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Original Article

The potential of calcitriol gel in accelerating the healing of traumatic oral ulcers

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الملخص

أهداف البحث: هدفت هذه الدراسة إلى تقييم تأثيرات هلام الكالسيتريول الموضعي في شفاء القرحات الفموية الرضّية، من خلال تقييم الاستجابات الالتهابية والتكاثرية والمولِّدة للأوعية في نموذج حي من الجرذان.

طرق البحث: أُحدثت قرحات رضّية في جرذان ويستار، ثم قُسمت الحيوانات إلى مجموعة معالجة بهلام الكالسيتريول الموضعي ومجموعة ضابطة. قُيّم شفاء القرحات في عدة نقاط زمنية، وهي الأيام 4 و6 و8، باستخدام تلوين الهيماتوكسيلين والإيوزين، وتلوين ماسون ثلاثي الألوان، والكيمياء النسيجية المناعية. وشملت المعايير المقيمة ارتشاح البلاعم واللمفاويات، وتكاثر الخلايا الليفية، وترسب الكولاجين، وتكوّن الشعيرات الدموية، وتعبير عامل نمو بطانة الأوعية الدموية.

النتائج: أظهرت القرحات المعالجة بالكالسيتريول، مقارنةً بالمجموعة الضابطة، ارتشاحًا أعلى بدرجة معنوية للبلاعم في اليوم 6 واليوم 8، إضافة إلى أعداد أعلى بدرجة معنوية من اللمفاويات في اليوم 6 واليوم 8. كما كان تكاثر الخلايا الليفية، وترسب الكولاجين، وكثافة الشعيرات الدموية أعلى بدرجة معنوية في مجموعة العلاج مقارنةً بالمجموعة الضابطة. وبلغ تعبير عامل نمو بطانة الأوعية الدموية ذروته في اليوم 6، مما يشير إلى تعزيز النشاط المولِّد للأوعية.

الاستنتاجات: عزّز هلام الكالسيتريول الموضعي شفاء القرحات الفموية الرضّية بدرجة معنوية من خلال تعديل الاستجابات الالتهابية، وتحفيز نشاط الخلايا الليفية، وتنشيط تكوّن الأوعية الدموية. وتدعم هذه النتائج إمكانية استخدامه عاملًا علاجيًا في ترميم المخاطية الفموية.

الكلمات المفتاحية: إعادة الظهارة؛ القرحة الفموية الرضّية؛ تكوّن الأوعية الدموية؛ عملية الشفاء؛ هلام الكالسيتريول

Abstract

Objectives: This study was aimed at evaluating the effects of topical calcitriol gel on traumatic oral ulcer healing, by assessing inflammatory, proliferative, and angiogenic responses in an in vivo rat model.

Methods: Traumatic ulcers were induced in Wistar rats (*Rattus norvegicus*), and the animals were divided into a topical calcitriol gel treatment group and a control group. Ulcer healing was assessed at multiple time points (days 4, 6, and 8) with hematoxylin and eosin staining, Masson's trichrome staining, and immunohistochemistry. The evaluated parameters included macrophage and lymphocyte infiltration, fibroblast proliferation, collagen deposition, capillary formation, and vascular endothelial growth factor (VEGF) expression.

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Results: Calcitriol-treated ulcers, compared with those in the control group, demonstrated significantly greater macrophage infiltration on day 6 ($p = 0.045$) and day 8 ($p = 0.031$), as well as significantly higher lymphocyte counts on day 6 ($p = 0.033$) and day 8 ($p = 0.021$). In addition, fibroblast proliferation, collagen deposition, and capillary density were significantly greater in the treatment group than the control group ($p < 0.05$). VEGF expression peaked on day 6 ($p = 0.023$), thus indicating enhanced angiogenic activity.

Conclusions: Topical calcitriol gel significantly enhanced healing in traumatic oral ulcers by modulating inflammatory responses, promoting fibroblast activity, and stimulating angiogenesis. These findings support its potential as a therapeutic agent for oral mucosal repair.

Keywords: Angiogenesis; Calcitriol gel; Healing process; Re-epithelialization; Traumatic oral ulcer

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Introduction

Traumatic ulcers are among the most common oral mucosal lesions, and are characterized by epithelial discontinuity extending beyond the basement membrane into the lamina propria. These lesions are caused by mechanical, thermal, or chemical trauma, and affect as much as 62.2% of the global population.[1] The healing of traumatic ulcers follows a coordinated sequence involving hemostasis, inflammation, proliferation, and remodeling.[2] Macrophages and lymphocytes play critical roles during the inflammation response by orchestrating cytokine release and transitioning from pro-inflammatory (M1) to anti-inflammatory (M2) phenotypes,[2] particularly in the remodeling phase. In later stages, fibroblasts facilitate collagen synthesis, extracellular matrix deposition, and re-epithelialization.[3,4] Meanwhile, angiogenesis, driven by factors such as vascular endothelial growth factor (VEGF), ensures adequate oxygenation and nutrient delivery for tissue regeneration.[5,6]

Although most cases resolve spontaneously within 10–14 days, the associated pain often disrupts essential functions such as speech and mastication, and substantially affects quality of life.[7,8] Although current treatments, including topical corticosteroids and oral antiseptics such as chlorhexidine, are effective, their prolonged use may result in adverse effects including oral candidiasis, burning sensations, xerostomia, and an altered sense of taste.[9–11]

Recent advances in oral ulcer management include the exploration of biomaterial-based and bioengineered therapeutic strategies to enhance drug retention and modulate the wound microenvironment. For example, novel delivery platforms such as nanogel systems, bioactive films, and plasma-assisted therapies have been investigated to regulate inflammation, stimulate fibroblast activity, and accelerate mucosal regeneration.[12–15] Although these approaches have shown promising regenerative potential, they often require complex fabrication processes and material engineering, thus potentially limiting their translational feasibility in routine clinical practice. Therefore, a need exists for simpler bioactive therapeutic agents that directly regulate key molecular pathways involved in oral mucosal wound healing.

Calcitriol, the hormonally active form of vitamin D, has gained attention as a potential wound-healing agent, because of its broad biological activity, high receptor affinity, and favorable bioavailability with respect to that of other vitamin D analogues.[16] Vitamin D exerts its biological effects primarily through the vitamin D receptor (VDR), a nuclear receptor expressed in diverse tissues including the oral mucosa. Given that VDR is expressed in oral keratinocytes, fibroblasts, endothelial cells, and various immune cells, vitamin D signaling might have important roles in maintaining mucosal homeostasis and regulating tissue repair processes.[17–19] After activation, the calcitriol–VDR complex modulates the transcription of genes involved in immune regulation, epithelial proliferation, and angiogenesis. After synthesis via sequential hydroxylation of cholecalciferol, calcitriol binds VDR and regulates gene transcription involved in immune modulation, epithelial proliferation, and angiogenesis.[20–22] Experimental studies have demonstrated that calcitriol exerts anti-inflammatory effects by suppressing pro-inflammatory cytokines and enhancing antimicrobial peptide expression, while also promoting angiogenic signaling pathways associated with VEGF.[23,24]

Topical drug delivery offers particular advantages for the management of oral mucosal lesions, including localized therapeutic action, minimal systemic exposure, and avoidance of first-pass metabolism.[25,26] However, the oral cavity is a challenging environment for topical therapy, because its continuous salivary flow, mechanical movement, and mucosal turnover might limit drug residence time at lesion sites.[27,28] To overcome these limitations, mucoadhesive polymer-based formulations have been widely explored to enhance drug retention and prolong local therapeutic effects.[29] Carboxymethyl cellulose (CMC), a hydrophilic polymer with recognized mucoadhesive and gel-forming properties, is frequently used as a topical delivery vehicle in oral mucosal applications to improve formulation stability and mucosal contact time.[30,31]

Although topical calcitriol has shown clinical benefits in conditions such as oral mucositis and recurrent aphthous stomatitis,[32,33] evidence of its mechanistic effects on immune cell infiltration, fibroblast activity, collagen deposition, and VEGF-mediated angiogenesis in traumatic oral ulcer healing remains limited. Therefore, this in vivo study was aimed at investigating the effects of topical calcitriol gel on traumatic oral ulcer healing by evaluating key inflammatory, proliferative, and angiogenic markers involved in the wound-healing process.

Materials and Methods

Preparation of topical calcitriol gel

A 0.0003% topical calcitriol gel was prepared with calcitriol powder (GlpBio®, Montclair, CA, USA; GC13852). Calcitriol was first dissolved in an ethanol stock solution and subsequently incorporated into a 3% CMC gel base with continuous mixing until a homogeneous dispersion was

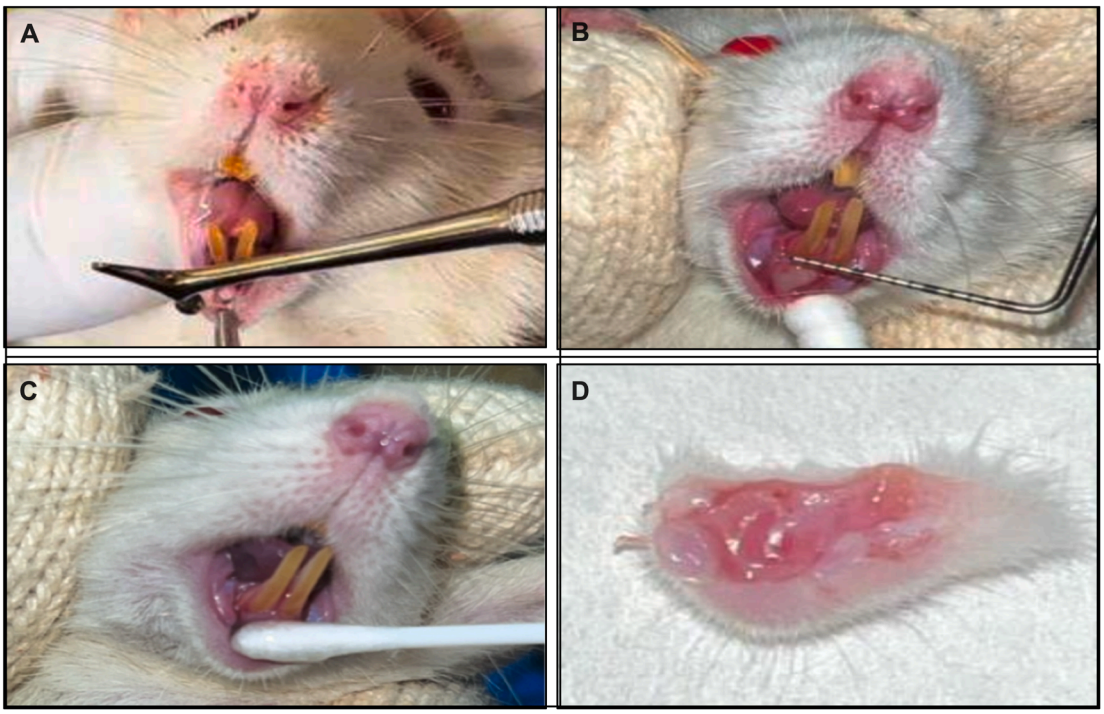


Figure 1: Procedure for traumatic ulcer induction and topical treatment application in Wistar rats. (A) Induction of traumatic ulcers on the lower labial mucosa with a heated instrument. (B) Ulcer measurement post-induction. (C) Application of gel to the ulcer site. (D) Tissue sample excision showing the ulcer surface.

achieved. The concentration of calcitriol (0.0003%) was selected according to previous literature investigating its use in similar topical applications.[34] The final formulation was stored in appropriate containers under cool conditions until use.

Animal and experimental design

Three-month-old male Wistar rats (*Rattus norvegicus*), weighing approximately 200 g and exhibiting no observable abnormalities, were used in this study. The necessary sample size was determined with Federer's formula $(n-1)(t-1) \geq 15$, where n represents the number of animals per group, and t represents the number of experimental groups. A minimum of four animals were calculated to be required per group. To account for a potential dropout rate of 10%, the sample size was adjusted to a final sample size of five animals per group at each observation time point. The animals were housed in standard laboratory cages (three to five rats per cage) under a controlled temperature of 20–25 °C. They were given rice husk bedding, and provided with food and water *ad libitum*.[35]

Anesthesia and traumatic ulcer induction

The rats were anesthetized with a combination of ketamine and xylazine, administered intraperitoneally or intramuscularly at 0.05–0.1 ml/10 g body weight, with acepromazine and saline as needed.[34,36] Ulcer formation was induced by gentle application of a 2 mm burnisher, which had been heated over a Bunsen burner for 30–60 s, to the rat's labial mucosa for 1 s.[37,38] Although the exact temperature was not directly measured, the procedure was performed under standardized conditions to ensure consistency and reproducibility. Ulcer formation was confirmed within 24–48 h. Most ulcers measured 2–5 mm in diameter. Ulcers exceeding 10 mm in diameter were excluded from analysis (Figure 1).

Calcitriol treatment protocol

The animals were randomly assigned to the treatment or control group. In the treatment group, topical calcitriol gel was applied to the lower labial mucosa twice daily for 3, 5, or 7 days with sterile cotton buds.[35] The control group received CMC gel according to the same application method and schedule.[35,39,40] During topical application, the animals were gently restrained by the operator, with the forelimbs secured between the fingers, the head tilted posteriorly, and the lower lip retracted to ensure accurate gel delivery.[36]

Tissue collection and histological preparation

After the animals were euthanized with a lethal dose of ether, biopsies of the lower labial mucosa were collected on days 4, 6, and 8 after the initial treatments. Tissue samples included both the ulcerated region and surrounding mucosa. Specimens were fixed in 10% buffered formalin (pH 7.4), and the fixation process was repeated with fresh solution. After fixation, the tissues were rinsed, dehydrated with a Graded ethanol series, cleared in xylene, embedded in paraffin, and sectioned to a thickness of 5 μm.[37]

Histological evaluation of inflammatory cells, fibroblasts, and angiogenesis

For hematoxylin and eosin (H&E) staining, paraffin sections were deparaffinized in three changes in absolute xylene (4 min each), rehydrated in a graded ethanol series (100%, 95%, 90%, and 70%; 4 min each), and rinsed in distilled water. Sections were stained with hematoxylin for 2 min, differentiated with 1% acid alcohol, treated with 2% potassium acetate, counterstained with eosin for 2 min, dehydrated, and mounted with Dibutylphtalate Polystyrene Xylene (DPX).[41] Cell counts for macrophages, lymphocytes, fibroblasts, and newly formed blood vessels were performed manually at 400× magnification under a Nikon H600 L light microscope equipped with digital imaging.

Assessment of collagen deposition

Masson's trichrome staining was performed to evaluate collagen deposition. Sections were deparaffinized, rehydrated, treated with pre-warmed Bouin's solution at 60 °C for 45 min, then rinsed until clear. Subsequently, the sections were stained with phosphomolybdic acid for 10 min followed by methyl blue for 5 min, differentiated with 1% acetic acid, dehydrated, cleared, and mounted. In ImageJ software, collagen density was quantified by division of the observed collagen area (in μm^2) by the total measurement area in Masson's-trichrome-stained sections.[42]

Immunohistochemical analysis of VEGF expression

Lower labial mucosa sections were processed for immunohistochemical analysis with rabbit polyclonal anti-VEGF antibodies (Bioenzy: BZ-082059DD-AP) at 1:150 dilution after antigen retrieval at 60 °C for 60 minutes. VEGF-positive endothelial cells were identified by brown chromogenic staining of flattened, elongated cells forming a single-layer lining of vascular lumens under 40× magnification. VEGF-expressing endothelial cells appeared brown under an immuno-electron microscope (Olympus, Japan) at 200× magnification. Quantitative analysis was conducted to determine the mean endothelial cell count obtained independently by two blinded examiners.

Statistical analysis

Statistical analyses were performed in SPSS version 29. Data are presented as mean ± standard deviation. Normality was assessed with the Shapiro–Wilk (or Kolmogorov–Smirnov) test, and homogeneity of variance was determined with Levene's test. For normally distributed data with equal variances, one-way ANOVA followed by Tukey's HSD post-hoc test was applied, whereas the Games–Howell test was used for unequal variances. Non-parametric data were analyzed with the Kruskal–Wallis test followed by Mann–Whitney pairwise comparisons. Multiple comparisons were controlled for with appropriate post-hoc tests for parametric data. However, no formal adjustment was applied for non-parametric pairwise comparisons, which we acknowledge as a study limitation.

Results

Traumatic oral ulcer model of the labial mucosa in Wistar rats

Histopathological examination of the negative control group confirmed the successful induction of mucosal ulceration (Figures 2 and 8). At 400× magnification, the ulcerated tissue exhibited clear epithelial disruption with loss of surface integrity, accompanied by dense inflammatory cell infiltration within the underlying connective tissue. Higher-magnification images further revealed the predominance of inflammatory cells and disorganized tissue architecture in the ulcer regions. These findings validated the establishment of the ulcer model in the negative control group and provided a baseline for comparison of both treatment groups.

Macrophage counts

Topical application of calcitriol gel, compared with the CMC control, significantly increased macrophage infiltration in traumatic oral ulcers in *Rattus norvegicus* (Figures 3 and 8). Quantitative analysis (Table 1) demonstrated consistently higher macrophage counts in the treatment group (3.00 ± 1.00 on days 4 and 6; 3.40 ± 0.55 on day 8), than the control group (1.25 ± 0.50 on day 4; 1.80 ± 0.45 on day 6; and 2.40 ± 0.55 on day 8). The Kruskal–Wallis test indicated a significant overall difference between groups ($p = 0.009$), and significant pairwise differences were observed on day 6 ($p = 0.045$) and day 8 ($p = 0.031$).

Lymphocyte infiltration

Topical application of calcitriol gel to traumatic oral ulcers affected lymphocyte infiltration to the mucosal tissue (Figures 3 and 8). Lymphocyte counts were consistently higher in the calcitriol-treated group (2.40 ± 0.55 on day 4; 3.40 ± 0.55 on day 6; and 3.80 ± 0.84 on day 8) than the control group (1.75 ± 0.50 on day 4; 2.20 ± 0.84 on day 6; and 2.80 ± 0.45 on day 8). The Kruskal–Wallis test indicated a significant overall difference between groups ($p = 0.008$), and significant pairwise differences on day 6 ($p = 0.033$) and day 8 ($p = 0.021$) (Table 1).

Fibroblast counts

At all observation time points, mean fibroblast proliferation (Figures 4 and 8) was consistently higher in the calcitriol-treated group (30.60 ± 4.69 on day 4; 55.76 ± 23.25 on day 6; and 80.10 ± 25.62 on day 8) than the control group (27.10 ± 12.95 on day 4; 44.60 ± 14.80 on day 6; and 62.32 ± 11.86 on day 8). One-way ANOVA revealed a significant overall difference between groups ($p = 0.001$). However, Games–Howell post hoc analysis indicated no statistically significant pairwise differences between groups at the individual time points (Table 1).

Collagen density

Mean collagen density (Figures 5 and 8) was consistently higher in the calcitriol treated group (33.30 ± 2.77 on day 4;

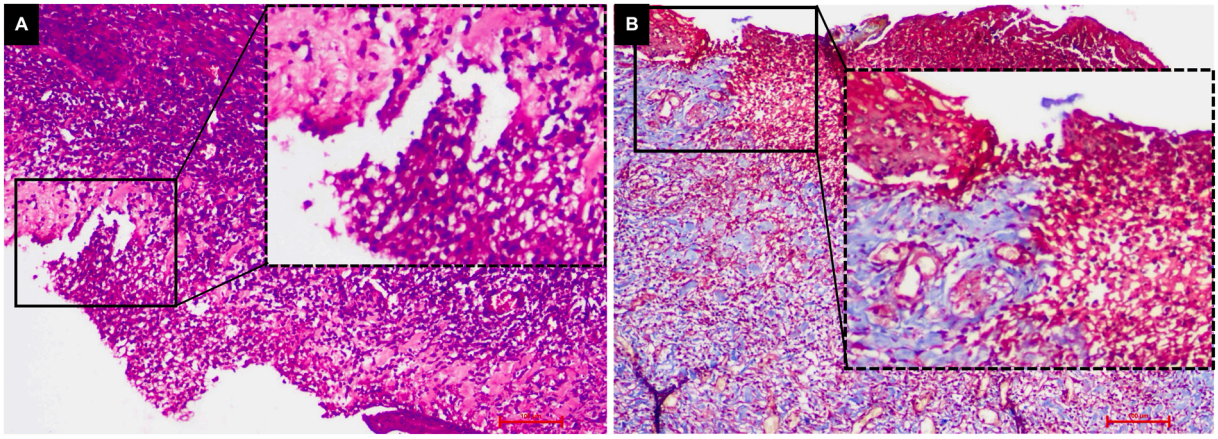


Figure 2: Histopathological features of the labial mucosa in *Rattus norvegicus* in the negative control group. (A, B) Representative hematoxylin and eosin (H&E)-stained sections of labial tissue showing characteristic features of ulceration at 400× magnification, including epithelial disruption and inflammatory cell infiltration. Insets provide higher-magnification views of the selected regions, highlighting cellular and structural changes within the ulcer area. Scale bars are shown in red.

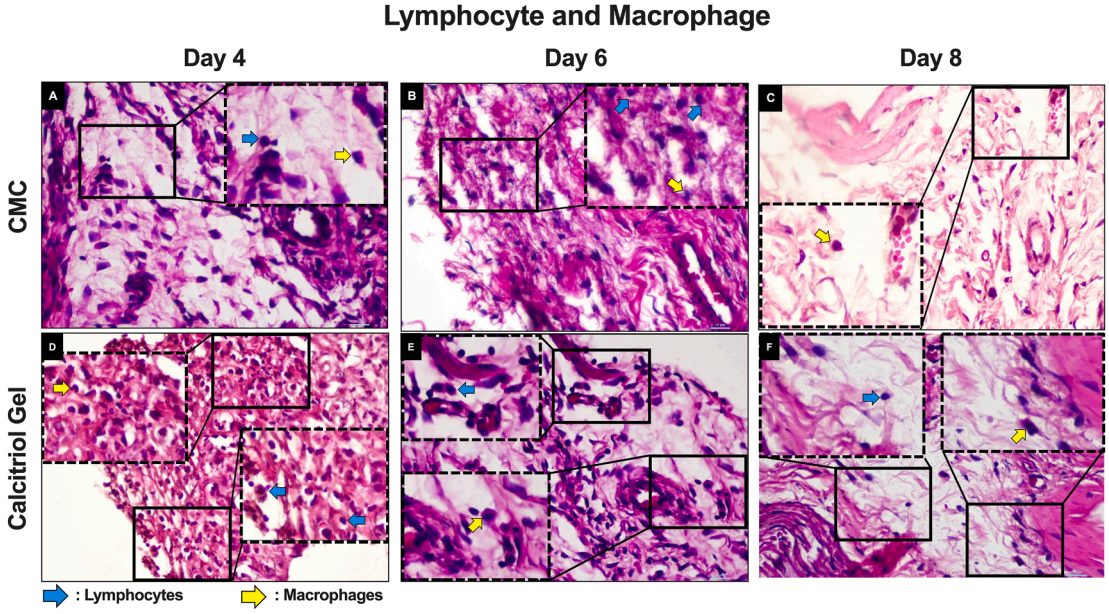


Figure 3: Histological sections of traumatic ulcers in the labial mucosa in *Rattus norvegicus,* stained with hematoxylin and eosin (H&E, 400× magnification). Macrophages are indicated by yellow arrows, whereas lymphocytes are indicated by blue arrows. Top row: CMC group specimens on day 4 (A), day 6 (B), and day 8 (C). Bottom row: calcitriol-CMC group specimens on day 4 (D), day 6 (E), and day 8 (F). Insets provide higher-magnification views of the selected regions. Scale bars are shown in blue.

39.44 ± 8.90 on day 6; and 50.41 ± 13.15 on day 8) than the control group (19.66 ± 4.55 on day 4; 33.23 ± 12.68 on day 6; and 43.45 ± 8.72 on day 8). One-way ANOVA showed significant overall differences between groups ($p < 0.001$). Tukey's post-hoc test revealed no significant pairwise differences between groups on the same day, except within each treatment group between days 4 and 8 ($p = 0.041$) (Table 1).

Blood vessel counts

Blood vessel counts (Figures 6 and 8) were higher in the calcitriol-treated group (2.60 ± 0.55 on day 4; 3.80 ± 0.84 on day 6; and 4.20 ± 0.84 on day 8) than the control group (2.25 ± 0.50 on day 4; 2.40 ± 0.55 on day 6; and 3.20 ± 0.84 on day 8). The Kruskal–Wallis test showed a significant overall difference between groups ($p = 0.007$), and Mann–Whitney post hoc analysis confirmed a significant difference on day 6 ($p = 0.021$) (Table 1).

VEGF expression

Mean VEGF expression (Figures 7 and 8) was higher in the calcitriol-treated group (3.40 ± 0.55 on day 4; 4.89 ± 1.00 on day 6; and 5.20 ± 1.00 on day 8) than the control group (2.75 ± 0.25 on day 4; 3.00 ± 0.45 on day 6; and 4.00 ± 0.45 on day 8). The Kruskal–Wallis test indicated

Table 1: Summary of multiple comparison analysis of wound healing parameters.

Parameter	Time (Day)	Group	n	Mean ± SD	Pairwise Test	p-value
Macrophages	4	Control	5	1.25 ± 0.50	Mann–Whitney	0.023
		Calcitriol gel	5	3.00 ± 1.00		
	6	Control	5	1.80 ± 0.45	Mann–Whitney	0.045
		Calcitriol gel	5	3.00 ± 1.00		
	8	Control	5	2.40 ± 0.55	Mann–Whitney	0.031
		Calcitriol gel	5	3.40 ± 0.55		
Lymphocytes	4	Control	5	1.75 ± 0.50	Mann–Whitney	0.107
		Calcitriol gel	5	2.40 ± 0.55		
	6	Control	5	2.20 ± 0.84	Mann–Whitney	0.033
		Calcitriol gel	5	3.40 ± 0.55		
	8	Control	5	2.80 ± 0.45	Mann–Whitney	0.045
		Calcitriol gel	5	3.80 ± 0.84		
Fibroblasts	4	Control	5	27.10 ± 12.95	Games–Howell	0.992
		Calcitriol gel	5	30.60 ± 4.69		
	6	Control	5	44.60 ± 14.80	Games–Howell	0.933
		Calcitriol	5	55.76 ± 23.25		
	8	Control	5	62.32 ± 11.86	Games–Howell	0.784
		Calcitriol gel	5	80.10 ± 25.62		
Collagen	4	Control	5	19.66 ± 4.55	Tukey HSD	0.150
		Calcitriol gel	5	33.30 ± 2.77		
	6	Control	5	33.23 ± 12.68	Tukey HSD	0.814
		Calcitriol	5	39.44 ± 8.90		
	8	Control	5	43.45 ± 8.72	Tukey HSD	0.778
		Calcitriol gel	5	50.41 ± 3.15		
Blood vessels	4	Control	5	2.25 ± 0.50	Mann–Whitney	0.322
		Calcitriol gel	5	2.60 ± 0.55		
	6	Control	5	2.40 ± 0.55	Mann–Whitney	0.021
		Calcitriol	5	4.20 ± 0.84		
	8	Control	5	3.20 ± 0.84	Mann–Whitney	0.100
		Calcitriol	5	3.10 ± 0.98		
VEGF	4	Control	5	2.75 ± 0.50	Mann–Whitney	0.107
		Calcitriol gel	5	3.40 ± 0.55		
	6	Control	5	3.00 ± 1.00	Mann–Whitney	0.023
		Calcitriol	5	5.20 ± 0.84		
	8	Control	5	4.00 ± 1.00	Mann–Whitney	0.082
		Calcitriol	5	3.89 ± 1.17		

a significant overall difference between groups, and Mann–Whitney post hoc analysis demonstrated a significant difference on day 6 ($p = 0.023$) (Table 1).

Discussion

Topical application of calcitriol gel enhanced key cellular and molecular events involved in the healing of traumatic oral ulcers, as evidenced by increased inflammatory cell infiltration, fibroblast proliferation, collagen deposition, angiogenesis, and VEGF expression across multiple healing phases. Traumatic ulcers in the oral cavity pose substantial clinical challenges because of their anatomical location, and the exposure of the tissue to repeated mechanical irritation and microbes. These aspects potentially prolong the healing process and exacerbate pain and functional impairment. Recent advances in topical therapeutics have highlighted the potential of calcitriol, the active form of vitamin D_3, as a promising agent in modulating wound healing responses, particularly for treating inflammatory and autoimmune skin disorders, including recurrent aphthous ulcers.[43]

Calcitriol acts primarily by directly binding the VDR, and subsequently orchestrating inflammation and diverse cellular processes including immune regulation, angiogenesis, and tissue remodeling. This investigation of the histological and immunohistochemical changes in traumatic ulcer healing after topical calcitriol gel application demonstrated an enhanced inflammatory cell response across multiple healing phases. The concentration of calcitriol (0.0003%) in this study was selected according to previous literature investigating similar materials and applications[34] and therefore is suitable for topical use. The absence of histological signs of local irritation or an adverse tissue response suggested that the tested concentration was well tolerated. However, because only a single concentration was evaluated, the optimal therapeutic range could not be determined.

Histological sections stained with H&E staining at 400× magnification revealed denser and more widespread macrophage infiltration in calcitriol-treated than control ulcers across all time points (Figure 3). This finding aligns with the critical roles of macrophages in wound healing, particularly during the acute inflammatory response and early proliferative phases. Macrophages facilitate phagocytosis, cytokine production, and growth factor release, thereby clearing apoptotic cells and debris, and initiating tissue repair. The observed elevation, particularly on day 8,

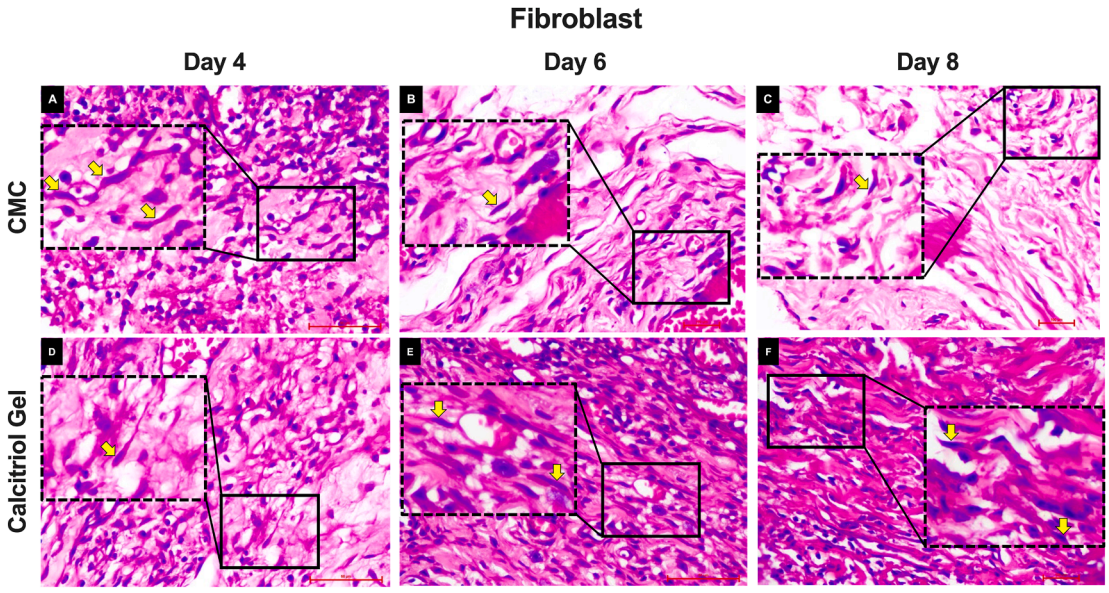


Figure 4: Histological sections of traumatic ulcers in the labial mucosa in *Rattus norvegicus*, stained with hematoxylin and eosin (H&E, 400× magnification). Fibroblasts are indicated by yellow arrows. Top row: CMC group specimens on day 4 (A), day 6 (B), and day 8 (C). Bottom row: calcitriol-CMC group specimens on day 4 (D), day 6 (E), and day 8 (F). Insets provide higher-magnification views of the selected regions. Scale bars are shown in red.

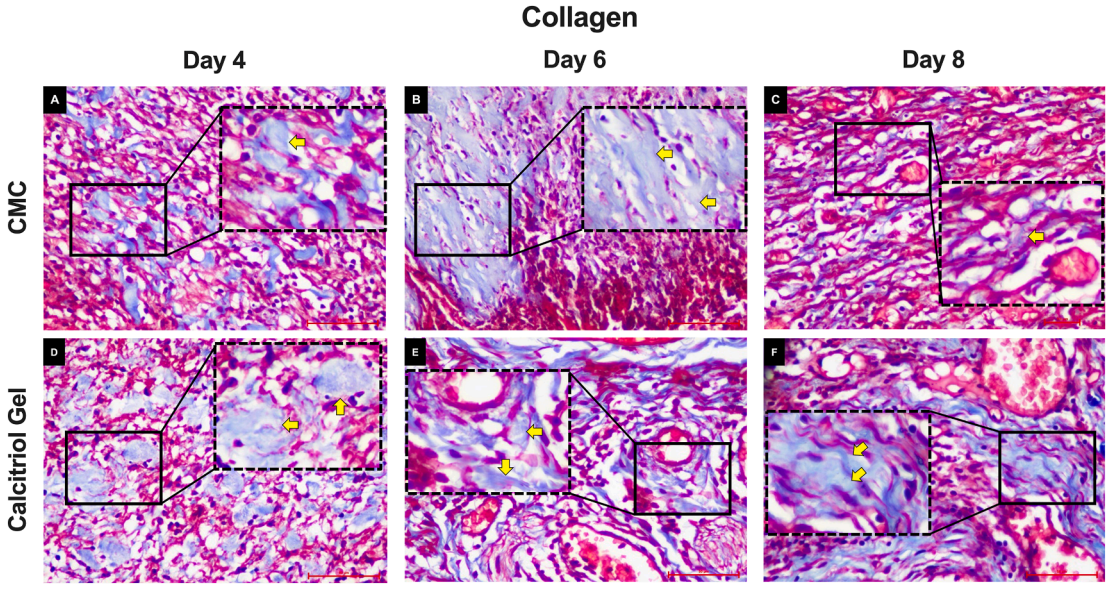


Figure 5: Histological sections of traumatic ulcers in the labial mucosa in *Rattus norvegicus*, stained with Masson's trichrome staining, 400× magnification. Collagen depositions are indicated by arrows. Top row: CMC group specimens on day 4 (A), day 6 (B), and day 8 (C). Bottom row: calcitriol-CMC group specimens on day 4 (D), day 6 (E), and day 8 (F). Insets provide higher-magnification views of the selected regions. Scale bars are shown in red.

suggested enhanced immunomodulation and a potential shift toward M2 macrophage polarization in the repair phase.

Calcitriol's interaction with VDR is known to suppress pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α, while promoting anti-inflammatory mediators including IL-10 and TGF-β, thus forming a microenvironment conducive to resolution and regeneration.[2,3,21] This immunomodulatory effect was further supported by the denser lymphocyte infiltration observed in the calcitriol-treated group than the control group (Figure 3), thus indicating active adaptive immune engagement. Lymphocytes orchestrate cytokine signaling and matrix remodeling, and calcitriol has been shown to modulate T-cell proliferation and differentiation via VDR-mediated pathways. Moreover, calcitriol enhances the synthesis of LL-37, an antimicrobial peptide that supports epithelial regeneration,[19] thus contributing to a balanced inflammation process and improved immune cell recruitment.

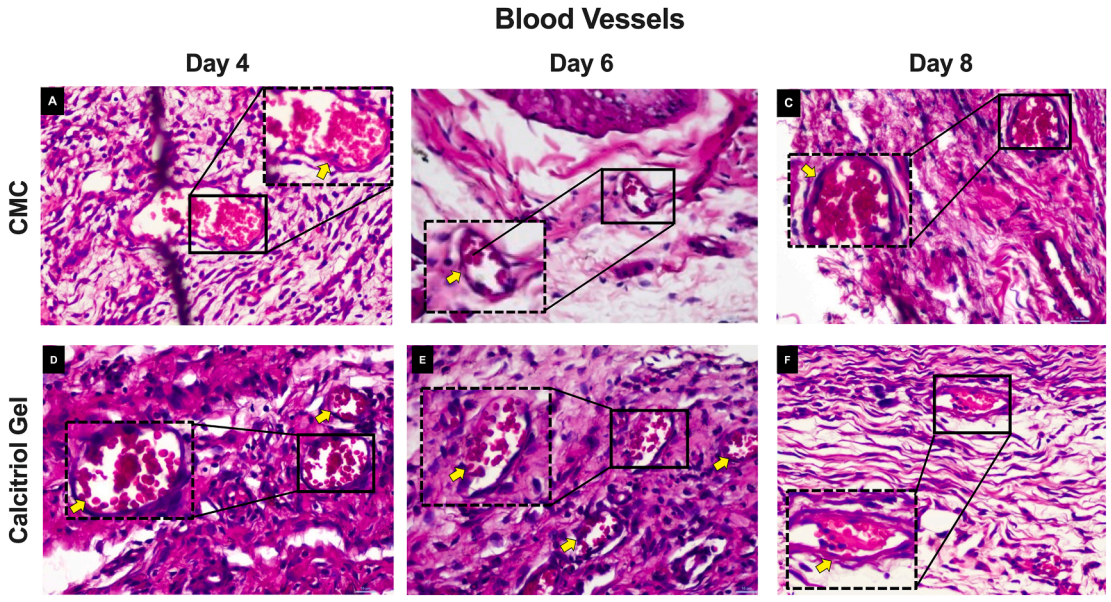


Figure 6: Histological sections of traumatic ulcers in the labial mucosa in *Rattus norvegicus*, stained with hematoxylin and eosin (H&E, 400× magnification). Blood vessels are indicated by arrows. Top row: CMC group specimens on day 4 (A), day 6 (B), and day 8 (C). Bottom row: calcitriol-CMC group specimens on day 4 (D), day 6 (E), and day 8 (F). Insets provide higher-magnification views of the selected regions. Scale bars are shown in blue.

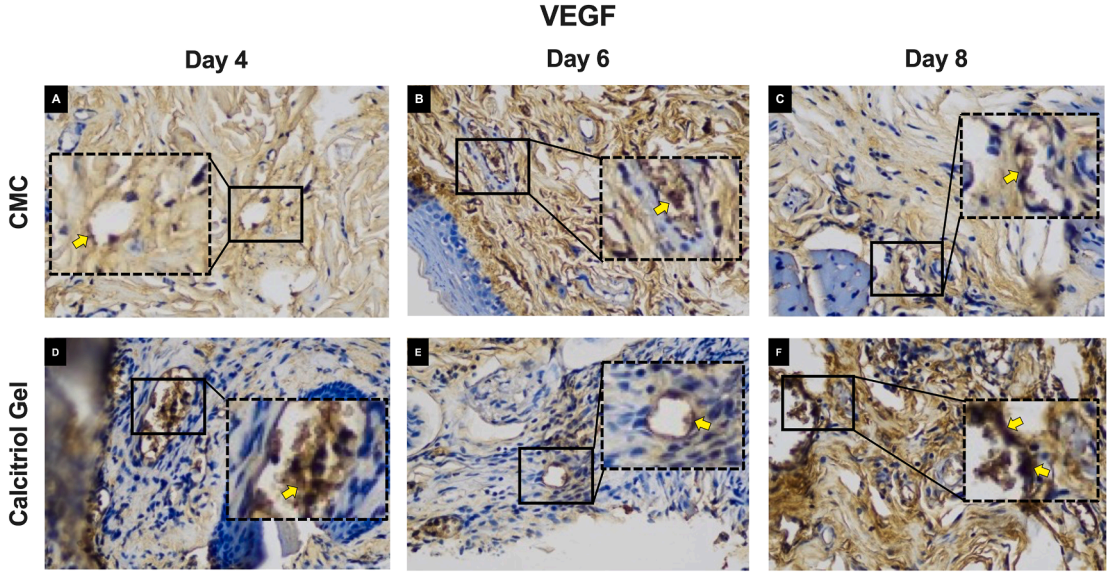


Figure 7: Histological sections of traumatic ulcers in the labial mucosa in *Rattus norvegicus*, stained with immunohistochemistry, 400× magnification. VEGF expression on endothelial cells is indicated by arrows. Top row: CMC group specimens on day 4 (A), day 6 (B), and day 8 (C). Bottom row: calcitriol-CMC group specimens on day 4 (D), day 6 (E), and day 8 (F). Insets provide higher-magnification views of the selected regions.

H&E-stained sections indicated that the spindle-shaped fibroblasts progressively increased in both groups over time but had visibly greater abundance in calcitriol-treated than control wounds (Figure 4). Fibroblasts are critical in the proliferative phase, and are responsible for synthesizing extracellular matrix components and replacing provisional fibrin with granulation tissue. Calcitriol is likely to enhance fibroblast activity by suppressing pro-inflammatory cytokines and promoting M2 macrophage-derived regenerative signals such as TGF-β.[44,45] In addition, increased LL-37 expression induced by calcitriol might stimulate fibroblast proliferation through downstream signaling cascades.[20]

Despite these promising mechanisms, the absence of statistically significant pairwise differences suggested that the dosing regimen or application frequency used in this study might not have been optimal, in agreement with evidence from other comparable mucosal models.[46] Future studies

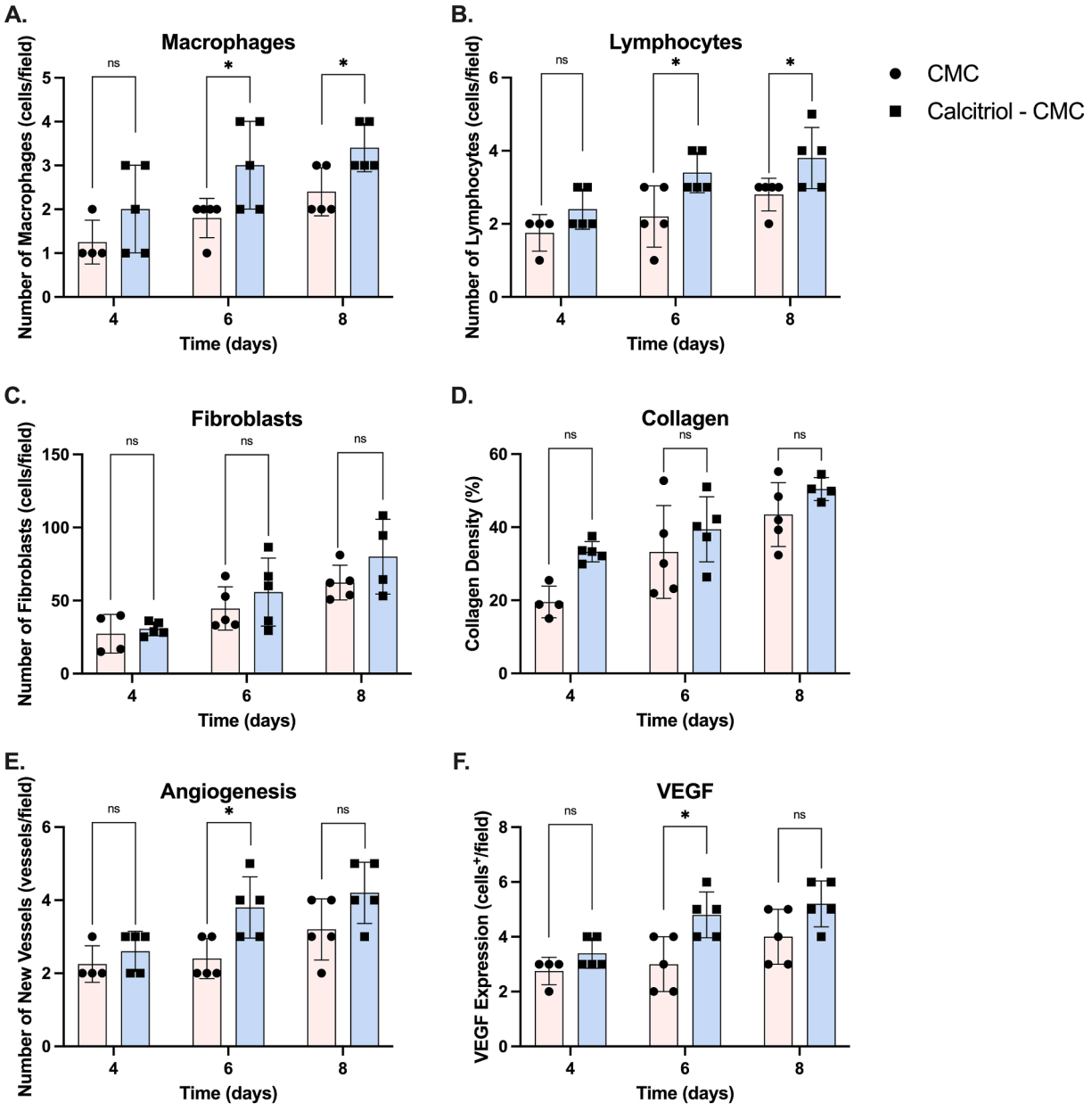


Figure 8: Bar graphs showing average (A) macrophage numbers, (B) lymphocyte numbers, (C) fibroblast numbers, (D) collagen density, (E) blood vessels, and (F) VEGF expression in the labial mucosa tissues in *Rattus norvegicus* after three, five, and seven days of treatment. The groups included a treatment group receiving topical calcitriol gel and a control group receiving CMC gel. Values in the calcitriol group were higher than those in the control group in all comparisons.

should explore dose-response relationships, the ideal dosing frequency, and sustained-release formulations to maximize therapeutic efficacy.

Masson's trichrome staining revealed denser and more organized collagen deposition in calcitriol-treated wounds than control wounds (Figure 5). Collagen is essential for structural integrity and tissue remodeling, and its synthesis is tightly regulated by fibroblast activity. Calcitriol not only promotes fibroblast function but also upregulates tissue inhibitors of metalloproteinases (TIMP-1) and inhibits matrix metalloproteinases, which degrade extracellular matrix components. Calcitriol indirectly stabilizes collagen accumulation by regulating the extracellular matrix.[47] Calcitriol has been reported to decrease MMP-1 and MMP-10 expression, thus further supporting extracellular matrix preservation.[3] Enhanced TGF-β induction also contributes to fibroblast differentiation and collagen synthesis,[21] and consequently facilitates the transition from inflammation to remodeling.

In calcitriol-treated ulcers, compared with control ulcers, H&E-stained sections at 400× magnification revealed more numerous and well-organized capillaries by day 6 and the presence of mature lumen walls at day 8 (Figure 6). This pattern corresponded to the angiogenesis timeline, which typically peaks between days 5–7 during the proliferative phase.[48] Adequate vascularization is essential for nutrient

delivery, waste removal, immune cell trafficking, and epithelial regeneration. Calcitriol might therefore support angiogenic processes and neovascularization during traumatic oral ulcer healing.

Immunohistochemical staining further confirmed enhanced VEGF expression in endothelial cells of calcitriol-treated ulcers, with peak levels on day 6 (Figure 7). Calcitriol has been reported to influence VEGF expression through VDR-mediated signaling in endothelial and immune cells, in a process potentially involving PI3K/Akt and MAPK/ERK pathways.[22] Moreover, calcitriol has been suggested to promote M2 macrophage polarization, which in turn might contribute to paracrine VEGF release and angiogenic activity. Although we did not directly investigate the underlying molecular pathways, the observed increase in VEGF expression suggested that topical calcitriol might support angiogenic processes during oral ulcer healing. VEGF expression was quantified through blinded cell counting and statistical analysis; however, because immunohistochemistry is a semi-quantitative method, additional approaches such as densitometric analysis or western blotting would provide more objective validation in future studies.

This study has several limitations. First, the experimental design included evaluation of only a single calcitriol concentration and application frequency, which might not have been the optimal therapeutic regimen. Because inter-rater reliability for manual cell counting was also not formally assessed, inter-observer variability might have influenced the findings. Future studies incorporating dose–response analysis and cytotoxicity evaluation are warranted to define the optimal and safe concentration range for clinical application.

In addition, molecular signaling pathways were inferred from histological and immunohistochemical findings rather than directly quantified. Topical calcitriol treatment was associated with increased inflammatory cell infiltration in the early healing phase, enhanced fibroblast activity, greater collagen deposition, and elevated VEGF expression. Therefore, it might have roles in regulating inflammatory, proliferative, and angiogenic processes during mucosal wound healing. However, because macrophage phenotypes were not specifically characterized, any interpretations regarding polarization remain speculative. Moreover, the precise molecular mechanisms underlying these effects cannot be definitively established from our findings. Future studies using molecular approaches such as RNA sequencing, quantitative PCR, or western blotting are warranted to further elucidate the gene and protein expression profiles involved in calcitriol-mediated immune modulation, macrophage polarization, extracellular matrix remodeling, and angiogenic signaling.

In this study, calcitriol was incorporated into a 3% CMC gel base. The 3% concentration was selected according to previous publications and preliminary formulation studies demonstrating suitable viscosity and stability for topical application.[34] Although the mucoadhesive properties were not directly evaluated in this study, the CMC-based gel probably facilitated localized delivery of calcitriol at the wound site. Future studies should further investigate the mucoadhesive performance and residence time of calcitriol formulations in the oral cavity.

Conclusion

Topical calcitriol gel has potential in promoting the healing of traumatic oral ulcers by influencing key processes involved in wound repair, including inflammatory regulation, tissue regeneration, and angiogenesis. In this study, calcitriol treatment was associated with increased macrophage and lymphocyte activity, enhanced fibroblast proliferation, improved collagen organization, and elevated VEGF expression, thereby suggesting enhanced organization of the healing response. These effects might have been associated with mechanisms involved in immune regulation and tissue remodeling; however, the underlying molecular pathways, including potential involvement of VDR signaling and macrophage polarization, were not directly evaluated in this study. Further studies are needed to optimize formulation parameters and dosing strategies, and to elucidate the molecular mechanisms underlying these effects.

Authors contributions

Conceptualization: DR, NFA, DSE, RKB. Data Curation: RKB, ARF, ZKR, MAD. Formal Analysis: RKB, NTF, ARF, ZKR, MAD. Funding Acquisition: DR. Investigation: RKB, NTF, ARF, ZKR, MAD. Methodology: DR, DSE, NFA, FYM, AEP, ASP. Project Administration: RKB, DR, AS. Supervision: DR, NFA, FYM, AEP. Validation: DSE. Visualization: RKB. Writing—Original draft preparation: RKB. Writing—Review & Editing: AS. All authors have reviewed and approved the final manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical approval

This study was ethically approved by the Health Research Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga (997/HRECC.FODM/VIII/2023, 973/HRECC.FODM/VIII/2023, and 1430/HRECC.-FODM/XII/2023).

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