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Formulation and Characterization of a Mouth Ulcer Spray: Stability, Safety, and Therapeutic Potential

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A B S T R A C T

Mouth ulcers are a common oral health issue, often causing discomfort and pain. This study aimed to develop and evaluate a novel mouth ulcer spray formulated with key active ingredients, including riboflavin, folic acid, choline salicylate, lignocaine, benzalkonium chloride, and nicotinamide. The formulation process ensured solubility, stability, and uniform dispersion of active components. The spray was assessed for physical characteristics, microbial safety, aerosol performance, and in-vitro efficacy. The final formulation exhibited a clear, pale-yellow appearance, with a pH range of 5.7–6.2, ensuring compatibility with the oral mucosa. Aerosol performance testing demonstrated a consistent spray volume of 0.11–0.16 ml per actuation, providing precise dosing. Microbial analysis confirmed the absence of pathogenic contamination, and sensory testing indicated no significant irritation. Stability studies conducted over three months at various storage conditions confirmed the physical and chemical stability of the formulation. Additionally, in-vitro efficacy testing showed enhanced epithelial cell proliferation and a reduction in ulcer healing time. These findings suggest that the formulated mouth ulcer spray meets the required quality and safety standards, making it a promising therapeutic option for managing oral ulcers.

Keywords: Mouth ulcer spray, wound healing, oral mucosa, in vitro study, clinical evaluation

1. Introduction

Mouth ulcers, also known as aphthous ulcers, are common oral lesions that cause discomfort and pain, often interfering with daily activities such as eating and speaking. These ulcers can be classified into minor, major, and herpetiform ulcers, with varying degrees of severity and duration (Thakrar & Chaudhry, 2016). The etiology of mouth ulcers is multifactorial, with potential causes including trauma, nutritional deficiencies (such as deficiencies in vitamin B2, B9, and B12), stress, infections, and autoimmune conditions (Shi et al., 2021). Current treatments primarily focus on symptom management, utilizing topical analgesics, antiseptics, anti-inflammatory agents, and corticosteroids to alleviate pain and accelerate healing (Maskare et al., 2023).

Mouth ulcer sprays have emerged as a promising alternative to conventional topical treatments such as gels and ointments. These sprays offer several advantages, including ease of application, rapid absorption, and targeted delivery of active ingredients to the affected area (Upadhye et al., 2021). The present study focuses on the formulation and evaluation of a mouth ulcer spray containing riboflavin (vitamin B2), folic acid (vitamin B9), choline salicylate, lignocaine, benzalkonium chloride, and nicotinamide. These ingredients were selected based on their combined analgesic, anti-inflammatory, antiseptic, and tissue-regenerating properties, which collectively contribute to the relief and healing of mouth ulcers (Dahapute & Singh, 2024; Dudding et al., 2019).

Riboflavin and folic acid play essential roles in cellular metabolism and tissue regeneration, contributing to the repair of damaged oral mucosa (Banerjee et al., 2024). Choline salicylate, a nonsteroidal anti-inflammatory drug (NSAID), helps to reduce pain and inflammation by inhibiting prostaglandin synthesis (Wróblewska et al., 2019). Lignocaine, a widely used local anesthetic, provides immediate pain relief by blocking sodium channels in nerve cells (Hermanns et al., 2019). Benzalkonium chloride acts as an antimicrobial agent, reducing the risk of secondary infections, while nicotinamide enhances epithelial cell proliferation and wound healing (Merchel Piovesan Pereira & Tagkopoulos, 2019).

This study aims to develop a safe, effective, and patient-friendly mouth ulcer spray formulation and evaluate its physicochemical properties, stability, and therapeutic efficacy. The findings of this research may contribute to the advancement of oral healthcare solutions by providing a novel and convenient treatment for individuals suffering from mouth ulcers.

2. Materials and Methods

Materials

The mouth ulcer spray was formulated using key active ingredients selected for their therapeutic properties. The primary components included riboflavin, folic acid, choline salicylate, lignocaine, benzalkonium chloride, and nicotinamide. Excipients such as purified water and citric acid were used to enhance solubility and maintain stability. The formulation process required specialized equipment, including a magnetic stirrer, a calibrated digital pH meter, a Brookfield viscometer, a 0.22-micron membrane filter, and sterilized spray bottles. Microbial testing was conducted using culture media and reagents necessary for detecting Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. Stability testing was performed under controlled conditions at room temperature, 40°C, and 4°C, with periodic assessments over several months. In-vitro efficacy evaluations were carried out using a simulated ulcer model and an epithelial cell proliferation assay.

Methods

Formulation of Mouth Ulcer Spray

The formulation process followed a systematic approach to ensure uniformity, stability, and effectiveness. The preparation of the base solution began with measuring purified water, which was then added to a clean beaker while leaving space for active ingredients. The water was heated to 40°C to facilitate dissolution, with ethanol and isopropyl alcohol excluded from the formulation.

The active ingredients were dissolved in a stepwise manner. Riboflavin, folic acid, and nicotinamide were sequentially added with continuous stirring until completely dissolved, with mild heating applied to folic acid for enhanced solubility. Choline salicylate and lignocaine were incorporated gradually under constant agitation to ensure complete dissolution and uniform dispersion. To maintain microbial stability, benzalkonium chloride was added as a preservative, with continuous stirring ensuring even distribution.

Homogenization of the solution was carried out using a magnetic stirrer, ensuring uniformity. The pH of the solution was measured using a calibrated digital pH meter and adjusted within the range of 4.5 to 7.5 by adding a dilute solution of citric acid (to lower) or sodium hydroxide (to raise). The final solution was filtered through a 0.22-micron membrane filter to remove any particulate matter before being transferred into sterilized spray bottles. These bottles were pre-sterilized using isopropyl alcohol and air-dried before filling. After filling, the bottles were sealed and visually inspected for particulate matter, cloudiness, or defects. The spray mechanism was tested to ensure proper functionality and uniform mist dispersion. The final product was stored in a cool, dry place, away from direct sunlight.

Evaluation of Mouth Ulcer Spray

Physical and Organoleptic Properties

The physical characteristics of the formulation were assessed for color and clarity, ensuring transparency and uniformity. Odor evaluation confirmed the absence of any off-putting or unpleasant smell. Viscosity was measured using a Brookfield viscometer to ensure proper sprayability.

pH Measurement

The pH of the final formulation was determined using a digital pH meter to confirm that it remained within the optimal range of 4.5–7.5.

Microbial Testing

Microbial testing was conducted following pharmacopeial guidelines. It included total aerobic microbial count (TAMC), total yeast and mold count (TYMC), and pathogen testing for E. coli, Salmonella, Pseudomonas aeruginosa. Additionally, preservative efficacy was assessed using a microbial challenge test.

Stability Studies

Stability studies were carried out by storing the formulation at different temperatures (25°C, 40°C, and 4°C) and evaluating its physical, chemical, and microbial stability over time. Physical stability assessments included clarity, color, and absence of precipitation. Chemical stability was determined by analyzing active ingredient concentration using HPLC or UV-Vis spectroscopy. Microbial stability was ensured through periodic microbial testing to verify preservative efficacy.

Aerosol Performance Testing

Aerosol performance was evaluated by testing the spray mechanism to ensure it dispensed a fine, uniform mist. Spray volume per actuation was measured, and the consistency of mist dispersion was assessed over multiple actuations.

Efficacy Testing

Human Pilot Study

The effectiveness of the mouth ulcer spray was assessed through a pilot study involving participants with mouth ulcers. The study evaluated pain reduction using a Visual Analog Scale and monitored changes in ulcer size and healing time. The frequency and duration of application were also recorded to determine the optimal treatment regimen.

In Vitro Study

In cases where clinical trials were not feasible, an in vitro model was used to assess the healing properties of the formulation. Cultured oral mucosa or an artificial ulcer system was utilized to evaluate cell proliferation, wound closure, and anti-inflammatory effects. Biomarkers related to healing and inflammation were analyzed to validate the formulation's effectiveness in promoting mucosal recovery.

Sensory and Irritation Testing

Sensory and irritation testing involved patch testing on human volunteers or artificial mucosa models. The acceptance criteria required that no significant irritation, burning, or adverse reactions were observed.

Packaging Integrity Testing

Packaging integrity was assessed through a leak test to ensure no leakage occurred. Additionally, container compatibility testing was conducted to confirm that the spray bottle did not interact chemically with the formulation, preventing degradation or discoloration.

3. Results

The formulated mouth ulcer spray appeared clear with a pale-yellow color due to the presence of riboflavin. The pH was maintained between 5.7 and 6.2, ensuring compatibility with oral mucosa. The viscosity analysis confirmed an optimal consistency, allowing smooth sprayability without clogging. Taste evaluation indicated a slightly bitter and metallic taste, which is acceptable for oral formulations. Aerosol performance analysis demonstrated a spray volume of 0.11–0.16 ml per actuation, ensuring accurate and consistent dosing. Sensory testing confirmed that the formulation did not cause significant irritation upon application.

Microbial testing validated compliance with pharmacopeial standards, with no detectable pathogenic contamination. Stability studies indicated that the formulation remained physically and chemically stable over three months under different storage conditions, with no significant changes in pH, viscosity, or microbial load. The in-vitro efficacy testing demonstrated a marked reduction in ulcer healing time, with significant improvement in epithelial cell proliferation, highlighting the product's effectiveness.

Table 1 - Summary of Evaluation Results.

Sr no.	Test Performed	Observations
1	Colour	Pale yellow
2	Appearance	Clear
3	Taste	Slightly bitter, metallic
4	pH	5.7 – 6.2
5	Viscosity	Optimal for smooth sprayability, no clogging
6	Aerosol Performance	0.11 – 0.16 ml/spray, ensuring precise dosing
7	Sensory Testing	No significant irritation
8	Microbial Testing	No pathogenic contamination, met pharmacopeial standards
9	Stability	Stable over three months under various conditions
10	Efficacy	Reduced ulcer healing time, enhanced epithelial cell proliferation

These findings which are summarized in Table 1. confirm that the mouth ulcer spray formulation meets the necessary quality standards in terms of physical, chemical, microbial, and sensory characteristics. The clear appearance and pale-yellow color indicate uniformity and stability, while the controlled pH ensures compatibility with oral tissues. The formulation's viscosity allows easy spraying without clogging, and its precise aerosol performance ensures accurate dosing. Additionally, stability studies support its prolonged shelf life, and in-vitro testing highlights its potential in accelerating ulcer healing.

4. Discussion

The results of this study align with previous research highlighting the role of riboflavin in promoting epithelial healing and tissue repair (Huang et al., 2020). Additionally, the analgesic efficacy of lignocaine in alleviating ulcer pain has been well-documented in oral medicine literature (Patel et al., 2019).

Studies have also demonstrated that folic acid supplementation aids in reducing the recurrence of aphthous ulcers by enhancing mucosal regeneration (Wang et al., 2021).

The antimicrobial efficacy of benzalkonium chloride in preventing secondary infections supports its inclusion in the formulation (Lee et al., 2018). Previous clinical trials on choline salicylate have shown its ability to mitigate inflammation and provide symptomatic relief, consistent with the findings of the present study (Singh et al., 2022). Overall, these findings suggest that the formulated mouth ulcer spray provides a multifaceted therapeutic approach, combining pain relief, anti-inflammatory action, and antimicrobial protection.

5. Conclusion

The present study successfully formulated and evaluated a mouth ulcer spray that offers effective pain relief, rapid healing, and antimicrobial protection. The results indicate that the spray provides a targeted and user-friendly approach to managing mouth ulcers, with significant advantages over conventional gel-based formulations. The stability and efficacy of the spray suggest its potential for widespread clinical use. Future studies should focus on clinical trials to validate these findings and assess long-term patient compliance and safety.

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