazilian Society of Laboratory Animal Sciences (SBCAL). Wistar rats were supplied by Laboratory Animals Breeding and Commerce (ANILAB; Paulínia, Brazil) and kept at the Animal Experimentation Vivarium of São Francisco University in individual cages, at 22 ± 3 °C on a 12 h light/dark cycle, with free access to a standard diet and ad libitum water.

A total of 24 male rats (Wistar) were used, for a sample of 24 adult animals (3 months old, weighing between 274 and 300 g). The animals were randomly divided into 6 groups (n = 4, each), where they constituted untreated groups (Control) and treated groups (DPG): animals that were not treated (control groups, 3, 7, and 14 days) and animals that were treated with DPG (DPG groups, 3, 7, and 14 days). We want to be clear that all of the animals had surgery on the same day (day 0).

### *3.3. Excision Wound Model*

The animals were subjected to an anesthetic procedure with 2% xylazine hydrochloride (Xilazin®, Syntec, Santana de Parnaíba, Brazil) (10 mg.Kg$^{-1}$) associated with 5% dextrocetamine hydrochloride (Ketamin®, Cristália, Itapira, Brazil) (25 mg.Kg$^{-1}$), prepared by combining 0.5 mL of xylazine (10 mg) with 0.5 mL of ketamine (25 mg) to a volume of 1.0 mL, which was administered intraperitoneally (1.0 mL.Kg$^{-1}$).

After being anesthetized, the respective animals were positioned on appropriate tables in the horizontal prone position, and skin antisepsis with chlorhexidine 2% (RIOHEX®, Rioqumica, So José do Rio Preto, Brazil), followed by 0.5% alcoholic chlorhexidine (RIOHEX®, Rioqumica, So José do Rio Preto, Brazil), was performed as part of the pre-surgical preparation [132]. With the help of a scalpel (handle and blade number 15), Mezenbaum scissors curve, and anatomical forceps, each animal—having already been identified by its group—had one circular excision of the skin made in the median plane of the dorsal region, which was constrained in depth by the muscular aponeurosis. This was accomplished by precisely measuring the 2 cm diameter of each excision using a caliper and a plastic circular mold (Universal Digimess 100003) [132]. Thereafter, animals were housed individually and monitored in properly disinfected cages to prevent infection or further damage to the wounds after recovering from anesthesia.

### *3.4. Topical Treatment*

Wounds in the treated groups (DPG) were treated once a day for 3, 7, and 14 days at the same time and by the same researcher. This was performed with topical application of 2% DPG cream (in the amount of 0.1 mL/each animal) using cotton-tipped flexible shafts (Cotonetes®—Johnson & Jonhson, Brazil) and without the use of bandages. For this purpose, 0.1 mL of cream was measured using an insulin-type syringe (Slip without needle—Injex®). Treatment started 24 h after the surgical procedure in the treated groups (DPG). The wounds in the untreated groups (Control) did not receive any intervention during the entire experimental period (3, 7, and 14 days) (Figure 5).

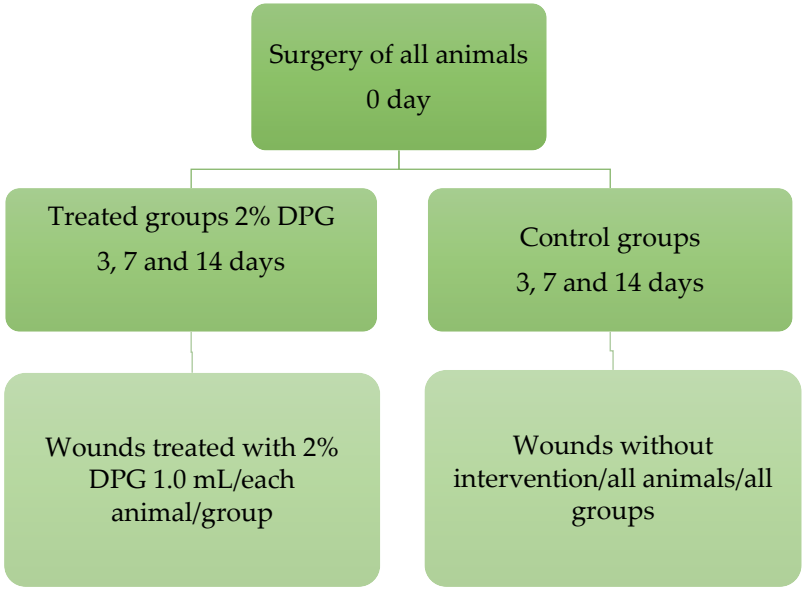



**Figure 5.** Schematic diagram of the experimental design.

### *3.5. Collection, Storage, and Processing of Samples*

Then, according to each experimental group, skin samples (scar tissue) were excised and gathered in due time (3, 7, and 14 days) for histological and gene expression analyses. For this, the animals, under the effect of the 3% isoflurane anesthetic, were submitted to

active euthanasia by sodium thiopental (Thiopentax®, Cristália, Itapira, Brazil) at a dosage of 100 mg.Kg$^{-1}$ [133].

For microscopic analysis, the skin samples were fixed in 10% formaldehyde solution (Labsynth®, Diadema, Brazil) for 24 h, fixed at the extremities in cork, dehydrated in increasing concentrations of ethanol (Labsynth®), clarified in xylene (Labsynth®), embedded in paraffin (Labsynth®), and submitted to microtomy (Lupetec MRPO3, São Carlos, Brazil). The slides (5 μm thick sections) were deparaffinized in two xylene baths (10 min each) hydrated in decreasing concentrations of ethanol (100%, 95%, 80%, and 70%) and distilled water, and stained with hematoxylin–eosin (HE) for semi-quantitative analysis of inflammatory parameters (inflammatory exudate and active hyperemia) and proliferative parameters (granulation tissue and reepithelialization). The modified and adapted inflammatory/proliferative score scales were used, as well as Masson's Trichrome (MT) for quantitative analysis of the total collagen. After staining, the slides were dehydrated in increasing concentrations of ethanol (70%, 80%, 95%, and 100%) and xylene, then mounted with synthetic balsam from Canada.

The samples collected for gene expression analysis were identified and stored in individual tubes for processing and analysis: in RNAlater stabilization solution at room temperature for 24 h, in a refrigerator for another 24 h, frozen at −20 °C for 1–2 weeks, and, finally, frozen at −80 °C. Additionally, the following practices were used for the collection: The entirety of each animal's scar tissue was removed, and from each sample taken, a tiny flap was taken for gene expression analysis without including any potential crusts.

### *3.6. Macroscopic Analysis*

Wound areas were measured on days 1, 3, 7, and 14 of experimentation with the aid of a pachymeter (Universal Digimess 100003) [132]. Wound areas were measured (large versus small diameter). Subsequently, from the values found, the diameter of the circumference of each wound was calculated using the formula ($C = 3.14 \times r^2$).

### *3.7. Microscopic Analysis—Inflammatory and Proliferative Scores*

Through the modified and adapted inflammatory score grading scale, the following scores were assessed for microscopic anatomopathological analysis (semi-quantitative): histological characteristics related to the inflammatory process (presence of leukocyte infiltration/inflammatory exudate and active hyperemia) and characteristics related to tissue proliferation (presence of granulation tissue and re-epithelialization).

Hematoxylin–eosin (HE) was used for semi-quantitative analysis of inflammatory parameters (inflammatory exudate and active hyperemia) and proliferative parameters (granulation tissue and re-epithelialization) through the modified and adapted inflammatory/proliferative score scales and with Masson's Trichrome (MT) for quantitative analysis of total collagen [134]. Briefly, for the HE technique, the slides were stained with hematoxylin for 5 min, washed in running water, stained with eosin for 3 min, and, finally, washed in running water. For the MT technique, the slides were kept for 1 h in Bouin's solution in the oven, cooled, washed in running water until completely clear, washed in distilled water 3 times, and treated with Biebrich's Scarlet solution for 3 min. Again, they were washed in running distilled water, kept in phosphotungstic acid for 8 min, washed in running distilled water, kept in aniline blue for 3 min, and, finally, washed in running water once again.

Each of the parameters (inflammatory exudate, active hyperemia, granulation, and re-epithelialization) were stratified as: 0 = absent; 1 = mild; 2 = moderate; and 3 = intense, according to the changes found using HE (Tables 2 and 3) [134]. Each parameter in each experimental group was analyzed separately by an experienced collaborating pathologist who was unaware of the experimental groups to which the animals belonged. For this purpose, the slides (HE) were analyzed in 3 different fields, and the value adopted for each parameter analyzed for each animal in the group was the mean value found after

reading three different fields of the lesion area. The final value assigned to each sample analyzed in each experimental group was the mean value obtained by adding the values of each parameter [134]. The 500× final magnification data were used for the analysis.

**Table 2.** Inflammatory score graduation scale [134].

| Degree | Score | Inflammatory Exudate | Active Hyperemia |
|---|---|---|---|
| Absent | 0 | No leukocyte tissue infiltration (0%) | No tissue hyperemia (0%) |
| Light | 1 | Leukocyte infiltration (<50%) | Presence of hyperemia (<50%) |
| Moderate | 2 | Leukocyte infiltration (≥51%) | Presence of hyperemia (≥51%) |
| Intense | 3 | Abundant leukocyte infiltration (≥75%) | Abundant hyperemia (≥75%) |

**Table 3.** Grading scale of proliferative parameters [134].

| Degree | Score | Granulation Tissue | Re-Epithelialization |
|---|---|---|---|
| Absent | 0 | Neoformed tissue absent (0%) | Re-epithelialization tissue absent (0%) |
| Light | 1 | Presence of neoformed tissue (<50%) | Presence of re-epithelialization tissue (<50%) |
| Moderate | 2 | Presence of neoformed tissue (≥51%) | Presence of re-epithelialization tissue (≥51%) |
| Intense | 3 | Abundant neoformed tissue (≥75%) | Presence of re-epithelialization tissue (≥75%) |

The total collagen content was analyzed by quantitative reading on the slides in three different fields. The computer-assisted image analysis program was used. The selected image was captured by a video camera previously coupled to an optical microscope (Eclipse DS50—Nikon Inc., Osaka Japan) and then analyzed by the program NIS-Elements (Nikon Inc., Japan) [134]. By means of color histograms, the software determines the color intensity of each area selected for measurement, transforming the chosen color into a numerical expression for each selected field of view. Using the color histogram in the RGB (red, green, blue) system, the blue color was selected, the intensity of which was captured by the number of pixels containing the color and then converted into a numerical value. The final value considered for each measured field of each sample was represented by the average of the values found after the evaluation of three different fields [134]. For both analyses, a final magnification of 500× was used.

### *3.8. RNA Extraction and Reverse Transcription Quantitative PCR (qPCR)*

A portion of the lesion samples from each animal was collected in RNAlater and frozen at −80 °C. Total RNA was extracted from skin tissues using Trizol® reagent (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. After extraction, ~100 ng of RNA was used for cDNA synthesis using the High-Capacity cDNA Archive Kit (Applied Biosystems), following the manufacturer's recommendations.

q PCR was performed using a 7300 real-time PCR System (Applied Biosystems), and threshold cycle numbers were determined using RQ Study Software (Applied Biosystems). Reactions were performed in triplicate, and threshold cycle numbers were averaged. The reaction mixture was prepared using Power Up SYBR® Green Master Mix (Applied Biosystems). The reaction was cycled with preliminary Uracil–DNA glycosylase was treated for 2 min at 50 °C with a denaturation step for 2 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing for 15 s, and primer extension at 72 °C for 15 s. This was followed by melting point analysis of the double-stranded amplicons consisting of 40 cycles of 1 °C decrement (15 s each) beginning at 95 °C. The first derivative of this plot, dF/dT, is the rate of change of fluorescence in the reaction, and a significant change in fluorescence accompanies the melting curve of the double-stranded PCR products. A plot of –dF/dT vs. temperature displays these changes as distinct peaks. *Cox-2, Tnf-α*, *Nf-kb*, *Il-1α*, *Irak-2*, *Il-8rb*, *Il-10*, *Vegf*, *Col-α1*, and *18s* (Table 4) expressions were examined and normalized to a constitutive gene (*18s*).

**Table 4.** Sequence of primers used in qPCR.

| Gene | Primers Sequence 5′-3′ |
|---|---|
| *Cox-2* | FW: AACAACATTCCCTTCCTTCG<br>RV: AAGTTGGTGGGCTGTCAATC |
| *Tnf-α* | FW: GGGCTCCCTCTCATCAGTT<br>RV: TTGCTACGACGTGGGCTAC |
| *Nf-kb* | FW: CAGCTCTTCTCAAAGCAGCA<br>RV: AGCCTTCTCCCAAGAGTCG |
| *Il-1α* | FW: GGCCATAGCCCATGATTTAG<br>RV: TGATGAACTCCTGCTTGACG |
| *Irak-2* | FW: TCAAGAGGCTCAGGGAGGT<br>RV: CCCAGCAGAGGTAGGATGTT |
| *Il-8rb* | FW: ATCTTTGCTGTGGTCCTCGT<br>RV: GGTCTCCTTGATCAGCTTGG |
| *Il-10* | FW: AGCCTTGCAGAAAACAGAGC<br>RV: GCCTTTGCTGGTCTTCACTC |
| *Vegf* | FW: CGGAGAGCAACGTCACTATG<br>RV: GCTGCAGGAAGCTCATCTCT |
| *Col-1* | FW: GGAATGAAGGGACACAGAGG<br>RV: AGGCTCTCCCTTAGGACCAG |
| *18s* | FW: CGCGGTTCTATTTTGTTGGT<br>RV: CGGTCCAAGAATTTCACCTC |

FW (forward), RV (reverse).

### *3.9. Statistical Analysis*

Data are expressed as the mean ± S.E.M. Comparisons among groups of data were made using one-way ANOVA followed by the Dunnett Multiple Comparisons test. An associated probability ($p$-value) of <5% was considered significant.

## 4. Conclusions

Our findings have led us to the additional conclusion that DPG reduces inflammation by promoting skin wound healing through modulation of various mechanisms and signaling pathways, such as: anti-inflammatory, through modulation of pro- and anti-inflammatory cytokine expression; promotion of new granulation tissue, angiogenesis, and tissue re-epithelialization, indicated by modulation of VEGF and stimulation of collagen synthesis; and contributing to tissue re-epithelization. Our research supports topical DPG's ability to reduce inflammation and promote healing, suggesting that this substance has potential as a therapeutic agent, particularly for cutaneous inflammatory disorders and illnesses.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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## 4. CONCLUSÃO

Relativo ao trabalho de revisão bibliográfica, as evidências de todos os estudos experimentais abordados sugerem que os compostos bioativos (G, GA e DPG) obtidos do alcaçuz possuem propriedades anti-inflamatórias e antioxidantes que afetam os distúrbios gastrointestinais por meio de diferentes mecanismos de ação. Isso fornece um histórico interessante para entendermos como esses compostos agem e contribuem para o desenvolvimento de estratégias terapêuticas naturais e para o estabelecimento de modelos de pesquisa. Além disso, mais pesquisas são necessárias para determinar o mecanismo de ação em diferentes atividades biológicas. Ensaios clínicos com G, GA e DPG também são necessários para validar esses efeitos farmacológicos, para estabelecer esses compostos como fármacos promissores e para preencher algumas lacunas em relação à sua segurança e características toxicológicas.

Relativo ao trabalho experimental, nossos achados nos levaram à conclusão adicional de que o DPG reduz a inflamação ao promover a cicatrização de feridas na pele por meio da modulação de vários mecanismos e vias de sinalização, tais como: anti-inflamatório, por meio da modulação da expressão de citocinas pró e anti-inflamatórias; promoção de novo tecido de granulação, angiogênese e reepitelização tecidual, indicada pela modulação do VEGF e estimulação da síntese de colágeno; e contribuindo para a reepitelização tecidual. Nossa pesquisa apóia a capacidade do DPG tópico de reduzir a inflamação e promover a cicatrização, sugerindo que essa substância tem potencial como agente terapêutico, principalmente para distúrbios e doenças inflamatórias cutâneas.

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