PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

NATHÁLIA SOARES DE ALMEIDA

EFEITOS DO TRATAMENTO PRECOCE E CONTÍNUO DE RESVERATROL ASSOCIADO A DEXAMETASONA EM FERIDAS CUTÂNEAS DE RATOS.

Presidente Prudente - SP
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Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências da Saúde – Área de concentração: Ciências da Saúde.

Orientadora:
Profa. Dra. Ana Clara Campagnolo Gonçalves Toledo

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Presidente Prudente, 9 de março de 2021.

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"É que tem mais chão nos meus olhos do que cansaço nas minhas pernas,

mais esperançanos meus passos do que tristeza nos meus ombros,

mais estrada no meu coração do que medo na minha cabeça."

(Cora Coralina)

LISTA DE SIGLAS

AFf	- Área da Ferida final
AFi	- Área da Ferida inicial
ANOVA	- Análise de Variância
APS	- Persulfato de Amônio
AT	- Azul de Toluidina
BSA	- Albumina do Soro Bovino
C	- Controle
CEUA	- Comissão de Ética no Uso de Animais
CI	- Intervalo de Confiança
Comp	- Comprimento
D+R	- Dexametasona associado ao Resveratrol
Dexa	- Dexametasona
h	- Horas
HCL	- Ácido clorídrico
HE	- Hematoxina Eosina
Kg	- Kilogramas
KGF	- Fator de crescimento de Queratonócitos
Larg	- Largura
mg	- Miligrama
ml	- Mililitro
MMP-2	- Metaloproteinase-2
MMP-9	- Metaloproteinase-9
MMPs	- Metaloproteinases
PI	- Pele Íntegra
Pro-MMP-2	- Pro-Metaloproteinase-2
Pro-MMP-9	- Pro-Metaloproteinase-9
Resv	- Resveratrol
SDS	- Dodecil Sulfato de Sódio
TEMED	- Tetramethylethylenediamine
vs	- Versus

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ARTIGO

Efeitos do tratamento precoce e contínuo de resveratrol associado à dexametasona em feridas cutâneas de ratos
Effects of early and continuous treatment of resveratrol associated with dexamethasone in rat cutaneous wounds

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O trabalho está apresentado sob a forma de artigo, segundo as normas do periódico o qual serásubmetido: Advances in Skin & Wound Care, 1.355, A3

RESUMO

OBJETIVO: Verificar os efeitos da associação do uso de resveratrol e dexametasona em tatamento profilático e contínuo no processo de cicatrização de feridas cutâneas de ratos. **MÉTODO**: Foram utilizados 60 ratos machos (*Wistar),* divididos randomicamente em quatro grupos experimentais (grupos: n=15): Controle (C), Dexametasona (Dexa), Resveratrol (Resv) e Dexametasona associado ao Resveratrol (D+R). A lesão cutânea foi realizada após o 35º dia de tratamento com as substâncias citadas. Os tratamentos foram realizados continuamente até 3º, 7º e 14º dias pós-lesão. Para cada etapa, os animais foram pesados e a ferida foi medida. Após os animais foram eutanasiados, as lesões cutâneas removidas, fixadas e encaminhadas para técnicas histológicas rotineiras. A quantificação de mastócito (Azul de toluidina), Fibroblastos (Hematoxilina Eosina) Colágeno tipo I e III (Picrossírius) e metanoloproteinases 2 e 9 por análise bioquímica. Análise estatística utilizada foi ANOVA two-way com pós-teste de Bonferroni e nível de significância $p<0.05$. **RESULTADOS:** o grupo Dexa demonstrou perda significativa de peso final nos períodos de 3 dias vs 7 dias e 3 dias vs 14 dias. A contração da ferida mostrou resultados significativos para todos os grupos. A quantificação de mastócitos foi significativa somente para o grupo controle. Para fibroblastos houve resultados significativos para todos os grupos que receberam tratamento. Colágeno tipo III demonstrou resultados signifivcativos para o grupo controle. As atividades de Pro-MMP-2 demontraram valores significativos para o grupo controle, MMP-2 para controle e dexa, Pro-MMP-9 para os grupos dexa e D+R assim como para MMP-9. Os demais resultados não foram significativos. **CONCLUSÃO:** os tratamentos preventivos da lesão usando a associação de resveratrol e dexametasona e o ativo resveratrol isolado mostraram-se positivos para as fases de cicatrização frente aos demais grupos. Além disso, o ativo natural resveratrol não demonstrou efeitos colaterais ou alérgicos aos animais, o que pode indicar uma promissora substância na cicatrização de feridas.

Palavras-chave: Antioxidantes; Cicatrização de feridas; Dexametasona; Fibroblastos; Metalopreteinases; Resveratrol.

ABSTRACT

OBJECTIVE: To verify the effects of the association of the use of resveratrol and dexamethasone in prophylactic and continuous treatment in the healing process of rat skins. METHODS: Sixty male rats (Wistar) were randomly divided into four experimental groups (groups: n=15): Control (C), Dexamethasone (Dexa), Resveratrol (Resv) and Dexamethasone associated with Resveratrol (D+R). The lesion was performed after the 35th day of treatment with the aforementioned substances. The treatments were carried out continuously until the 3rd, 7th and 14th day-injury. For each step, the animals were taken and the wound was measured. After the maintenance of their animals, they were used for animals and domestic animals, the animals used as technical accessories, were used for animals and domestic animals. The quantification of mast cells (Toluidine Blue), Fibroblasts (Hematoxylin Eosin), Collagen type I and III (Picrosirius) and methanoloproteinases 2 and 9 by biochemical analysis. Statistical analysis used was two-way ANOVA with Bonferroni post-test and significance level $p<0.05$. RESULTS: The Dexa group had significant final weight loss in the periods of 3 days vs 7 days and 3 days vs 14 days. Wound contraction showed results for all groups. Mast cell quantification was significant only for the control group. For fibroblasts there were results for all groups that received treatment. Type III collagen project significant results for control group. The activities of ProMMP-2 showed values of -2 for the control group, MMP- for control and dexa, Pro-MMP-9 for the dexa and D+ groups as well as for MMP-9. The other results were not results. CONCLUSION: the preventive treatments of the lesion using the association of resveratrol and dexamethasone, the active resveratrol alone and separate groups were positive for the healing phases to the others. In addition, the active veratrol does not indicate natural effects or allergic effects to animals, which can be a promising substance in wound healing.

Keywords: Antioxidants; Wound healing; Dexamethasone; Fibroblasts; Metallopreteinases; Resveratrol.

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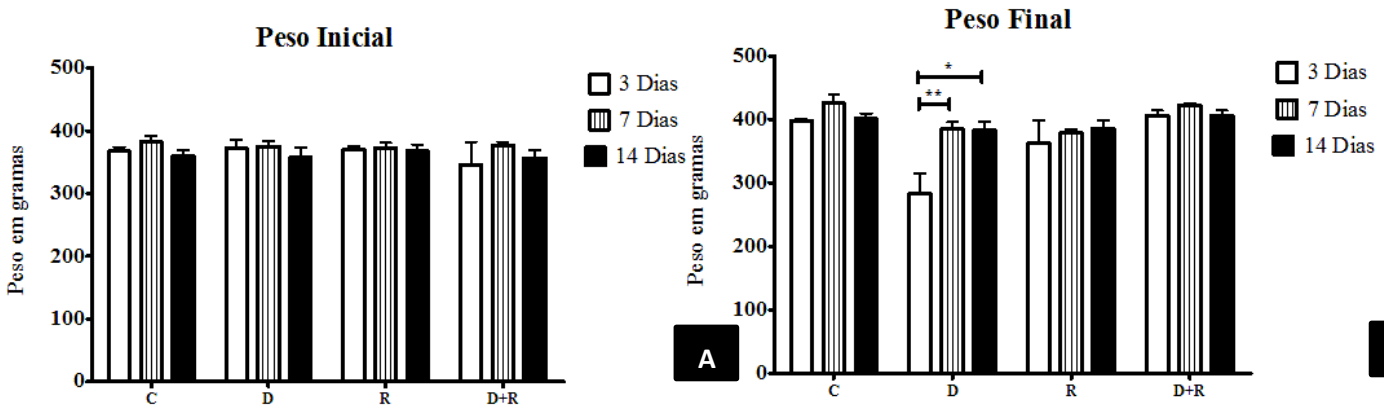

Figura 3: Peso dos animais. **A:** Peso inicial e **B:** Peso final.

C: Controle; Dexa: Dexametasona; Resv:Resveratrol e D+R: Dexametasona + Resveratrol.

*Diferença entre 3 dias vs 14 dias; **Diferença entre 3 dias vs 7 dias.

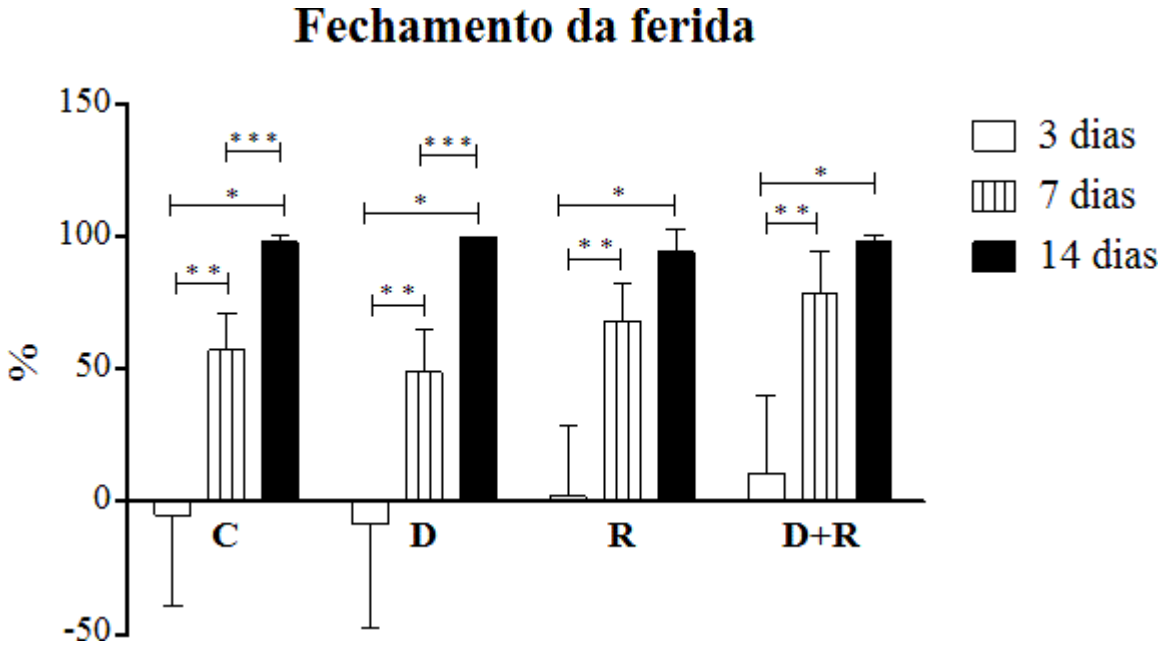

Figura 4: Porcentagem do fechamento da ferida

C: Controle; Dexa: Dexametasona; Resv: Resveratrol e D+R: Dexametasona + Resveratrol.

*Diferença entre 3 dias vs 14 dias; **Diferença entre 3 dias vs 7 dias e ***Diferença entre 7 dias vs 14 dias.

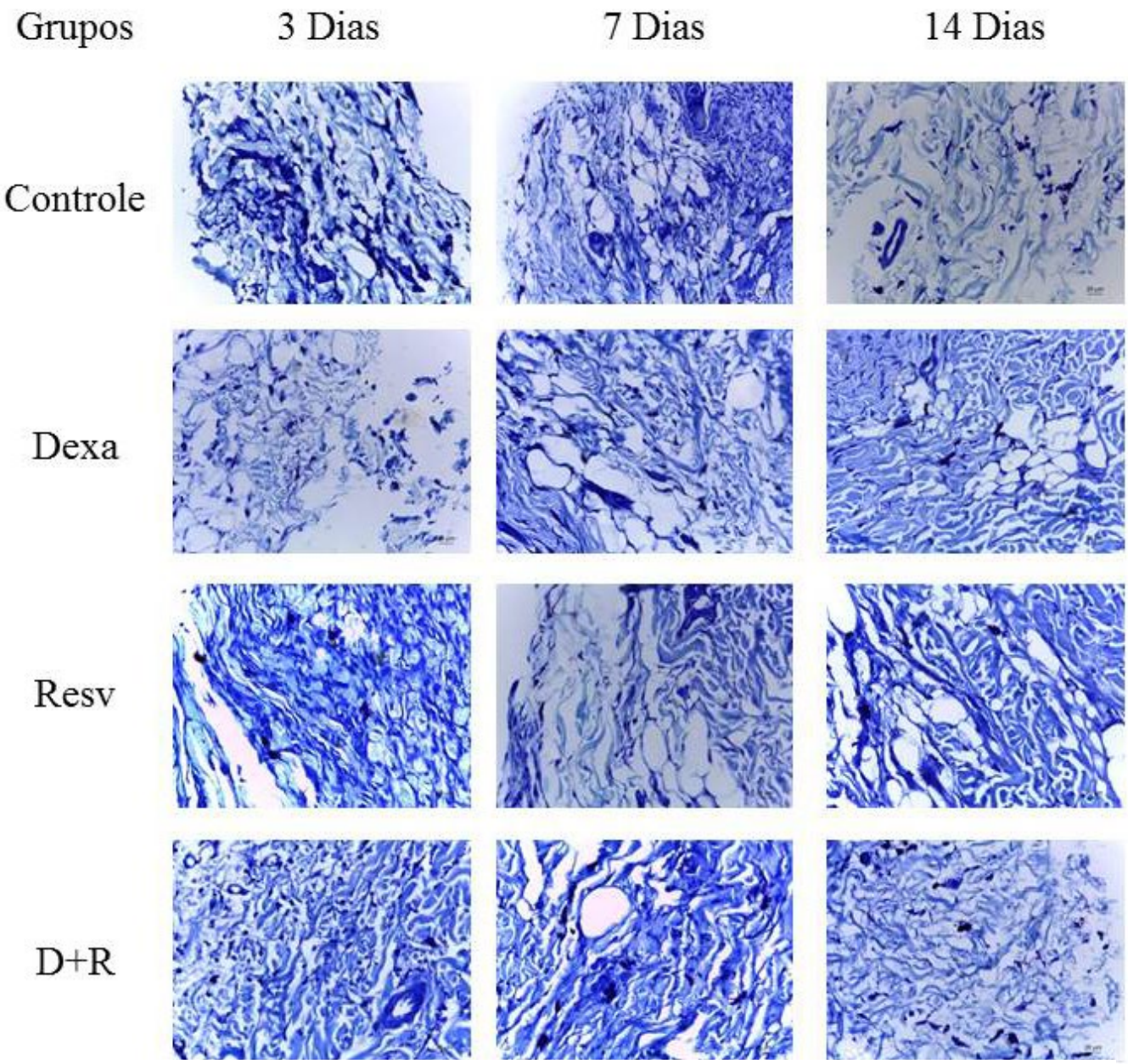

Figura 5 – Fotomicrografias representativas do 3°, 7º e 14º dia de pós-lesão, coloração Azul de Toluidina

Legenda: Dexa (Dexametasona); Resv (Resveratrol); D+R (Dexametasona+Resveratrol)

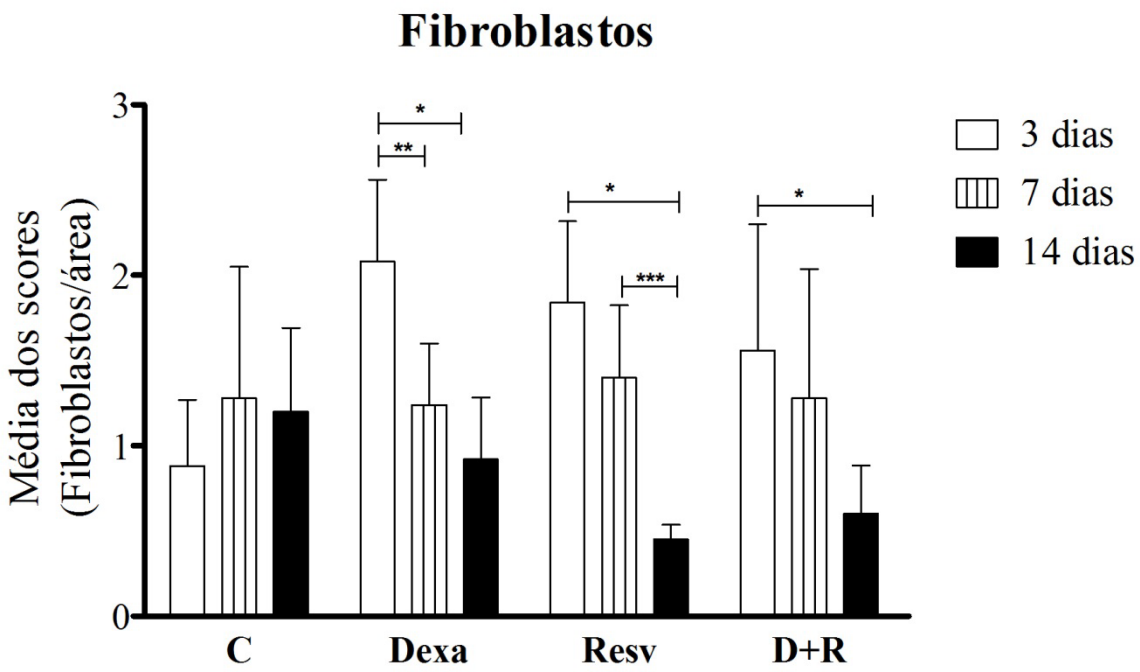

Figura 6: Quantificação dos fibroblastos.

C: Controle; Dexa: Dexametasona; Resv:Resveratrol e D+R: Dexametasona + Resveratrol.

*Diferença entre 3 dias vs 14 dias; **Diferença entre 3 dias vs 7 dias e ***Diferença entre 7 dias vs 14 dias.

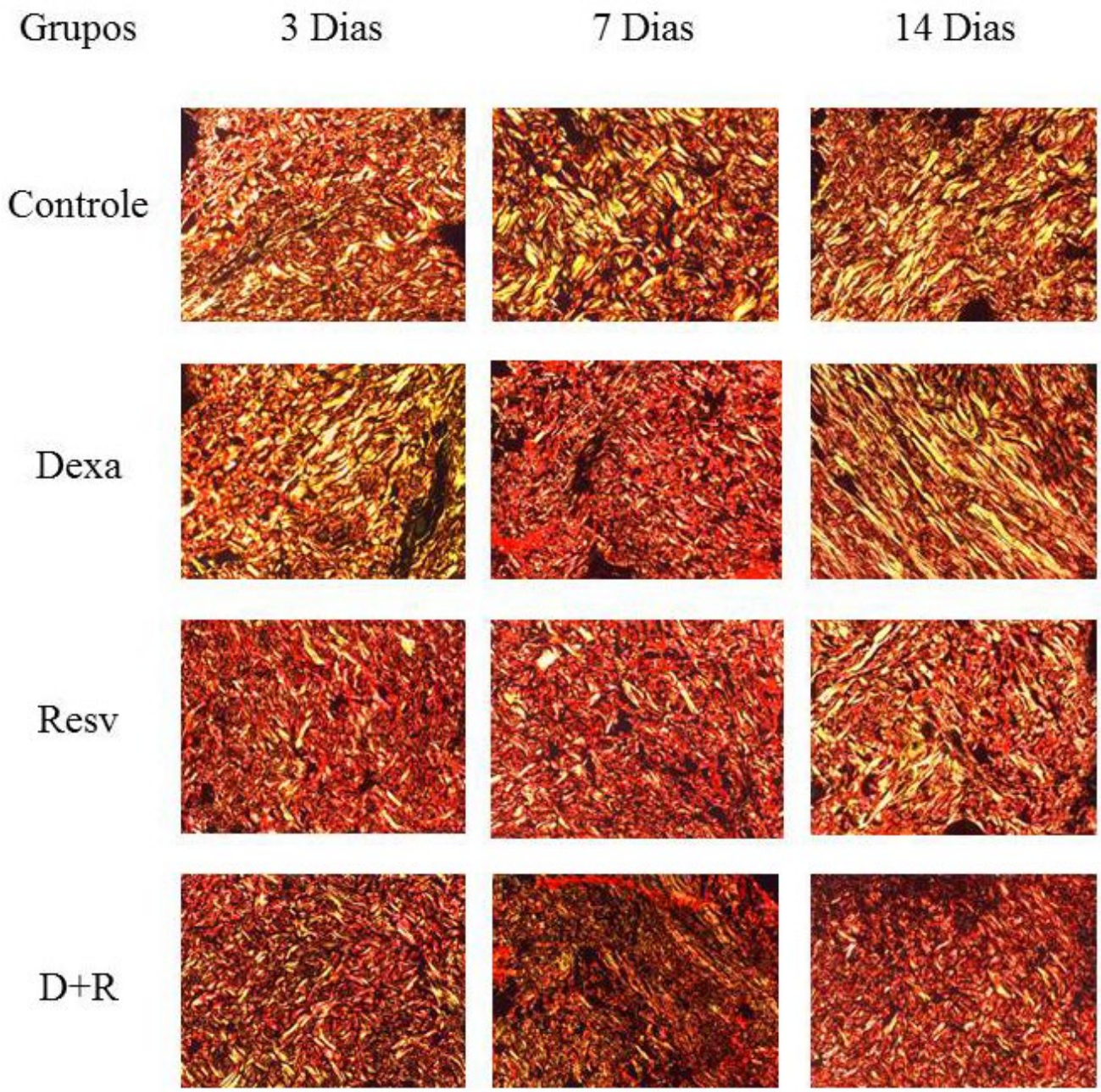

Figura 7 – Fotomicrografias representativas do 3°, 7º e 14º dia pós-lesão, coloração Picrossirius

Legenda: Dexa (Dexametasona); Resv (Resveratrol); D+R (Dexametasona+Resveratrol).

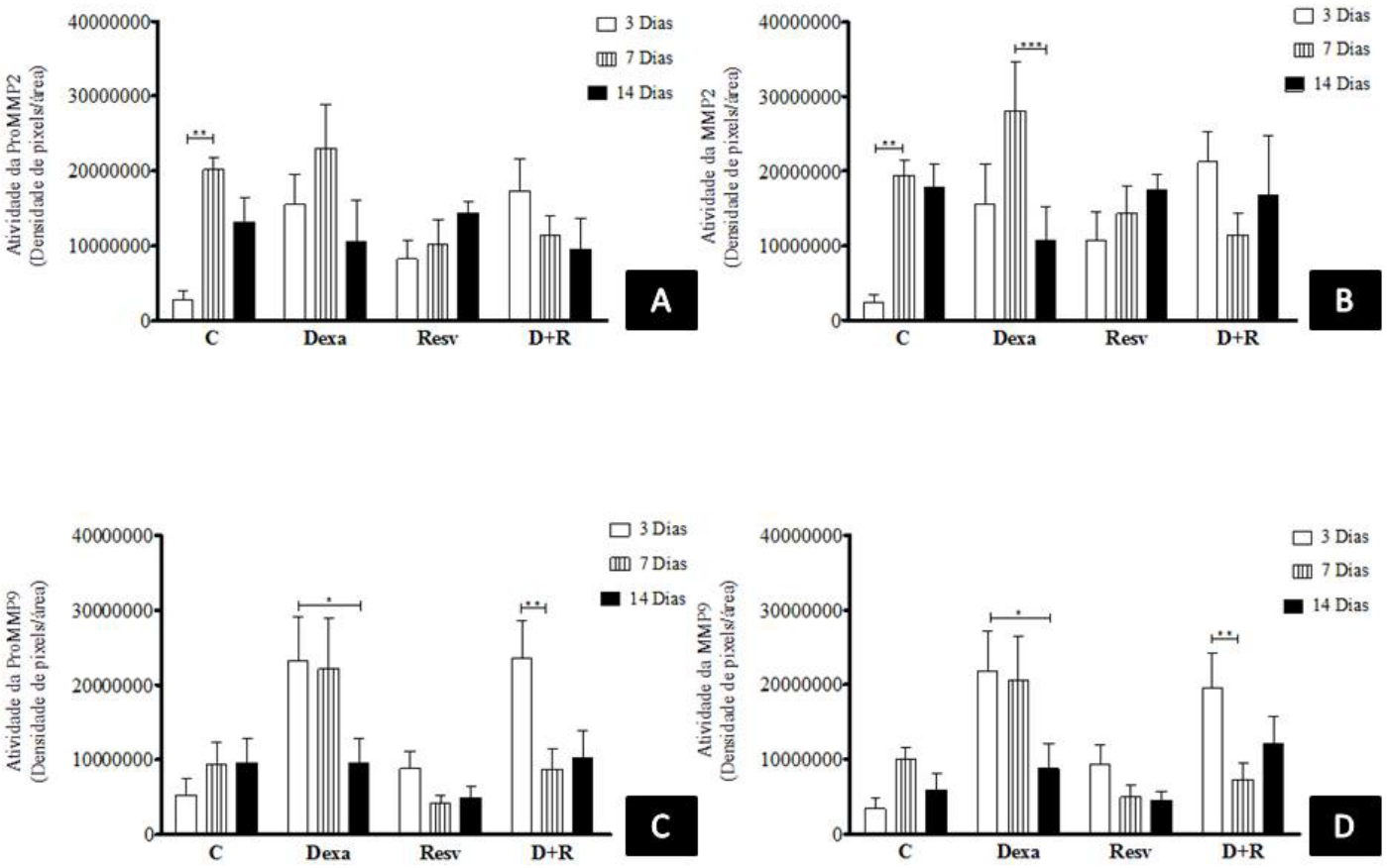

Figura 8: Quantificação da atividade das MMPs. **A:** Atividade da Pro-MMP-2; **B:** Atividade da MMP-2; **C:** Atividade da Pro-MMP-9; **D:** Atividade da MMP-9.

Legenda: C: Controle; Dexa: Dexametasona; Resv: Resveratrol e D+R:Dexametasona + Resveratrol.

*Diferença entre 3 dias vs 14 dias; **Diferença entre 3 dias vs 7 dias e ***Diferença entre 7 dias vs 14 dias.

Tabela 1 – Quantificação demastócitos

Momentos	Tratamentos	Mastócitos
3 Dias	Controle	0,620 ± 0,286* [-0.9477 – 1.308]
	Dexa	0,840 ± 0,577 [-1.048 – 1.208]
	Resv	1,140 ± 0,635 [-0.9877 – 1.268]
	D+R	0,800 ± 0,367 [-0.8077 – 1.448]
7 Dias	Controle	0,800 ± 0,187 [-0.3077 – 1.948]
	Dexa	0,920 ± 0,335 [-1.288 – 0.9677]
	Resv	1,280 ± 0,449 [-1.208 – 1.048]
	D+R	1,120 ± 0,536 [-1.208 – 1.048]
14 Dias	Controle	1,620 ± 0,622* [-0.1277 – 2.128]
	Dexa	0,760 ± 0,493 [-1.208 – 1.048]
	Resv	1,200 ± 0,524 [-1.068 – 1.188]
	D+R	1,040 ± 0,673 [-0.8877 – 1.368]

Legenda: Dexa (Dexametasona); Resv (Resveratrol); D+R (Dexametasona+Resveratrol).
Média, desvio padrão e intervalo de confiança. *diferençasignificativa entre 3 dias vs 14 dias (controle).

Tabela 2 – Análise de Colágeno

Momentos	Tratamentos	Colágeno Total	Colágeno tipo III	Colágeno tipo I
3 Dias	Controle	1,7076 ± 0,021 [-0.1064 – 0.05402]	1,7148 ± 0,120*# [-0.2944 – -0.02000]	1,7724 ± 0,057 [-0.03955 – 0.1507]
	Dexa	1,7018 ± 0,050 [-0.03562 – 0.1248]	1,6698 ± 0,074 [-0.2300 – 0.04440]	1,7814 ± 0,067 [-0.1135 – 0.07675]
	Resv	1,6918 ± 0,055 [-0.06602 – 0.09442]	1,5078 ± 0,024 [-0.1092 – 0.1652]	1,7816 ± 0,059 [-0.09615 – 0.09415]
	D+R	1,6892 ± 0,032 [-0.1300 – 0.03042]	1,535 ± 0,021 [-0.1132 – 0.1612]	1,8334 ± 0,026 [-0.06215 – 0.1281]
7 Dias	Controle	1,6814 ± 0,064 [-0.05802 – 0.1024]	1,5576 ± 0,035* [-0.1090 – 0.1654]	1,828 ± 0,059 [-0.1179 – 0.07235]
	Dexa	1,7464 ± 0,0063 [-0.1318 – 0.02862]	1,577 ± 0,062 [-0.1524 – 0.1220]	1,763 ± 0,020 [-0.03955 – 0.1507]
	Resv	1,706 ± 0,042 [-0.08522 – 0.07522]	1,5358 ± 0,033 [-0.08940 – 0.1850]	1,7806 ± 0,072 [-0.05395 – 0.1363]
	D+R	1,6394 ± 0,073 [-0.02182 – 0.1386]	1,559 ± 0,081 [-0.1690 – 0.1054]	1,8664 ± 0,085 [-0.1455 – 0.04475]
14 Dias	Controle	1,7036 ± 0,065 [-0.08422 – 0.07622]	1,5858 ± 0,080# [-0.2662 – 0.008203]	1,8052 ± 0,059 [-0.06235 – 0.1279]
	Dexa	1,6948 ± 0,041 [-0.08722 – 0.07322]	1,5618 ± 0,063 [-0.2452 – 0.02920]	1,8186 ± 0,031 [-0.05795 – 0.1323]
	Resv	1,701 ± 0,023 [-0.07102 – 0.08942]	1,5836 ± 0,052 [-0.06140 – 0.2130]	1,8218 ± 0,024 [-0.05495 – 0.1353]
	D+R	1,6978 ± 0,025 [-0.07162 – 0.08882]	1,5272 ± 0,031 [-0.1450 – 0.1294]	1,816 ± 0,022 [-0.1125 – 0.07775]

Legenda: Dexa (Dexametasona); Resv (Resveratrol); D+R (Dexametasona+Resveratrol). Média, desvio padrão e intervalo de confiança.

*diferença significativa entre 3 dias vs 7 dias (controle) e # diferença significativa entre 3 dias vs 14 dias (controle).

ANEXO – Normas de Submissão Advances in Skin & Wound Care

INSTRUCTIONS FOR AUTHORS

Advances in Skin & Wound Care, a peer-reviewed, interprofessional journal, publishes quantitative and qualitative studies, continuing education articles, scholarly reviews of the literature, reports on innovative treatments, case series, case studies, and practical articles for skin and wound care professionals. Published articles translate knowledge into practice for all professionals involved in skin and wound management: physicians, nurses, dermatologists, surgeons, podiatrists, physical and occupational therapists, dietitians, infection control practitioners, pharmacists, biomedical engineers, researchers, academics, and administrators.

The journal welcomes submissions of original articles in all areas of skin and wound management. Manuscripts are considered for publication only if the work has not been published and is not under consideration for publication by another periodical. Manuscript submissions must be accompanied by an author profile form. All authors must reveal their affiliation(s) with organizations that have a direct or indirect financial interest in the editorial content or products that are discussed in the manuscript. Authors must state all relevant conflicts of interest in the manuscript, including financial, consultant, institutional, and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared.

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Manuscript Submission

All manuscripts must be submitted electronically to www.lwwesubmissions.com.

Review Process

All submitted papers are subject to a double-blind peer review. To facilitate blinding, the body of the manuscript should not include any identifiable information, including but not limited to author names/initials, facility names, or specific locations. The initial review process takes 2 to 3 months. Reviewer comments will be shared with the authors. If substantive revision is necessary, the manuscript will be returned to the authors for rework based on feedback from the Editor(s)-in-Chief and peer reviewers. Accepted manuscripts are subject to editorial revision for clarity, punctuation, grammar, syntax, and conformity to journal style.

The corresponding author will receive a copy of the final manuscript for review prior to publication.

ARTICLE TYPES

Original Investigation

Original Investigations are scientific feature articles that create original data and translate new findings into practice for improved patient care. These articles should be no more than 6,000 words, and the reference list should be limited to 50 or fewer key recent references.

Continuing (Medical) Education (CE/CME)

A CE/CME article should review the recent literature and key concepts with an interpretation of the evidence for clinical practice. These articles should be written by practicing healthcare professionals and not employees of companies with conflicts of interest on the subject. The submission should be evidence-based and may follow a case format. The CE/CME may reflect scientific evidence, expert opinion, and/or patient preference. The presented data should discuss the impact of the author conclusions/recommendations on healthcare systems (when appropriate). These articles should be no more than 4,500 words, and the reference list should be limited to 25-40 key recent references. The article must also include Practice Points: 4 or 5 bullets that summarize the main clinical takeaways of the article.

Literature Reviews

Reviews that provide a complete overview of the literature on any topic related to skin and wound care will be considered and will be subject to peer review. The text should include a structured abstract of no more than 250 words under the following headings: Objective, Data Sources, Study Selection, Data Extraction, Data Synthesis, Conclusions.

Case Series

A Case Series offers readers practical information and contains reports of a seriesof cases that have interesting outcomes of care. Case Series are any articles describing the specifics of more than one case, up to a dozen patients. Clinical images to accompany the Series are encouraged. Case Series must clearly state within the body of the manuscript that written informed consent was provided byeach subject of the case to publish the case details and associated images, if any. Submissions without this statement will be immediately returned to the author.

Case Report

A Case Report offers readers practical information about a single case that may be applicable to clinical care more broadly. The case may detail a rare condition/diagnosis or an unusual outcome; clinical images to accompany the case are encouraged. Case Reports must clearly state within the body of the manuscript that written informed consent was provided by the subject of the case to publish the

case details and associated images, if any. Submissions without this statement will be immediately returned to the author.

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Advances in Skin & Wound Care encourages this type of professional exchange. Letters are reviewed by the Editor(s)-in-Chief for suitability before publication. They must be signed and will be subject to editing for style and length. Letters that question, criticize, or respond to a previously published paper will be sent to the author of that paper for a reply. Letters should be between 500 and 1,500 words in length, and should be submitted to ASWCedit@wolterskluwer.com, NOT Editorial Manager, with the subject line "Letter to the Editor."

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- **Title Page:** Should include the following:
  - The article title
  - The full name of each author, all credentials including highest academic degree, and current job/position title and affiliation, including city and state (eg, Jamie Smith, PhD, RN, Associate Professor, School of Medicine, Arizona State University, Tempe,Arizona, USA) listed in publication order.
  - Please note that **Advances in Skin & Wound Care** does not designate a corresponding author for its features.
  - Any conflicts of interest and funding received for this work from any of the following organizations: National Institutes of Health (NIH), Wellcome Trust, Howard Hughes Medical Institute (HHMI), and other(s). Funding disclosures should be specific and clearly state whatsupport was provided, eg, study materials, travel reimbursement, editorial support, etc.
- **Abstract:** A structured abstract of no more than 250 words included within the body of the manuscript should contain the following headings: Objective,Methods, Results, Conclusions, and 6 Keywords in alphabetical order.
- **Text:** Do not number manuscript sections. The body of the manuscript mayinclude the following:
  - Introduction (including a brief literature review, statement of the problem/clinical relevance, study objective)
  - Methods (including study design, setting, participants, inclusion/exclusion criteria, materials, procedure, etc.)
  - **Regardless of type, every manuscript involving living beings must include an ethics section that addresses institutional review/approval and/or participant/guardian consent, even if the study was exempt. Submissions that do not address this will be immediately returned to the author.**
  - Results
  - Discussion (including implications for clinical practice and limitationssections)

- Conclusions

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Authors must use AMA (American Medical Association) style for references. Do not use endnotes in the text. Number each reference consecutively from the first time it is cited in the text (do not use the author's name in the text; references must be numbered). Use superscript numbers, placed after the punctuation. List all authors when there are 6 or fewer; for 7 or more, list the first 3 followed by "et al." Authors are responsible for the accuracy of all relevant citations.

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Authors should incorporate the following style considerations into theirmanuscripts before submission:

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- Do not use the first person (we, us, our); instead, third person should be used (study authors, researchers, investigators, etc).
- Spell out all abbreviations at first mention. Abbreviations should only be used for terms that appear three or more times in the text.
- Patient-first language must be used, ie, persons with diabetes, NOTdiabetics.
- Please note the following statistical/special character conventions: $P < .05$;n = 580; χ^2 test; TNF-α

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