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Nanoemulsão tópica de canabidiol e quercetina sobre inflamação e barreira cutânea em dermatite atópica experimental

Nanoemulsión tópica de cannabidiol y quercetina sobre inflamación y barrera cutánea en dermatitis atópica experimental

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ABSTRACT

Atopic dermatitis is a chronic inflammatory skin disease characterized by epidermal barrier dysfunction and dysregulated immune responses. This study developed and evaluated a topical nanoemulsion containing cannabidiol (CBD) and quercetin (QU) in Pentravan® in an experimental rat model. Twenty-eight male Wistar rats were allocated into four groups (n=7):

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Pentravan® (GI), Pentravan® + 1% CBD (GII), Pentravan® + 1% QU (GIII), and Pentravan® + 1% CBD + 1% QU (GIV). Therapeutic efficacy was assessed by clinical examination, adapted SCORAD, infrared thermography, transepidermal water loss (TEWL), and histopathology over four weeks. Mixed ANOVA showed a significant group effect for all five SCORAD components ($p \leq 0.007$), with GIV showing the lowest scores; Kruskal-Wallis confirmed differences at T4 for all five components ($p \leq 0.017$). GIV showed the lowest skin temperature at all time points, differing from GI at T1 ($p=0.030$) and T2 ($p=0.048$), and the greatest reduction in dermal infiltrate, spongiosis, and exocytosis. The topical CBD-quercetin nanoemulsion showed therapeutic potential in experimental atopic dermatitis.

Keywords: Dermatitis, Atopic; Cannabidiol; Quercetin; Emulsions; Skin Absorption.

RESUMO

A dermatite atópica é uma doença inflamatória crônica caracterizada por disfunção da barreira cutânea e resposta imune desregulada. Este estudo desenvolveu e avaliou uma nanoemulsão tópica contendo canabidiol (CBD) e quercetina (QU) em Pentravan® em modelo experimental em ratos Wistar. Vinte e oito ratos machos foram distribuídos em quatro grupos (n=7): Pentravan® (GI), Pentravan® + CBD 1% (GII), Pentravan® + QU 1% (GIII) e Pentravan® + CBD 1% + QU 1% (GIV). A eficácia foi avaliada por exame clínico, SCORAD adaptado, termografia, perda de água transepidérmica (TEWL) e histopatologia durante quatro semanas. A Mixed ANOVA mostrou efeito significativo de grupo nos cinco componentes do SCORAD ($p \leq 0,007$), com os menores escores no GIV; o Kruskal-Wallis confirmou diferenças em T4 nos cinco componentes ($p \leq 0,017$). O GIV apresentou a menor temperatura cutânea em todos os tempos, diferindo do GI em T1 ($p=0,030$) e T2 ($p=0,048$), e

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a maior redução de infiltrado dérmico, espongiose e exocitose. A nanoemulsão tópica de CBD e quercetina demonstrou potencial terapêutico na dermatite atópica experimental.

Palavras-chave: Dermatite Atópica; Canabidiol; Quercetina; Emulsões; Absorção Cutânea.

RESUMEN

La dermatitis atópica es una enfermedad inflamatoria crónica caracterizada por disfunción de la barrera cutánea y respuesta inmunitaria desregulada. Este estudio desarrolló y evaluó una nanoemulsión tópica con cannabidiol (CBD) y quercetina (QU) en Pentravan® en un modelo experimental en ratas Wistar. Veintiocho ratas macho fueron distribuidas en cuatro grupos (n=7): Pentravan® (GI), Pentravan® + CBD 1% (GII), Pentravan® + QU 1% (GIII) y Pentravan® + CBD 1% + QU 1% (GIV). La eficacia se evaluó mediante examen clínico, SCORAD adaptado, termografía, pérdida de agua transepidérmica (TEWL) e histopatología durante cuatro semanas. El Mixed ANOVA mostró efecto significativo de grupo en los cinco componentes del SCORAD ($p \leq 0,007$), con las menores puntuaciones en el GIV; el Kruskal-Wallis confirmó diferencias en T4 en los cinco componentes ($p \leq 0,017$). El GIV presentó la menor temperatura cutánea en todos los tiempos, difiriendo del GI en T1 ($p=0,030$) y T2 ($p=0,048$), y la mayor reducción de infiltrado dérmico, espongiosis y exocitosis. La nanoemulsión tópica de CBD y quercetina demostró potencial terapéutico en la dermatitis atópica experimental.

Palabras clave: Dermatitis Atópica; Cannabidiol; Quercetina; Emulsiones; Absorción Cutánea.

INTRODUCTION

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Atopic dermatitis (AD) is a chronic, pruritic, multifactorial inflammatory skin disease characterized by epidermal barrier alterations and immune dysregulation. In both humans and dogs it markedly compromises quality of life and shows a high recurrence rate, being frequently associated with the atopic march[1,2].

In Brazil, the International Study of Asthma and Allergies in Childhood (ISAAC) estimated an atopic dermatitis prevalence between 11.7% and 13.8% among children and adolescents, with a significant impact on quality of life[3]. The disease results from the interaction between epidermal barrier dysfunction and a predominantly Th2 immune response. Alterations in filaggrin and ceramide levels increase transepidermal water loss (TEWL) and permeability to allergens, promoting IL-4, IL-13, and IL-31 production and favouring Staphylococcus sp. colonization, which perpetuates inflammation[4,5]. Although corticosteroids, calcineurin inhibitors, and Janus kinase inhibitors are effective, their limitations drive the search for alternative therapies. Cannabidiol (CBD) exerts anti-inflammatory and immunomodulatory effects through interaction with CB1 and CB2 receptors, TRPV1 channels, and PPAR-γ receptors[6,7], whereas quercetin exerts antioxidant, anti-inflammatory, and mast cell-stabilizing actions[8].

However, the low solubility and limited skin permeation of these compounds may restrict their efficacy. Nanoemulsions and the transdermal vehicle Pentravan® represent strategies to increase the stability, permeation, and bioavailability of active compounds[9,10].

Given the scarcity of studies on the association of CBD and quercetin in nanostructured systems, this study developed and evaluated a topical nanoemulsion containing these compounds in an experimental model of atopic dermatitis, investigating their effects on skin inflammation and barrier function. To our knowledge, this is the first study to assess this combination in a nanostructured topical system using an integrated clinical, thermographic, functional, and histopathological approach.

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MATERIALS AND METHODS

Chemical characterization of the cannabidiol-enriched oil

The cannabidiol (CBD)-enriched corn oil was supplied by Associação Abrace Esperança (Paraíba, Brazil), judicially authorized to cultivate Cannabis sativa and produce derivatives. Chemical characterization was performed by gas chromatography coupled to mass spectrometry (GC-MS) using a certified analytical standard for CBD identification. Samples were previously filtered, diluted in chloroform, and analysed using caffeine as internal standard. All analyses were carried out at the Laboratory of Phytochemical Bioprospecting (LaBioFito) of the Universidade Federal Rural de Pernambuco.

Experimental design

This was a preclinical, in vivo, controlled, randomized, and comparative study using an experimental model of atopic dermatitis induced in 28 male Wistar rats (200-250 g). Animals from the Central Animal Facility of the Universidade Federal Rural de Pernambuco (UFRPE) were kept under controlled temperature, a 12-hour light/dark cycle, and free access to water and feed. All procedures were approved by the Animal Use Ethics Committee of UFRPE (CEUA/UFRPE; protocol No. 4130150822).

Experimental induction of atopic dermatitis

Experimental atopic dermatitis was induced in the cervicodorsal region by topical application of analytical-grade acetone (1 mL/animal/day) for three consecutive days, after trichotomy

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and subepidermal infiltration of 2% lidocaine, following Barcelos et al.[11] with adaptations. Animals were then allocated into four groups (n=7) by simple randomization using a random number sequence generated in R: Group I (Pentravan®), Group II (Pentravan® + 1% CBD), Group III (Pentravan® + 1% quercetin), and Group IV (Pentravan® + 1% CBD + 1% quercetin). Treatments were applied topically once daily for four consecutive weeks. Clinical, thermographic, and histopathological assessments were performed by evaluators blinded to group allocation.

Development of the formulations

Formulations were developed as nanoemulsions containing CBD-enriched corn oil and/or quercetin incorporated into the transdermal vehicle Pentravan®, in order to favour compound stability, solubilization, and skin permeation.

Formulations were standardized at 1% for each active compound and prepared by homogenization for 10 to 15 minutes until complete dispersion of the components. Compositions were as follows: Group I: Pentravan® (50 g); Group II: 1% CBD (2.5 g CBD + 47.5 g Pentravan®); Group III: 1% QU (2.5 g quercetin + 47.5 g Pentravan®); Group IV: 1% CBD + 1% QU (2.5 g CBD + 2.5 g quercetin + 45 g Pentravan®).

Formulations were stored in sterile containers in a dry, cool, light-protected environment. The preparation methodology is covered by patent application No. BR 10 2025 013585 0.

Clinical-dermatological evaluation

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Treatment response was assessed weekly for four weeks through standardized digital photographs obtained with a smartphone (iPhone 15 Pro Max®) under controlled lighting, distance, and positioning.

Inflammatory areas were manually delimited in graphic software and quantified in mm^2. In addition, a SCORAD index adapted for experimental assessment of skin inflammation was used, considering erythema, edema, exudation, crusts, and xeroderma. Each parameter was scored from 0 to 3. The index was adapted from the human SCORAD by retaining the extent (A) and intensity (B) components and omitting the subjective component (C, pruritus and sleep loss), which cannot be obtained in animals; the final score was calculated as $A/5 + 7B/2$.

Thermographic evaluation

Infrared thermography was performed with a FLIR E60® camera (320 × 240 pixels; thermal sensitivity < 0.05 °C). Animals were positioned 10 cm from the equipment in a controlled environment at 24-25 °C and 40-50% relative humidity.

Images were obtained weekly over four weeks. Quantitative analysis was performed on the seven animals of each group at T1, T2, and T3, using the maximum temperature of the region of interest; the T4 series was retained for qualitative documentation only, as technical failures during acquisition compromised part of the frames at that time point and prevented their inclusion in the statistical analysis.

Evaluation of skin barrier function

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Skin barrier function was monitored weekly by transepidermal water loss (TEWL) using a portable Dermeter® analyser (Estek®). Measurements were taken in triplicate per animal under the same environmental conditions used for thermography, and the mean value was considered at each experimental time point.

Dermatohistopathological evaluation

For skin fragment collection, animals were anaesthetized with 2% xylazine and 10% ketamine, followed by local anaesthesia with 2% lidocaine. Excisional biopsies were performed with a 4 mm punch in the cervicodorsal region.

Collections were performed weekly for four weeks. Fragments were fixed in 10% formalin, routinely processed, and stained with haematoxylin and eosin (H&E).

Morphometric analysis considered epidermal parameters (spongiosis, exocytosis, and atrophy) and dermal parameters (inflammatory infiltrate, floating collagen, and perivascular, periglandular, and perifollicular distribution), using a semiquantitative 0-3 scale. Histopathological readings were unavailable for two animals of Group III at T2. As the repeated-measures model requires complete series, these two animals were excluded from the histopathological analyses, which were run with 26 animals (Group III, n=5; all other groups, n=7). No imputation was performed. All other variables were analysed with the full sample of 28 animals.

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At the end of the experiment, animals were euthanized in accordance with CONCEA guidelines using ketamine (25 mg/kg) and xylazine (10 mg/kg) intramuscularly, followed by thiopental (100 mg/kg, intraperitoneally) for anaesthetic deepening[12].

Statistical analysis

Data were submitted to descriptive (mean and standard deviation) and inferential statistical analysis. Normality was assessed by the Shapiro-Wilk test and homogeneity of variances by the Levene test. Repeated-measures ANOVA (Mixed ANOVA) was applied with Group (4 levels) and Time as factors, followed by the Tukey HSD post-hoc test. As a non-parametric alternative, the Kruskal-Wallis test was applied per time point with Dunn-Bonferroni post-hoc. Mann-Whitney was used for complementary pairwise comparisons. Effect size was calculated as Cohen's d. Analyses were performed in R (v.4.4.1; stats and DescTools packages), with α=5%.

Use of artificial intelligence: large language models (GPT-4 and Claude) were used solely in the data analysis stage, to assist in the interpretation and cross-checking of statistical outputs. They were not used to generate text, data, or bibliographic references. All results and references were verified by the authors against the primary sources.

RESULTS

Physicochemical characterization of the nanoemulsions

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Formulations showed mean particle size in the nanometre range, from 262.9 ± 13.79 nm to 300.1 ± 4.55 nm. The formulation containing only Pentravan® showed the largest mean particle size (300.1 ± 4.55 nm), while the formulation with 1% quercetin showed the smallest (262.9 ± 13.79 nm). Formulations containing CBD alone (297.5 ± 3.73 nm) and combined with quercetin (270.7 ± 5.85 nm) showed intermediate values.

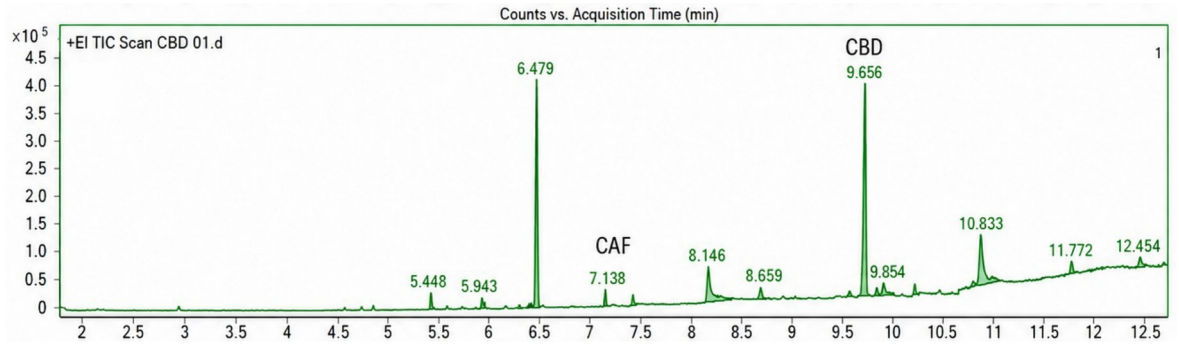
Zeta potential ranged from -44.5 ± 0.57 mV to -40.2 ± 0.52 mV. The formulation containing only Pentravan® showed the most negative value (-44.5 ± 0.57 mV), while formulations containing active compounds showed a gradual reduction of this parameter (1% CBD: -43.0 ± 0.88 mV; 1% QU: -41.4 ± 0.93 mV; 1% CBD + 1% QU: -40.2 ± 0.52 mV). The polydispersity index (PDI) ranged from 0.08 to 0.33, indicating homogeneous particle distribution across the formulations.

No statistically significant differences were observed among formulations regarding the physicochemical parameters evaluated (Kruskal-Wallis, $p>0.05$).

Chromatographic characterization of the cannabidiol-enriched corn oil

Gas chromatography-mass spectrometry (GC-MS) confirmed the presence of cannabidiol in the enriched corn oil used in the formulations. The chromatogram showed a peak compatible with CBD according to the retention time and spectrum of the analytical standard employed (Supplementary Figure S1). The peak corresponding to the internal standard (caffeine) was also identified, demonstrating the adequacy of the analytical method.

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Supplementary Figure S1. Chromatographic characterization of the cannabidiol-enriched corn oil. Gas chromatography–mass spectrometry (GC–MS) profile of the CBD-enriched corn oil used in the formulations, showing the peak compatible with cannabidiol according to the retention time and mass spectrum of the analytical standard, and the internal-standard (caffeine) peak. Source: prepared by the authors.

Clinical-dermatological evaluation of skin inflammation

The clinical evolution of skin lesions over the four experimental time points (T1-T4) is shown in Figure 1.

Group I (Pentravan®) showed persistence of inflammatory signs throughout the experimental period, with only slight lesion reduction. Group III (1% QU) behaved similarly, with residual alterations maintained. Animals treated with CBD (Group II) showed progressive reduction of inflammatory signs, with more evident improvement from the intermediate time points onwards. Group IV (1% CBD + 1% QU) showed the most pronounced lesion reduction over the period evaluated, with a marked decrease of inflammatory parameters at the final time points.

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Overall, the pattern of lesion evolution differed among experimental groups, with a more pronounced response in groups treated with CBD-containing formulations, especially when combined with quercetin.

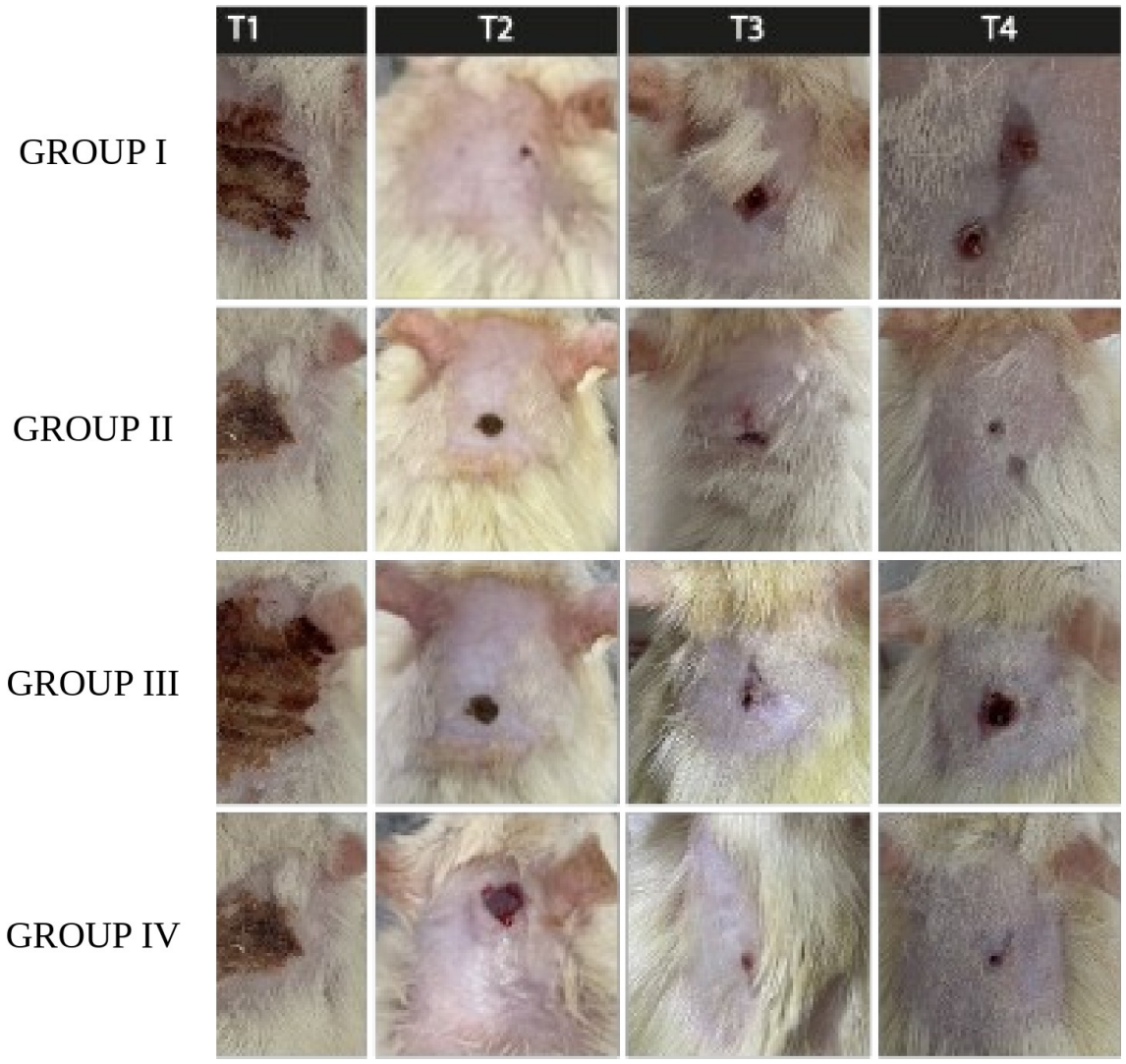


Figure 1. Clinical evolution of skin lesions in the experimental groups over the four evaluation time points. Source: prepared by the authors.

Evaluation of inflammatory parameters by the SCORAD index

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Inflammatory parameters assessed by the adapted SCORAD index were analysed separately for each of the five components (Figure 2). Mixed ANOVA revealed a significant effect of Group for erythema ($F(3,24) = 5.64$; $p = 0.005$; ges = 0.169), edema ($F(3,24) = 7.29$; $p = 0.001$; ges = 0.205), exudation ($F(3,24) = 7.14$; $p = 0.001$; ges = 0.210), crusts ($F(3,24) = 7.67$; $p < 0.001$; ges = 0.189), and xeroderma ($F(3,24) = 8.13$; $p < 0.001$; ges = 0.184). The effect of Time was significant and of large magnitude for all five parameters ($p < 0.001$; ges from 0.184 to 0.210 for Group and from 0.72 to 0.79 for Time, with Greenhouse-Geisser correction applied where sphericity was violated). The Group × Time interaction was significant for erythema ($p = 0.033$), edema ($p = 0.029$), exudation ($p = 0.015$), and crusts ($p = 0.037$), but not for xeroderma ($p = 0.076$).

Because the normality assumption was not met, the Kruskal-Wallis test was also applied at each time point. Significant differences among groups were found at T4 for all five components: erythema ($H = 10.14$; $p = 0.017$), edema ($H = 12.90$; $p = 0.005$), exudation ($H = 15.73$; $p = 0.001$), crusts ($H = 14.15$; $p = 0.003$), and xeroderma ($H = 15.28$; $p = 0.002$); differences were also present at earlier time points for edema (T1 and T3), exudation (T1 and T3), crusts (T3), and xeroderma (T1, T2, and T3). Dunn-Bonferroni post-hoc consistently identified Group IV as having lower scores than Group I: erythema at T4 ($p = 0.035$), edema at T3 ($p = 0.013$) and T4 ($p = 0.021$), exudation at T3 ($p = 0.022$), crusts at T3 ($p = 0.002$) and T4 ($p = 0.010$), and xeroderma at T2 ($p = 0.005$), T3 ($p = 0.018$) and T4 ($p = 0.008$). Group IV also scored lower than Group III for edema (T4, $p = 0.048$), exudation (T4, $p = 0.008$), and xeroderma (T4, $p = 0.036$).

Across all five components the lowest scores were consistently recorded in Group IV and the highest in Groups I and III. Parametric and non-parametric analyses converged: the group

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effect was significant for every parameter, and the pairwise differences concentrated at the final time points, supporting a progressive and treatment-dependent reduction of inflammatory signs, most pronounced in the group treated with the CBD + quercetin combination.

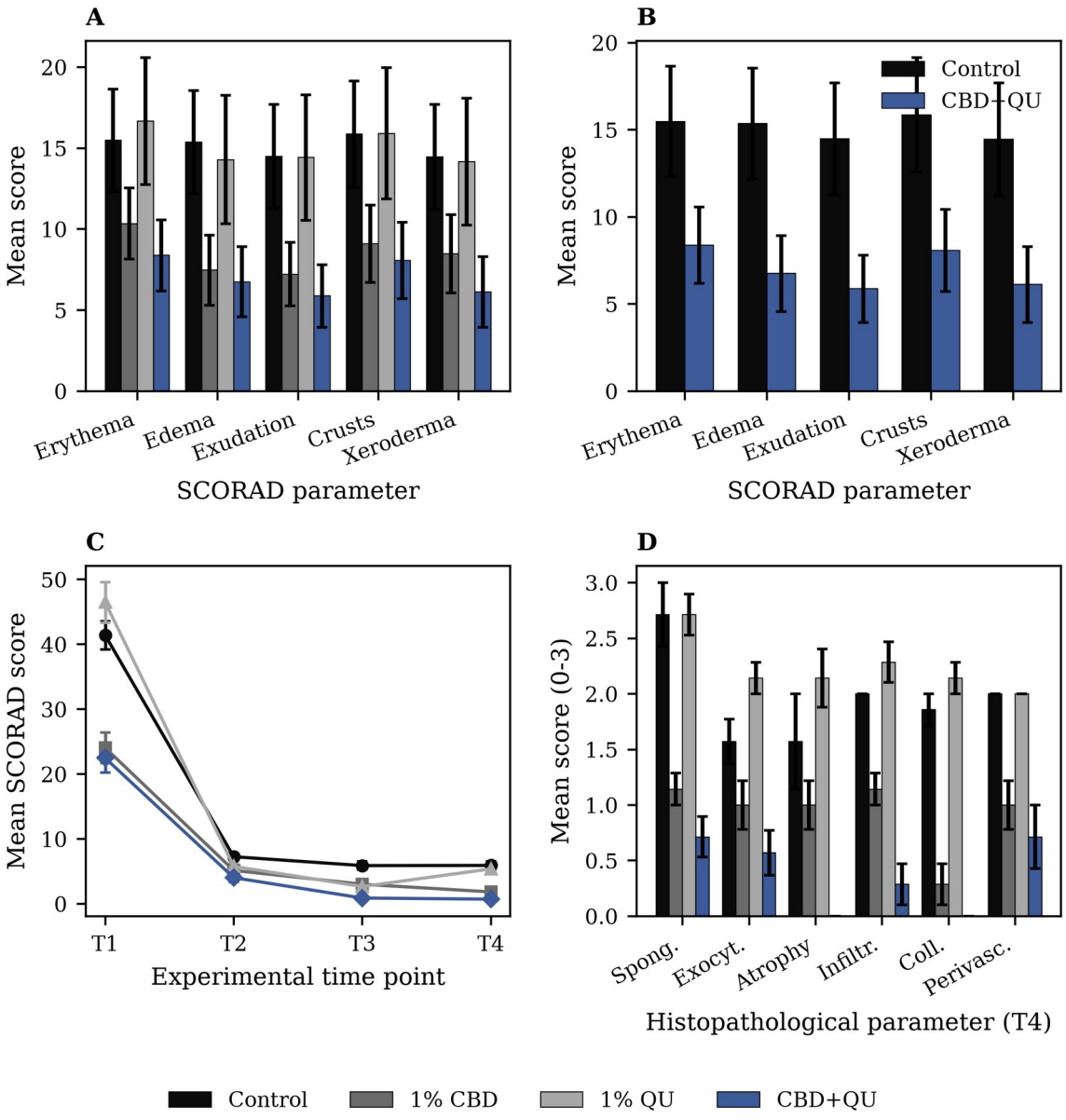


Figure 2. Inflammatory parameters across experimental groups. (A) Mean SCORAD scores by parameter and group; (B) negative control versus CBD+QU; (C) SCORAD evolution over the four time points; (D) histopathological parameters. Source: prepared by the authors.

Thermographic evaluation of skin inflammation

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Skin surface temperature was quantified in seven animals per group at three time points (T1–T3). Mean values (± SD) were, for Group I, 37.20 ± 0.67 °C, 36.54 ± 0.98 °C, and 35.56 ± 1.14 °C; for Group II, 36.61 ± 0.83 °C, 35.30 ± 1.28 °C, and 35.14 ± 1.24 °C; for Group III, 35.71 ± 0.45 °C, 36.01 ± 0.56 °C, and 35.14 ± 2.49 °C; and for Group IV, 35.33 ± 0.34 °C, 34.61 ± 0.45 °C, and 33.43 ± 1.11 °C (Figure 3A and 3B). Mixed ANOVA revealed a significant effect of Group ($F(3,24) = 13.60$; $p < 0.001$; ges = 0.321) and of Time ($F(2,48) = 10.27$; $p < 0.001$; ges = 0.236), with no significant interaction ($F(6,48) = 0.85$; $p = 0.796$). Since the normality assumption was not met in all group × time strata, the non-parametric alternative was also applied: Kruskal-Wallis confirmed differences among groups at T1 ($H = 18.29$; $p < 0.001$) and T2 ($H = 12.61$; $p = 0.006$), but not at T3 ($H = 6.98$; $p = 0.073$). Dunn-Bonferroni post-hoc showed lower temperatures in Group IV than in Group I at T1 ($p < 0.001$) and T2 ($p = 0.005$), and than in Group II at T1 ($p = 0.016$).

Group IV showed the lowest mean temperature at every time point and the largest overall decrease (−1.90 °C from T1 to T3), whereas Group I remained the warmest throughout. Groups II and III showed intermediate values with a transient rise at T2. This pattern is consistent with a more pronounced reduction of local inflammatory activity in the group treated with the CBD + quercetin combination. Figure 4 presents representative thermographic images of one animal per group at each of the four time points; the temperatures displayed within those frames correspond to the upper limit of the thermal scale of each individual image and are therefore illustrative of the qualitative thermal pattern, not group means, and were not used in any statistical analysis.

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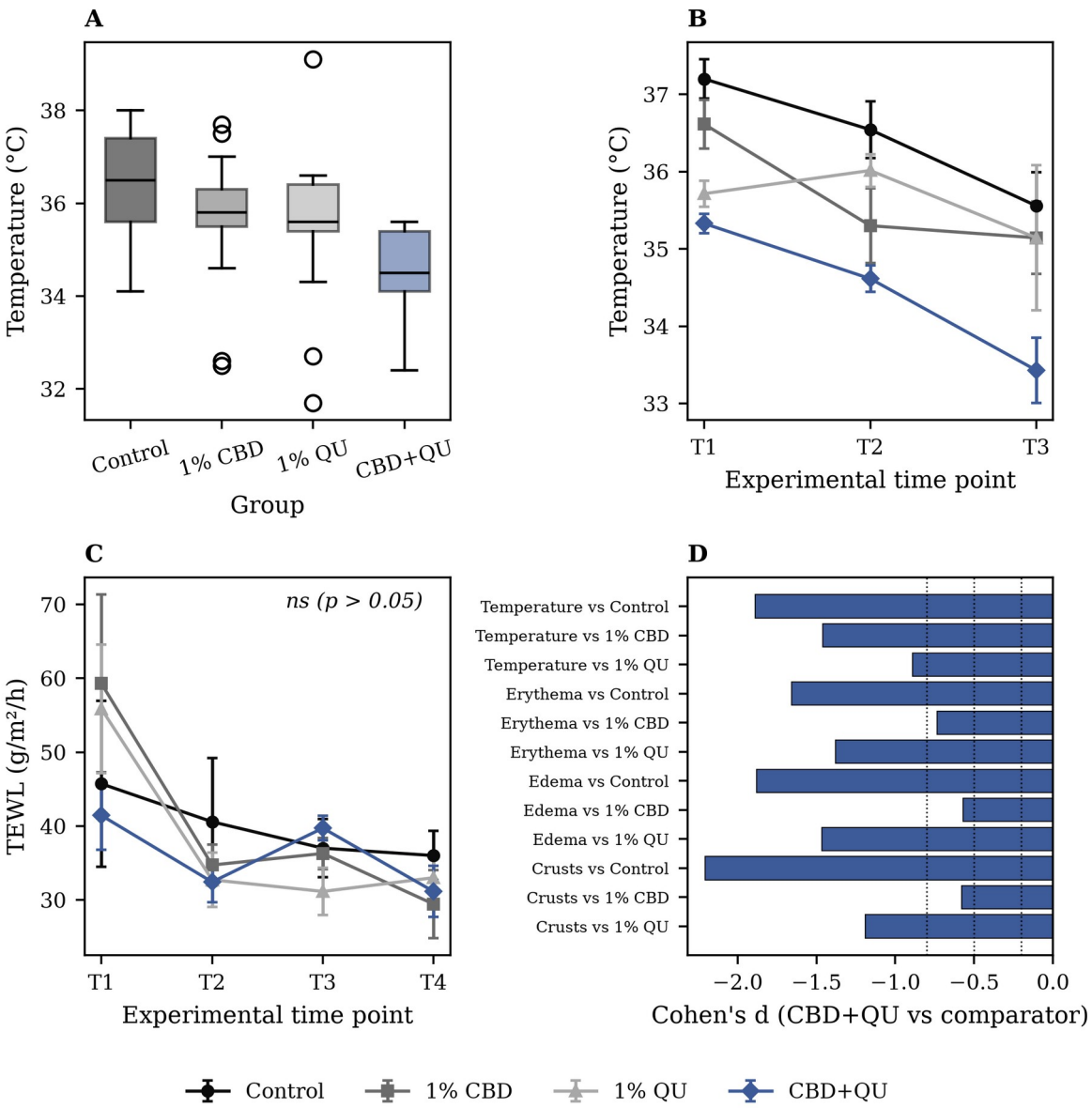


Figure 3. Skin barrier and thermal parameters. (A) Skin temperature by group; (B) temperature over the evaluation time points; (C) transepidermal water loss (TEWL) over the four time points; (D) effect sizes (Cohen's d) for comparisons against the CBD+QU group.

Source: prepared by the authors.

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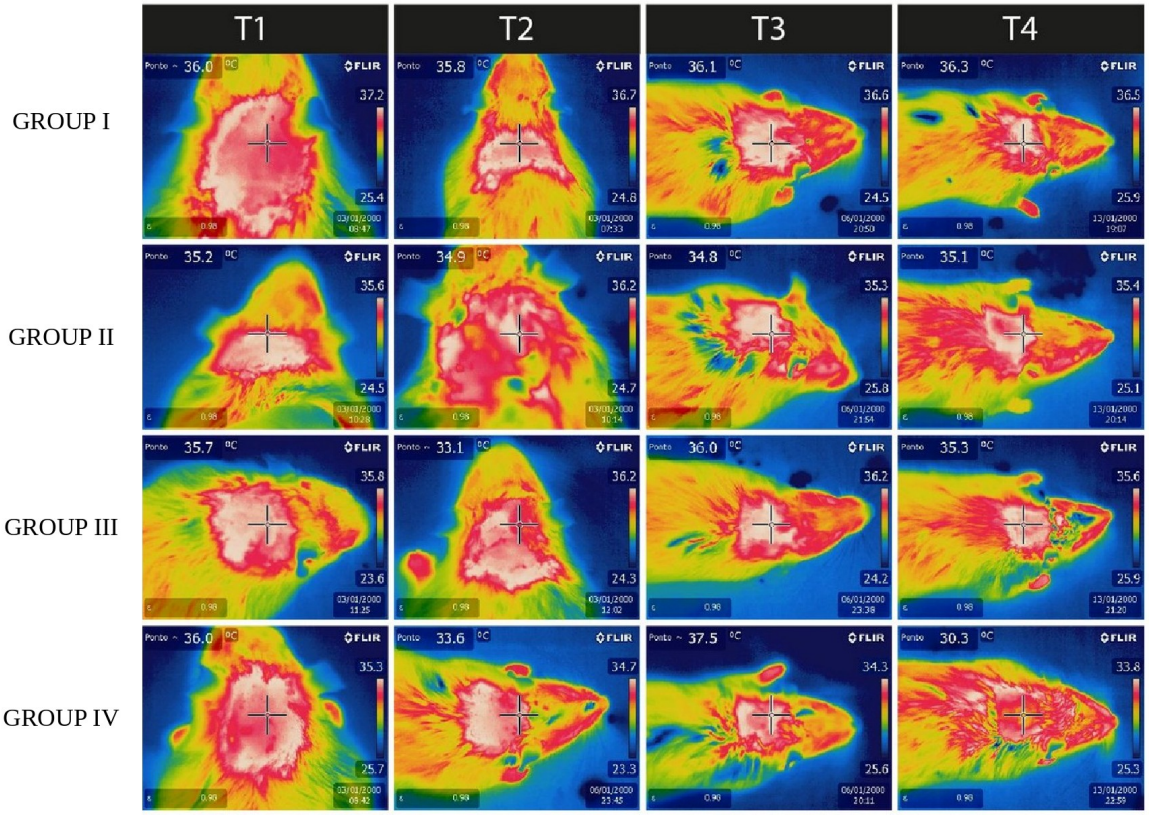


Figure 4. Representative thermographic images of the inflamed region in the experimental groups across the four evaluation time points (one animal per group and time point). The temperature shown in each frame corresponds to the upper limit of its own thermal scale and is illustrative; it does not represent the group mean. Source: prepared by the authors.

Dermatohistopathological evaluation

Dermatohistopathological alterations observed in the experimental groups are shown in Figure 5.

At T1 all groups showed intercellular spongiosis, exocytosis of inflammatory cells, and dermal inflammatory infiltrate with a predominantly perivascular and perifollicular distribution. Across T2-T4 these alterations decreased progressively: Group I retained dermal infiltrate and slight epidermal disorganization until the end, Group III showed an initial

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reduction followed by persistence of infiltrate at T4, and Groups II and IV showed the most marked improvement.

Semiquantitative scores were analysed for each of the six histopathological parameters. Because readings were unavailable for two animals of Group III at T2, these subjects were excluded from the repeated-measures model, which was therefore fitted with 26 animals (Group III, n=5). Mixed ANOVA revealed a significant effect of Group for all six parameters: spongiosis ($F(3,22) = 9.94$; $p < 0.001$; ges = 0.325), exocytosis ($F(3,22) = 25.57$; $p < 0.001$; ges = 0.460), epidermal atrophy ($F(3,22) = 11.83$; $p < 0.001$; ges = 0.278), inflammatory infiltrate ($F(3,22) = 66.14$; $p < 0.001$; ges = 0.717), floating collagen ($F(3,22) = 77.20$; $p < 0.001$; ges = 0.668), and perivascular infiltrate ($F(3,22) = 30.37$; $p < 0.001$; ges = 0.532). Inflammatory infiltrate and floating collagen showed the largest effect sizes of the whole study. The effect of Time was significant for all parameters ($F(3,66)$ from 3.17 to 26.94; $p \leq 0.030$), and the Group × Time interaction was significant for all except perivascular infiltrate ($F(9,66) = 1.70$; $p = 0.133$).

The non-parametric analysis confirmed this pattern and localized it in time. At T4, Kruskal-Wallis showed significant differences among groups for spongiosis ($H = 20.67$; $p < 0.001$), exocytosis ($H = 17.33$; $p < 0.001$), atrophy ($H = 17.17$; $p < 0.001$), inflammatory infiltrate ($H = 23.01$; $p < 0.001$), floating collagen ($H = 24.11$; $p < 0.001$), and perivascular infiltrate ($H = 19.09$; $p < 0.001$). Dunn-Bonferroni post-hoc at T4 showed lower scores in Group IV than in Group I for spongiosis ($p = 0.002$), atrophy ($p = 0.021$), inflammatory infiltrate ($p = 0.001$), floating collagen ($p = 0.003$), and perivascular infiltrate ($p = 0.006$); and lower than in Group III for spongiosis ($p = 0.002$), exocytosis ($p < 0.001$), atrophy ($p < 0.001$), inflammatory infiltrate ($p < 0.001$), floating collagen ($p < 0.001$), and perivascular infiltrate ($p = 0.006$). For

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inflammatory infiltrate and floating collagen, Group IV already differed from Group I at T1 ($p < 0.001$ for both) and remained lower at every subsequent time point.

Overall, inflammatory parameters decreased in all groups, with differences in the intensity and speed of resolution. Group III (1% QU) was the only group in which several parameters did not decrease consistently, remaining close to control values at T4, whereas Groups II and IV showed the lowest scores across all six parameters.

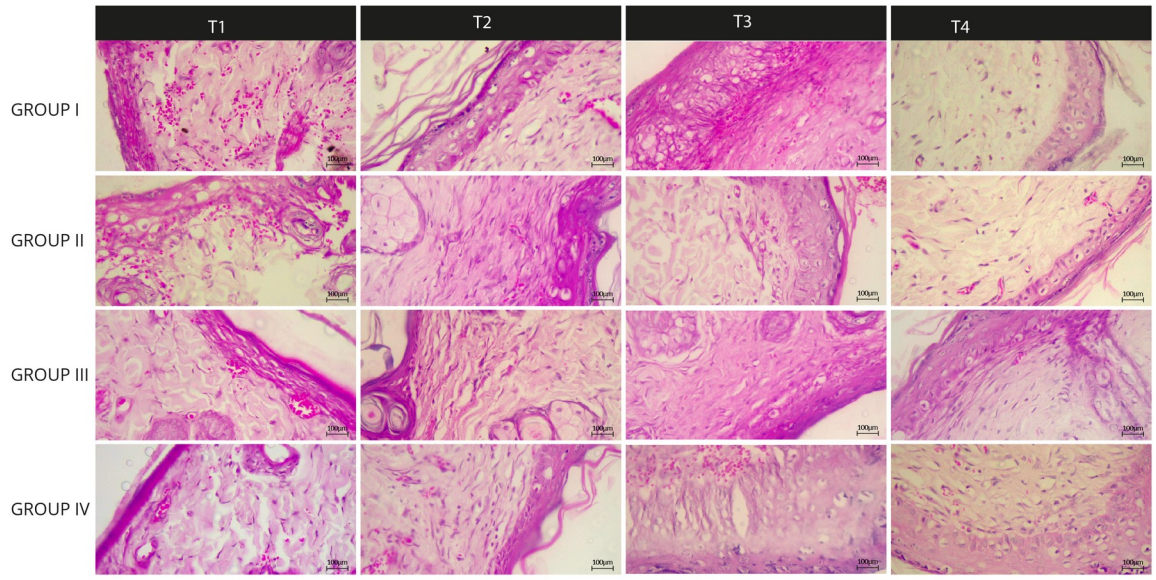


Figure 5. Dermatohistopathological evaluation of the experimental groups at the different analysis time points (H&E). Source: prepared by the authors.

Evaluation of transepidermal water loss (TEWL)

Transepidermal water loss (TEWL) varied among groups across the experimental time points, with a general downward trend during follow-up (Figure 3).

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At T1 all groups showed elevated TEWL, highest in Groups II and III and lowest in Group IV. Values fell in all groups at T2 and converged. At T3 Group II rose slightly and Group IV showed a transient increase, while Groups I and III kept decreasing. At T4 values fell again: Group II was lowest, followed by Groups IV and III, with Group I highest.

Overall, results showed a reduction in transepidermal water loss throughout the experiment in all groups, although with intermediate oscillations between time points. Differences among groups were not statistically significant.

DISCUSSION

The results of this study show that the topical nanoemulsion containing cannabidiol (CBD), quercetin (QU), and Pentravan® had physicochemical characteristics compatible with stable colloidal systems, in addition to a relevant therapeutic effect on the clinical, functional, thermographic, and histopathological parameters associated with experimental atopic dermatitis. Overall, the CBD-quercetin combination performed better than the isolated treatments, suggesting a complementary effect between the compounds in modulating skin inflammation and restoring epidermal barrier function.

Physicochemical characterization showed that all formulations had particle size in the nanometre range, low polydispersity, and high zeta potential, indicating adequate homogeneity and colloidal stability. These parameters are fundamental for nanostructured topical systems, since they influence formulation stability, active compound distribution, and the potential for interaction with the skin surface[13]. The reduction in particle size after incorporation of CBD and quercetin may relate to interfacial reorganization of the system, a phenomenon previously described in formulations containing lipophilic compounds[14,15].

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Moreover, maintenance of zeta potential values more negative than −40 mV reinforces the electrostatic stability of the nanoemulsions[16], while the low PDI values, especially in the quercetin formulation, suggest homogeneous particle distribution. These findings indicate that simultaneous incorporation of CBD and quercetin did not compromise the nanoarchitecture of the system.

GC-MS analysis confirmed the identity and chemical stability of the CBD incorporated into the corn oil, with no evidence of detectable degradation. This is relevant because stability of the active compound is essential for an adequate interpretation of the biological effects observed. The analytical confirmation obtained is consistent with Gurley et al.[17] and reinforces the importance of internal standards for methodological reliability[18]. The absence of degradation products also suggests that the lipid vehicle may have contributed to protecting the CBD, as described by Jeong et al.[19] and O'Sullivan et al.[20]. The clinical and histological results can therefore be attributed with greater confidence to the activity of the incorporated compounds.

Clinically, the treated groups showed reduction of the inflammatory parameters assessed by SCORAD, including erythema, edema, exudation, crusts, and xeroderma. However, the response was more pronounced in the group treated with CBD + quercetin, with the group effect reaching significance for all five components in both the parametric and the non-parametric analyses, and the pairwise differences concentrating at the final time points. The anti-inflammatory action of CBD may be explained by its interaction with CB1/CB2 receptors and TRPV1 channels, as well as by modulation of pro-inflammatory cytokines such as TNF-α and IL-6[21,22]. In addition, studies suggest that CBD may contribute to restoring the skin barrier, which is particularly relevant in atopic dermatitis[23]. Quercetin, in turn, shows antioxidant and anti-inflammatory activity related to inhibition of the COX-2, 5-LOX, and NF-κB pathways[24]. Nevertheless, the less stable response observed in the group treated with

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quercetin alone may be associated with its low bioavailability and pharmacokinetic limitations[25].

The superiority of the combined treatment suggests complementary effects on different pathways of skin inflammation, in line with the “entourage effect”, in which bioactive compounds act jointly to potentiate the therapeutic response[26], and with evidence that quercetin suppresses NF-κB-dependent cytokine production in keratinocytes[27]. Here this was reflected in a more consistent reduction of clinical scores and better lesion resolution in the CBD + quercetin group; interindividual variability was not formally compared between groups. These results align with studies in dogs and humans pointing to potential benefits of cannabinoids in atopic dermatitis, although that literature still shows important methodological limitations[28,29].

Thermographic evaluation reinforced the clinical findings, showing lower skin temperature in the CBD + quercetin group, which differed significantly from the control at T1 and T2 and showed the largest decrease across the period analysed. Thermography has been described as a sensitive, non-invasive tool for monitoring cutaneous inflammatory processes, allowing identification of thermal changes associated with local inflammatory activity[30,31]. The thermal modulation observed may relate to the action of CBD on inflammatory pathways and TRPV1 channels[22,32]. It should be noted, however, that the supporting evidence for TRPV1 involvement derives largely from non-cutaneous models, so this mechanism remains a hypothesis in the skin context. The less stable response of quercetin alone may again reflect its pharmacokinetic limitations[25]. It should also be noted that the group difference was no longer significant at T3 in the non-parametric analysis, so the thermographic evidence supports an early, rather than a sustained, anti-inflammatory effect.

The reduction of transepidermal water loss (TEWL) in the treated groups indicates progressive improvement of epidermal barrier function, a central aspect of the

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pathophysiology of atopic dermatitis[5]. CBD showed relatively stable performance in this parameter, possibly related to its action on lipid homeostasis and the cutaneous inflammatory response[33]. Quercetin contributed partially to barrier recovery, although with lower stability over time. The CBD + quercetin combination showed behaviour compatible with dynamic re-epithelialization processes. Although a complete return to baseline levels did not occur, the general downward trend in TEWL suggests progressive functional restoration of the skin barrier. It must be stressed, however, that differences among groups in TEWL did not reach statistical significance, so this interpretation remains exploratory.

The dermatohistopathological findings provided morphological support for the clinical, thermographic, and functional results. The initial alterations observed — intercellular spongiosis, exocytosis of inflammatory cells, and dermal inflammatory infiltrate with a predominantly perivascular and perifollicular distribution — are compatible with classic models of atopic dermatitis[11]. Throughout treatment, a progressive reduction of these alterations was observed, with a more evident response in the CBD + quercetin group. CBD showed relevant immunomodulatory activity associated with reduction of cytokines and inflammatory cell recruitment[33]. The combination with quercetin appears to have amplified this effect, probably through the combination of antioxidant and anti-inflammatory mechanisms[34].

The reduction of spongiosis and exocytosis in the treated groups indicates lower infiltration of inflammatory cells into the epidermis. These findings agree with the spongiotic model described by Graham and Wilkinson[35] and Tanei and Hasegawa[36], in which inflammation involves T lymphocytes and inflammatory epidermal dendritic cells. The CBD + quercetin combination achieved the best control of these parameters, suggesting a greater capacity to modulate epidermal inflammation. Likewise, the reduction of epidermal atrophy in the treated

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groups, especially in the combined group, indicates structural recovery of the epidermis, possibly related to decreased keratinocyte apoptosis and restoration of cell adhesion[36].

A reduction of dermal inflammatory infiltrate was also observed in the treated groups, indicating lower recruitment of lymphocytes and dendritic cells, as described in the pathophysiology of spongiotic dermatitides[37,38]. The CBD + quercetin group showed the greatest reduction of this infiltrate, suggesting more efficient modulation of the cutaneous immune response. The decrease of floating collagen in the treated groups indicates extracellular matrix reorganization and reduced dermal inflammation, a finding compatible with alterations described in inflammatory dermatitides[37,39]. In addition, the reduction of perivascular, periglandular, and perifollicular infiltrate reflects lower inflammatory activity in the dermal and adnexal microenvironment, a pattern characteristic of atopic dermatitis[36].

Taken together, the results indicate that the formulation containing CBD + quercetin delivered through Pentravan® acted in an integrated manner on different components of experimental atopic dermatitis: clinical inflammation, local hyperthermia, barrier dysfunction, and epidermal and dermal histopathological alterations. The convergence between clinical, functional, and morphological findings strengthens the interpretation that the combination has greater therapeutic potential than the isolated compounds.

Some limitations should be considered. The sample size (n=7 per group), defined according to the 3Rs principle and to CEUA/UFRPE recommendations, limits statistical power to detect small effects, and no a priori power calculation was performed. The experimental model reproduces important aspects of skin inflammation and barrier dysfunction but does not fully encompass the immunological complexity of spontaneous atopic dermatitis in humans or dogs. TEWL showed high intragroup variability, which is expected in inflamed skin and reduced the power to detect differences between groups for this parameter. Finally, the histopathological scoring used a semiquantitative scale, which is less precise than

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morphometric quantification. Further studies should assess long-term stability, skin permeation, local safety, dose-response, and efficacy in natural clinical models.

Thus, the topical nanoemulsion containing CBD, quercetin, and Pentravan® represents a therapeutic possibility for the management of inflammatory dermatitides, particularly through the combination of physicochemical stability, anti-inflammatory activity, functional improvement of the skin barrier, and histopathological recovery. From a collective health perspective, atopic dermatitis affects 11.7% to 13.8% of Brazilian children and adolescents[3], and the limitations of prolonged corticosteroid use reinforce the public health relevance of developing safe, low-cost topical alternatives that can be produced by compounding pharmacies within the national health system.

CONCLUSION

The topical nanoemulsion containing cannabidiol, quercetin, and Pentravan® showed adequate physicochemical stability and greater efficacy in reducing skin inflammation compared with the isolated treatments. Clinical, thermographic, and dermatohistopathological findings converge to indicate a complementary effect between the compounds, evidencing their therapeutic potential as a topical strategy for atopic dermatitis; changes in skin barrier function measured by TEWL, however, did not differ significantly among groups. Further studies are needed to confirm safety and efficacy in clinical models.

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Conflict of interest

The authors declare that the preparation methodology described is covered by patent application No. BR 10 2025 013585 0, of which they are inventors. No other conflicts of interest are declared.

Ethics statement

All procedures involving animals were approved by the Animal Use Ethics Committee of the Universidade Federal Rural de Pernambuco (CEUA/UFRPE; protocol No. 4130150822) and conducted in accordance with the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and with the ARRIVE guidelines. The study did not involve human subjects.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

AKL Araújo: conceptualization, resources, methodology, investigation, formal analysis, writing – original draft. R Miranda: methodology, resources, investigation, formal analysis, writing – review & editing. AHJ Silva: formal analysis, data curation, software, visualization, writing – review & editing. AJ Alves: supervision, validation, writing – review & editing. VA Silva Junior: supervision, validation, writing – review & editing. All authors approved the final version to be published and are accountable for all aspects of the work.

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