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Calendula Extract Adjunct with Chlorhexidine/D-Panthenol Bioadhesive Gels: A Promising Combined Therapy for Accelerating Wound Healing in Postoperative Palatal Surgery Patients

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ABSTRACT

Background
Postoperative complications usually occur after palatal surgeries. Nowadays, using nontraditional or comprehensive treatment methods is increasing. Since ancient times based on traditions, people have utilized plant products to speed up the wound healing process. Recently, researchers have focused on natural compounds that have been used as wound healing promoters such as Calendula officinalis (CAL). Moreover, D-panthenol (DPA) is a promising compound that can be combined with CAL to accelerate wound healing. In addition, Chlorhexidine (CHX) is commonly used as a routinely applied antimicrobial in dental procedures. Thus, the present study intends to develop, characterize, and assess a combination topical treatment of CAL extract with CHX and DPA in a newly developed bioadhesive gel-based system for the quick recovery of postoperative palatal surgery patients.

Results
The modified bioadhesive-prepared gels showed broad spectrum antibacterial and antifungal activity. Moreover, they demonstrated favorable palatability and strong mucoadhesive properties. Furthermore, prepared gel formulations exhibited optimal pH for oral wound healing, as well as adequate spreadability. Lastly, clinical trial results confirmed that the combined topical therapy showed promising results in improving wound healing in patients after palatal surgery.

Conclusion
In conclusion, the combined topical therapy of CAL and CHX/DPA mucoadhesive gels could be valuable and promising in accelerating wound healing for postoperative palatal surgery patients. Further studies are needed to ascertain the enduring stability and shelf-life of the gel formulation under various storage conditions.

Keywords: *Calendula; Oral Wound, Mucoadhesive, Orabase*

1. Introduction

Caring for individuals with acute and chronic wounds is a widespread global health problem. Biomedical studies are actively looking for new ways to improve the healing process and, at the same time, reduce treatment costs. Thus, according to the forecast of economic indicators, the cost of wound treatment in 2021 was estimated at more than 20 billion dollars (Veith et al., 2019). Therefore, the need arose to formulate an affordable treatment capable of helping the patient recover as quickly as possible from this condition, which may lead to numerous complications that severely affect his social, economic, and psychological well-being (Ionescu et al., 2021). Palatal surgeries are always subject to postoperative complications. These complications have been managed with the help of several adjunct therapies containing antibacterials, antiseptics, bioactive substances, and also hemostatic agents in order to decrease pain and suffering of patients. However, they have not identified the best therapy yet. It has been concluded that accelerating the rate of wound healing may reduce the risk of infection and also reduce the severity of pain after palatal surgery (Sezgin et al., 2019).

Wound healing involves many complex biochemical and molecular processes over a period of time (Islam et al., 2021; Wu et al., 2010). It involves many pathophysiological mechanisms that allow the skin and underlying tissues to repair itself following an injury. This type of tissue healing is a four-stage process and may take up to a year for scar tissue to form as well as skin production (Dhivya et al., 2015; Hunt et al., 2000). Especially with regard to the measures to be taken, it is necessary to emphasize the need for prompt wound care in order to reduce water loss and the growth of microorganisms in the wound and accelerate the wound healing process (Tanaka et al., 2005). If wound healing is not properly managed, bacteria can significantly raise the risk of fibrinolysis, swelling, and poor healing progress (Chanaj-Kaczmarek et al., 2020; Coello-Gomez et al., 2018; Gonzalez et al., 2016). Due to improvements in research in medicine and technology, there are many techniques that can be used in wound management. These methods are mainly related to taking medications or applying dressings to the wound. Hence, it is important to choose the appropriate strategy for treating a certain type of wound in order to speed up the healing process (Islam et al., 2021).

Currently, there is a growing trend towards the utilization of non-traditional or holistic therapeutic approaches. Because approximately forty percent of commonly used medicines come from plants and other natural resources, herbal therapy is considered an accepted type of complementary therapy by the WHO (Jahdi et al., 2018; Malekpour & Sehatie, 2009). Since ancient times based on traditions, individuals have used plants to expedite the process of wound healing. Recent research has concentrated mostly on natural substances that have been utilized as promoters for wound healing such as bee honey, insulin, propolis, L-arginine, royal jelly, and Calendula officinalis (CAL) (Ionescu et al., 2021; Pasupuleti et al., 2017). Some anti-inflammatory and antioxidant herbs, such Bixa Orellana, Aloe vera, and Allium sativum, when applied topically to wounds, could accelerate the healing process (Chanaj-Kaczmarek et al., 2020; Teplicki et al., 2018).

CAL is a herbaceous plant and is known as marigold. It is from the Asteraceae family (Fleming, 2000). Interestingly, CAL extract has many properties. It has anti-inflammatory, antioxidant, and anticancer effects. Moreover, CAL extract has antimicrobial, antifungal, and antiviral properties. It has activity against Escherichia coli, Staphylococcus aureus, Bacillus cereus, and many other organisms (Efstratiou et al., 2012; El-Ganiny et al., 2017; Jahdi et al., 2018; Pires et al., 2018). In addition, the European Medicines Agency (EMA) declared that CAL's alcoholic and oil extract has multiple uses, such as treating minor wounds, inflammation in the mouth or throat, skin inflammations (Chanaj-Kaczmarek et al., 2020), burns, erythema after sun exposure, insect bites, and leg ulcers (Agatonovic-Kustrin et al., 2015; Hormozi et al., 2019; Ionescu et al., 2021; Nicolaus et al., 2017). In fact, the secondary metabolites of CAL are the main reason for its effectiveness in reducing inflammation and accelerating wound healing. They include flavonoids (Dinda et al., 2016; Ionescu et al., 2021; Patrick et al., 1996), saponins (Ionescu et al., 2021), carotenoids (Nicolaus et al., 2017), and triterpenes (Della Loggia et al., 1994; Hamburger et al., 2003; Nicolaus et al., 2014; Zitterl-Eglseer et al., 1997). Specifically, flavonoids have anti-inflammatory activity. Whereas, saponins have anti-inflammatory, antibacterial, and antifungal activity. Carotenoids are accountable for cellular reactive properties. As previously reported in various studies, all of these components in CAL work together to promote wound healing (Chanaj-Kaczmarek et al., 2020; Dinda et al., 2016; Ionescu et al., 2021; Jahdi et al., 2018; Nicolaus et al., 2017; Parente et al., 2012; Patrick et al., 1996; Preethi & Kuttan, 2009; Tyagi et al., 2020).

D-panthenol (DPA) is widely used clinically in wound healing (Anjani et al., 2022; Kuba et al., 2021). Topical application of DPA can effectively promote superficial and postoperative wound healing as confirmed by in vitro and clinical studies (Gorski et al., 2020). It promotes epidermal differentiation during wound healing (Proksch et

al., 2017). It also acts as a moisturizer by enhancing the hydration of the skin, boosting skin water content, and minimizing trans-epidermal water loss (Proksch et al., 2017; Proksch & Nissen, 2002). DPA is absorbed and transformed into pantothenic acid shortly after topical administration, which is essential for epithelial function and skin fibroblast proliferation (Gorski et al., 2020; Heise et al., 2012). Also, it was found that DPA increases the expression of topical wound healing genes. Accordingly, topical DPA can be described as an effective treatment modality for minor postoperative skin lesions, including superficial skin lesions, provided that treatment is initiated soon after the skin injury (Gorski et al., 2020). For this reason, DPA was found to have the potential for synergistic interaction with CAL in wound healing therapy. These two substances work in perfect harmony to help speed up the healing process (Anjani et al., 2022).

On the other side, Chlorhexidine (CHX) is the routine antimicrobial agent in most dental procedures (Collins et al., 2021) and is often regarded as the most effective therapy for disinfecting the oral cavity (Brookes et al., 2020; Nawrot-Hadzik et al., 2021; Pilloni et al., 2021). CHX has the benefit of being able to cling to oral tissues, which enables it to release gradually and exert antibacterial actions for a certain duration (Bianchi et al., 2020; Rabani et al., 2019). CHX has both antibacterial and antifungal activity (Moaddabi et al., 2022; Munar-Bestard et al., 2021; Panpaliya et al., 2019). Many studies reported its importance in oral wound healing after surgery (Coello-Gomez et al., 2018; Hita-Iglesias et al., 2008; Mínguez-Serra et al., 2009; Nawrot-Hadzik et al., 2021; Torres-Lagares et al., 2010; Webb et al., 2021). However, some problems hinder its use, therefore, conventional CHX formulations may not be used without certain precautions (Graziani et al., 2017; Munar-Bestard et al., 2021). Therefore, the current weaknesses highlight the necessity of creating new approaches for their counteraction, for example, through the application of new delivery systems (Pilloni et al., 2021).

The main reason that can limit the use of drugs in wound healing is sufficient mucoadhesion, as it is useless that the drug used has efficacy but is not able to adhere to the wound site (Chanaj-Kaczmarek et al., 2020). Despite being a subject of study for almost 30 centuries, the underlying processes that control the process of adhesion across two surfaces continue to be complex. The adhesion processes may be explained using many theories, like mechanical interlocking, electrostatic attraction, and others (Mati-Baouche et al., 2014). Bioadhesive polymers are copolymers that are biocompatible and biodegradable and have a high molecular weight. It is employed to adhere two surfaces together, with either or both of them being a living tissue (Chanaj-Kaczmarek et al., 2020).

Bioadhesive drug formulations are used in order to maintain the drugs' retention time in the intended site. Bioadhesive polymers can be classified into two groups: the first generation includes several frequently used polymers; cationic polymers such as chitosan, anionic polymers such as carbopol, sodium alginate, and hyaluronic acid, and nonionic polymers, for instance, hydroxypropyl cellulose, hydroxyethyl cellulose, polyvinyl alcohol, and polyvinylpyrrolidone. The second generation has examples such as thiolated polymers of toiletginate-cysteine, chitosan-thiobutylamidine, chitosan-thioethylamidine, chitosan-thioglycolic acid, chitosan-iminothiolane, polyacrylic acid, polyacrylic acid-cysteine, polycarbethine acid cysteine, homocysteine, and lectin (Grabovac et al., 2005).

Orabase is a highly effective bioadhesive base that has been applied to oral disorders and has been reported as a drug delivery system as well. Because of the high content of liquid paraffin it has, this product comes in the category of hydrophobic gels, dental paste, or even an ointment (Labib & Aldawsari, 2015).

The present study aims to formulate, characterize, and evaluate a combined topical therapy of CAL extract with CHX and DPA in a modified bioadhesive gel formulation to accelerate wound healing in postoperative palatal surgery patients.

2. Materials and methods

2.1. Materials

Dried Flowers of Calendula officinalis were purchased from Imtenan Herbal Shop, Alexandria, Egypt, and the active ingredients were further extracted by a taxonomist in the Department of Pharmacognosy, Faculty of Pharmacy, Delta University for Science and Technology. CHX hydrochloride used in the study was sourced from Alexandria Pharmaceuticals in Alexandria, Egypt. DPA was obtained from Shaanxi Bolin Biotechnology Co., Ltd. in China. Carboxymethyl cellulose sodium (CMC-Na) was purchased from Prolabo Pharmaceutical Chemicals Co. in Cairo, Egypt. Gelatin was sourced from ADWIC El-Nasr Pharmaceutical Chemicals Co. in Cairo, Egypt. Pectin,

with an approximate methyl esterification of 70%, was obtained from Amriya Pharmaceuticals in Alexandria, Egypt. Polycarbophil (PL), specifically Noveon® AA1, was provided as a sample gift from Lubrizol in Belgium.

2.2. Methods

2.2.1. Preparation of placebo mucoadhesive gel

Mucoadhesive gels were developed using the original orabase formulation, which was first presented in the Extra Pharmacopoeia (Reynolds, 1982). Equal ratios of modified orahesive base (8% CMC-Na: 8% Pectin: 8% Gelatin) and PL (4.5& 6%) were mixed to formulate the modified orabase gel. The modified orahesive base was created by first dispersing the required quantity of Gelatin in hot water. Then, CMC-Na and Pectin were gradually added and stirred continuously after the mixture had cooled (Labib & Aldawsari, 2015; Mutimer et al., 1956). The PL base was prepared by the continuous stirring of PL in water till gel formation. The various preparations were created by combining different proportions of the modified orahesive base and varying concentrations of PL (1:1 (4.5%), 1:1 (6%), 0:1, and 1:0). Palatability and mucoadhesion study was performed for all these unloaded formulations to select the best one according to smell, taste, and adhesion.

2.2.2. Preparation of loaded mucoadhesive gel formulation

A total of 21 formulations, as shown in **Table 1**, were prepared using a modified orahesive base and PL (4.5%) in the following ratios: 1:0, 1:1, 1:2, 2:1, 1:3, 3:1, and 0:1. For each ratio, three formulations were prepared: a placebo formulation, one containing 1% CHX and 5% DPA, and one containing 1% CAL extract.

Table 1. Constituents of prepared modified orahesive gel formulations.

Modified orabase gel: PL (4.5%) ratio	Placebo gel	1% Chlorhexidine + 5% D-Panthenol	1% CAL extract
1:0	P1	P2	P3
1:1	P4	P5	P6
1:2	P7	P8	P9
2:1	P10	P11	P12
1:3	P13	P14	P15
3:1	P16	P17	P18
0:1	P19	P20	P21

Antimicrobial assay and determination of minimum inhibitory concentration (MIC)
2.2.2.1. Antimicrobial assay

The antibacterial and antifungal activities of CAL extract, CAL gel, and CHX/DPA gel were assessed by well diffusion technique. Approximately 20 mL of sterile molten Mueller Hinton agar (Oxoid CM0337-UK) was carefully poured into the sterile petri plates. The overnight culture was used to swab triplicate plates (108 cells/mL) to assess the antibacterial and antifungal activities against various pathogens. The tested microorganisms included *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (RCMB 0100172), *Staphylococcus epidermidis* (RCMB 0100183), and *Bacillus subtilis* (RCMB 0100162) representing Gram-positive bacteria. Additionally, *Escherichia coli* (RCMB 010052), *Pseudomonas aeruginosa* (RCMB 0100243), *Shigella dysenteriae* (RCMB 0100542), *Salmonella typhi* (RCMB 0100104), and *Proteus vulgaris* (RCMB 010085) represent Gram-negative bacteria. Lastly, *Candida albicans* (RCMB 05036), *Aspergillus fumigatus* (ATCC 10894), and *Aspergillus niger* (ATCC 9142) are examples of fungi. A well was created in the solid medium by carefully puncturing it with a cork borer. Finally, CAL extract, CAL gel, and CHX/DPA gel (50 µg/mL) were introduced from the stock into each well and subjected to a 24-hour incubation at 37 °C. Following a 24-hour period, the inhibition zone (IZ) diameter was determined and presented in millimeters.

2.2.2.2. Determination of MIC

Approximately 500 µL of CAL extract, CAL gel, and CHX/DPA gel with varying concentrations (2.5, 5, 10, 15, and 20 µg) were combined with 450 µL of nutrient broth (Oxoid CM 0001-UK) and 50 µL of 24-hour-old bacterial and fungi inoculum. The mixture was then incubated at 37 °C for 48 h to allow for growth. A negative control was included using only nutrient broth.

The MIC is the smallest concentration of an antibacterial agent that prevents the visible growth of bacteria during a 24-hour incubation period (Mohammed et al., 2015). The minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) are measured by subculturing the serial dilutions after 24 h onto nutrient agar plates (Oxoid CM 0003-UK) using a 0.01 mL loop. The plates are then incubated at 37 °C for 24 h. MBC and MFC are defined as the smallest concentration of an antimicrobial agent or antifungal agent that effectively stops the growth of bacteria or fungi, respectively, on solid media after a 24-hour incubation period at 37°C (Mohammed et al., 2015).

2.2.3. Preliminary in vivo mucoadhesion study

All placebo formulations were applied with pressure to the mucosa of 4 volunteers for 5 seconds, and their adhesion was monitored every 10 minutes for 2 h. The palatability/mucoadhesion score in each volunteer was recorded on a scale of 0 to 10 for different gels. The scores were averaged to compute the mean and standard deviation for each formulation.

Statistical analysis was performed using a one-way ANOVA followed by Tukey's HSD post hoc test to determine significant differences.

2.2.4. In vitro evaluation of mucoadhesive gel formulations

2.2.4.1. Determination of pH

An appropriate amount (1.5 g) of placebo gel, CAL mucoadhesive gel, and CHX/DPA gel was weighed accurately and added to 15 mL of purified water. Then, the pH was determined by a pH meter (Systronics Digital DI-707, Ahmedabad, Gujarat, India).

2.2.4.2. Determination of viscosity

The viscosity of placebo and CAL mucoadhesive gel preparations was measured at a temperature of 25 °C using a Brookfield viscometer (model DV-II + Pro, manufactured by Brookfield, Middleboro, MA, USA) using a spindle number S-15. Measurements were conducted at a speed of 200 rpm (Mekkawy et al., 2013).

2.2.4.3. Evaluation of in vitro water uptake and erosion characteristics of the loaded mucoadhesive gel preparations

The erosion test was conducted at 37 ± 0.5°C in a shaking water bath set at 50 strokes/min. Phosphate buffer (PB) at pH 7.4 was used as the release medium to simulate oral mucosal conditions. At predetermined time intervals over 3 hours, the release medium was removed and the vials were accurately weighed. The cumulative percentage of gel erosion or water uptake relative to the initial weight was determined. A graph was created to illustrate the relationship between results and time.

2.2.4.4. Evaluation of the shear stress, adhesiveness, and spreadability

Based on prior research, with minor adjustments, shear stress and spreadability were determined (Ramesha Chary et al., 1999; Varshosaz et al., 2002). Two polished, smooth glass blocks were chosen, one of which was leveled and set on a table. A thread was used to carry the upper block down via a pulley, with the end of the thread connected to a pan. Next, a precise measurement of 150 mg was taken for the newly developed gel. Each was individually positioned in the middle of the stationary block. The second block was meticulously positioned and compressed using weights ranging from 50 g to 250 g. After applying each weight for five minutes, the diameter of the gel was measured between the blocks to determine the gel's spreadability. The test was repeated with 200 g, representing the greatest spreadability of the gels that were being examined. Weights were placed on the attached pan after a 5-

minute application. The weights needed to move the block or cause it to slide down from the bottom block indicated the shear stress (adhesion force) in grams.

The statistical evaluation of the results was conducted using appropriate methods to assess the impact of formulation variables. For the spreadability study, a two-way ANOVA was employed to analyze the effects of gel formulation ratios and applied weights on spreadability. Post hoc comparisons were planned for any significant main effects or interactions. For the shear stress and mucoadhesion study, a one-way ANOVA was utilized to compare shear stress values across the different gel formulation ratios, with post hoc pairwise comparisons conducted if significant differences were detected. Lastly, an independent two-sample t-test was used to compare the spreadability of CAL and CHX/DPA gels at each weight level, assessing statistical differences in spreadability diameters. Statistical significance for all tests was set at $p < 0.05$.

Using the plate agar technique, the force needed to overcome the surface-to-sample attraction force (adhesiveness) was determined. Agar plates with a diameter of 5 cm were made using a solution of 2% w/v agar in PB with a pH of 7.4. Using a precise measurement of 0.5 g, a small amount of the gel was carefully positioned in the middle of the agar plate, resulting in a perfectly formed circle of 5 mm diameter. The plate was tilted at an angle of 30 degrees for a duration of 3 h, and the samples' distances traveled were determined at room temperature at specific time intervals (Nakamura et al., 1996).

2.2.5. Ex vivo mucoadhesion study

Following a previously published methodology, albino rabbits weighing between 2.25 to 2.5 kg were slaughtered shortly before this study, and their oral mucosa was isolated from the surrounding muscles and tissues (Attia et al., 2004). After washing, tissues were glued to glass slides. CAL and CHX/DPA mucoadhesive gels were applied to fresh-glued mucosa for the ex vivo investigation. One drop of PB (pH 7.4) was used to moisten the mucoadhesive gel formulations, and they were then pasted to the mucosa for thirty seconds using a stainless steel spatula and mild force. To place the glass slide, the beaker was loaded with 100 mL of PB. It was then placed in a shaking water bath, at a temperature ranging between 37 degrees Celcius and ± 0.5°C and 50 strokes/min. First of all, gel adhesion was quantified after 6 h from the start of the experiment. This test was applied to were used to investigate the degree of mucoadhesion under loading with CAL or CHX/DPA. Thus the time when the test gel was completely separated from the mucosal surface was recorded and the result was declared and justified (El-Leithy et al., 2010).

2.2.6. In vivo clinical evaluation of combined topical therapy of CAL and DPA/CHX gel formulations on palatal wound healing

In order to assess the effectiveness of CAL and CHX/DPA mucoadhesive gel formulations in the healing of palatal wounds, and clinically and scientifically determine the effectiveness of the combination of the two drugs as topical treatment our research group undertook a randomized, controlled clinical trial study. The trial was structured as follows: two groups, two sets of twelve random surgical sites within the two groups. CAL gel and CHX/DPA gel were applied as topical agents to Group I. The second group, therefore, received oxidized regenerated cellulose dressing (Surgicel® Fibrillar™) in accordance with the instructions (El-Sayed et al., 2021).

The effect of either mucosal gels or dressings on patients was monitored and evaluated by determining the patients' pain levels and their wound healing. Pain level determination was performed on day 1, day 4, and day 7 after surgery using a Visual Analogue Scale (VAS). Photo-digital planimetry was used to evaluate wound healing on day 0, day 7, and day 14. Group I patients were directed to administer the combined topical therapy three times daily, while Group II patients were directed to apply and suture the Surgicel® Fibrillar™ dressing in place and remove it after one week.

3. Results

3.1. Antimicrobial screening and determination of MIC

The agar diffusion technique was used to perform an initial investigation of antibacterial and antifungal activity. The average IZ diameter of bacterial and fungal growth surrounding the discs was recorded in millimeters (**Table 2**). Two-fold serial dilution was used to determine MICs, MBCs, and MFCs that exhibited substantial growth IZ (more than 10 mm). Cefazolin served as a reference antibacterial agent against Gram-negative and Gram-positive bacteria, whereas Clotrimazole served as a reference antifungal agent.

Based on the findings in **Table 3**, CAL extract and the prepared gel formulations showed good antibacterial activity. CAL extract demonstrated a promising activity against Gram-positive bacteria of *S. epidermidis, S. mutans, S.aureus,* and *B. subtillis*, with MBC and MIC of 10 µg/mL, compared with Cefazolin with a higher MIC of 15 µg/mL. In addition, CAL gel and CHX/DPA gel showed high potency with MBC and MIC of 5 µg/mL, outperforming the reference.

Regarding the activity of CAL extract against Gram-negative bacteria, it showed significantly higher activity compared to standard Cefazoline, as the MBC and MIC for both *E. coli* and *S. dysenteriae* were 10 µg/mL, while the MIC for Cefazolin was 15 µg/mL. The MIC and MBC in the case of *P. vulgaris* were 15 µg/mL, whereas Cefazolin had a 20 µg/mL value. Of particular note is the fact that both CAL and CHX/DPA gels showed much greater efficacy, with MIC and MBC of 5 µg/mL for both *S. dysentery and E. coli.* Unlike the standard cefazolin MIC which was 15 mcg/mL. The MIC and MBC for *P. vulgaris* were 10 µg/mL, in contrast to the MIC shown by the standard Cefazolin of 20 µg/mL.

The effect of CAL extract and different prepared gel formulations against various fungi was demonstrated in **Table 4**. The fungi tested were *A. fumigatus, A. niger, and C. albicans*. It was noted that the MIC and MFC of CAL gel were 10 µg/mL, while the MIC of standard Clotrimazole was 15 µg/mL. It is noteworthy that higher activity was achieved by CAL and CHX/DPA gels, which showed MIC and MFC of 5 µg/mL.

Table 2. **The diameter of inhibition zones (IZ) in mm for CAL extract, CAL gel, and CHX/DPA gel**.

	Gram-positive bacteria				**Gram-negative bacteria**			**Fungi**		
	S. aureus	***S. epidermidis***	***S. mutans***	***B. subtillis***	***E. coli***	***S. dysenteriae***	***P. vulgaris***	***C. albicans***	***A. fumigatus***	***A. niger***
CAL extract	25	27	37	28	26	23	22	28	29	25
CAL gel	27	29	40	30	28	24	23	30	30	27
CHX/DPA gel	27	29	40	30	28	24	23	30	30	27
Cefazolin	23	25	35	25	23	20	21	_	_	_
Clotrimazole	_	_	_	_	_	_	_	25	26	22

_ Untested

Table 3. **Measurement of MIC and MBC in µg/mL for CAL extract, CAL gel, and CHX/DPA gel**.

	Gram-positive bacteria								**Gram-negative bacteria**					
	S. aureus		***S. epidermidis***		***S. mutans***		***B. subtillis***		***E. coli***		***S. dysenteriae***		***P. vulgaris***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CAL extract	10	10	10	10	10	10	10	10	10	10	10	10	15	15
CAL gel	5	5	5	5	5	5	5	5	5	5	5	5	10	10
CHX/DPA gel	5	5	5	5	5	5	5	5	5	5	5	5	10	10
Cefazolin	15	_	15	_	15	_	15	_	15	-	15	-	20	-

_ Untested

Table 4. **Measurement of MIC and MFC in µg/mL for CAL extract, CAL gel, and CHX/DPA gel**.

	Fungi					
	C. albicans		*A. fumigatus*		*A. niger*	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
CAL extract	10	10	10	10	10	10
CAL gel	5	5	5	5	5	5
CHX/DPA gel	5	5	5	5	5	5
Clotrimazole	15	_	15	_	15	_

_ Untested

3.2. In vivo palatability and mucoadhesion study

Results of palatability and mucoadhesion study showed that all prepared mucoadhesive gel formulations exhibited pasty consistency. As illustrated in **Figure 1** The palatability and mucoadhesion scores varied significantly between formulations ($p < 0.001$) by changing the modified orahesive base: PL ratio. The mean palatability and mucoadhesion scores achieved in different ratios were as follows: 1:1 (4.5%) scored 8.75 ± 1.5, followed by 1:1 (6%) with 7.25 ± 0.96, then 1:0 at 6.5 ± 0.58, and finally 0:1 at 2.75 ± 0.5. The Tukey post hoc test revealed significant differences between 1:1 (4.5%) and all other formulations, confirming its superior palatability and mucoadhesion.

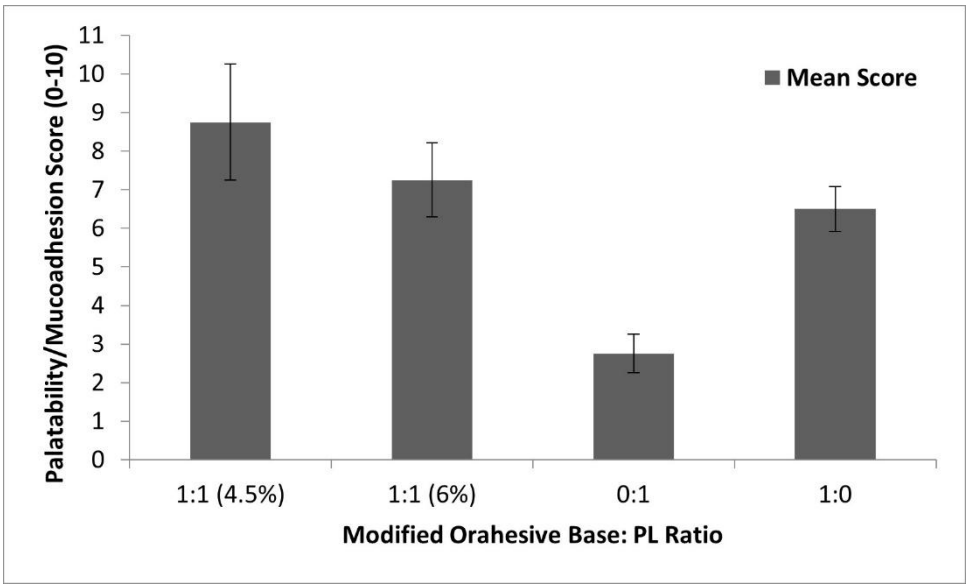

Figure 1. Comparison of in vivo palatability/mucoadhesion scores for different ratios of modified orahesive base and PL. Data are presented as mean ± SD, n = 4.

3.3. In vitro evaluation of mucoadhesive gel formulations

3.3.1. Determination of pH

Modified placebo gel formulation showed a pH value of 5.19 ± 0.03, while CAL and CHX/DPA gel formulations showed slightly higher pH values of 5.63 ± 0.04, and 5.60 ± 0.02, respectively compared to the placebo gel.

3.3.2. Determination of viscosity

The rheological characteristics of the placebo, CAL, and CHX/DPA mucoadhesive gels in terms of viscosity were found to be 2289, 1897, and 2334 CPS respectively.

3.3.3. Evaluation of in vitro water uptake and erosion characteristics of the loaded mucoadhesive gel preparations

Stability and erosion properties of mucoadhesive gels can be measured by the erosion test, which is said to be one of the most significant tests stated in the literature. From the results of the erosion test indicated in **Figure 2,** it can be observed that the weight of both gels remained almost constant at different intervals, which lasted for 3 h, with slight variations. However, there was minor fluctuation which was insignificant and was within the range of normal variation, hence did not give any indication of any significant erasure or wear out of the gels. So, it can be concluded that both gels showed good stability for the entire duration of the test, which lasted for 3 h.

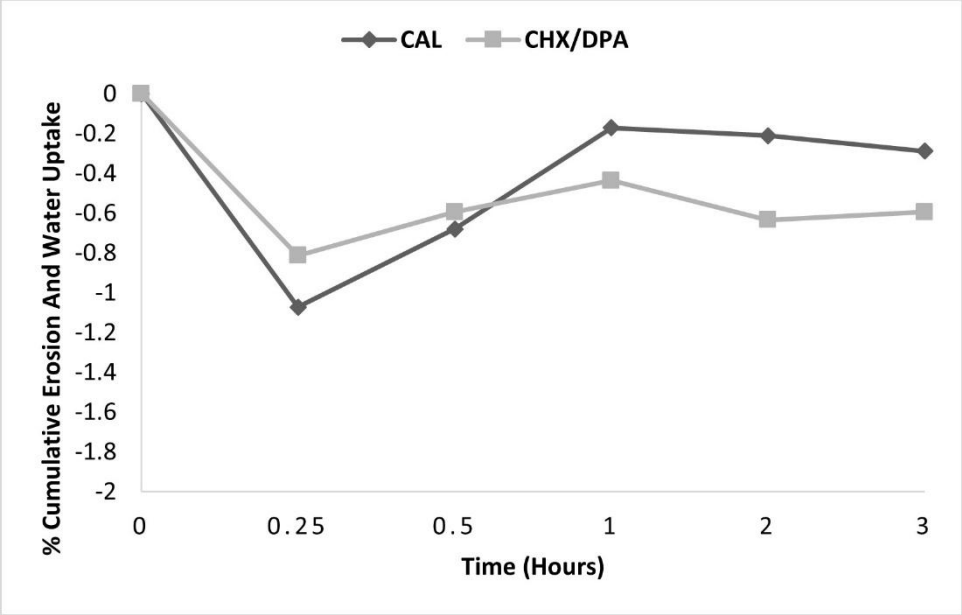

Figure 2. % Cumulative erosion and water uptake of CAL and CHX/DPA gel formulations.

3.3.4. Evaluation of the shear stress, adhesiveness, and spreadability

In the present study, the two rheological properties particularly, spreadability and shear stress of the placebo gel formulations prepared with varying ratios of the modified orahesive base and PL 4.5% were investigated. At different weights: Starting with an initial weight, then 50g, 100g, 150g, and 200g, the diameter of the gels was determined and consequently the shear stress was computed.

Referring to spreadability, the two-way ANOVA revealed significant main effects for formulation ratio on spreadability ($p < 0.05$). The spreadability as illustrated in **Figure 3** changes as follows: As the proportion of PL content rises, the initial weight or the spreadability too rises. Surprisingly the 1:0 ratio that is the pure modified orahesive base has an initial spreadability of 1.5 cm and for the 0:1 ratio (pure PL) it was 3.1 cm of initial spreadability, indicating better spreadability with higher PL content.

The one-way ANOVA for shear stress indicated significant differences across the formulation ratios ($p < 0.05$). As shown in **Figure 4,** the results reveal the fact that as the PL content increases, the shear stress decreases and the mucoadhesion property decreases. The evidence is that the 1:0 ratio showed the highest shear stress of 360 g for the modified pure base and thus the strongest mucoadhesion property. Then, a high value of mucoadhesive capacity and shear stress was also achieved at 340.8 g with a ratio of 1:1. However, the lowest shear stress was shown with the 0:1 ratio (pure PL) that recorded a value of 111.5 g, thus the lowest mucoadhesion property among all the prepared gel formulations.

Additionally, a spreadability test was performed for both CAL and CHX/DPA mucoadhesive gels. Independent t-tests comparing CAL and CHX/DPA gels at each weight level revealed significant differences ($p < 0.05$). The results presented in **Figure 5** indicated that CAL mucoadhesive gel had higher spreadability compared to the CHX/DPA gel at all weights tested. Specifically, the diameter of CAL gel after 5 minutes of spreading ranged from 1.43 to 1.53 cm, while the diameter of the CHX/DPA gel ranged from 1.13 to 1.3 cm. Furthermore, the results also suggest that the weight applied has little effect on spreadability, as the diameter measurements remained relatively consistent across all weights tested. Regarding the agar plate test, there was displacement was observed for the prepared gels.

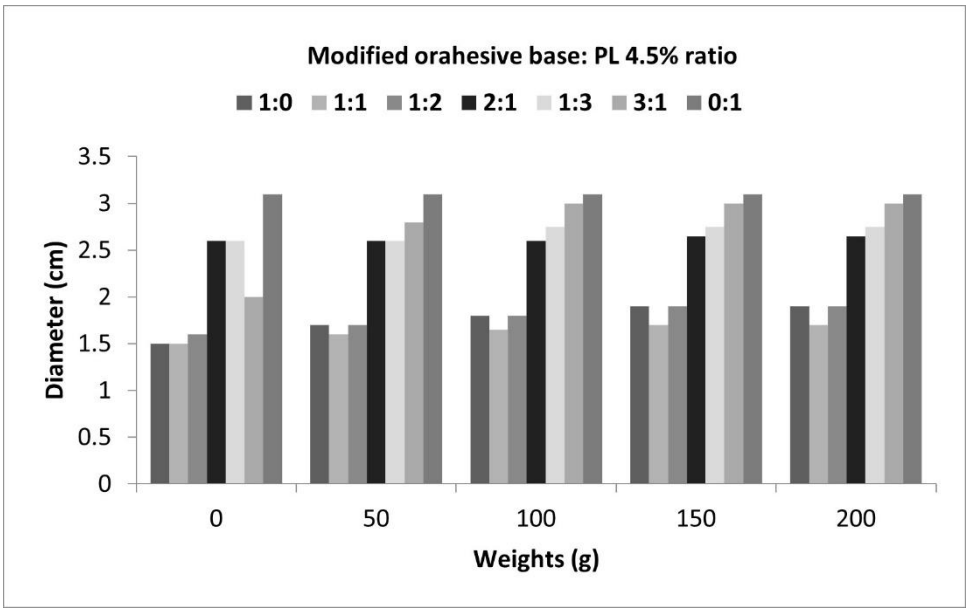

Figure 3. Spreadability study of placebo mucoadhesive gel formulations prepared using different ratios of modified orahesive base and PL.

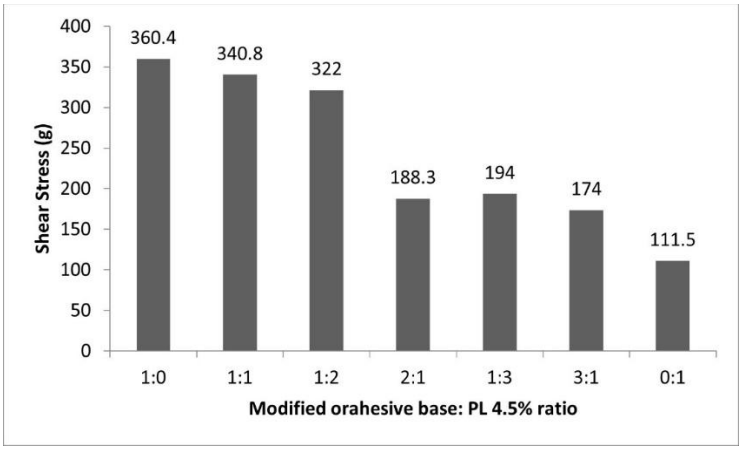

Figure 4. Shear stress study of placebo mucoadhesive gel formulations prepared using different ratios of modified orahesive base and PL.

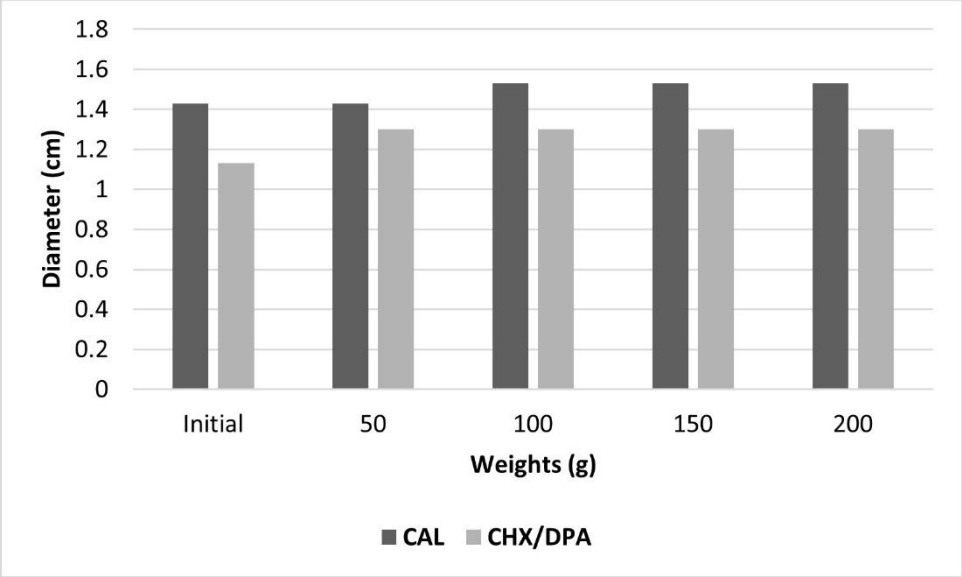

Figure 5. Spreadability study of CAL and CHX/DPA mucoadhesive gels.

3.4. Ex vivo mucoadhesion study

sIn this study, the mucoadhesion of two formulations, CAL gel and CHX/DPA gel, was evaluated using an ex vivo model mucosa. CAL gel formulation demonstrated strong adhesiveness to the oral mucosa for more than 6 h, whereas CHX/DPA gel formulation was completely detached after 4 h.

3.5. In vivo clinical evaluation of combined topical therapy of CAL and DPA/CHX gel formulations on palatal wound healing

The observations in this study demonstrated that the pain level decreased significantly in the patients of the first group and this is evident from their VAS scores. The first group also showed an excellent positive result in terms of wound healing. This is evidenced by significant wound closure and substantial reduction in wound area that occurred between day 7 and day 14 after surgery in the first group.

4. Discussion

4.1. Antimicrobial screening and determination of MIC

Following the National Committee for Clinical Laboratory Standards (NCCLS), a test compound can be identified as bacteriostatic or bactericidal against a specific organism. A test substance is found to be bactericidal if its MIC equals the MBC. On the other hand, if the MBC is greater than the MIC, the test substance is confirmed to be bacteriostatic. Likewise, if MIC equals MFC, the test substance is regarded as a fungicide. Accordingly, as shown in **Tables 3** and **4**, CAL extract, CAL gel, and CHX/DPA gel exhibit bactericidal activities against both Gram-negative and Gram-positive bacteria, along with fungicidal activity, demonstrating a broad spectrum of both antibacterial and antifungal activities.

4.2. In vivo palatability and mucoadhesion study

Palatability and mucoadhesion study showed that the modified orahesive base: PL ratios had an impact on the tolerability, acceptance, and mucoadhesion scores of the volunteers. The selected concentration was the 1:1 ratio (4.5% PL), as it demonstrated superior results in terms of mucoadhesion, comfort, and satisfaction regarding taste and odor, as detailed in **Figure 1**.

4.3. In vitro evaluation of mucoadhesive gel formulations

4.3.1. Determination of pH

It was found that the pH values of the mucoadhesive gels can have an impact on their performance in various applications, including oral wound healing. In general, the pH of a mucoadhesive gel should be close to the pH of the target tissue to minimize irritation and promote healing (Maslii et al., 2020; Rençber et al., 2022; Wahyuni et al., 2024). The measured pH was slightly lower in the case of placebo gel which was 5.19 ± 0.03 compared to CAL and CHX/DPA gels which was 5.63 ± 0.04 and 5.60 ± 0.02, respectively. This could be attributed to the loading of the placebo-modified mucoadhesive gel with either CAL or CHX/DPA, which results in little change in pH. Nevertheless, all pH measurements are suitable because they fall within the reported acceptable range for oral topical application, which is 4.1 to 7.9 (Toma et al., 2021; Wahyuni et al., 2024).

It was reported that CAL extract is often used to treat wounds because it has antibacterial, anti-inflammatory, and antioxidant effects. In addition, several studies have demonstrated the beneficial impact of CAL adhesive gels in accelerating oral wound healing (Cedillo-Cortezano et al., 2024; Nakamura-García et al., 2022). The prepared loaded adhesive gels would be ideal for wound healing than the placebo gel, as their pH values were slightly higher than the placebo gel. This higher pH is compatible with the pH of the oral mucosa, which ranges from 5.5 to 7.5. As a result, this optimal pH would promote the growth of new tissue while preventing bacterial growth, thus accelerating oral wound healing (Ahmed et al., 2021; Pagano et al., 2020; Toma et al., 2021).

4.3.2. Determination of viscosity

As mentioned in other studies, the simple addition of plant extract or any other compound to a gel may change the viscosity of the gel. For the same reason, the lower viscosity of the CAL gel compared to that of the placebo gel might have been due to the replacement of the semisolid CAL extract in the placebo gel. Moreover, the difference in the viscosity of the CHX/DPA gel compared to the placebo gel can be attributed to the solid constituent the CHX and DPA which was mixed into the placebo gel (Mohammadi, 2008; Wahyuni et al., 2024).

4.3.3. Evaluation of in vitro water uptake and erosion characteristics of the loaded mucoadhesive gel preparations

Mucoadhesive gels have been employed in a number of tissue repair, wound healing, and drug delivery approaches (Dawoud et al., 2019; Fathalla et al., 2022). Hence, the response of mucoadhesive gels is principally focused towards how well they can maintain their integrity at the mucosal site. Since the results of the erosion test confirmed that the prepared gels are stable for the entire duration of the test, they will be useful in application where there is prolonged adhesion to mucosal tissue.

4.3.4. Evaluation of the shear stress, adhesiveness, and spreadability

Another factor to be discussed with regard to mucoadhesive gels is their spreadability. A mucoadhesive gel with acceptable spreadability can easily be applied to the wound site and it can adhere nicely to the mucosal tissue to stay there for a long period of time. This property is favorable for wound healing (Syed et al., 2022; Wahyuni et al., 2024). According to the outcomes described in the current work, these formulations are quite useful when it comes to the possibilities of their application in oral wound healing.

From the results presented in **Figures 3** and **4**, the differences in the observed spreadability (initial weight) and the shear stress can be discussed in terms of the ratio of the modified orahesive base to PL used in the formulations and this observation is aligned with that previously reported (Andrews et al., 2009; Berretta et al., 2013). Results showed that as the proportion of PL content rises, the initial weight or the spreadability too rises. This finding is consistent with previously published research (Bassi da Silva et al., 2018).

Shear stress measures the mucoadhesion strength and the capacity of the gel to disfigure or move as soon as force is applied. Shear stress is directly proportional to the mucoadhesive properties, the higher the shear stress, the better the mucoadhesive property of the prepared formulation (Andrews et al., 2009; Bassi da Silva et al., 2018; Berretta et al., 2013). From the results presented, we could conclude that as the PL content increases, the shear stress decreases and the mucoadhesion property decreases. These results are in agreement with the other published study (Bassi da Silva et al., 2018). In conclusion, in a specific ratio of 1:1 case, the spreadability test was quite better, the shear stress values were adequate, and mucoadhesive properties were also significantly higher proving that the cohesiveness and adhesiveness of the formulations were just in balance and harmony.

Concerning the increased spreadability of the CAL mucoadhesive gel compared to CHX/DPA gel, it may be attributed to the presence of CAL semisolid extract. This may have contributed to the gel's ability to spread more easily, which could lead to better performance in promoting oral wound healing (Wahyuni et al., 2024).

It was also observed that spreadability wasn't affected by changing the applied weights. This suggests that the spreadability of the gels may be more dependent on their composition and formulation rather than the weight applied during application (Syed et al., 2022). Moreover, this finding demonstrates that the application with the least stress will have the highest spreadability effect on the prepared gel (Labib & Aldawsari, 2015).

It was noted that the spreadability values of the prepared gels (1.43–1.53 cm for CAL gel and 1.13–1.3 cm for CHX/DPA gel) are lower than that reported in the literature for oral mucoadhesive gels (2.5–8.0 cm) (Bassi da Silva et al., 2018; Charyulu et al., 2013; Pintea et al., 2022; Thorat et al., 2015; Venugopal et al., 2023; Wahyuni et al., 2024). This reduction is attributed to the deliberate optimization of viscosity and cohesive strength, which enhances mucoadhesion and retention on the mucosal surface. Lower spreadability correlates with increased viscosity and cohesive strength, which are critical for the formulation's clinical efficacy.

These findings were supported by the fact that no displacement was observed on agar plates. Accordingly, these tests confirmed good adhesiveness of the prepared gel formulations, and this may predict an excellent in vivo mucoadhesive profile.

4.4. Ex vivo mucoadhesion study

Mucoadhesion is a critical property for oral wound healing formulations as it ensures prolonged contact between the gel and the oral mucosa, facilitating the delivery of active ingredients and promoting wound healing (Sabale et al., 2014).

The prolonged adhesion determined by CAL gel indicates the potential of the CAL formulation to remain in contact with the wound site, allowing for sustained release of therapeutic agents and facilitating the healing process. The findings of this study are consistent with previous research highlighting the mucoadhesive properties of CAL-based formulations (Emre et al., 2018; Sabale et al., 2014). CAL extract has been shown to possess adhesive properties due to the presence of polysaccharides, such as arabinogalactans and pectins, which interact with the mucosal surfaces (Sabale et al., 2014; Schmidgall et al., 2000). Moreover, the anti-inflammatory and wound healing effects of CAL extract have been extensively reported, making it a promising ingredient for oral wound healing formulations (Chanaj-Kaczmarek et al., 2020; El-Sayed et al., 2021; Ferreira et al., 2023; Leach, 2008; Preethi & Kuttan, 2009).

It is worth noting that the choice of the base material and the specific ratio used in the formulations can significantly impact the mucoadhesive properties. In this study, we specifically employed a modified orahesive base combined with 4.5% PL in a 1:1 ratio for all formulations. This particular ratio was selected based on our previous mucoadhesion study suggesting its potential to enhance mucoadhesive properties. By utilizing the 1:1 ratio of modified orahesive base to PL, we aimed to optimize the mucoadhesive behavior of the formulations and achieve a balanced combination of adhesive strength and formulation stability.

4.5. In vivo clinical evaluation of combined topical therapy of CAL and DPA/CHX gel formulations on palatal wound healing

These results from the clinical trial support and reinforce the prominent role of the combined therapy of CAL and CHX/DPA gel in wound healing during the second week after surgery. The results of this study further confirm that the reduction in wound size that occurred in the group treated with CAL-based gel is mainly attributed to CAL and its notable anti-inflammatory and wound healing properties, thereby making it significantly potent and effective during later stages after surgery. Besides, the coadministration of CHX/DPA gel provided additional antimicrobial, hydration, and moisturizing effects that resulted in enhanced overall efficacy. This suggests that the combined therapy of CAL and CHX/DPA gels could be a valuable approach in clinical settings, especially for accelerating and enhancing wound healing after palatal surgery (El-Sayed et al., 2021).

5. Conclusion

This study discussed in detail the successful preparation and evaluation of a herbal delivery system which is a modified bioadhesive base gel loaded with CAL extract and CHX/DPA. The prepared bioadhesive gels showed strong mucoadhesive properties and thus would remain for a long time at the application site. They also showed a wide spectrum of both antibacterial and antifungal activity.

The mucoadhesive gel formulations were prepared using different ratios of modified bioadhesive base and PL, then characterized and showed differences in shear stress, mucoadhesion, and spreadability. The 1:1 ratio was selected as it showed good spreading properties, high shear stress, and favorable mucoadhesion. Moreover, this selected ratio showed the best satisfaction regarding odor and taste.

The stability of the gel on mucosal surfaces was confirmed by a 3-hour erosion test, indicating its potential benefit for extended residence periods in mucus-lined tissues. The pH of the mucoadhesive gel formulations was within the appropriate range making it suitable for wound healing in the oral cavity. The slightly higher pH value of the CAL mucoadhesive gel compared to the placebo-modified orahesive gel may be advantageous for promoting oral wound healing.

Moreover, the CAL mucoadhesive gel exhibited strong mucoadhesion to the model mucosa for more than 6 h, while the CHX/DPA gel formulation detached after 4 h. This highlights the potential of the CAL gel to facilitate the sustained release of therapeutic agents and enhance wound healing.

Furthermore, a randomized controlled clinical trial confirmed that the combined topical therapy of CAL with CHX/DPA showed promising results in improving wound healing in patients after palatal surgery. In conclusion, the combined topical therapy of CAL and CHX/DPA mucoadhesive gels could be valuable and promising in accelerating wound healing for postoperative palatal surgery patients. Further studies are needed to ascertain the enduring stability and shelf-life of the gel formulation under various storage conditions.

Disclosure

The authors declare that they have no conflicts of interest in this work.

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List of abbreviations

CAL: Calendula Officinalis

CMC-Na: Carboxymethyl Cellulose Sodium

CHX: Chlorhexidine

DPA: D-Panthenol

EMA: European Medicines Agency

IZ: Inhibition Zone

MBC: Minimal Bactericidal Concentration

MFC: Minimal Fungicidal Concentration

MIC: Minimum Inhibitory Concentration

NCCLS: National Committee for Clinical Laboratory Standards

PB: Phosphate Buffer

PL: Polycarbophil

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