



ORIGINAL RESEARCH

EFFECT OF PHYTOCOMPOSITION ON MUCOSAL REGENERATION IN EXPERIMENTAL PROSTHETIC STOMATITIS

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Abstract

The rapid and uneven atrophy of soft tissues and bone structures associated with the use of removable dentures, combined with local inflammatory processes, can damage the neurovascular bundles in the maxillofacial region. This leads to the formation of painful and bleeding wound surfaces on the oral mucosa, which compromise local mucosal defenses and act as portals for infection. For this purpose, solutions derived from plant materials have long been used for disinfection.

An experimental study was performed on 60 sexually mature male Wistar rats, aged 8-9 months, with a mean body weight of 350 ± 54 g. Atrophic stomatitis was induced using our proprietary model. A phytosolution—comprising aloe hydrolat and juniper essential oil—was applied topically to the affected oral mucosa. The animals were divided into groups based on the duration of phosphoric acid exposure on the oral mucosa (30 seconds and 90 seconds).

The resulting mucosal alterations were characterized by edema, vasodilation, and lymphocytic infiltration of the lamina propria. Topical application of aloe hydrolat was found to reduce edema and inflammation, whereas juniper essential oil promoted accelerated re-epithelialization of the mucosal lesions.

Keywords: regeneration, experiment, phytochemistry, denture stomatitis

INTRODUCTION

The increase in average life expectancy has led to a growing number of individuals living with partial or complete edentulism. As many patients are unsuitable for or cannot afford implant-supported prostheses, they rely on conventional removable dentures¹. Atrophy of the oral mucosa is a common complication associated with such prosthetics. The accelerated and uneven resorption of the alveolar bone and overlying soft tissues, coupled with chronic local inflammation, can compromise neurovascular bundles in the maxillofacial region. This may subsequently necessitate complex secondary surgical procedures and hinder patient

rehabilitation².

Approximately 30% of removable denture wearers report symptoms including xerostomia (dry mouth), a burning sensation, pruritus, dysgeusia (altered taste), and difficulties with phonation and swallowing dry foods—collectively termed xerostomia syndrome. A characteristic feature is a compensatory increase in fluid intake, particularly during meals, highlighting the clinical need for interventions to maintain oral hydration. For centuries, daily oral rinsing with warm aqueous extracts has been a common practice for disinfection. These include decoctions and infusions prepared from botanicals such as oak bark, sage leaves, chamomile and

marigold flowers, St. John's wort, and string, among others.^{3,4,5}

The development of mucositis, presenting as erosive-ulcerative lesions, results in painful wound surfaces on the oral mucosa. These lesions cause pain, bleeding, and inflammation, impair the local mucosal barrier function, and create portals for secondary infection. Patients suffering from persistent mucositis with severely delayed epithelialization experience significant impairments in speech, mastication, and deglutition. This can lead to psychological sequelae and a spectrum of social dysfunctions indicative of a markedly diminished quality of life. Affected individuals often exhibit decreased self-confidence, social withdrawal, impaired interpersonal communication, and an inability to maintain daily activities, resulting in poor social adaptation^{3,5,6}.

Consequently, there is a pressing need to identify novel, effective, and safe therapeutic agents. In this context, phytotherapeutic preparations represent a highly promising avenue of research.

MATERIAL AND METHODS.

The experimental study was conducted at the Central Research Laboratory of the "Genetic and Cellular Biotechnologies" Engineering Center, Medical Institute named after S.I. Georgievsky, V.I. Vernadsky Crimean Federal University. The study was approved by the Ethics Committee of the Medical Institute named after S.I. Georgievsky, V.I. Vernadsky Crimean Federal University (protocol No. 2 dated 02.02.2026).

The study was performed using 60 healthy, sexually mature white male Wistar rats aged 8-9 months, with a body weight of 350±54 g and no external signs of disease. The animals underwent a quarantine period in the vivarium and were maintained on a standard diet. All procedures involving the experimental animals (white rats) were performed in accordance with the requirements of the Russian Federation Federal Law No. 4979-1 dated 14.05.1993 "On Veterinary Medicine" (as amended on 02.07.2021).

To experimentally assess the combined use of a phytosolution for the treatment and prevention of denture-related stomatitis (K12.12), a mixture of an antiseptic agent (aloe hydrolat) and a natural-based preparation (juniper essential oil) was applied to the affected area of the oral mucosa. Aloe Hydrolat is a product derived from Aloe vera (*Aloe arborescens* Mill., asphodel seeds *Asphodelaceae*), obtained by steam distillation at the production facility of Federal State Scientific Budgetary Institution of Science "Nikitsky Botanical Garden - National Scientific Center" (FSBI "NBS-NNC") using the "Alpha-Ether" apparatus

designed for processing essential oil-bearing plant materials (herbal, floral, grain, root types) via steam distillation⁷.

Juniper essential oil was obtained by steam distillation of *Juniperus communis* L. It is a light-yellow liquid rich in monoterpenes, including α -pinene, β -pinene, 3-carene, and β -phellandrene⁸.

Experimental Model Chemical mucositis was induced by applying a 35% orthophosphoric acid solution to the maxillary mucosa in the region of the transitional fold corresponding to teeth 3–4². Animals were divided into eight groups (n = 3 per group) according to exposure time and treatment regimen:

1. Control 1: 30 s exposure, no treatment
2. Control 2: 90 s exposure, no treatment
3. Experimental 1.1: 30 s exposure + juniper oil
4. Experimental 1.2: 30 s exposure + aloe hydrolat
5. Experimental 1.3: 30 s exposure + mixture
6. Experimental 2.1: 90 s exposure + juniper oil
7. Experimental 2.2: 90 s exposure + aloe hydrolat
8. Experimental 2.3: 90 s exposure + mixture

After exposure, acid residues were rinsed off with distilled water.

Histological and Morphometric Analysis

Biomaterial sampling was performed under general inhalation anesthesia (isoflurane). Tissue samples were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin. Microscopic evaluation was performed using a Leica DM2000 microscope. Morphometric analysis was conducted using ImageJ software. Measurements included lesion area, epithelial layer thickness, lamina propria thickness, vascular area, and lymphocyte count. Statistical analysis was performed using MS Excel; the Mann–Whitney U test was applied with significance set at $p < 0.05$.

RESULTS

The mucosa of the lips and gingiva in healthy rats is represented by stratified squamous keratinized epithelium, consisting of four layers: basal, spinous, granular, and corneal. The lamina propria of the mucosa forms high papillae and is based on dense irregular connective tissue rich in collagen fibers and smooth myocytes. Capillaries and solitary lymphoid cells are located between the fibers (Fig. 1a).

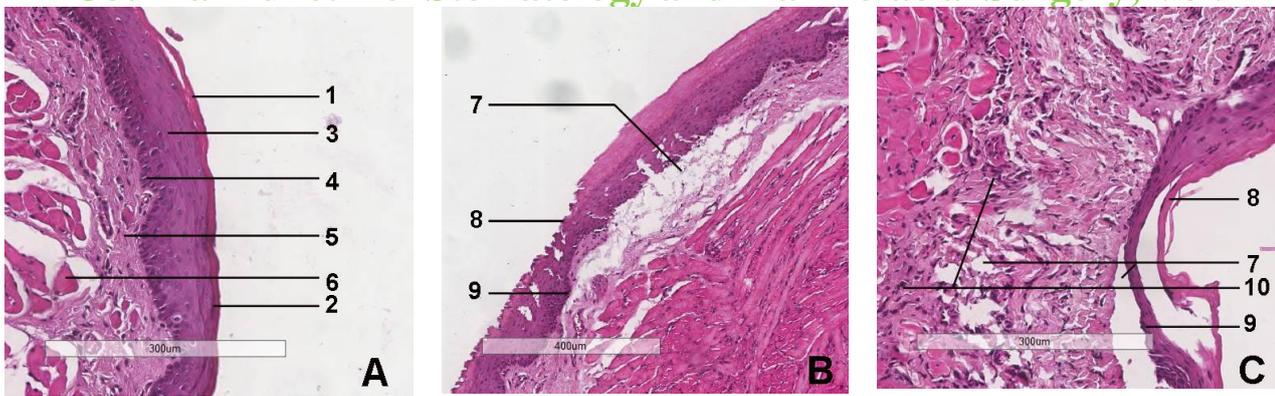


Figure 1. Oral mucosa of control and experimental rats. A – Healthy mucosa. B – Mucosa following a 30-second orthophosphoric acid burn. C – Mucosa following a 90-second acid burn.

1 – keratinized layer (stratum corneum), 2 – granular layer (stratum granulosum), 3 – spinous layer (stratum spinosum), 4 – basal (germinative) layer (stratum basale), 5 – lamina propria of the mucosa, 6 – smooth muscle fibers, 7 – edema of the lamina propria, 8 – desquamation of the keratinized layer, 9 – degeneration of the germinative layer, 10 – lymphoid infiltration.

Following a 30-second exposure to OPA, degeneration of keratinocytes, compaction of the spinous cell layers, disappearance of their keratohyalin granules, clumping of cells in the keratinized layer, and its desquamation were observed. The lamina propria was dehydrated; collagen fibers were compacted and acquired a basophilic stain, with abundant lympholeukocytic infiltration present (Fig. 1b, Table 1).

Table 1. Morphometric parameters of the mucosa in experimental animals after a 30-second exposure to orthophosphoric acid (Me(Q1-Q3))

| Group | 1 | 1.1 | 1.2 | 1.3 |
|--|---------------------------|----------------------------|--------------------------|---------------------------|
| Area of damage, mm ² | 0.54 (0.51-0.73) | 0.06 * (0.04-0.08) | 0.36 * (0.28-0.44) | 0.19 * (0.18-0.19) |
| Thickness of the keratinized layer, µm | 0 (0-0) | 9.27* (7.38-10.10) | 9.45* (8.26-10.29) | 13.03* (9.90-15.77) |
| Thickness of the spinous layer, µm | 51.71 (47.35-65.62) | 51.77 (47.22-62.98) | 23.46 * (22.23-25.05) | 14.74* (12.61-15.19) |
| Thickness of the basal layer, µm | 16.01 (12.19-20.05) | 8.53* (7.78-11.44) | 5.11* (3.29-6.15) | 4.14* (3.00-4.72) |
| Thickness of the lamina propria, µm | 147.21 (137.53-196.36) | 122.78* (115.45-140.21) | 72.72* (64.44-81.34) | 145.13 (135.21-168.57) |
| Relative area of the edema, % | 28.00 (20.02-28.09) | 13.00 * (10.84-18.60) | 6.25* (3.80-7.69) | 17.39* (15.73-17.72) |
| Relative area of the blood vessels, % | 8.16 (5.62-10.16) | 2.41* (2.00-4.44) | 1.12* (1.10-1.27) | 3.78* (3.26-4.60) |
| Number of lymphocytes per HPF | 27.00 (25.25-27.75) | 35.50 * (33.25-38.25) | 17.50 * (15.25-22.75) | 23.00 (21.25-32.75) |

* Differences from the control group are significant at p<0.05.

Following a 90-second exposure to OPA, more severe damage to the mucosal lining was observed. Specifically, over a significant area, degeneration of cells in all epithelial layers was present, with areas of complete desquamation and foci of necrosis in the lamina propria. The orientation and structure of collagen fibers were altered, with a large number of various leukocytic infiltrations between them. Areas adjacent to the damaged site were hyperemic and edematous (Fig. 1c, Table 2).

Table 2. Morphometric parameters of the mucosa in experimental animals after a 90-second exposure to orthophosphoric acid (Me(Q1-Q3))

| Group | 2 | 2.1 | 2.2 | 2.3 |
|--|------------------------|----------------------------|----------------------------|--------------------------|
| Area of damage, mm ² | 0.92 (0.76-0.93) | 0.23 (0.19-0.27) | 0.07* (0.07-0.09) | 0.20 (0.20-0.20) |
| Thickness of the keratinized layer, μm | 2.41 (1.92-4.70) | 1.16 (0.51-2.68) | 9.67* (7.86-11.17) | 1.97 (0.67-4.23) |
| Thickness of the spinous layer, μm | 8.39 (6.99-9.64) | 32.70* (24.00-38.81) | 35.25* (33.30-42.65) | 47.88* (41.02-63.04) |
| Thickness of the basal layer, μm | 6.45 (5.33-7.89) | 21.10 * (18.82-22.98) | 17.25* (13.84-20.72) | 18.23 * (15.82-19.05) |
| Thickness of the lamina propria, μm | 87.19 (81.62-97.13) | 239.10* (225.96-276.11) | 181.01* (175.84-197.59) | 91.29 (80.11-129.55) |
| Relative area of the edema, % | 8.54 (7.14-8.60) | 14.86 * (13.10-21.15) | 7.10 (6.02-7.25) | 17.81* (11.94-18.87) |
| Relative area of the blood vessels, % | 2.04 (2.04-2.78) | 5.00 (5.20-6.76) | 3.0 (1.15-4.10) | 7.46* (5.66-7.55) |
| Number of lymphocytes per HPF | 28.5 (23.5-42.5) | 39.00* (34.50-45.25) | 35.50 (30.00-39.25) | 11.50* (10.00-12.75) |

* Differences from the control group are significant at p<0.05.

The application of aloe, essential oil, and their mixture in cases of 30-second OPA exposures had a positive effect on the condition of the oral mucosa. Under the influence of aloe, the layer of basal cells completely recovered, and all other epithelial layers proliferated, displacing the damaged cells (Fig. 2a). Edema in the lamina propria decreased. The use of the essential oil also resulted in mucosal regeneration; however, cells in the spinous layer were characterized by the presence of a rounded vacuole surrounding the nucleus (Fig. 2b). Upon application of the mixture of the two preparations, no differences from healthy mucosa were identified.

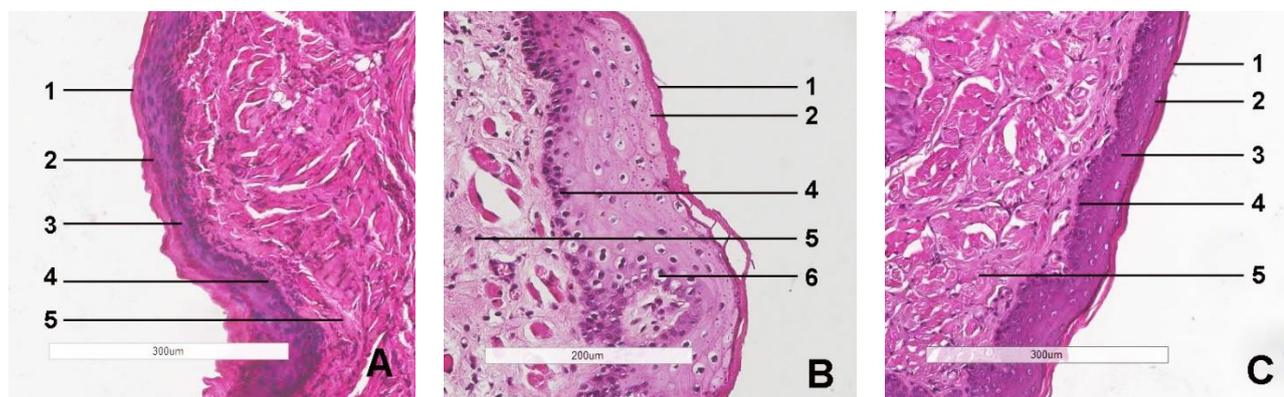


Figure 2. Oral mucosa of rats following a 30-second burn and therapeutic application. A – Application with aloe hydrolyt. B – Application with juniper oil. C – Application with a mixture of hydrolyt and oil. 1 – keratinized layer (stratum corneum), 2 – granular layer (stratum granulosum), 3 – spinous layer (stratum spinosum), 4 – basal (germinative) layer (stratum basale), 5 – lamina propria of the mucosa, 6 – vacuolated cells of the spinous layer.

The regeneration after the 90-second OPA exposures was more challenging due to the greater severity and area of damage. The application of the mixture had almost no effect. Epithelial defects, edema, and necrosis of the lamina propria persisted. The application of the oil resulted in more successful regeneration; however, cells in the spinous layer also exhibited vacuoles around their nuclei, and the necrotic masses at the injury site remained, with the epithelium retaining a degenerative appearance.

The application of aloe exerted the most favorable effect, reducing edema in the lamina propria and accelerating the regeneration of the epithelial germinative layer.

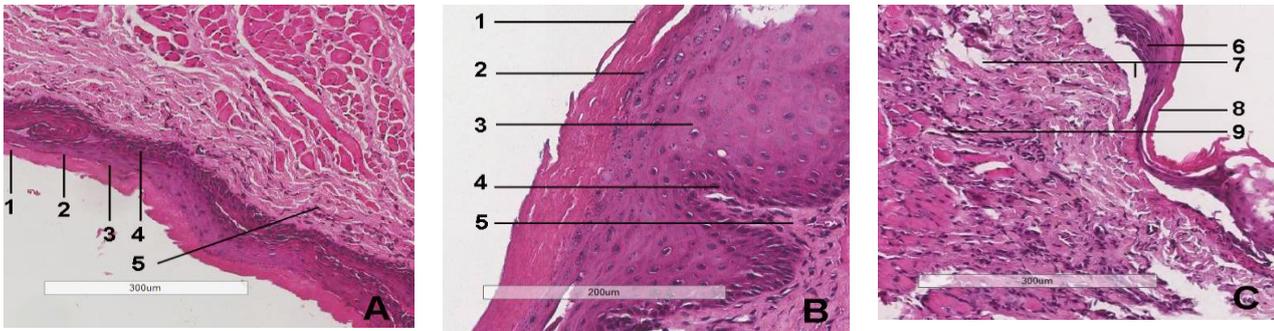


Figure 3. Oral mucosa of rats following a 90-second burn and therapeutic applications. A – Applications with aloe hydrolat. B – Applications with juniper oil. C – Applications with a mixture of hydrolat and oil. 1 – keratinized layer (stratum corneum), 2 – granular layer (stratum granulosum), 3 – spinous layer (stratum spinosum), 4 – basal (germinative) layer (stratum basale), 5 – lamina propria of the mucosa, 6 – dystrophy of the basal and spinous epithelial layers, 7 – edema, 8 – desquamation of the keratinized layer, 9 – lymphoid infiltration of the lamina propria.

Correlation analysis revealed that the lesion area in the untreated control groups showed the strongest positive correlation with the initial chemical burn area ($r = 0.804, p < 0.05$), suggesting impaired intrinsic reparative capacity. In contrast, a moderate negative correlation was identified between the burn area and topical application of juniper essential oil alone ($r = -0.387, p < 0.05$), irrespective of exposure duration. Treatment with aloe hydrolat demonstrated a moderate positive correlation with the thickness of the stratum corneum ($r = 0.392, p < 0.05$), which may indicate promoted terminal differentiation (keratinization) of epithelial cells. No other treatment group exhibited a significant correlation with this parameter. A moderate positive correlation was observed between lamina propria thickness and the application of juniper essential oil ($r = 0.346, p < 0.05$), potentially indicative of a more pronounced fibrotic or sclerotic response. Aloe hydrolat application was associated with a strong negative correlation with the severity of tissue edema ($r = -0.555, p < 0.05$), confirming its potent anti-edematous and anti-inflammatory effect. Consistently, this group also showed the lowest level of reactive vascularization in the burn area ($r = -0.431, p < 0.05$).

Lymphocytic infiltration correlated positively with juniper essential oil monotherapy ($r = 0.448, p < 0.05$). Conversely, the combined treatment (oil + hydrolat) showed a moderate negative correlation with lymphocyte count ($r = -0.44, p < 0.05$). This disparity suggests that while juniper oil alone may exacerbate inflammation, potentially through cytolytic effects on damaged mucosa, its combination with aloe hydrolat mitigates this response, leading to reduced inflammatory intensity and subsequent connective tissue deposition. The thickness of the epithelial basal layer and the degree of lymphocytic infiltration emerged as the most dynamically regulated parameters across all groups, underscoring their pivotal role in mucosal regeneration.

Both lymphocyte count and basal layer thickness were significantly influenced by the duration of acid exposure. However, the lack of a strong correlation between these two parameters, coupled with the statistically non-significant effect of aloe hydrolat on inflammation during the proliferative phase ($p > 0.05$), indicates that this agent has limited efficacy in modulating the fibrotic cascade that leads to excessive connective tissue formation at the burn site (Fig. 4).

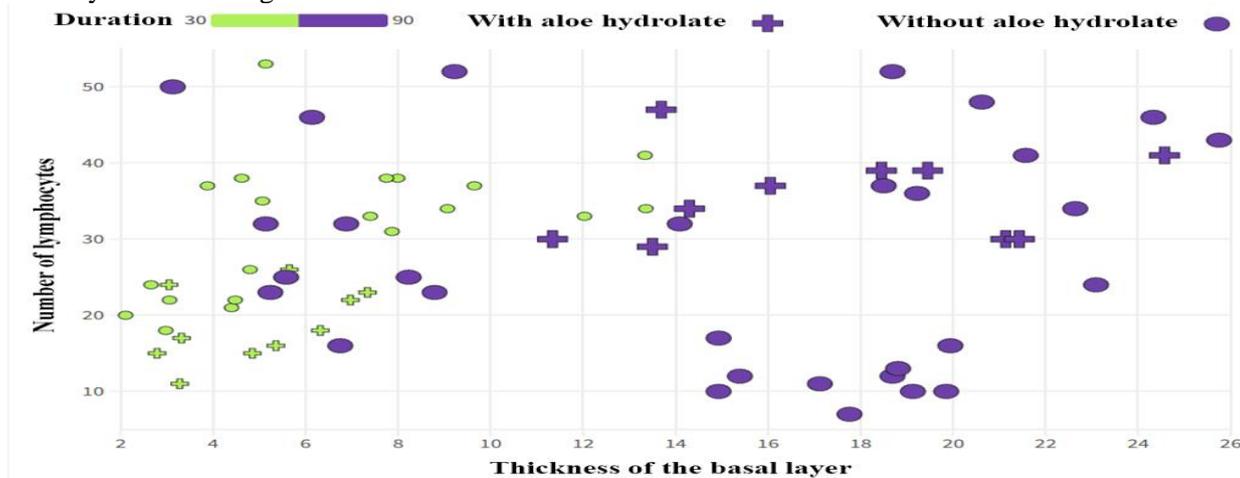


Figure 4. The relationship between the thickness of the basal layer and the number of lymphocytes against the background of aloe hydrolat application.

Both the lymphocyte number and the thickness of the basal layer were higher than in the group treated with aloe hydrolat, regardless of the exposure time to the acidic agent. This potentially indicates that the application of essential oil alone results in an intensified inflammatory process during the exudation phase, which consequently translates into the increased growth of connective tissue during the proliferation phase (Fig. 5).

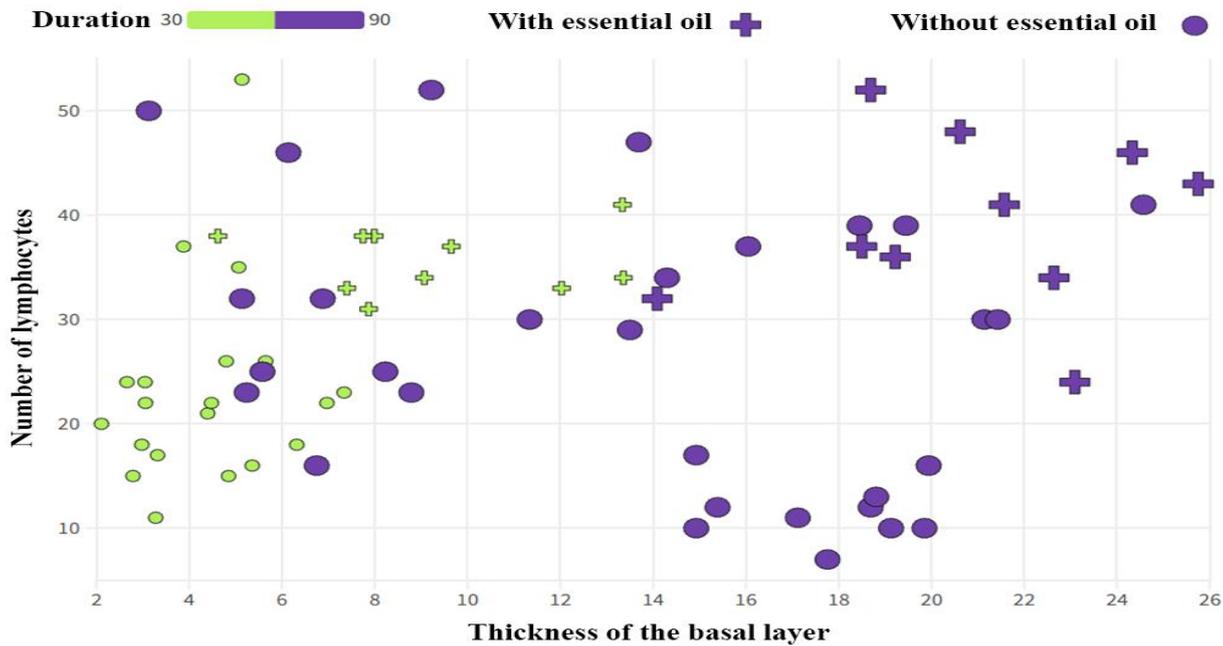


Figure 5. The relationship between the thickness of the basal layer and the number of lymphocytes against the background of essential oil application.

The values obtained in the group where the mixture of aloe hydrolat and essential oil was used indicate a lower intensity of the inflammatory process (based on lymphocyte number) compared to the separate application of essential oil, and a higher intensity compared to the application of aloe hydrolat alone. Similar trends are observed regarding the volume of connective tissue formed following the inflammatory process (Fig. 6).

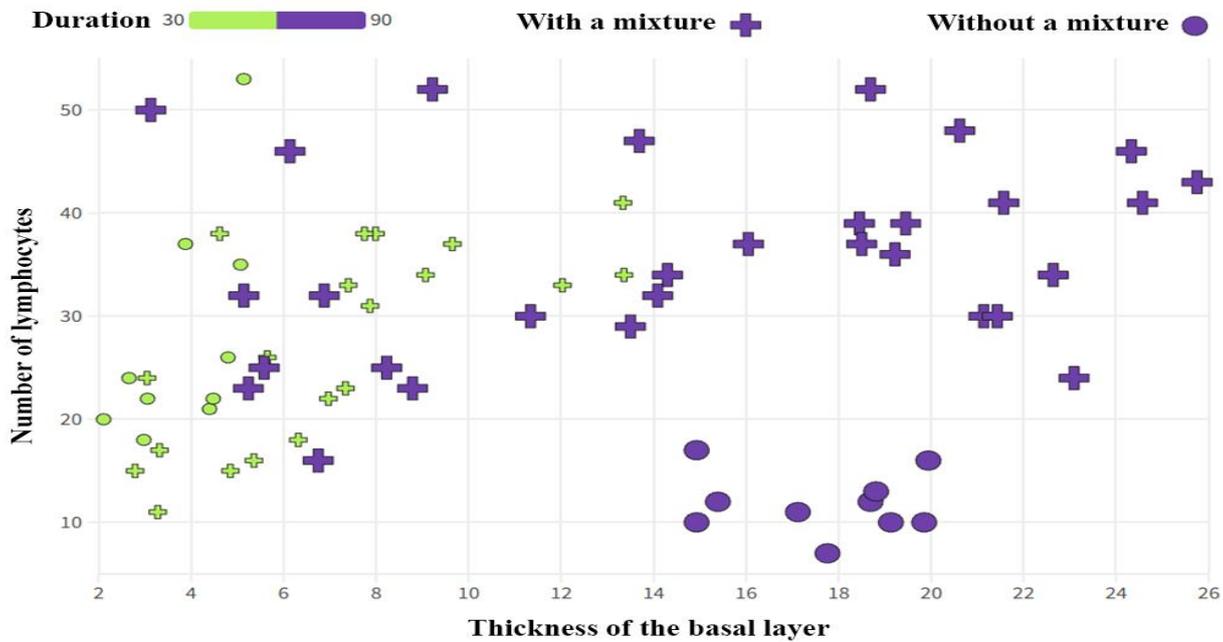


Figure 6. The relationship between the thickness of the basal layer and the number of lymphocytes against the background of the mixture of aloe hydrolat and essential oil application.

The therapeutic effect of plant-based preparations can be based on the presence of a large number of diverse biologically active substances within them. The component composition of the hydrolat is represented by volatile compounds: eucalyptol (0.03 mg/dm³), E-nerolidol (0.085 mg/dm³), α -bisabolol (0.085 mg/dm³), thymol (0.04 mg/dm³), α -bisabolon oxide A (0.09 mg/dm³), and isocalamendiol (0.045 mg/dm³). The hydrolat exerts an antimicrobial effect against strains of pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*⁷. Juniper oil has a local analgesic effect and stimulates reparative and regenerative processes due to its high content of α -pinene, β -pinene, 3-carene, and β -phellandrene⁸.

DISCUSSION

The mucosal alterations induced by OPA application share fundamental inflammatory pathways with clinical mucositis, manifesting as edema, vasodilation, and lymphocytic infiltration of the lamina propria. We have documented analogous histopathological findings using an alternative mucositis model^{9,10}.

Prosthetic stomatitis remains a highly prevalent complication in removable denture wearers, characterized by chronic mucosal erythema, inflammation, and compromised mucosal barrier function. It is a multifactorial condition involving mechanical irritation, microbial biofilms (particularly *Candida* species), and host immune responses¹¹. Despite advances in denture materials and hygiene protocols, clinical management continues to rely heavily on antifungal agents, disinfection regimens, and, increasingly, alternative modalities such as photodynamic therapy and phytotherapeutic agents¹².

The present experimental study provides novel evidence that a phytocomposition combining aloe hydrolat and juniper essential oil accelerates mucosal regeneration following chemically induced prosthetic stomatitis. Histologically, treated tissues exhibited reduced inflammatory cell infiltration, improved epithelial integrity, and more organized connective tissue architecture compared with untreated controls. These findings are consistent with the known anti-inflammatory, antioxidant, and wound-healing properties of phytochemicals derived from plant sources, which have been explored in clinical and preclinical contexts for stomatitis and oral mucosal conditions.

In clinical practice, herbal and plant-derived agents such as propolis, green tea, and essential oils have been evaluated as adjuncts or alternatives to conventional antifungal drugs. A systematic review of randomized controlled trials reported that phytotherapy modalities, while heterogeneous in formulation and quality, often demonstrated similar efficacy to standard antifungals in alleviating signs of denture stomatitis, with favorable patient tolerance and fewer adverse effects¹³⁻¹⁵. However, the certainty of the evidence remains low due to variability in study design and outcome measures, underscoring the need for more standardized research.

Our results indicate that aloe hydrolat significantly decreased mucosal edema and inflammation, supporting in vitro and in vivo evidence that Aloe species contain compounds (e.g., acemannan, polysaccharides) capable of modulating local immune responses and enhancing wound repair. These properties may be linked to improved epithelial proliferation and re-establishment of mucosal barrier function observed in the treated rats. This aligns with previous reports of Aloe and related botanicals promoting mucosal healing in oral ulcerative conditions, suggesting translational potential in denture wearers.

The juniper essential oil component appeared to stimulate re-epithelialization more prominently than aloe alone, which may be attributed to the terpenoid composition known for antimicrobial and regenerative effects. While juniper oil's specific mechanisms require further molecular investigation, its use in promoting epithelial migration and mitigating microbial colonization is supported indirectly by the broader literature on plant essential oils in oral mucosal therapy.

Importantly, the combination of aloe hydrolat and juniper oil did not outperform monotherapy in all parameters. This observation may reflect complex interactions between anti-inflammatory and proliferative signaling pathways that are not simply additive. It underscores that combined phytotherapy regimens must be carefully optimized; simultaneous modulation of inflammation and epithelial growth might require staged application or dose adjustments rather than concurrent use.

The translational relevance of this study lies in its reflection of clinical challenges in managing denture stomatitis in patients with compromised mucosal defenses. Current non-pharmacologic interventions such as denture hygiene optimization, antifungal rinses, and fixation creams have demonstrated variable efficacy¹⁶⁻¹⁸. Phytotherapeutic approaches present an attractive adjunct due to their broad bioactivity, lower likelihood of inducing resistance, and potential for integration into routine oral care regimens. Chemical injury induced by orthophosphoric acid reproduced key inflammatory and degenerative features characteristic of prosthetic mucositis, including edema, vascular dilation, and lymphocytic infiltration. Aloe hydrolat demonstrated a

pronounced anti-edematous and anti-inflammatory effect, whereas juniper essential oil primarily stimulated epithelial regeneration. The combined phytocomposition produced a moderate effect, suggesting partial attenuation of inflammatory activity by aloe components.

Correlation analysis indicated that aloe hydrolat most effectively reduced edema and vascular reactivity, while juniper oil increased inflammatory cell recruitment but promoted reparative epithelial responses. These findings support a staged therapeutic approach rather than simultaneous application.

Limitations of this study include its experimental design, which, while enabling controlled assessment of tissue responses, may not fully capture the complexity of human prosthetic stomatitis involving microbial biofilms and host immune variability. Future research should incorporate microbiological profiling, evaluation of clinical symptom regression, and controlled clinical trials to confirm the efficacy of plant-based therapies in denture wearers. Additionally, mechanistic studies using molecular biomarkers of healing and inflammation would further elucidate the regenerative pathways modulated by these phytocomponents.

In conclusion, the findings support the therapeutic potential of aloe hydrolat and juniper essential oil in enhancing mucosal regeneration and reducing inflammation in experimental prosthetic stomatitis. These results align with emerging evidence for natural therapies in denture stomatitis and provide a scientific basis for further clinical investigation. Ultimately, integrating phytotherapeutic agents into evidence-based clinical protocols may expand treatment options and improve outcomes for patients with prosthesis-associated mucosal disorders.

CONCLUSION

Thus, the application of aloe hydrolat leads to a reduction in edema and inflammation, while the use of juniper essential oil promotes faster re-epithelialization of oral mucosal lesions. It is likely that their sequential application will be most effective, whereas combined use results in a mucosal condition somewhat inferior to that observed in groups where the test substances were applied alone.

DECLARATIONS .

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Competing Interests

The no competing interests .

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