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Avaliação do Uso de Modelos Murinos BALB/c para Estudos de Cicatrização de Lesões de Pele Induzidas

Uberlândia-MG
2024

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Reuniu-se na Sala de Reuniões da PROPP (bloco H), Campus Umuarama, da Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Ciências Veterinárias, assim composta: Professores Doutores: Matias Pablo Juan Szabó (FAMEV/UFU); Alessandra de Almeida Ramos (Fiocruz/SBDA); Murilo Vieira da Silva (FAMEV/UFU), orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da mesa, Dr(a). Murilo Vieira da Silva, apresentou a Comissão Examinadora e o candidato(a), agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

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RESUMO

O emprego de agentes dermatológicos tópicos desempenha um papel essencial no tratamento de diversas condições cutâneas em animais, abrangendo dermatites, infecções, queimaduras e feridas. Esses medicamentos atuam de maneira eficaz na redução da inflamação local, proporcionando alívio aos sintomas como edema, rubor, dor e prurido. Essa abordagem contribui para o bem-estar animal e acelera o processo de cicatrização. A pesquisa de moléculas terapêuticas, incluindo fármacos para estes fins dermatológicos, exige a realização de testes pré-clínicos e clínicos, sendo imprescindível incluir experimentos com modelos animais para assegurar a viabilidade comercial. Os camundongos da linhagem BALB/c são amplamente empregados em estudos dermatológicos devido às suas características genéticas e imunológicas estabelecidas. O presente estudo busca esclarecer a relevância e a viabilidade do modelo BALB/c em pesquisas dermatológicas. Para alcançar esse objetivo, foram induzidas lesões dérmicas para avaliar a eficácia de produtos tópicos destinados a cães e gatos, comparando seus efeitos com o uso de solução fisiológica tópica. Os resultados revelaram que camundongos BALB/c tratados com solução fisiológica apresentaram uma recuperação cutânea superior em comparação com aqueles tratados com produtos comerciais comumente utilizados na prática clínica de animais de companhia. A condução do estudo envolveu avaliações diárias, registros fotográficos, medições da área da lesão, avaliação histológica e quantificação de citocinas. Esses achados suscitam questionamentos sobre a escolha indiscriminada da linhagem BALB/c em ensaios clínicos, sublinhando a importância da seleção criteriosa de modelos experimentais na pesquisa dermatológica. Destaca-se a necessidade de uma abordagem cautelosa ao escolher tais modelos experimentais quando há a intenção de extrapolar fármacos e tratamentos para outras espécies ou linhagens.

Palavras-chave: modelo de experimentação animal; linhagem; dermatologia; extrapolação interespécie; seleção de modelo experimental; testes em modelos murinos

ABSTRACT

The use of topical dermatological agents plays an essential role in the treatment of various skin conditions in animals, including dermatitis, infections, burns, and wounds. These medications effectively reduce local inflammation, providing relief for symptoms such as edema, redness, pain, and itching. This approach contributes to animal well-being and accelerates the healing process. The research of therapeutic molecules, including drugs for dermatological purposes, requires preclinical and clinical testing, making it imperative to include experiments on animal models to ensure commercial viability. BALB/c mice are widely employed in dermatological studies due to their established genetic and immunological characteristics. This study aims to clarify the relevance and viability of the BALB/c model in dermatological research. To achieve this goal, dermal lesions were induced to assess the effectiveness of topical products intended for dogs and cats, comparing their effects with the use of topical physiological solution. Results showed that BALB/c mice treated with physiological solution exhibited superior skin recovery compared to those treated with commercially used products in the clinical practice of companion animals. The study involved daily assessments, photographic records, lesion area measurements, histological evaluation, and cytokine quantification. These findings raise questions about the indiscriminate selection of the BALB/c strain in clinical trials, emphasizing the importance of careful model selection in dermatological research. It underscores the need for a cautious approach when choosing such experimental models, especially when there is an intention to extrapolate drugs and treatments to other species or strains.

Keywords: animal experimentation model; lineage; dermatology; interspecies extrapolation; experimental model selection; tests in murine models.

SUMÁRIO

CAPÍTULO 1

1 INTRODUÇÃO

Os medicamentos de uso tópico na medicina veterinária desempenham uma função crucial no tratamento de várias condições de pele em animais (AHLSTROM et al., 2010). Sua atuação é particularmente destacada no manejo de condições dermatológicas localizadas, como dermatites, eczemas, queimaduras e feridas. Esses medicamentos desempenham um papel significativo na redução da inflamação local, atenuando a resposta inflamatória na pele e proporcionando alívio dos sintomas associados, como vermelhidão, inchaço, dor e prurido. Essa abordagem contribui para melhora do bem-estar do animal e acelerar o processo de cicatrização (SAUVÉ, 2019).

Na medicina veterinária, a praticidade de aplicação desempenha, sem dúvida, um papel relevante no desenvolvimento de medicamentos. Nesse contexto, a formulação comumente adotada para medicamentos tópicos, como cremes, pomadas ou loções, facilita e torna mais conveniente a sua administração (SEMIGHINI et al., 2023), além disso, a minimização dos efeitos colaterais sistêmicos representa um fator crucial ao buscar o desenvolvimento de produtos tópicos (MULISSA et al., 2015). Essa característica pode ser vantajosa em animais que não toleram bem medicamentos orais ou que apresentam condições médicas tornando-os sensíveis aos efeitos colaterais sistêmicos (BRAZZINI et al., 2002; MILLS et al., 2006).

Entretanto, para que um fármaco seja desenvolvido e comercializado, necessita anteriormente passar por ensaios pré-clínicos e clínicos, muitas das vezes em modelos de experimentação animal, como por exemplo o camundongo (*Mus musculus*), modelo amplamente utilizados na literatura com objetivo de entendimento de processos fisiopatológicos que ocorrem em humanos e animais (KARAMANI et al., 2021). Tais animais possuem ciclo de vida curto (reposta rápida), são dóceis, de fácil manejo e manipulação genética acessível. Ainda, para esta espécie se encontra uma rica variedade de reagentes no mercado, possibilitando a realização de testes com as mais diversas técnicas e alta qualidade (EHRET et al., 2017).

Os camundongos da linhagem BALB/c são comumente utilizados como modelos experimentais devido às suas características genéticas e imunológicas bem estabelecidas, associadas à facilidade de manejo em laboratório e rápido ciclo de vida. Essa linhagem tem sido amplamente utilizada em pesquisas dermatológicas, desempenhando um papel essencial na compreensão dos mecanismos subjacentes a diversas doenças, incluindo psoríase, dermatite atópica e câncer de pele, além de lesões em tecidos cutâneos. (YADAV et al., 2023). Estes animais apresentam resolução espontânea do processo de lesão dérmica em até 11/12 dias, o que traz agilidade na pesquisa e vai de encontro com os princípios de bem-estar animal além de reforçar ser um modelo experimental apropriado para estudos dérmicos (BARTON et al., 1991).

Diante do exposto, neste projeto objetiva-se elucidar a contribuição de modelos murinos para a compreensão e viabilidade de estudos dermatológicos, bem como a avaliação da eficácia de produtos comerciais comumente indicados para uso tópico em cães e gatos, comparando seus efeitos com o uso tópicos de solução fisiológica NaCl 0,9% de maneira a analisar mecanismos fisiológicos e patológicos de reparação tecidual.

As análises foram realizadas através do acompanhamento diário com registros fotográficos, medição da recuperação tecidual em mm² através de software de processamento de imagens, avaliação histológica em áreas de transição entre pele lesionada e não lesionada e quantificação por teste imunoenzimático (ELISA) de citocina pró-inflamatória envolvida no processo de recuperação tecidual.

2 REVISÃO BIBLIOGRÁFICA

A pele, o maior órgão do corpo, constitui uma membrana fibroelástica que desempenha um papel vital em diversas funções, incluindo hidratação, regulação térmica, reconhecimento imunológico e percepção sensorial. Além disso, atua como uma barreira física, oferecendo proteção contra radiação ultravioleta, produtos químicos e patógenos (MEDELLIN-LUNA et al., 2019; TOTTOLI et al.,2020). Devido à exposição frequente a agressões externas, este órgão torna-se suscetível a lesões que comprometem sua integridade, interferindo no desenvolvimento normal de suas funções (GUARÍN-CORREDOR et al., 2013).

Como resposta a essas lesões, é acionado o mecanismo de cicatrização de feridas, um processo biológico complexo e multifatorial responsável por restaurar a integridade do tecido e restabelecer a homeostase local (IBRAHIM et al., 2018; CORRÊA et al., 2017). Esse processo compreende uma série de estágios em cascata, embora sobrepostos, que podem ser divididos em três fases principais: inflamatória, proliferativa e de remodelação. Essas fases exigem uma sinalização altamente coordenada e precisa de diversas células, como queratinócitos, fibroblastos e células endoteliais, que produzem citocinas, fatores de crescimento e colágeno (JACOB et al., 2015; IYYAM et al., 2010).

Durante essas etapas, ocorrem eventos distintos, como a fase inflamatória, que representa uma fase crucial no processo de cicatrização, durante a qual ocorre a limpeza do tecido através da fagocitose, liberação e ativação de citocinas, fatores de crescimento, bem como a participação de células imunes como macrófagos e células dendríticas além da angiogênese para promover a vascularização da área lesada (LI et al., 2007). Durante os processos de recuperação tecidual, ocorre também a fase celular, onde ocorre a granulação do tecido; a fase de contração da ferida, estreitando as bordas da área afetada; formação de colágeno; epitelização e cicatrização. A progressão coordenada desses eventos é crucial para o sucesso do processo de cicatrização de feridas (IBRAHIM et al., 2018).

Embora o processo de autorregeneração da pele seja geralmente rápido e eficiente, lesões de aspecto extenso podem comprometer a cicatrização normal da pele (PEREIRA et al., 2016). Tais eventos podem resultar em alterações que interferem no processo de cicatrização, prolongam o dano tecidual, aumentam o risco de infecção e estendem o período de reparo. Isso

pode levar ao desenvolvimento de condições patológicas, como feridas crônicas e úlceras de pressão (TOTTOLI et al.,2020; IYYAM et al., 2010). Apesar dos avanços no tratamento de feridas, o manejo clínico eficaz continua sendo um desafio significativo, pois, lesões cutâneas necessariamente envolvem inflamação e frequentemente infecções microbianas. Portanto, uma terapia ideal não apenas deve promover uma cicatrização rápida, com contração eficiente da ferida, epitelização acelerada e ganho adequado de resistência, mas também deve possuir propriedades anti-inflamatórias, antibacterianas e cicatrizantes (MIRHAJ et al., 2022), para isso, é necessário o desenvolvimento de produtos farmacêuticos, sendo indispensável a realização de testes em modelos de experimentação animal.

Pesquisas envolvendo modelos animais têm uma longa história, com relatos documentados de experimentos remontando ao século V a.C. Contudo, observa-se um aumento significativo na frequência de sua utilização desde o século XIX (FERNANDES et al., 2017). A maioria das instituições de pesquisa científica utiliza animais não humanos como modelos experimentais, visando aprofundar a compreensão das doenças e explorar possíveis opções de tratamento (LAFOLLETTE, 2020). A premissa central por trás da experimentação animal é sua contribuição benéfica para a pesquisa biomédica. Várias razões fundamentam a importância do uso de animais nesse contexto. Uma delas reside na partilha de processos biológicos entre animais humanos e não humanos, aliada a notáveis semelhanças anatômicas em vertebrados, que possuem grandes semelhanças na distribuição de órgãos como pulmões, coração, rins e fígado. Essas similaridades tornam certos animais particularmente adequados para experimentos e para fornecer treinamento básico em ciências biológicas e biomédicas (FRANCO; N.H., 2013).

Mesmo animais evolutivamente distantes dos mamíferos, como *Drosophila melanogaster*, zebrafish (*Danio rerio*) e *Caenorhabditis elegans*, compartilham semelhanças fisiológicas e genéticas, tornando o uso destes uma ferramenta valiosa para o avanço da ciência médica (LAFOLLETTE, 2020). Alguns exemplos podem ser citados como na avaliação da eficácia e segurança de fármacos e tratamentos médicos potenciais, facilitando a identificação de potenciais efeitos colaterais indesejados, como defeitos congênitos, infertilidade, toxicidade, danos ao fígado e efeitos cancerígenos (REGEMBERG et al., 2009). É importante ressaltar que não apenas os seres humanos se beneficiam dessas pesquisas e testes, uma vez que muitos

medicamentos e tratamentos desenvolvidos para humanos são rotineiramente aplicados na rotina da medicina veterinária, contribuindo para a promoção de vidas mais longas e saudáveis para diversos animais (LAIRMORE et al., 2015). Destaca-se a importância das descobertas utilizando animais de laboratório para animais de companhia como cães e gatos, pois testes medicamentosos para drogas direcionadas a tais espécies são inicialmente analisados em sua fase pré-clínica e clínica em animais de laboratório devido as desvantagens do uso de animais de companhia como modelos experimentais; e as diversas utilidades no uso de modelos murinos para tais fins, como disponibilidade, fácil manipulação e manutenção acessível (LAIRMORE et al., 2015).

A maioria desses modelos são roedores murinos, especialmente em estudos sobre doenças infecciosas e investigação imunológica. Essa preferência é evidenciada por números impactantes, como o fato de que, apenas na União Europeia em 2011, 75% de todos os animais utilizados para "fins experimentais e outros fins científicos" eram camundongos (*Mus spp.* 61%) e ratos (*Rattus spp*. 14%) de acordo com a Comissão ao Conselho e Parlamento Europeu (2013). Animais de companhia apresentam diversas limitações potenciais na pesquisa científica como a propensão dos cães ao vômito, dificultando a administração de certos medicamentos (GANDERUP et al., 2012; SCHMITT et al., 2015) e a diversidade genética, podendo resultar em variações fisiológicas e metabólicas específicas de raça. Já a desvantagem mais significativa reside nas questões éticas envolvidas no uso de cães e gatos como modelos não roedores de experimentação, por se tratar de espécies que carregam fatores afetivos e empáticos por seres humanos (GILMORE & GREER, 2015).

A elaboração de novas drogas geralmente necessita de testes pré-clínicos, e, se tratando de medicamentos dermatológicos, é indispensável um modelo de experimentação animal confiável, já que ensaios in vitro é incapaz de simular de maneira fidedigna os processos fisiológicos da pele (KARAMANI et al., 2021). Além disso, ajudam a compreender seus mecanismos de ação, bem como na análise de relações farmacodinâmicas, toxicidade e farmacocinética (MCGONIGLE & RUGGERI, 2014), sendo os modelos murinos, os principais responsáveis por proporcionar insights valiosos e contribuir para a identificação de novos medicamentos (BOCHEŃSKA et al., 2017). Desta forma, o modelo mais utilizado para ensaios

científicos (figura 1), incluindo pesquisas dermatológicas, são camundongos (*Mus musculus*) (EHRET et al., 2017).

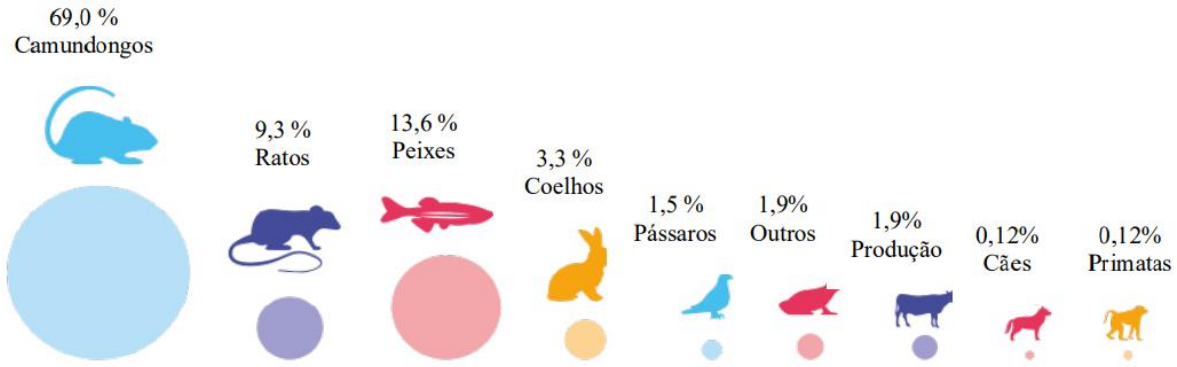

Figura 1 - Principais grupos de animais utilizados em pesquisas. Fonte: Adaptado de German Primate Center – Disponível em <https://www.dpz.eu/en/unit/about-experimental-animalresearch/zahlen-und-fakten/tierversuchszahlen-in-deutschland.html>

Camundongos, principalmente indivíduos endogâmicos, são caracterizados de acordo com a definição da endogamia, sendo aqueles "gerados por meio de pelo menos 20 gerações consecutivas de acasalamentos entre irmãos ou entre pai e prole", ou "rastreáveis até um único par ancestral na 20ª geração ou em gerações subsequentes", conforme estabelecido pela "Nomenclatura de Camundongos Endogâmicos" definida pelo Comitê de Nomenclatura Genômica de Camundongos. É relevante observar que, embora 20 gerações de endogamia não resultem em alelos totalmente fixados em todo o genoma, a maioria dos fenótipos não apresenta diferenças notáveis após esse ponto (CHIA et al., 2005).

Existem diversas populações endogâmicas, frequentemente denominadas linhagens, que possuem benefícios no seu uso em experimentos por serem geneticamente altamente homogêneas, claramente definidas, além disso, informações detalhadas sobre tais linhagens estão acessíveis no banco de dados Mouse Phenome (GRUBB et al., 2014) ou no International Mouse Phenotyping Consortium (KOSCIELNY et al., 2014).

Dentre as principais linhagens endogâmicas utilizadas em ensaios científicos, está a linhagem BALB/c, uma cepa de camundongo albino de origem laboratorial, da qual se originaram várias sub-cepas amplamente reconhecidas. Com mais de 200 gerações desde a sua introdução em Nova York, em 1920, esta linhagem distribui-se globalmente e destacou-se como

uma das mais amplamente empregadas em experimentação animal (BLAKE et al., 2021), incluindo estudos dermatológicos (HAY et al., 1983).

Sendo assim, o tecido cutâneo desses animais mostra-se como uma ferramenta crucial para avaliar in vivo o tratamento de cicatrização de lesões na pele (POSTEN et al., 2005), no entanto, para que pesquisas e estudos sejam relevantes e confiáveis, é necessário compreender os complexos processos fisiopatológicos de cada linhagem e sua aplicabilidade para uma espécie a ser testada, pois, moléculas testadas podem ter resultados diferentes quando aplicadas e comparadas entre espécies ou linhagens(RYDEL et al., 2019).

Dentre todas as possibilidades, uma porção restrita de fármacos consegue percorrer com êxito todas as etapas clínicas até alcançar a fase de comercialização. A principal causa de insucesso nos testes clínicos reside na ineficácia, evidenciando limitações na previsibilidade da pesquisa pré-clínica. Portanto, a confiabilidade de um ensaio é essencial, sendo imprescindível que o modelo experimental atenda a todos os parâmetros necessários, demandando que o pesquisador possua conhecimento aprofundado sobre a linhagem (BAEDEKER et al., 2020).

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animals

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Article

Evaluation of the Use of BALB/c Murine Models for Studies on Induced Skin Lesion Healing

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Simple Summary: Topical dermatological medications are crucial for treating various skin conditions in animals, such as infections, burns, and wounds. These medications alleviate symptoms of local inflammation, such as swelling, pain, and itching, promoting the well-being of animals and accelerating the healing process. The development of medications requires laboratory testing before potential commercialization, necessitating evaluations in animal experimental models. BALB/c mice are widely used in dermatological research due to their well-known physiological, genetic and immunological characteristics. This study aims to elucidate the contribution and viability of BALB/c in dermatological studies. Skin lesions were induced in animals to assess the efficacy of topical products marketed for dogs and cats, comparing their effects with the use of topical saline solution. The results indicate that BALB/c mice treated with saline solution exhibited superior skin recovery compared to those treated with commonly used commercial products in the clinical routine for dogs and cats. Daily analyses, photographic records, lesion area measurements, histological evaluation, and cytokine quantification were conducted. These findings raise questions about the indiscriminate selection of the BALB/c lineage for any scientific study, emphasizing the importance of appropriate experimental models in dermatological research and highlighting the need for a cautious approach in choosing experimental models.

Abstract: The use of topical dermatological medications is crucial for treating various skin conditions in animals, such as infections, burns, and wounds. These medications act effectively on local inflammation, alleviating symptoms like edema, pain, and itching, promoting animal well-being, and accelerating healing. The search for therapeutic molecules requires both preclinical and clinical tests, necessitating evaluations in animal experimental models to achieve market readiness. BALB/c mice are extensively utilized in dermatological research due to their well-established genetic and immunological characteristics. This study aims to elucidate the contribution and viability of the BALB/c in dermatological studies. Dermal lesions were induced to evaluate the efficacy of topical products marketed for dogs and cats, comparing their effects with the use of topical saline solution. Results indicate that BALB/c mice treated with saline solution exhibited superior cutaneous recovery compared to those treated with commonly used commercial products in the clinical routine for dogs and cats. Daily analyses, photographic records, lesion area measurements, histological evaluation, and cytokine quantification were conducted. These results raise questions about the indiscriminate selection of the BALB/c lineage in clinical trials, emphasizing the importance of appropriate experimental models in dermatological research and underscoring the need for a cautious approach in choosing experimental models.

Keywords: animal experimentation model; lineage; dermatological tests; interspecies extrapolation; drug development; dermatology; experimentation; selection of experimental model

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1. Introduction

Topical dermatological medications in veterinary medicine are crucial for treating various skin conditions in animals [1], particularly in the management of dermatitis, eczema, infections, burns, and wounds. These medications effectively reduce local inflammation, alleviating symptoms such as redness, swelling, pain, and itching, contributing to animal well-being and accelerating healing [2].

The development and production of molecules for therapeutic purposes, including dermatological drugs, can promote the minimization of systemic side effects in the formulation of topical products [3], offering a more focused approach to the affected area compared to systemic administration methods [4]. Additionally, it requires preclinical and clinical testing to achieve market readiness, involving evaluations in experimental animal models [5].

Mice (*Mus musculus*) are widely used models to understand pathophysiological processes in both humans and animals due to their docility, ease of handling, short life cycle, and availability of specific reagents [6]. The BALB/c strain is commonly employed in dermatological research due to its well-established genetic and immunological characteristics, proving effective in understanding skin diseases [7]. The BALB/c mouse exhibits spontaneous resolution of dermal lesions within 11/12 days, making it an efficient experimental model aligned with animal welfare principles [8].

Thus, this project aims to elucidate the contribution and viability of BALB/c murine models in dermatological studies, as well as evaluate the efficacy of topical products for treating wounds in dogs and cats in these models. The study seeks to draw parallels regarding the reliability of using this strain for developing products that will be extrapolated to other species. Daily analyses, photographic records, lesion area measurements, histological evaluation, and cytokine quantification by ELISA will be performed during the tissue recovery process.

2. Materials and Methods

2.1. Ethical Aspects in the Use of Animals in Research

All studies involving mice were previously approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Uberlândia (CIAEP No. 02.0105.2019), responsible for overseeing the study.

In accordance with the Brazilian Guide for the Production, Maintenance, or Use of Animals in Teaching or Scientific Research Activities from the National Council for Animal Experimentation Control - CONCEA, this study protocol includes the use of humane endpoints at four levels:

i) Adoption of treatment to relieve pain, discomfort, or distress;
ii) Interruption of a painful procedure;
iii) Exclusion of the animal from the study; or,
iv) Humane euthanasia of the animal.

2.2. Study Locations

The clinical phase will be conducted at the Central Animal Facility of the Rodent Biotechs Network of UFU (Federal University of Uberlândia). Address: Rua Ceará – S/N – Bloco 4U – Campus Umuarama; ZIP Code: 38405-320, Uberlândia, MG.

The analytical/laboratory phase will take place at the Laboratory of Biotechnology in Experimental Models – LABME, Federal University of Uberlândia. Address: Rua Ceará – S/N – Bloco 8G - Campus Umuarama; ZIP Code: 38405-320, Uberlândia, MG.

2.3. Experimental Animals

For the development of this research, BALB/c mice (*Mus musculus*) were used, young adults, males, and females in equal proportions, aged between 6 to 8 weeks, weighing between 20 to 25 grams, and presenting a body condition score between 2 to 4. They showed no alterations in respiratory patterns, no skin or cutaneous annexes lesions, and no signs of pain and stress. All mice were born and kept throughout the experimental period at REBIR/UFU, in groups of five animals per cage. Each cage was labeled with the group identification, and each animal was individually marked from 1 to 5 with a permanent pen on the tail. The marking was touched up daily at the time of lesion measurement.

2.4. Housing, Facilities, General Management, Feeding, and Hydration

The animals were housed in the experimental section of the Central Animal Facility of the Rodent Biotechs Network of UFU, under specific pathogen-free conditions, with a 12-hour light/dark cycle and free access to irradiated NUVILAB CR-1 feed and filtered and autoclaved water. They were kept in microisolators, following the management protocols of the central biotech facility of REBIR, with bedding and water changes once a week. Each microisolator housed 5 animals, placed in ventilated racks that allowed individual ventilation control per cage (15 changes/hour). The ventilated racks were maintained in temperature-controlled rooms (22°C ± 1°C). Daily monitoring of the animals was conducted by veterinarians and a team of researchers with experience in rodents.

Housing and management conditions complied with the guidelines outlined in Chapter 2 "Rodents and Lagomorphs" of the Brazilian Guide for the Production, Maintenance, or Use of Animals in Teaching or Scientific Research Activities [9].

2.5. Experimental Design

For this study, 30 mice (BALB/c strain), comprising 15 males and 15 females, were selected on experimental day D-3 (three days prior to the administration of veterinary products and saline solution). As depicted in Figure 1, those meeting the defined inclusion

criteria without falling under any exclusion or discontinuation criteria on the day of product administration (D0) underwent the process of stratification and randomization. At this point, a completely randomized design determined the 30 animals that effectively constituted the assay and were subsequently allocated to form three experimental groups, each containing 10 mice, comprising 5 males and 5 females.

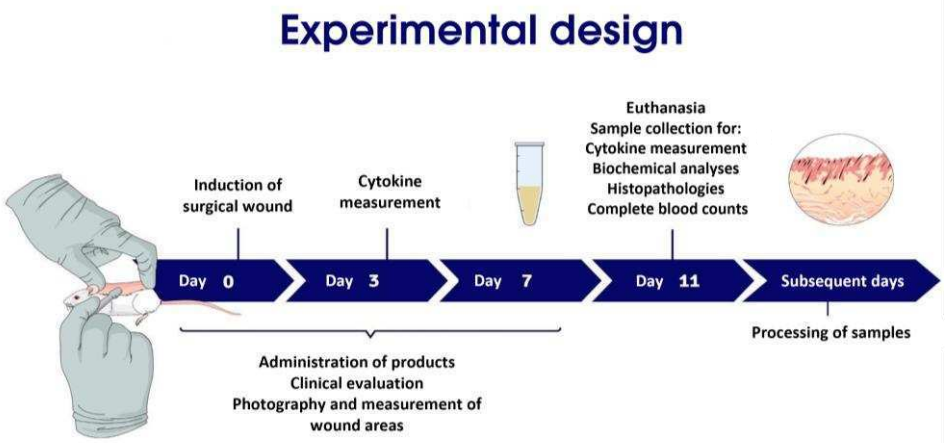

Figure 1. Experimental design of the scientific assay. Source: Original image (2021) - image created with *Mind the Graph*®.

The number of animals selected for effective participation in the assay aimed to meet the minimum viable number for obtaining reliable data to achieve the study's objectives. This was based on previous studies using animal models and followed the standards accepted by international journals for publishing such studies [10].

The experimental groups were divided into: Group 01: *Saline solution* (n= 4 males and 5 females), Group 02: *Aurigen*® (n= 5 males and 5 females) and Group 03*: Dermotrat*® (n= 5 males and 5 females). The sample size (n) for male mice in group 01 decreased from 5 individuals to 4 individuals after the death of the animal during the anesthetic induction for the cutaneous lesion on Day 0.

The selection and randomization of animals were performed using a spreadsheet in Microsoft Excel® or a similar software, attached to the raw data of the assay. This document was identified by initials/signature and dated by the study investigator, containing information about all pre-selected animals.

2.6. Criteria for Inclusion, Exclusion, and Removal

Table 1. Criteria for Inclusion of Animals in the Study.

Profile and Category:	BALB/c Mice – Males and Females
Body Weight:	Animals between 20 and 25 g
Physiological Conditions:	No alterations in respiratory patterns, no skin and cutaneous annexes lesions, no signs of pain and stress.

Table 2. Criteria for Exclusion of Animals from the Study.

Weight reduction greater than 10% of the initial weight at any time during the experiment.

Presenting any skin and fur alterations not associated with the protocol.
Behavior associated with stress.
Clinical signs of pain referring to the classification: Obviously present "2" according to Akintola (2017) [11].

Animals that met the inclusion criteria and did not fall under any exclusion criteria could potentially be removed during the study. The criteria for determining the removal of an animal are listed in the table below.

For this work, isogenic mice with specific pathogen-free (SPF) sanitary standards were used, meaning they exhibit around 99% homozygosity in genes, making them very similar in terms of age, with the age among the animals varying by only one week. Moreover, the SPF standard provides these animals with health assurance, rendering the batches more homogeneous.

The pre-selection of animals that comprised the study groups was based on the individual assessment of all animals when they were allocated to the experimental area by the breeding sector. This pre-selection was conducted by the team of researchers involved in the project.

The animals underwent a 3-day acclimatization period to the study conditions, between the moment of pre-selection and the start of the administration of the tested products (D0). The breeding sector provided animals with an approximate age of 6 weeks. Acclimatization was carried out solely by changing the housing (from breeding to experimentation), but within the same building. The start of acclimatization was identified by the pre-selection record when the animals were identified and evaluated for health to be included in the study. During this period, the animals were observed at least once a day to check their health, and observations were recorded on an appropriate form.

2.7. General Health Observations (GHO)

A team member, experienced in species handling and familiar with their behavior and health, was tasked with conducting General Health Observations (GHO) on all animals at least twice a day throughout the experimental period. GHO involved observing the overall physical appearance and behavior, abnormalities in food and water consumption, urine and feces appearance, as well as other parameters indicative of the animals' health. If necessary, at the investigator's discretion, the animal would be segregated and kept isolated from the study participants until fully recovered.

Normal or abnormal observations were recorded on a specific form. If any abnormality was identified, a veterinarian would be responsible for examining the animal and documenting the findings on a specific form: if the record was before the administration of the products, it should be made through a Study Note; if it occurs after the administration of the products, it constitutes an Adverse Event, and the record should follow the guidelines outlined for AE.

2.8. Clinical Examinations

All animals underwent a clinical evaluation at the time of their pre-selection to determine their overall health status. The data from these assessments were recorded on a specific form. The table below lists the scores or ranges of normality that were used for evaluating the parameters comprising the clinical examinations.

Table 4. Definition of scores or ranges of normality applicable to the assessment of the animals' clinical parameters.

Parameter	**Score Determination (bold) or Presentation of Normal Range**

Respiratory Pattern	**1** (normal); **2** (dyspnea).
Animal Behavior	**1** (normal); **2** (altered, describe any changes).
Body Position	**1** (normal – full extension of the body while walking); **2** (body curved while walking); **3** (other alteration, describe if any).
Grooming	**1** (shiny and aligned fur); **2** (piloerection); **3** (piloerection, dull and dirty fur).
Eyes	**1** (bright, wide open, and without discharge); **2** (half-open and without discharge); **3** (half-open or closed and with discharge).
Body Condition Score (BCS)	**1** (very thin); 2 (thin); **3** (ideal); **4** (overweight); **5** (obese).

Source: Burkholder et al., 2012 [12].

The respiratory pattern was determined through observation. An animal breathing within the expected rhythm without difficulty was considered normal, while those exhibiting respiratory distress were deemed dyspneic.

Hydration assessment via cutaneous turgor involved pinching a fold of anterior thoracic skin encompassing cutaneous tissue. The skin's return time to its normal state was classified according to the table above.

Animal behavior was observed daily, noting any abnormal behaviors such as stereotypies, signs of pain, or others, which were considered and recorded.

Body position was also analyzed through observation. Animals walking with a stretched body (in an anatomical position) were classified as normal. Those walking with hyperkyphosis were considered with a curved body. Animals with different body positioning conditions had specific records.

Grooming evaluation involved observing the animal's self-cleaning. If the fur was aligned and clean, it received a score of 1. If the fur was raised, it received a score of 2. If the fur was dirty and messy, it received a score of 3.

Eye observation fell into three possible scores. Bright, clean, and wide-open eyes were considered normal (score 1). Half-open eyes without discharge received a score of 2, while half-open or closed eyes with any type of discharge received a score of 3.

The BCS will be further described below but essentially involved observing the visibility of pelvic bones and the spine, the muscle mass covering these bones, and the layer of body fat enveloping the animal.

2.9. Weighing of the Animals, Scale Calibration, and Body Condition Score

The animals were weighed when the groups were formed (D0), approximately at 6 weeks of age.

The Body Condition Score (BCS) was assessed on a scale of 1 to 5, where 1 is very thin, 3 is ideal, and 5 is obese, according to Burkholder (2012) [12]. This scale involves the

observation and palpation of bones and muscles, especially the vertebrae and pelvis, to report the levels of body condition. Each of the scores is described below: (1) Very thin – Pelvic bones and vertebrae are very prominent, with very little muscle mass; (2) Thin – Pelvic bones and vertebrae are evident, with little muscle coverage; (3) Ideal – Bones are not evident but palpable with slight pressure; (4) Overweight – Waist is not evident, and pelvic bones and vertebrae are palpable only with firm pressure and (5) Obese – The mouse is smooth and bulky, and the bone structure is not palpable.

2.10. Administration of the Veterinary Product

The veterinary products *Aurigen*® and *Dermotrat*® (selected for being commonly used drugs in veterinary clinical routine) and saline solution were topically administered to the animals without prior sanitation in the wound area for a period of 7 (seven) consecutive days, from D0 to D6, immediately after the induction of wounds, covering the entire injured area, according to the dosage of the investigational products for each experimental group:

- Group 01 (Saline Solution): NaCl 0.9% saline solution was administered directly to the animals on the skin, with the administration of one drop per area of up to 5 cm², covering the entire lesion area, twice a day, with an interval of approximately 12 hours.
- Group 02 *Aurigen*®: *Aurigen*® was administered directly to the animals on the skin, with the administration of one drop per area of up to 5 cm², covering the entire lesion area, twice a day, with an interval of approximately 12 hours.
- Group 03 *Dermotrat*®: *Dermotrat*® was administered directly to the animals on the skin, with the administration of one drop per area of up to 5 cm², covering the entire lesion area, twice a day, with an interval of approximately 12 hours.

If the healing process occurred in less time, which is possible due to the accelerated metabolism of the species, the study could be stopped and recorded in a protocol amendment or protocol deviation form.

2.11. Concomitant Medications

The administration of an anesthetic necessary for the induction of inflammation and sample collection was protocolized. Additionally, if the application of any endpoint for intense pain relief was deemed necessary, analgesic medications would be used.

2.12. Inflammation Induction

For inflammation induction on D0, the animals were anesthetized with isoflurane (4% for induction and 2% for maintenance). Under anesthesia, trichotomy and antisepsis of the dorsal region were performed with 70% v/v alcohol, with an approximate extension of 6 cm in length and 4 cm in width, caudally to an imaginary line passing through the lower edge of the ears. Once trichotomy was performed, the animals underwent a circular incision of 6 mm in diameter using surgical material at the center of the shaved area, employing a sharp metallic punch. The skin and subcutaneous tissue were removed, though without reaching the musculature of the dorsal region where the products were applied.

2.13. Assessment of Anti-inflammatory Efficacy

For the assessment of the product's anti-inflammatory efficacy, an experimental inflammation model was utilized, involving the induction of skin wounds using a punch. It is known that all cutaneous wounds, from surgical incisions to puncture wounds, trigger a tissue repair process through a complex mechanism coordinated by multiple cellular and molecular events that interact simultaneously and synergistically [13,14]. This tissue repair process can be didactically divided into stages, including inflammation, proliferation, and tissue remodeling [15,16]. The first stage, inflammation, was chosen as the model for evaluating the action of different products in BALB/c mice.

The anti-inflammatory efficacy was confirmed through the comparison of groups via histological analysis of the lesion areas and adjacent regions; Daily photographs with measurements of the evolution of the recovery process; IL-12 cytokine profile in the animals' serum. Additionally, to monitor the safety of product administration, absence of concomitant infections, and ensure the health of the animals throughout the study, a complete blood count was conducted as a complementary examination.

2.14. Histological Analysis of Lesion and Adjacent Areas

Skin samples were collected from the dorsal region of mice in all groups on the last day. The tissues were fixed in 10% phosphate-buffered formalin for 24 hours and then placed in 70% alcohol until they underwent the paraffin embedding process. After embedding, the organs were cut (5 μm thickness) and deposited on microscopic slides and subsequently stained with hematoxylin and eosin for the evaluation of the inflammatory infiltrate. In assessing the inflammatory score, an examination was conducted for the presence of cells and inflammatory indicators in the tissue (such as a connective tissue, congested vessels, hypertrophy, and hyperplasia of scar tissue). Scores ranging from 1 to 3 were used for the analyses, with 1 indicating mild inflammation when few inflammatory indicators were observed, 2 indicating moderate inflammation, and 3 denoting intense inflammation when a significant presence of inflammatory signs was observed.

2.15. Daily Photography with Measurement of Recovery Process Evolution

The wound dimensions were measured using a digital caliper and photographed from D0 to D6. The wound area was calculated according to the equation: Wound area (mm2) = π.R.r, where π = 3.1416, R = cranio-caudal radius, and r = latero-lateral radius [17].

For photographic recording, the camera was fixed at the standard distance of 10 cm to make comparisons between groups and healing times. Wound dimension data and confirmation of photographs were recorded in appropriate forms.

2.16. Cytokine Profile

IL-12p40 concentrations were measured in serum samples from mice in all groups on D3. Cytokine quantification was performed using commercial ELISA kits, conducted according to protocols recommended by the manufacturer (BD Biosciences, San Diego, USA).

Briefly, 96-well high-affinity plates (Corning Laboratories Inc, New York, USA) were coated with specific anti-mouse capture antibodies for IL-12p40 and incubated overnight at 4°C. Subsequently, to block nonspecific sites, 10% fetal bovine serum in 0.01M PBS (pH 7.2) was added to the plate for 1 hour. After blocking, standard curves of respective cytokines in serial dilutions and test samples were added to the plates and incubated for 2 hours. For cytokine detection in samples, biotin-conjugated anti-mouse cytokine detection antibodies with streptavidin-peroxidase were added and incubated for 1 hour. Between each step, the plates were washed with 0.05% PBS-T (PBS-T). The reaction was developed with tetramethylbenzidine (TMB) followed by the addition of 2M sulfuric acid to stop the reaction. Optical density (OD) was determined at 450 nm using a microplate reader (SpectraMax M2e, Molecular Devices, USA). Cytokine concentrations were determined from the standard curve with known cytokine concentrations, and the results were expressed in pg/mL, according to the detection limits for each assay (IL-12p40 15.6 pg/mL). Collection records were made on appropriate forms.

2.17. Hematological Profile

The hematological profile was obtained from blood collected on D7, seven days after the surgical wound induction procedure. On this day, the animals were anesthetized with isoflurane (2 to 4.5% rate), and blood collection was performed via the retro-orbital route

using a glass capillary. The samples were placed in 0.5 ml tubes containing K2 EDTA and immediately homogenized after collection.

Subsequently, the samples were processed on an automatic veterinary hematology analyzer to obtain the following parameters: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), total leukocytes (WBC), and total platelets (PT).

Concurrently, blood smears were prepared and stained with a rapid 25-panoptic stain for a leukocyte differential count. Collection records were made on appropriate forms.

2.18. Conclusion of Study Participation

The anti-inflammatory efficacy assessment period was initially planned for 11 days, from D0 to D10, considering the literature suggesting a recovery period of 11 to 12 days for animals in Group 01. However, as mentioned earlier, the experiment could be terminated earlier than 12 days in the event of wound resolution in treated groups. Since there was rapid healing of lesions, the anti-inflammatory efficacy assessment period was shortened to 7 days, from D0 to D6. The early conclusion aligns with RESOLUTION No. 55, OF OCTOBER 5, 2022, from CONCEA, stating: "3.4.9. Any procedure involving animals must have its objective clearly defined and be completed as soon as the proposed results are obtained."

At the end of in vivo observations, the animals were euthanized for sample collection and analysis. For this purpose, they were anesthetized with a combination of 10% Ketamine Hydrochloride - 90 mg/kg and 2% Xylazine Hydrochloride - 10 mg/kg. Under anesthesia, total blood was collected from the retro-orbital plexus, and death was confirmed by cervical dislocation. Skin samples (transition between injured and non-injured areas) were then collected for histological analysis. Part of the blood was used for a complete blood count, and the remainder was centrifuged to obtain serum. In the serum, cytokine analyses were performed, as detailed in each item of the Anti-inflammatory Efficacy Assessment.

2.19. Adverse Events (AE)

An adverse event consists of any response that is harmful, unintentional, and may manifest as illness, abnormality, or injury observed in the animals participating in a clinical study after the administration of the product, whether or not it may be related to its use. Adverse events were classified as serious when resulting in death, posing a risk of death, or resulting in persistent or significant disability and/or incapacity. A non-serious adverse event, on the other hand, is any that occurs during the product administration phase and is not a serious adverse event. In the case of non-serious adverse events, the Investigator / team reported to the monitor(s) within 24 hours. In the case of a serious adverse event requiring immediate medical intervention, the monitor(s) were notified immediately, and the Investigator / team was to include a description of the case and proceed with the necessary diagnosis and/or treatment. In extreme cases requiring immediate action, this was to be performed while the Investigator is contacted. In the event of any adverse event, it was the Investigator's obligation to provide as much information as possible, including its likely diagnosis. If any emergency intervention (medication or surgery) was necessary, this had to be authorized in advance by the Investigator. The animal experiencing an adverse event was to be monitored until the complete resolution of the event. Regarding causality related to the tested products, adverse events were classified as follows:

-Probable: the adverse event fits with the pharmacological/toxicological profile of the product, with no other plausible explanation for the event;

-Possible: the adverse event fits with the pharmacological/toxicological profile of the product, but there is another plausible explanation for the event;

-Inconclusive: when there is not enough information to establish a causal relationship with the product;

-Improbable: there is enough information to ensure that the investigational veterinary product did not cause the adverse event.

If an adverse event was observed, it was the Investigator's obligation to ensure proper registration in an appropriate form, which must contain information regarding:

-Classification of the severity of the adverse event (serious or non-serious);

-Causality assessment (probable, possible, inconclusive, or improbable);

-Observed clinical signs (onset, duration, frequency, severity, and description);

-Results of any complementary tests (laboratory or other);

-Possible diagnosis;

-Procedures adopted for treatment (data on the product used, such as commercial name, active ingredient name and concentration; dose, administration frequency, and duration of treatment; route of administration);

-Evolution of the adverse event(s);

-Resolution of the adverse event.

2.20. Euthanasia or Mortality of Animals During the Study

At the conclusion of the evaluations to demonstrate anti-inflammatory efficacy on Day 7, the animals were euthanized. The procedure was performed under anesthesia and occurred after the collection of the samples scheduled for this day.

2.21. Statistical Analysis

The obtained data were analyzed using *GraphPad Prism version 8.0®* (GraphPad Software, San Diego, CA). Group data were using Two-way ANOVA, followed by Bonferroni's post-test. Values with $P < 0.05$ were considered statistically significant. The number of animals was estimated based on previous studies using an animal model, following international journal standards for publishing such studies [10]. Thus, 10 animals per group were requested.

3. Results

3.1. Evaluation of punch wound induction and daily monitoring

The progression of size and appearance of dermal lesions was assessed through photographs (all photographic records were taken from 10cm, with the assistance of a tripod fixed on D0) during the monitoring period, and wound areas were measured using *Archicad®* software. The results are presented in Figures 2 and 3, illustrating the evolution in males and females of each tested group, respectively.

The photos clearly depict the evolution of the group treated with saline solution, while in the other groups, there was the formation of "crusts" with relief at the edges of the lesion in a centripetal direction, some exhibiting exudate.

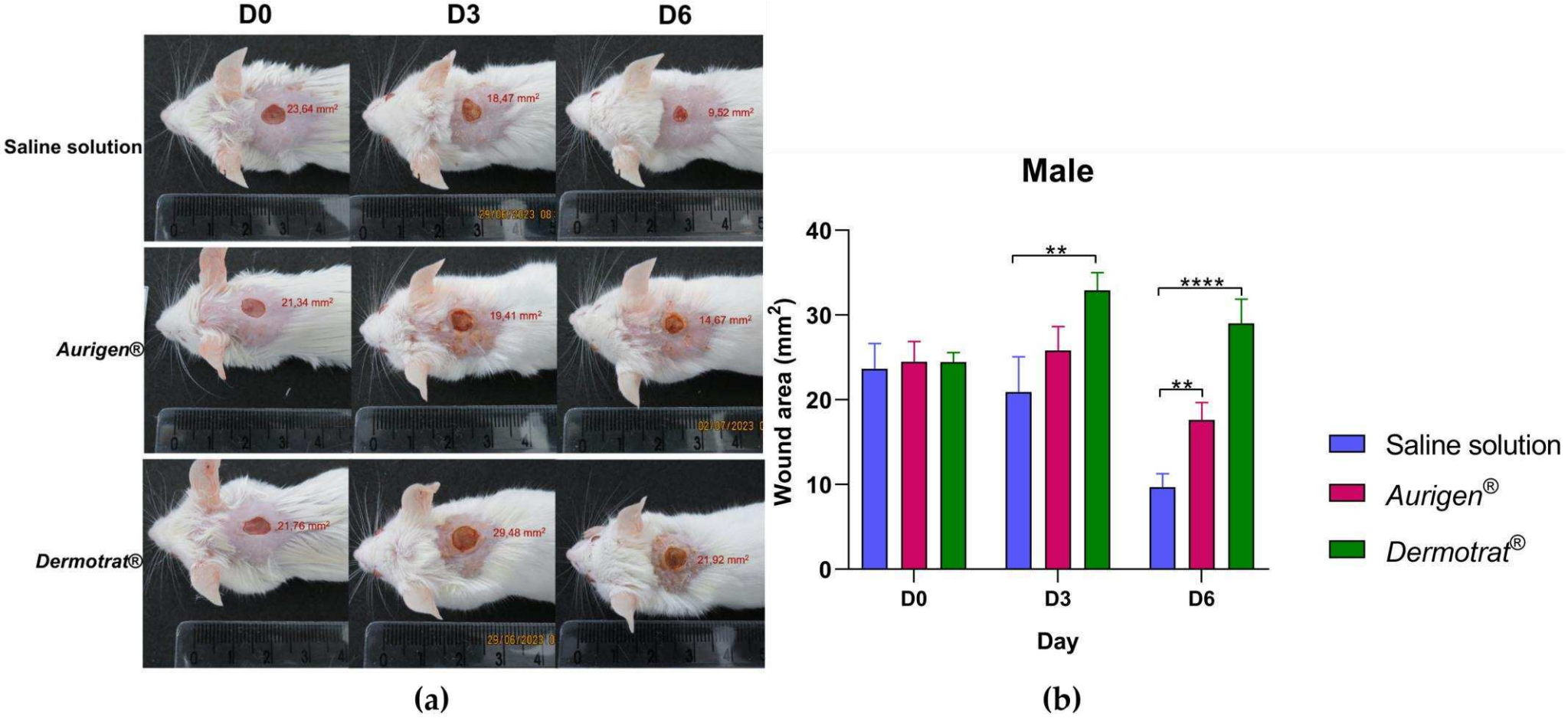

Figure 2. (a) Illustrative photographs depicting the progression of the wound in male animal groups on day 0, day 3, and day 6, with measurements in mm² using *Archicad*® software; (b) Average wound progression from day 0 to day 6 in the three tested male groups, measured in mm². Wound area measured in *GraphPad Software*®. Results were expressed as mean ± standard error of the mean (SEM) and are representative of two independent experiments. Differences between groups were analyzed using Two-way ANOVA test followed by Bonferroni multiple comparison post-test. Statistically significant differences (*$p < 0.05$; ** $p< 0.01$; *** $p<0.001$; **** $p<0.0001$).

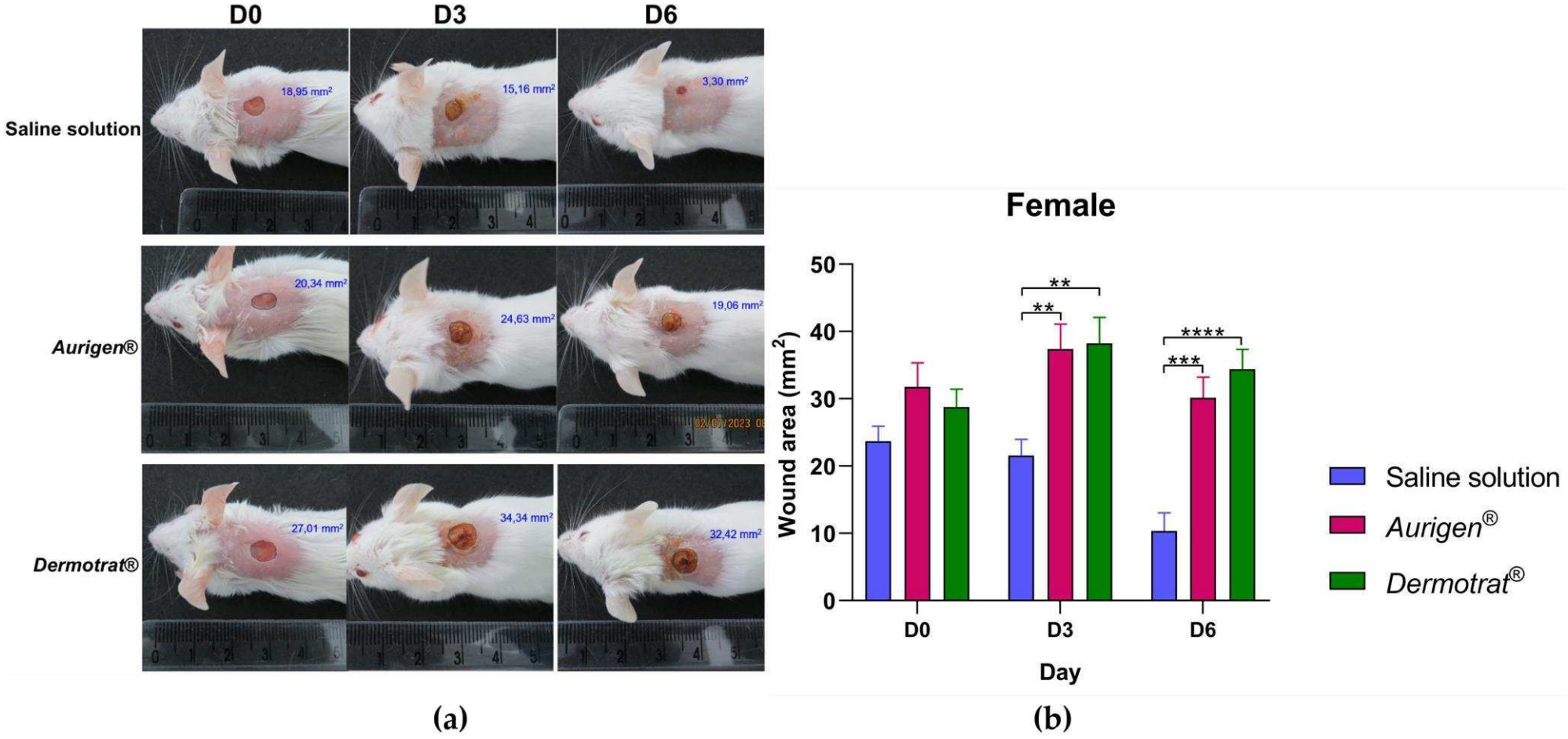

Figure 3. (a) Illustrative photographs depicting the progression of the wound in female animal groups on day 0, day 3, and day 6, with measurements in mm² using *Archicad*® software; (b) Average wound progression from day 0 to day 6 in the three tested female groups, measured in mm². Wound area measured *in GraphPad Software*®. Results were expressed as mean ± standard error of the mean (SEM) and are representative of two independent experiments. Differences between groups were analyzed using Two-way ANOVA test followed by Bonferroni multiple comparison post-test. Statistically significant differences (*$p < 0.05$; ** $p< 0.01$; *** $p<0.001$; **** $p<0.0001$).

3.2. Cytokine Profile

Using the Punch-induced inflammation model, we analyzed the production of IL-12, an important pro-inflammatory cytokine during the immune response. It was possible to observe a pronounced induction of IL-12 in the serum of animals in the group treated with saline solution, three and seven days after treatment. On the other hand, groups of animals treated with *Aurigen*® showed almost undetectable productions after three and seven days, while the *Dermotrat*® group exhibited a slight increase after seven days of treatment. (Figure 4).

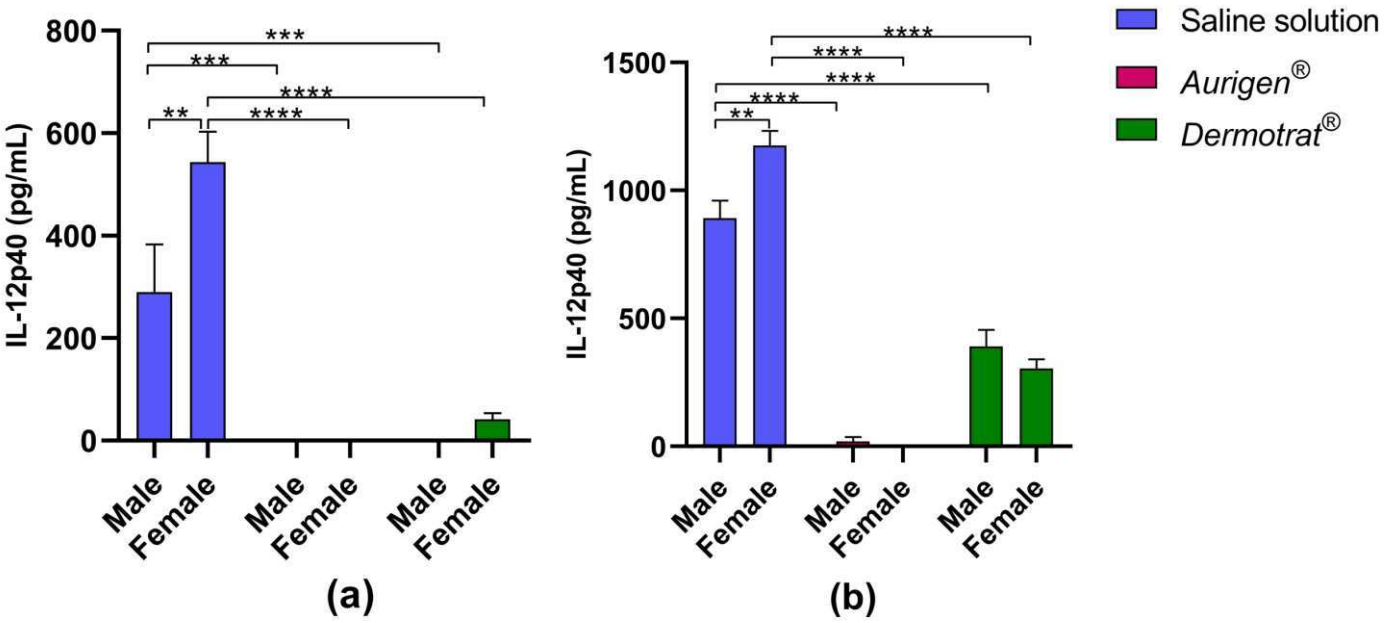

Figure 4. (a) IL-12 cytokine profile in the tested groups, quantified on day 3; (b) IL-12 cytokine profile in the tested groups, quantified on day 7. Results were expressed as mean ± standard error of the mean (SEM) and are representative of two independent experiments. Differences between groups were analyzed using Two-way ANOVA test followed by Bonferroni multiple comparison post-test. Statistically significant differences (*p < 0.05; ** p< 0.01; *** p<0.001; **** p<0.0001).

3.3. Hematological and Leukocyte Differential Profile

There were no significant changes in the erythrocyte profile of any of the groups, as shown in Figure 5. Hematological data demonstrate that none of the tested products had an impact on red blood cells. It is emphasized that the animals are maintained under pathogen-free conditions.

The leukocyte differential was performed by obtaining the relative values of leukocytes through the complete blood count, with the differential and obtaining the absolute values through the distinct counting of leukocytes in a blood smear. The values are presented in Figure 6, and it is possible to observe neutrophilia and relative lymphopenia due to neutrophilia in the group treated with *Aurigen*®, additionally, this group exhibited a reduction in the number of lymphocytes. The other leukocyte parameters are within the normal range, according to previous data from healthy animals at REBIR-UFU (Table S1 - Supplementary Material) for all groups.

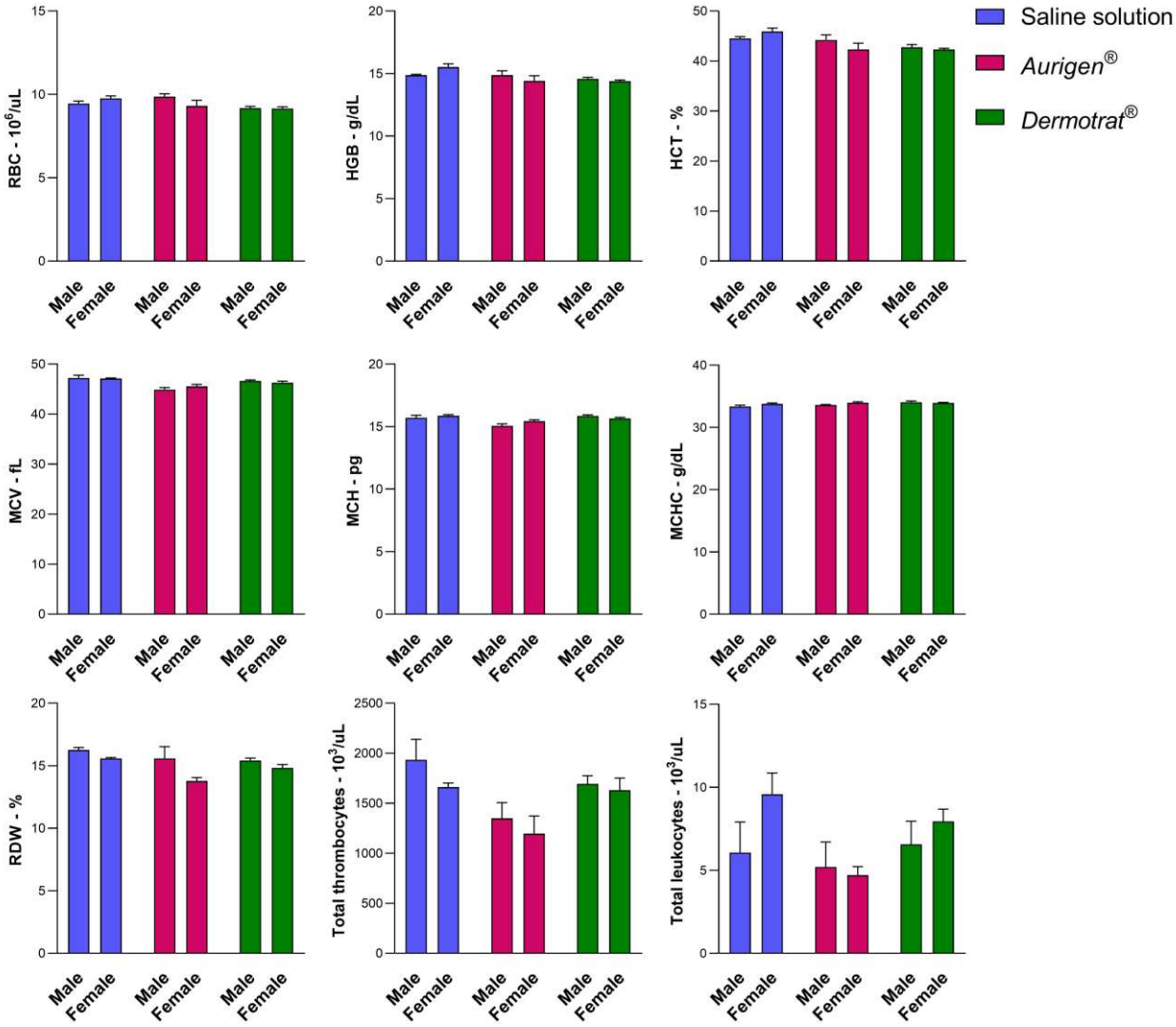

Figure 5. Hemogram Results - Analyzed parameters: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), total white blood cells (WBC), and total platelets (PT). Results were expressed as mean ± standard error of the mean (SEM) and are representative of two independent experiments. Differences between groups were analyzed using Two-way ANOVA test followed by Bonferroni multiple comparison post-test.

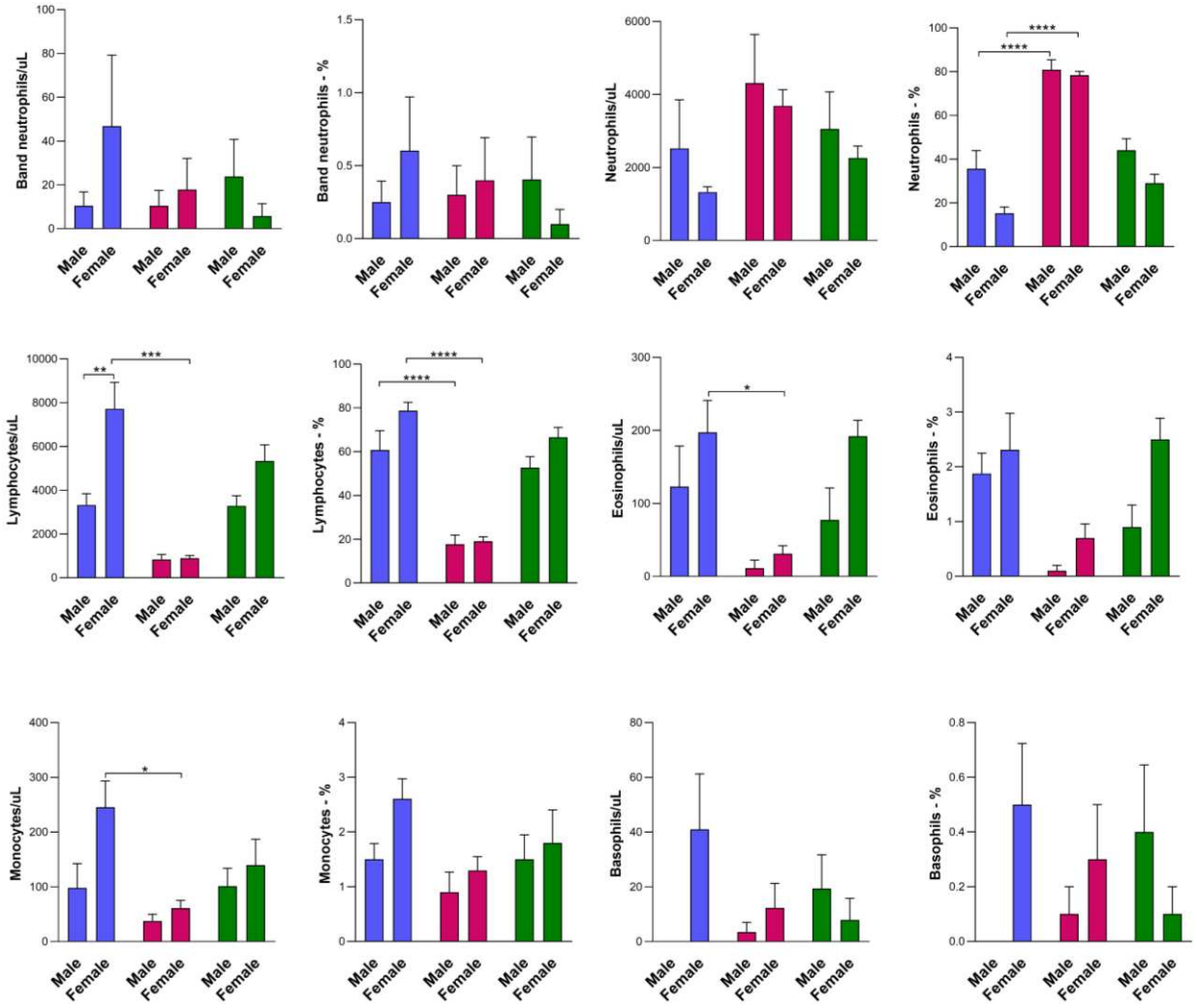

Figure 6. Results of leukocytic parameters obtained from all groups through leukogram (relative values) and a blood smear differential count (absolute values). Results were expressed as mean ± standard error of the mean (SEM) and are representative of two independent experiments. Differences between groups were analyzed using Two-way ANOVA test followed by Bonferroni multiple comparison post-test. Statistically significant differences (*p < 0.05; ** p< 0.01; *** p<0.001; **** p<0.0001).

3.4. Histological Analysis

It was possible to observe through the evaluations of histological slides that inflammatory infiltrates were present in the more superficial layers of the tissues. Although there were no significant differences between the experimental groups (Figure 7), reepithelialization was observed in groups 02 and 03, *Aurigen*® and *Dermotrat*® respectively. In the group treated with saline solution, animals showed complete healing, where the analyzed tissue appeared closer to normal, with preserved tissue structures, while the tissue structures of the other groups appear impaired (Figure 8).

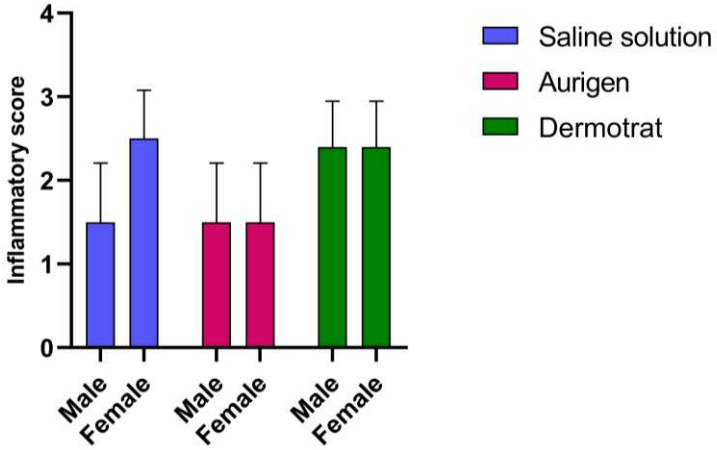

Figure 7. Inflammatory score analysis. Results were expressed as mean ± standard error of the mean (SEM) and are representative of two independent experiments. Differences between groups were analyzed using Two-way ANOVA test followed by Bonferroni multiple comparison post-test. Statistically significant differences (*p < 0.05; ** p< 0.01; *** p<0.001; **** p<0.0001).

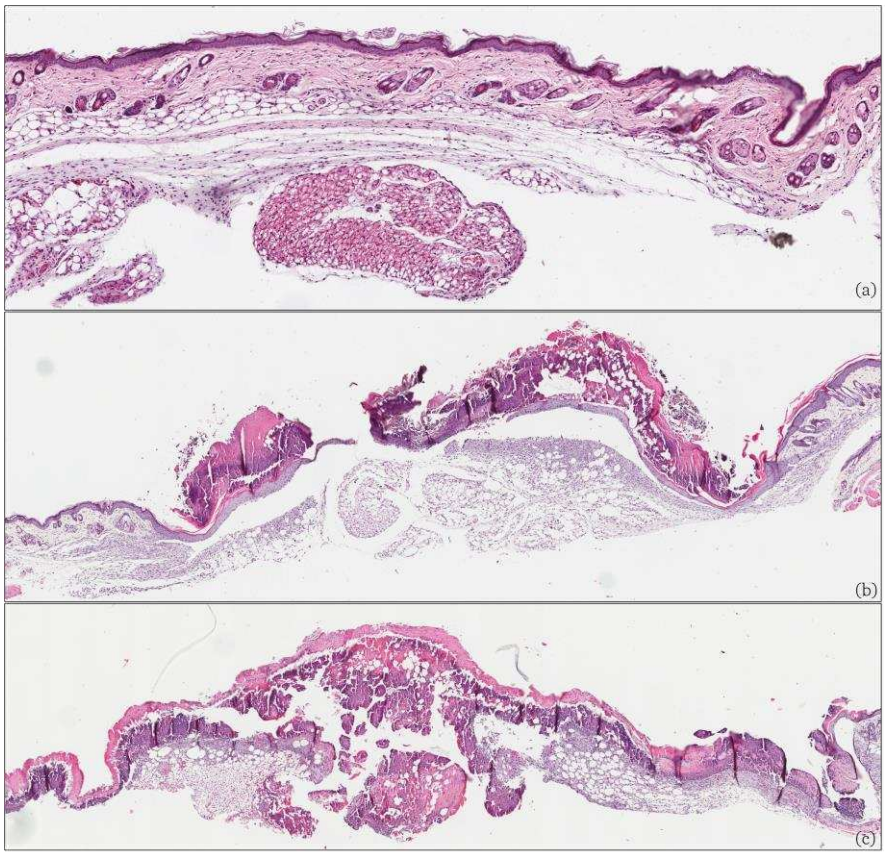

Figure 8. Photomicrographs representing histological slides of the dermal tissue from the injured area of Group 01 (a), Group 2 (b), and Group 3 (c).

4. Discussion

Mice are widely used as animal models in biological studies aimed at understanding human and veterinary health, leading to the development of new diagnostic and therapeutic approaches [18]. This includes demands for their use in pharmacological, oncological, and toxicological research, as well as drug efficacy studies [19]. This is due to their ease of breeding, short generation time, and the availability of inbred strains, established through at least 20 generations of sibling mating, such as the BALB/c and C57BL/6 strains [20].

The BALB/c strain has played a significant role in dermatological studies, contributing crucially to elucidating the mechanisms underlying various skin conditions such as psoriasis, atopic dermatitis, skin cancer, as well as cutaneous tissue injuries. This has contributed to the development of dermatological treatments and medications [7]. However, only a limited number of products tested on the BALB/c strain as an experimental model are developed to the final stages of an experiment, becoming a product ready for commercialization [21]. Therefore, this study aimed to assess whether BALB/c mice are suitable experimental models for the development of products related to skin healing and anti-inflammatory properties.

Analyzing the results of wound evolution in terms of diameter and area in mm^2, it becomes evident that the group treated with saline solution recovery significantly faster than the others, with better scar responses to dermal injury (Figures 1 and 2), followed by Group 2 treated with *Aurigen*® and finally Group 3 treated with *Dermotrat*®, both in males and females.

Observing the evident dermal recovery in the groups treated with physiological solution compared to the other groups, a raised hypothesis corroborates what has already been suggested by Ousey (2016) [22], that hydration is the most important external factor responsible for ideal healing. Moreover, some studies suggest that topical irrigation solutions such as saline solution, distilled water, or silver nitrate can create an environment conducive to rapid healing through mechanisms such as the regular removal of microscopic debris, exudate, microbial load reduction, inhibition of pro-inflammatory cytokines, and improvement in the general state of tissue regeneration [23,24].

The benefits of using saline solution in dermal injuries as described above can provide proper healing. However, the effectiveness of the other products (*Dermotrat*® and *Aurigen*®), in theory, should have favorable effects on the recovery of the tested animals. Both products are commercialized (having undergone preclinical and clinical testing) and are commonly used in the clinical routine of companion animals. They have the same compounds in their formulation (gentamicin, miconazole, and betamethasone) (Figure S1 and Figure S2 - Supplementary Material).

Gentamicin, due to its antimicrobial properties, plays a significant role in promoting wound healing by preventing and treating bacterial infections related to skin injuries. Its ability to cause premature termination of bacterial protein synthesis results in the effective eradication of pathogens, contributing to an environment conducive to cutaneous repair [25].

Miconazole, an antifungal agent, plays a crucial role in healing by combating cutaneous fungal infections. By inhibiting the synthesis of ergosterol in the fungal cell membrane, miconazole regulates the growth of these microorganisms, favoring the restoration of skin integrity during the healing process [26].

Betamethasone, a corticosteroid, can provide anti-inflammatory benefits. Its ability to modulate the immune response and reduce local inflammation contributes to

minimizing edema and discomfort associated with cutaneous lesions, preventing excessive animal manipulation (such as licking), creating a conducive environment for tissue repair [27].

With the benefits for dermal healing of the components of the tested products elucidated, we raise the question of why the groups treated with saline solution showed better results in tissue repair compared to groups treated with commercially available products with clarified effectiveness.

One possibility is that the components of commercialized products may have deleterious effects on skin repair, such as miconazole, which, by inhibiting ergosterol synthesis, induces the production of reactive oxygen species (ROS), causing oxidative stress and leading to cell damage and death. However, the accumulation of ROS can also be detrimental to the cells of the cutaneous tissue, potentially exerting anti-cicatricial effects [28].

Studies suggest that topical corticosteroids, such as betamethasone, another compound found in the tested products, may possess the ability to suppress the development of vascular tissue at an early stage. This can result in delayed wound healing by inhibiting the reepithelialization of keratinocytes, interfering with angiogenesis, impacting the formation of new blood vessels, and consequently depriving the scar tissue of oxygen. [29-30].Additionally, these compounds decrease collagen production by fibroblasts, compromising the quality of scar tissue [29]. This set of adverse effects culminates in the inhibition of the normal healing process and, consequently, impaired scar formation.

However, as these are products used routinely and have undergone preclinical and clinical tests before reaching commercialization, it is believed that they provide benefits to cutaneous tissue in the target species of the product (dogs and cats). They have undergone tests in such species to demonstrate their efficacy in dermatological treatment. However, in the strain used as an experimental model (BALB/c), widely tested for the development of dermatological drugs [7], they did not show beneficial effects for healing, especially compared to the group of animals treated with saline solution.

It is important to consider that there is an impact of the microbiota on the physiological responses of any species, and despite similarities, there are still significant physiological differences, as well as variations in the environment in which each species is maintained. The mice used for this study belong to a specific pathogen-free (SPF) laboratory, while dogs and cats typically live in environments with less control over microorganisms. As a result, their skin may be more exposed to bacteria and fungi, necessitating better control of microorganisms in these species [31].

The extrapolation of doses between species must also be considered. Misinterpreting allometric dose conversion and applying the safety factor can result in significant complications in dose calculation. Thus, determining the appropriate dose requires a careful evaluation of various elements, including body surface area, pharmacological, physiological, and anatomical factors, pharmacokinetic parameters, metabolic function, and receptor characteristics [32].

Statistical differences between the female and male genders were observed in analyses of cytokine quantification and absolute lymphocyte count within the group treated with saline solution; however, these differences did not interfere with the recovery of injuries between genders within the same group. Some studies emphasize a certain relevance in the expression of results between genders, which may vary depending on the scientific assay, such as studies on cognitive performance, stress physiopathology, behavioral studies, endocrinological studies, among others [33]. Therefore, it is incumbent upon the researcher responsible for selecting experimental models to be attentive to the type of study to be conducted and whether the results may differ between males and females.

In addition to visual assessments of healing, the combination of multiple wound assessment methods not only increases the reliability and validity of results but also deepens the understanding of the mechanisms underlying tissue repair. This includes methods

such as blood tests, histological analysis, and measurement of pro-inflammatory cytokines [34].

As shown in Figures 5 and 6, there were no significant hematological changes in the tested groups. However, when evaluating the cytokine profile (Figure 4), it can be observed that IL-12, an interleukin that plays an important role in promoting different immune responses [35], was markedly induced in animals in the groups treated with saline solution. In contrast, the tested groups with commercial products showed a significant reduction in the plasma presence of this cytokine. In addition to the cytokine profile, the histological profile indicated adequate healing nearing completion in the groups treated with saline solution. However, in the groups treated with *Aurigen*® and *Dermotrat*®, it was observed that the cutaneous tissue was still in the reepithelialization phase.

The results obtained from IL-12 cytokine measurement can be justified by the use of topical corticosteroids, which can cause the reduction of cellular infiltrates at injured sites and the inhibition of cytokines involved in inflammatory processes [36]. The inflammatory stage represents a crucial phase in the healing process, during which the release and activation of cytokines, growth factors, as well as the participation of immune cells such as macrophages and dendritic cells occur. These elements play essential roles in resealing the skin, facilitating the formation of epithelium to cover the wound surface, and in dermal restoration, including angiogenesis to promote vascularization of the injured area [37].

Studies indicate that IL-12 can induce a faster onset and higher metabolic activity in murine model wounded skin during the initial phases of healing. These findings suggest that the presence of this pro-inflammatory cytokine triggers a more robust inflammatory response, potentially beneficial for the overall progression of cutaneous wound healing [38]. Therefore, higher levels of pro-inflammatory cytokines may be directly related to the faster healing process in the saline solution-treated group.

However, studies indicate that elevated serum levels of pro-inflammatory cytokines may be related to increased pain [39], raising questions about the validity of rapid healing with increased pro-inflammatory cytokines, which may cause discomfort in the treated animal, going against ethical principles.

The raising of so many questions surrounding dermatological tests of products for companion animals carried out in one of the most widely used experimental model strains worldwide, BALB/c, brings reflection on the indiscriminate selection of this strain in clinical and preclinical trials. Therefore, for research and studies to be relevant and reliable, it is necessary to understand the complex physiopathological processes of each strain and its applicability to a tested species [40].

The presented results are directly related to the exploration and development of drugs, an extensive and intricate process that involves multiple stages, from the conception of a potential therapeutic approach to the availability of the drug on the market. This procedure encompasses the identification and validation of interactions between targets and functions, as well as between compounds and targets, in the early stages of basic research, with the discovery of pharmacological parameters during the preclinical phase of development and culminates in the verification of efficacy and safety through clinical trials [41].

Only a reduced number of selected drugs manage to successfully pass through all clinical phases until the moment of their commercialization. The main reason for the failure in clinical trials is a lack of efficacy, indicating limitations in the predictability of preclinical research. For a trial to be reliable, it is essential that the experimental model is suitable in all necessary parameters, with the researcher having knowledge about the strain [42].

The researcher or laboratory responsible for selecting the animal model for scientific assays must be meticulous and attentive to the species or strain's appropriateness. To

achieve this, an in-depth search in the scientific literature should be conducted, looking for similar studies employing the species or strain intended for study. Additionally, it is crucial to gather all relevant information about the selected strain, such as genetic description, developmental characteristics, and recommendations for its use in specific assays. This approach efficiently reduces the likelihood of errors during the pre-clinical phase [43].

Another important criterion in designing an experiment involving animal models is the preparation from its conception to the application of specific concepts and guidelines for reproducibility and experimental reliability. It is advisable to follow scientific guidelines such as ARRIVE (Animal Research: Reporting of In Vivo Experiments) and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) [44].

5. Conclusions

In summary, this study emphasizes the need to consider variable responses among experimental lineages when evaluating the efficacy of dermatological products. Despite the widespread use of commercial products in clinical practice, the results indicate that, in the BALB/c lineage, often employed in preclinical trials, saline solution demonstrated superior cutaneous recovery.

Factors such as inappropriate lineage selection, extrapolation of inappropriate doses, and potential adverse effects of certain components, such as miconazole and betamethasone, underscore the ongoing importance of understanding the complex interactions between products and healing processes. These findings underscore the significance of a cautious approach in selecting experimental models.

Supplementary Materials: The following supporting information may be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Leaflet of the product tested in group 02; Figure S2: Leaflet of the product tested in group 03; Table S1: Hematological parameters of BALB/c mice.

Author Contributions: Conceptualization, R.C.S. and M.V.S.; investigation, R.C.S., S.G.K., M.M.N., M.C.F.P, I.S.R., T.S.A., G.M.F., M.C.O.F.; L.S.M., G.G.B., F.B.F., I.L.L. and M.V.S., writing—original draft preparation, R.C.S.; writing—review and editing, F.B.F. and I.L.L.; supervision, M.V.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: : The experiment was carried out in accordance with the guidelines given by the Ethics Committee (22/2016 of 20 January 2016. I LKE in Krakow); the Regulation of the Ministry of Agriculture and Rural Development, item 778, Journal of Laws No. 116; and Instructions of the Chief Veterinary Officer, No. GIWz.400/AW-46/2010 of 23 August 2010.

Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.

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

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- ## Manuscript Submission Overview

- ### Types of Publications

Animals has no restrictions on the maximum length of manuscripts, provided that the text is concise and comprehensive. Full experimental details must be provided so that the results can be reproduced. *Animals* requires that authors publish all experimental controls and make full datasets available where possible (see the guidelines on **Supplementary Materials** and references to unpublished data).

Manuscripts submitted to *Animals* should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

- *Article:* Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides substantial new information. Authors should not unnecessarily divide their work into several related manuscripts, although short *Communications* of preliminary, but significant, results will be considered. The quality and impact of the study will be considered during peer review.
  Articles should have a main text (Abstract to Conclusion) of around 4000 words at minimum and should have more than 30 references. *Animals* has no restrictions on the maximum length of research manuscripts, provided that the text is concise and comprehensive.
  For all Western blot figures, densitometry readings/intensity ratio of each band should be included; the whole Western blot showing all bands and molecular weight markers should be included in the Supplementary Materials.

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- *Commentary:* Commentaries must provide an in depth, rather than superficial, review of a topic, showing a novel way of interpreting the scientific literature. Articles are usually identified as commentaries rather than scientific papers if they contain material which is deemed to be open to interpretation, rather than empirically proven. Commentaries must contain reasoned arguments, not just arguments expressing the emotions of the author (although the emotional responses from the public may be included as an argument). Commentaries must be concise, well argued, and erudite, as well as being written to a very high standard of English. All submissions will be submitted for peer review.

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contain reasoned arguments, not just arguments expressing the emotions of the author. Case studies must be concise, well argued, and erudite, as well as be written to a very high standard of English. All submissions will be submitted for peer review. Case reports should have a main text of around 2500 words.

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Authors are encouraged to use the **Microsoft Word template** or **LaTeX template** to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the Editorial Office **animals@mdpi.com**. Accepted file formats are:

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  - **Research manuscripts** should comprise:
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  - **Graphical Abstract:**

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- **Institutional Review Board Statement:** In this section, please add the Institutional Review Board Statement and approval number for studies involving humans or animals. Please note that the Editorial Office might ask you for further information. Please add "The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." OR "Ethical review and approval were waived for this study, due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans or animals. You might also choose to exclude this statement if the study did not involve humans or animals.

- **Informed Consent Statement:** Any research article describing a study involving humans should contain this statement. Please add "Informed consent was obtained from all subjects involved in the study." OR "Not applicable." for studies that did not involve humans. You might also choose to exclude this statement if the study did not involve humans.
  Written informed consent for publication must be obtained from identifiable human participants. For studies involving client-owned animals written informed consent must be obtained from the owner of the animals (or an authorized agent for the owner).

- **Data Availability Statement:** In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "**MDPI Research Data Policies**". You might choose to exclude this statement if the study did not report any data.

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- **Conflicts of Interest:** Authors must identify and declare any personal circumstances or interest that may be perceived as influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. *Animals* does not publish studies funded partially or fully by the tobacco industry. Any projects funded by industry must pay special attention to the full declaration of funder involvement. If there is no role, please state "The sponsors had no role in the design, execution, interpretation, or writing of the study". For more details please see **Conflict of Interest**.

- **References:** References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as **EndNote**, **ReferenceManager** or **Zotero** to avoid typing mistakes

and duplicated references. We encourage citations to data, computer code and other citable research material. If available online, you may use reference style 9. below.

- Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for **Endnote** and **Zotero** are available.

References should be described as follows, depending on the type of work:

- Journal Articles:

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.

- Books and Book Chapters:

2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196.
3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196.

- Unpublished materials intended for publication:

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- Unpublished materials not intended for publication:

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- Conference Proceedings:

7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

- Thesis:

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9. Title of Site. Available online: URL (accessed on Day Month Year).
Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as **WebCite**. Archived websites should be cited using the link provided as follows:
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See the **Reference List and Citations Guide** for more detailed information.

- **Preparing Figures, Schemes and Tables**

  - File for Figures and Schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

  - *Animals* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.

- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme 1, Figure 2, Scheme 2, Table 1, etc.).
- All Figures, Schemes and Tables should have a short explanatory title and caption.
- All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.
- Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

- **Original Images for Blots and Gels Requirements**

For the main text, please ensure that:

- All experimental samples and controls used for one comparative analysis are run on the same blot/gel.
- Image processing methods, such as adjusting the brightness or contrast, do not alter or distort the information in the figure and are applied to every pixel. High-contrast blots/gels are discouraged.
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- You have checked figures for duplications and ensured the figure legends are clear and accurate. Please include all relevant information in the figure legends and clearly indicate any re-arrangement of lanes.

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- **Supplementary Materials, Data Deposit and Software Source Code**

MDPI Research Data Policies

MDPI is committed to supporting open scientific exchange and enabling our authors to achieve best practices in sharing and archiving research data. We encourage all authors of articles published in MDPI journals to share their research data. Individual journal guidelines can be found at the journal 'Instructions for Authors' page. Data sharing policies concern the minimal dataset that supports the central findings of a published study. Generated data should be publicly available and cited in accordance with journal guidelines.

MDPI data policies are informed by **TOP Guidelines** and **FAIR Principles**.

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Data Availability Statements provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

Below are suggested Data Availability Statements:

- Data available in a publicly accessible repository
  The data presented in this study are openly available in [repository name e.g., FigShare] at [**doi**], reference number [reference number].
- Data available in a publicly accessible repository that does not issue DOIs
  Publicly available datasets were analyzed in this study. This data can be found here: [link/accession number]
- Data available on request due to restrictions eg privacy or ethical
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  Restrictions apply to the availability of these data. Data was obtained from [third party] and are available [from the authors/at URL] with the permission of [third party].
- Data sharing not applicable
  No new data were created or analyzed in this study. Data sharing is not applicable to this article.
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Data citation:

- [dataset] Authors. Year. Dataset title; Data repository or archive; Version (if any); Persistent identifier (e.g., DOI).

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository such as **GitHub** or uploading as supplementary information to the publication. The name, version, corporation and location information for all software used should be clearly indicated. Please include all the parameters used to run software/programs analyses.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable; however, we recommend that common, non-proprietary formats are used where possible. For more information on supplementary materials, please refer to **https://www.mdpi.com/authors/layout#_bookmark83**.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult **databib.org** or **re3data.org**. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal **Data** also accepts submissions of data set papers.

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New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

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- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the **GEO database** or in the NCBI's **Sequence Read Archive (SRA)**.
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All sequence names and the accession numbers provided by the databases must be provided in the Materials and Methods section of the article.

Deposition of Proteomics Data

Methods used to generate the proteomics data should be described in detail and we encourage authors to adhere to the "**Minimum Information About a Proteomics Experiment**". All generated mass spectrometry raw data must be deposited in the appropriate public database such as **ProteomeXchange**, **PRIDE** or **jPOST**. At the time of submission, please include all relevant information in the materials and methods section, such as repository where the data was submitted and link, data set identifier, username and password needed to access the data.

- **Research and Publication Ethics**
- **Research Ethics**
- **Research Involving Human Subjects**

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (**https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/**), revised in 2013. According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of

Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

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If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

- **Ethical Guidelines for the Use of Animals in Research**

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum,

the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (**arriveguidelines.org/**) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at **https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20Questionnaire.pdf**. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

1. NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: **https://www.animalethics.org.au/three-rs**
2. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: **https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf**
3. American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: **https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use**
4. European Animal Research Association. EU regulations on animal research. Available online: **https://www.eara.eu/animal-research-law**

- **Research Involving Cell Lines**

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

- **Research Involving Plants**

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the **Convention on Biological Diversity** and the **Convention on the Trade in Endangered Species of Wild Fauna and Flora**.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX, institute, city, country).

- **Clinical Trials Registration**

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) **guidelines** which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include **clinicaltrials.gov**, **the EU Clinical Trials Register** and those listed by the World Health Organisation **International Clinical Trials Registry Platform**.

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper without trial registration for further peer-review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 **checklist** and **flow diagram** as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (**http://www.consort-statement.org**) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

- **Dual Use Research of Concern**

MDPI follows the practical framework defined in **Guidance for Editors: Research, Audit and Service Evaluations** and introduced by the Committee on Publication Ethics (COPE). Research that could pose a significant threat, with broad potential consequences to public health or national security, should be clearly indicated in the manuscript, and potential dual-use research of concern should be explained in the cover letter upon submission. Potential areas of concern include but are not limited to biosecurity, nuclear and chemical threats, and research with a military purpose or application, etc. For these manuscripts to be considered for peer review, the benefits to the general public or public health must outweigh the risks. The authors have a responsibility to comply with relevant national and international laws.

- **Sex and Gender in Research**

We encourage our authors to follow the **'Sex and Gender Equity in Research – SAGER – guidelines'** and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full **guidelines** before submission.

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Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

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The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Animals* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Animals* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
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- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.

- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- The journal accepts exact translations of previously published work. All submissions of translations must conform with our **policies on translations**.
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Authors should not engage in excessive self-citation of their own work.

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Authors should not preferentially cite their own or their friends', peers', or institution's publications.

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In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on **citation manipulation** with recommendations for best practice.

- **Reviewer Suggestions**

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last three years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper. For detailed information regarding the qualifications and responsibilities of the reviewers, please visit **https://www.mdpi.com/reviewers**.

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Expanded and high-quality conference papers can be considered as articles if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper. *Animals* does not publish pilot studies or studies with inadequate statistical power.

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- Drafting the work or reviewing it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments. More detailed guidance on authorship is given by the **International Committee of Medical Journal Editors (ICMJE)**.

Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication. We reserve the right to request confirmation that all authors meet the authorship conditions.

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- Adequacy of reviewer comments and author response;
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Immediately after submission, the journal's Managing Editor will perform the technical pre-check to assess:

- Overall suitability of the manuscript to the journal/section/Special Issue;
- Manuscript adherence to high-quality research and ethical standards;
- Standards of rigor to qualify for further review.

The academic editor (i.e., the Editor-in-Chief in the case of regular submissions, the Guest Editor in the case of Special Issue submissions, or an Editorial Board member in the case of a conflict of interest and of regular submissions if the Editor-in-Chief allows) will be notified of the submission and invited to perform an editorial pre-check. During the editorial pre-check phase, the academic editor will assess the suitability of the submission with respect to the scope of the journal, as well as the overall scientific soundness of the manuscript, including the relevance of the references and the correctness of the applied methodology. Academic editors can decide to reject the manuscript, request revisions before peer-review, or continue with the peer-review process and recommend suitable reviewers.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

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  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.

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  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. A maximum of two rounds of major revision per manuscript is normally provided. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments. If the required revision time is estimated to be longer than 2 months, we will recommend that authors withdraw their manuscript before resubmitting so as to avoid unnecessary time pressure and to ensure that all manuscripts are sufficiently revised. (Please detail the revisions that have been made, citing line number and exact change, so that the editor can check the changes expeditiously. Simple statements like 'done' or 'revised as requested' will not be accepted, unless the change is simply a typographical error).

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  If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.

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  The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

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To help authors quickly find the correct identifiers for their materials, there is a single **website** where all resource types can be found and a ‘cite this’ button next to each resource, that contains a proper citation text that should be included in the methods section of the manuscript.

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CERTIFICADO

Certificamos que o projeto intitulado **"Avaliação em modelo murino da eficácia anti-inflamatória do produto F147 indicado para uso tópico em cães e gatos"**, protocolo nº **23117.038020/2023-46**, sob a responsabilidade de **Murilo Vieira da Silva** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADO** pela COMISSÃO DE ÉTICA NA UTILIZAÇÃO DE ANIMAIS (CEUA) da UNIVERSIDADE FEDERAL DE UBERLÂNDIA, em reunião **16 de Junho de 2023.**

(We certify that the project entitled **"Avaliação em modelo murino da eficácia anti-inflamatória do produto F147 indicado para uso tópico em cães e gatos"** protocol **23117.038020/2023-46**, under the responsibility of **Murilo Vieira da Silva** - involving the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata, for purposes of scientific research - is in accordance with the provisions of Law nº 11.794, of October 8th, 2008, of Decree nº 6.899 of July 15th, 2009, and the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and it was **approved** for ETHICS COMMISSION ON ANIMAL USE (CEUA) from FEDERAL UNIVERSITY OF UBERLÂNDIA, in meeting of **June 16th, 2023**).

Finalidade	( ) Ensino (**X**) Pesquisa Científica
Vigência do Projeto	Início: **26/06/2023** Término: **15/06/2024**
Espécie / Linhagem / Grupos Taxonômicos	**Camundongo Isogênico Balb/c**
Número de animais	**80 animais**
Peso / Idade	**20g a 25g/ 6 semanas**
Sexo	**Fêmea**
Origem / Local	**REBIR - UFU**
Local onde serão mantidos os animais:	**REBIR - UFU**

Documento assinado eletronicamente por **Luiz Fernando Moreira Izidoro**, **Coordenador(a)**, em 20/06/2023, às 11:38, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **4582079** e o código CRC **B0AB4746**.

ANEXO C – Figure S1: Leaflet of the product tested in group 2

Aurigen®

Uso Veterinário

Gel otológico cristalino e homogêneo.

Fórmula:

Cada 100 gramas contém:

Gentamicina (Sulfato) .... 300,00 mg

Dipropionato de Betametasona .... 122,00 mg

Miconazol .... 1.000,00 mg

Excipiente q.s.p. .... 100,00 g

Indicações:

Aurigen é um agente antibacteriano, antifúngico e anti-inflamatório de uso otológico indicado no tratamento de otites agudas ou crônicas causadas por bactérias e/ou fungos que acometem cães. Agentes etiológicos susceptíveis:

Bactérias - *Staphylococcus aureus, Trueperella pyogenes, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus pyogenes, Escherichia coli*. **Fungos -** *Microsporum canis, Malassezia pachydermatis, Trichophyton rubrum, Trichophyton mentagrophytes, Candida albicans*.

Farmacodinâmica:

A gentamicina é um antimicrobiano constituinte da classe de compostos aminoglicosídeos que exercem a sua ação bacteriana pela ligação irreversível a uma ou mais proteínas receptoras na subunidade 30S do ribossomo bacteriano, interferindo em vários mecanismos no processo de translação do RNA mensageiro, causando a terminação prematura da cadeia ou provocando a incorporação de um aminoácido incorreto no produto proteico. Esta proteína defeituosa formada leva à morte celular.

O miconazol é um composto azólico que exerce seu efeito antifúngico na membrana celular do fungo por inibir a síntese do ergosterol – esterol primário da membrana celular fúngica. A inibição de uma série de processos resulta na incapacidade de desmetilar os esteróis C14-metil e de reduzir a síntese do ergosterol.

O mecanismo clássico de ação hormonal esteroide, dentre eles a betametasona, começa com a permeação na membrana do esteroide e ligação subsequente aos receptores citossólicos. Essas proteínas provavelmente se originam de núcleos, mas em seguida migram para o citossol quando os glicocorticoides estão presentes. Na ligação, uma proteína conhecida como proteína "de choque térmico" (hsp90) é liberada e pode desempenhar um papel nas ações do hormônio. O complexo hormônio-receptor é, então, transportado para o núcleo, onde se liga aos elementos de resposta glicocorticoides (ERG) em vários genes e alteram sua expressão. O hormônio facilita a ligação da proteína receptora ao DNA. Em determinados tecidos, outras proteínas também devem ligar-se ao gene para permitir a expressão dos ERG particulares. A maioria das ações mediadas nuclearmente possui um início de efeitos farmacológicos dos esteroides, requerendo no mínimo várias horas para ocorrer. Os efeitos anti-inflamatórios são mediados por ligação direta do glicocorticoide ou do complexo glicocorticoide-receptor aos ERG na região promotora dos genes, ou por uma interação desse complexo com outros fatores de transcrição. Os glicocorticoides inibem muitas moléculas associadas à inflamação, como as citocinas, quimicinas, metabólitos do ácido araquidônico e moléculas de aderência.

Farmacocinética:

Quando os antibióticos são utilizados topicamente na terapia otológica, as concentrações atingidas no canal auditivo são maiores do que na terapia sistêmica, frequentemente uma bactéria considerada resistente pode ser sensível a estas altas concentrações. A eficácia da gentamicina, assim como qualquer combinação de antibióticos contendo aminoglicosídeo aplicada no canal auditivo, será maior após a limpeza da área acometida, eliminando-se o exsudato antes da aplicação. O miconazol é utilizado mais comumente por via tópica e, raramente, por via intravenosa sendo esta via restrita ao tratamento de infecções sistêmicas graves. A via parenteral apresenta ainda como desvantagem o curto tempo de meia-vida plasmática, devendo ser administrado a cada 8 horas. Os corticoides podem ser bem absorvidos em sítios locais de aplicação. A via tópica é útil em determinadas situações em que há necessidade de obter altas concentrações de corticoides em uma área restrita, com o mínimo de efeitos colaterais. Entretanto, quando administrados no canal auditivo, pode ser absorvido em quantidade suficiente para causar efeitos sistêmicos, de modo que tratamentos longos com esta droga devem ser considerados com cautela.

Dosagem e Modo de uso:

Antes da aplicação do produto, recomendamos a limpeza total do ouvido externo, removendo todas as sujidades e corpos estranhos, utilizando um produto específico.

Aurigen é um produto para uso tópico, e deve ser aplicado no canal auditivo externo, conforme orientação abaixo:

- Para cães com peso corporal de até 15 kg, deverão ser administradas 4 gotas do produto, 2 vezes ao dia (intervalos de 12 horas).
- Para cães com peso corporal de 15 kg ou mais, deverão ser aplicadas 8 gotas do produto, 2 vezes ao dia (intervalos de 12 horas).

Peso corporal	Dosagem	Frequência e duração do tratamento
Até 15 kg	4 gotas	2 vezes ao dia durante 7 a 9 dias
15 kg ou mais	8 gotas	

Após a aplicação de **Aurigen**, deve-se massagear o local cuidadosamente para que haja uma boa distribuição do produto no ouvido externo.

Aurigen deve ser administrado durante 7 a 9 dias consecutivos ou a critério do médico-veterinário.

Recomendamos a continuidade do tratamento por até 48 horas após o desaparecimento do quadro clínico.

A eficácia de antimicrobianos depende da sensibilidade dos microrganismos aos princípios ativos que compõem o produto e do atendimento adequado às recomendações do médico-veterinário que prescreveu o medicamento, como dose, tempo de tratamento, quantidade de aplicações por dia e limpeza das áreas afetadas.

Contraindicações e limitações de uso:

Não administrar em animais com histórico de hipersensibilidade aos componentes da formulação.

Não utilizar o medicamento com data de validade vencida.

Precauções em animais:

Obedecer ao modo de uso e dosagens preconizadas.

A administração de **Aurigen**, a partir do sétimo dia de tratamento, deve ser supervisionada, pois o uso excessivo do produto pode retardar a cicatrização das lesões oriundas da otite.

Utilizar com cautela em pacientes que apresentarem doença renal pré-existente, neonatos ou pacientes geriátricos, animais diabéticos e gestantes.

Preparações de betametasona são geralmente bem toleradas, mas a possível supressão do sistema imune aumenta a susceptibilidade do paciente à infecção.

Precauções em humanos:

Em caso de contato com os olhos ou pele, e ocorrência de irritação, lavar com água em abundância, se a irritação persistir consulte um médico, levando a embalagem completa do produto.

Durante a utilização do produto, proteger-se com luvas de borracha (luva nitrílica). Não manusear o produto com as mãos desprotegidas. Após a aplicação do produto, remover as luvas e lavar bem as mãos.

Não reutilizar as embalagens. Restos de produtos e de embalagens devem ser descartados conforme preconizado na legislação vigente, evitando a contaminação do meio ambiente.

Reações adversas:

Não são esperadas reações adversas com o uso do produto quando administrado conforme as indicações previstas em bula. Entretanto, conforme apontam relatos de literatura, reações de sensibilidade individual podem eventualmente ocorrer.

Estudos clínicos de segurança conduzidos com o produto demonstraram que o mesmo é seguro nas dosagens indicadas.

O uso de antibióticos aminiglicosídeos, dentre eles a gentamicina, pode acarretar nefrotoxicidade e ototoxicidade. Os aminoglicosídeos podem causar bloqueio neuromuscular, edema facial, neuropatia periférica e reações de hipersensibilidade. Raramente, sinais clínicos gastrointestinais, efeitos hepáticos e hematológicos são registrados. Pode ocorrer ototoxicidade em tratamentos prolongados, porém, a reversão desses quadros geralmente ocorre após a suspensão do tratamento.

Irritação causada por eritema, prurido e ocasionalmente exsudação podem raramente serem vistas com o uso do miconazol. É rara a ocorrência de reações adversa na aplicação tópica do miconazol, entretanto podem ocorrer queimação, prurido e irritação após aplicação tópica.

A via tópica é útil em determinadas situações em que há necessidade de obter altas concentrações de corticoides em uma área restrita, com o mínimo de efeitos colaterais. Por outro lado, sendo os glicocorticoides permeáveis à barreira cutânea, podem levar a supressão do eixo hipotálamo-hipófise-adrenal e ao aparecimento de efeitos adversos quando utilizados cronicamente, em áreas extensas ou que apresentem solução de continuidade.

Os efeitos adversos provenientes do uso sistêmico de corticosteroides incluem polifagia, polidipsia/poliúria, supressão do eixo hipotálamo-pituitária-adrenal, ulceração gastrointestinal, hepatopatia, diabetes, hiperlipidemia, diminuição do hormônio tireoidiano, diminuição da síntese proteica, prejuízo na cicatrização de feridas e imunossupressão.

Interações medicamentosas:

A gentamicina é inativada pela administração concomitante de carbenicilina. Não administrar o produto concomitantemente com relaxantes músculo-esqueléticos, pois aumenta a possibilidade de bloqueio neuromuscular. Pode haver um efeito sinérgico da gentamicina com antibióticos beta-lactâmicos.

Potencialmente, os cefalosporínicos (cefaloridine e cefalotina) podem causar neurotoxicidade adicional quando utilizado junto à gentamicina.

A utilização de diuréticos e gentamicina pode aumentar o seu potencial nefrotóxico e ototóxico. O uso de gentamicina concomitantemente a anestésicos gerais ou agentes bloqueadores neuromusculares podem potencializar o bloqueio neuromuscular. A combinação de anfotericina e miconazol parece ser menos efetiva do que quando usados separadamente.

O miconazol aumenta a atividade de clomipramina, carbamazepina e fenitoína. A inibição causada pelos azóis no sistema microssomal hepático de enzimas pode levar ao aumento de concentrações de drogas como ciclosporina, digoxina, fenitoína, quinidina, sulfonil-ureia, midazolam, cisaprida e warfarin quando estas drogas são co-administradas.

Fenitoína, fenobarbital e rifampicina aumentam o metabolismo de glicocorticoides.

Pode ocorrer hipocalemia quando glicocorticoides são administrados junto a anfotericina B ou diuréticos que causam perda de potássio.

Quando os glicocorticóides são utilizados junto à terapia com digitálicos há um aumento no risco da toxicidade digitálica na presença de hipocalemia.

A administração concomitante de glicocorticoides e ciclosporina leva a diminuição no metabolismo de ambas as drogas. Os glicocorticóides reduzem o metabolismo hepático da ciclosfosfamida.

O uso de glicocorticoides com drogas que induzem ulceração gastrointestinal pode aumentar o risco desse efeito adverso.

Estrógenos podem potencializar os efeitos dos glicocorticóides.

Conservar o produto em sua embalagem original, em local seco e fresco, em temperatura entre 15ºC a 30ºC, ao abrigo da luz solar direta, fora do alcance de crianças e animais domésticos.

Venda sob prescrição e aplicação sob orientação do médico-veterinário.

Responsável Técnica:
Dra. Caroline Della Nina Pistoni
CRMV/SP 24.508

Licenciado no Ministério da Agricultura sob nº 7.351 em 14/06/2000.

Proprietário e fabricante:
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Rod. Anhanguera SP 330 km 298
CEP: 14140 000 Cravinhos SP
CNPJ: 57.624.462/0001-05
www.ourofinosaudeanimal.com
Indústria brasileira

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ourofino saúde animal

ANEXO D – Figure S2: Leaflet of the product tested in group 3

Dermotrat® Creme

Uso Veterinário

Creme Dermatológico

Fórmula:
Cada bisnaga de 20 gramas contém:
Gentamicina (sulfato) ..... 0,04 g *
Miconazol (nitrato) ..... 0,40 g **
Valerato de Betametasona ..... 0,02 g
Excipiente q.s.p. ..... 20,00 g
(*) Equivalente a 0,34% p/p de Sulfato de Gentamicina
(**) Equivalente a 2,30% p/p de Nitrato de Miconazol

Indicações:
Dermotrat Creme é um agente antibacteriano, antifúngico e anti-inflamatório de uso tópico para afecções cutâneas causadas pelos agentes: *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Trueperella pyogenes, Streptococcus pyogenes, Proteus vulgaris* e *Proteus mirabilis, Microsporum canis, Trichophyton rubrum, Trichophyton mentagrophytes, Candida albicans* e *Malassezia pachydermatis*, que acometem cães e gatos.

Farmacodinâmica:
A gentamicina é um antimicrobiano constituinte da classe de compostos aminoglicosídeos que exercem a sua ação bacteriana pela ligação irreversível a uma ou mais proteínas receptoras na subunidade 30S do ribossomo bacteriano, interferindo em vários mecanismos no processo de translação do RNA mensageiro, causando a terminação prematura da cadeia ou provocando a incorporação de um aminoácido incorreto no produto proteico. Esta proteína defeituosa formada leva à morte celular.
O miconazol é um composto azólico que exerce seu efeito antifúngico na membrana celular do fungo por inibir a síntese de ergosterol - esterol primário da membrana celular fúngica. A inibição da enzima lanosterol C14 - desmetilase dependente de P450 resulta em depleção do ergosterol e acúmulo de esteróis C14 - metil na membrana citoplasmática alterando a fluidez da membrana e interferindo na função de barreira.
O mecanismo clássico de ação hormonal esteroide, dentre eles a betametasona, começa com a permeação na membrana do esteroide e ligação subsequente aos receptores citossólicos. Essas proteínas provavelmente se originam de núcleos, mas em seguida migram para o citossol quando os glicocorticoides estão presentes. Na ligação, uma proteína conhecida como proteína "de choque térmico" (hsp90) é liberada e pode desempenhar um papel nas ações do hormônio. O complexo hormônio-receptor é então transportado para o núcleo, onde se liga aos elementos de resposta glicocorticoides (ERG) em vários genes e alteram sua expressão. O hormônio facilita a ligação da proteína receptora ao DNA. Em determinados tecidos, outras proteínas também devem ligar-se ao gene para permitir a expressão dos ERG particulares. A maioria das ações mediadas nuclearmente possui um início de efeitos farmacológicos dos esteroides, requerendo no mínimo várias horas para ocorrer. Os efeitos anti-inflamatórios são mediados por ligação direta do glicocorticoide ou do complexo glicocorticoide-receptor aos ERG na região promotora dos genes, ou por uma interação desse complexo com outros fatores de transcrição. Os glicocorticoides inibem muitas moléculas associadas à inflamação, como as citocinas, quimicinas, metabólitos do ácido araquidônico e moléculas de aderência.

Farmacocinética:
A gentamicina quando utilizada topicamente pode ter a sua absorção percutânea retardada devido a sua grande estrutura molecular, carga positiva e capacidade de ligação ao pus. Após absorção, os aminoglicosídeos são distribuídos primariamente no fluído extracelular como os fluídos ascítico, pleural, do pericárdio, peritoneal, sinovial e de abscessos. São ligados a proteínas plasmáticas em pequena quantidade, menores que 20%. Não atravessam prontamente a barreira hematoencefálica ou penetram no tecido ocular. Níveis terapêuticos podem ser encontrados nos ossos, coração, bexiga e tecido pulmonar após dose parenteral. Tendem a se acumular em alguns tecidos sendo eles o conduto auditivo interno e os rins. São eliminados não-metabolizados do organismo em todas as espécies estudadas, sendo a eliminação feita através de filtração glomerular. As concentrações urinárias de gentamicina são relatadas por chegarem a 107+/- 33 µg/mL após 2,2 mg/kg e cada oito horas nos cães e 362 +/- 163 µg/mL três horas após a dose de 3 mg/kg nos gatos.
A administração de miconazol por via tópica é rapidamente absorvida podendo persistir por até quatro dias no estrato córneo. Entretanto, autores citam que o nitrato de miconazol é pouco absorvido através da pele quando aplicado topicamente. Estudo em porquinho da índia utilizando aplicação tópica de solução de nitrato de miconazol 1% na pele do abdômen avaliou a absorção percutânea e a absorção intracutânea. A concentração de miconazol no estrato córneo após duas horas da aplicação se mostrou em altos níveis, 1869 µg/g e 48 horas após a aplicação foi de 705 µg/g. A concentração do miconazol na epiderme também se mostrou em altos níveis após duas de aplicação 13,4 µg/g caindo para 7,6 µg/g após 48 horas. O miconazol atinge concentrações terapêuticas nos ossos, articulações e tecido pulmonar, entretanto a penetração no sistema nervoso central é mínima. São amplamente distribuídos no corpo podendo ser detectados na saliva, leite e cerúmen. As maiores concentrações dos imidazóis são encontradas no fígado, glândula adrenal, pulmões e rins. A biotransformação do miconazol ocorre no fígado por O-dealquilação e N-dealquilação. Apenas 1% deste antifúngico é excretado de maneira íntegra na urina.
Os corticoides são em geral prontamente absorvidos pelo trato gastrointestinal. Eles também podem ser bem absorvidos em sítios no local de aplicação.
A via tópica é útil em determinadas situações em que há necessidade de obter altas concentrações de corticoides em uma área restrita, com o mínimo de efeitos colaterais. Entretanto quando administrados por aplicação tópica, particularmente sobre grandes áreas, sobre bandagem oclusiva ou quando a pele está lesionada, quantidade suficiente de glicocorticoide pode ser absorvida para causar efeitos sistêmicos. O cortisol no plasma está ligado a mais de 90% de proteínas plasmáticas. Os 10% remanescentes do hormônio livre correspondem à fração ativa de acordo com a hipótese do hormônio livre. A betametasona é amplamente distribuída nos tecidos. O cortisol endógeno é removido da circulação pelo fígado onde é reduzido e conjugado a forma de glicuronídeos hidrossolúveis e sulfatos. O caminho metabólico da betametasona é similar aos outros corticosteroides e de forma geral são metabolizados principalmente no fígado, mas também pode ocorrer nos rins. Os metabólitos do cortisol após metabolismo hepático são excretados pela urina.

Dosagem e Modo de uso:
Dermotrat Creme é um produto para administração tópica, diretamente sobre as lesões da pele, devendo ser aplicado sobre toda a extensão da área a ser tratada, de maneira que se forme uma fina camada do produto sobre a lesão cutânea. Recomenda-se cortar o pelo e limpar a área a ser tratada antes da aplicação do produto, principalmente nas lesões de origem fúngica.
Deve ser aplicado por duas a três vezes ao dia, durante sete a dez dias, para tratamento de lesões cutâneas. O tratamento deverá ser continuado até 48 horas após o desaparecimento dos sinais clínicos ou a critério do médico-veterinário.
A eficácia de antimicrobianos depende da sensibilidade dos microrganismos aos princípios ativos que compõe o produto e do atendimento adequado às recomendações do médico-veterinário que prescreveu o medicamento, como dose, tempo de tratamento, quantidade de aplicações por dia e limpeza das áreas afetadas.

Contraindicações e limitações de uso:
Não administrar em animais com histórico de hipersensibilidade aos componentes da formulação.
Não utilizar medicamento com data de validade vencida.

Precauções em animais:
Obedecer ao modo de uso e dosagens preconizadas.
O produto **Dermotrat Creme** pode ser consumido em até 6 meses após abertura e primeiro uso.
Recomenda-se atenção especial no tratamento de animais com grandes áreas de pele desnuda ou queimaduras graves, pois uma fração maior do medicamento poderá ser absorvida.
Utilizar com cautela em pacientes que apresentarem doença renal pré-existente, neonatos ou pacientes geriátricos, animais diabéticos e gestantes.
Quando utilizados por período prolongado, corticosteroides tópicos podem causar alguns efeitos adversos localizados e sistêmicos, como atrofia e degeneração da camada epidérmica, devido sua atividade mineralocorticoide antiproliferativa em queratinócitos e fibroblastos, bem como Síndrome de Cushing ou hiperadrenocorticismo.

Precauções em humanos:
Perigo! Causa danos se ingerido. Não ingerir. Em caso de ingestão não induza o vômito. Lave a boca com água em abundância. Caso sinta indisposição, consulte um médico, levando a embalagem completa do produto.
Em caso de contato com os olhos ou pele, e ocorrência de irritação, lavar com água em abundância, se a irritação persistir consulte um médico, levando a embalagem completa do produto.
Durante a utilização do produto, proteger-se com luvas de borracha (luva nitrílica). Não manusear o produto com as mãos desprotegidas.
Após a aplicação do produto, remover as luvas e lavar bem as mãos.

Não reutilizar as embalagens. Restos de produtos e de embalagens devem ser descartados conforme preconizado na legislação vigente, evitando a contaminação do meio ambiente.
Produto tóxico para peixes. Não contaminar coleções de água de qualquer natureza.

Reações adversas:
Não são esperadas reações adversas com o uso do produto quando administrado conforme as indicações previstas pela Ourofino. Entretanto, conforme apontam relatos de literatura, reações de sensibilidade individual podem eventualmente ocorrer. Estudos clínicos de segurança conduzidos com o produto demonstraram que o mesmo é seguro nas dosagens e tempo de uso indicados. O uso de antibióticos aminoglicosídeos, dentre eles a gentamicina, pode acarretar nefrotoxicidade e ototoxicidade, entretanto, a ototoxicidade nos cães pode ocorrer após tratamento aminoglicosídeo sistêmico, mas após o uso tópico é aparentemente rara.
Os aminoglicosídeos podem causar bloqueio neuromuscular, edema facial, neuropatia periférica e reações de hipersensibilidade. Raramente, sinais clínicos gastrintestinais, efeitos hepáticos e hematológicos são registrados.
Irritação causada por eritema, prurido e ocasionalmente exsudação podem raramente serem vistas com o uso do miconazol.
É rara a ocorrência de reação adversa na aplicação tópica do miconazol, entretanto, podem ocorrer queimação, prurido e irritação após aplicação tópica. A via tópica é útil em determinadas situações em que há necessidade de obter altas concentrações de corticoides em uma área restrita, com o mínimo de efeitos colaterais. Por outro lado, sendo os glicocorticoides permeáveis à barreira cutânea, podem levar à supressão do eixo-hipotálamo-hipófise-adrenal e ao aparecimento de efeitos adversos, quando utilizados cronicamente, em áreas extensas ou que apresentem solução de continuidade.
Os efeitos adversos provenientes do uso sistêmico de corticosteroides incluem polifagia, polidipsia/poliúria, supressão do eixo hipotálamo-pituitária-adrenal, ulceração gastrintestinal, hepatopatia, diabetes, hiperlipidemia, diminuição do hormônio tireoidiano, diminuição da síntese proteica, prejuízo na cicatrização de feridas e imunossupressão.

Interações medicamentosas:
Pode haver um efeito sinérgico da gentamicina com antibióticos beta-lactâmicos. Potencialmente, os cefalosporínicos (cefaloridine e cefalotina) podem causar nefrotoxicidade adicional quando utilizado junto à gentamicina. A utilização de diuréticos e gentamicina pode aumentar o seu potencial nefrotóxico e ototóxico. O uso de gentamicina concomitantemente a anestésicos gerais ou agentes bloqueadores neuromusculares pode potencializar o bloqueio neuromuscular.
A combinação de anfotericina e miconazol parece ser menos efetiva que quando usados separadamente. O miconazol aumenta a atividade de clomipramina, carbamazepina e fenitoína.
A inibição causada pelos azóis no sistema microssomal hepático de enzimas pode levar ao aumento de concentrações de drogas como ciclosporina, digoxina, fenitoína, quinidina, sulfonil-ureia, midazolam, cisaprida e warfarin quando estas drogas são coadministradas. Fenitoína, fenibarbital e rifampicina aumentam o metabolismo de glicocorticoides.
Pode ocorrer hipocalemia quando glicocorticoides são administrados junto a anfotericina B ou diuréticos que causam perda de potássio.
Quando os glicocorticóides são utilizados junto à terapia com digitálicos há um aumento no risco da toxicidade digitálica na presença de hipocalemia. A administração concomitante de glicocorticoide e ciclosporina leva à diminuição no metabolismo de ambas as drogas.
Os glicocorticoides reduzem o metabolismo hepático da ciclofosfamida. O uso de glicocorticoides com drogas que induzem ulceração gastrintestinal pode aumentar o risco desse efeito adverso.
Estrógenos podem potencializar os efeitos dos glicocorticoides.

Conservar o produto em sua embalagem original, em local seco e fresco, em temperatura entre 15ºC e 30ºC, ao abrigo da luz solar direta, fora do alcance de crianças e animais domésticos.

Venda sob prescrição e aplicação sob orientação do médico-veterinário.

Responsável Técnica:
Dra. Caroline Della Nina Pistoni
CRMV/SP 24.508

Licenciado no Ministério da Agricultura sob nº 7.585 em 21/12/2000

Proprietária e fabricante:
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CNPJ: 57.624.462/0001-05
www.ourofinosaudeanimal.com
Indústria brasileira

Atendimento
16 3518 2025

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(seg. a sex. das 8h as 17h)

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ANEXO E – Table S1: Hematological parameters of BALB/c mice

	Machos				Fêmeas			
	Média	Intervalo de 95% de confiança			Média	Intervalo de 95% de confiança		
		Inferior	Superior	n		Inferior	Superior	n
RBC ($\times 10^6/\mu L$)	9,99	9,86	10,14	30	9,73	9,56	9,89	30
HGB (g/dL)	15,13	14,85	15,40	30	15,09	14,76	15,42	30
HCT (%)	45,84	45,10	46,58	30	45,51	44,62	46,41	29
MCV (fL)	45,91	45,47	46,34	30	46,61	46,06	47,16	30
MCH (pg)	15,07	14,93	15,21	30	15,46	15,28	15,65	30
MCHC (g/dL)	32,96	32,56	33,36	30	33,28	33,03	33,52	30
RDW (%)	12,39	12,22	12,56	29	13,08	12,87	13,29	29
WBC	7894	7069	8718	30	7203	6518	7889	29

(/μL)								
Basófilos	1	0	5	30	9	1	17	29
Eosinófilos	348	268	428	30	275	219	330	29
Monócitos	279	208	349	29	281	227	335	29
Bastonetes	129	86	171	30	140	96	184	29
Neutrófilos	1590	1423	1757	30	1258	1056	1460	28
Linfócitos	5333	4679	5988	30	4958	4477	5439	28
PLT (×10³/μL)	1298	1228	1367	30	1058	978	1137	17