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Formulation and Evaluation of Transdermal Gel with Wound Healing and Anti-inflammatory Properties Derived from *Calendula officinalis*

Deepak Prashar[1*], Anu Sharma[2], Akshay Kumar[2], Aaditya Guleria[2], Krishan Chand[2], Anita Kumari[2]

[1]Department of Pharmacy, LR Institute of Pharmacy, Jabli-Kyar, Solan HP India
[2]Department of Pharmacy, Shanti Niketan College of Pharmacy, Mandi (HP) India

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Abstract: *Calendula officinalis* (pot marigold) has been traditionally used in folk medicine for its prominent wound healing and anti-inflammatory properties. This study aimed to formulate and evaluate transdermal gel formulations (F1-F5) containing standardized *Calendula officinalis* extract. Five different formulations were prepared using various gelling agents and carriers to optimize the physicochemical and biological properties. The gels were evaluated for appearance, pH, viscosity, spreadability, *in vitro* permeation, stability, and anti-inflammatory activity. Results demonstrated that formulation F3, containing Carbopol 940 as the gelling agent, exhibited superior performance with optimized viscosity, spread ability, and consistent drug release profile. *In vitro* wound healing assay revealed significant fibroblast proliferation with F3 formulation, while anti-inflammatory studies showed inhibition of TNF-α and IL-6 cytokine production comparable to standard anti-inflammatory agents. Stability studies confirmed the robustness of the formulations under accelerated storage conditions. The findings validate the potential of Calendula derived transdermal gel as a promising therapeutic agent for topical wound healing and inflammatory skin conditions.
Keywords: Calendula officinalis, Transdermal gel, Wound healing, Anti-inflammatory, Topical delivery

Introduction

Skin injuries and inflammatory skin conditions represent a significant clinical burden affecting millions of individuals worldwide [1]. The complex process of wound healing involves multiple biological cascades including hemostasis, inflammation, proliferation, and remodeling phases [2]. Conventional treatment modalities often present limitations, including systemic absorption, side effects, and inadequate local delivery to the affected site [3]. Consequently, there is growing interest in plant derived therapeutics that offer enhanced efficacy with reduced adverse effects.

Calendula officinalis (Asteraceae family), commonly known as pot marigold, is a flowering plant with a rich historical background in traditional medicine across various cultures [4]. The plant has been used for centuries to promote wound healing, reduce inflammation, and treat various dermatological conditions [5]. The bioactive constituents of Calendula include flavonoids (particularly quercetin and isorhamnetin), terpenoids, triterpenes, carotenoids, and polysaccharides, which collectively contribute to its therapeutic properties [6, 7].

Figure 1: Flower of Calendula officinalis

Recent scientific investigations have substantiated the traditional uses of Calendula through multiple mechanisms of action [8]. The wound healing properties are mediated through enhanced fibroblast proliferation, increased collagen synthesis, and modulation of growth factors such as fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β) [9]. Furthermore, the anti-inflammatory effects are attributed to the inhibition of pro inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), through suppression of nuclear factor kappa B (NF-κB) signaling pathways [10]. Transdermal and topical gel formulations offer distinct advantages for localized drug delivery, including non-invasive administration, reduced systemic toxicity, improved patient compliance, and sustained therapeutic effects [11]. The development of an optimal transdermal gel requires careful selection of gelling agents, penetration enhancers, and preservatives to balance viscosity, spread ability, permeation rate, and stability [12]. This study was designed to formulate and comprehensively evaluate Calendula derived transdermal gels with optimized physicochemical properties and enhanced therapeutic efficacy.

Materials and Methods

Materials

Dried *Calendula officinalis* flowers were procured from botanical suppliers and authenticated by the Botany Department of the Research Institute. Carbopol-940, Carbopol-934, and HPMC K4M were obtained from Sigma-Aldrich (USA). Propylene glycol, isopropyl alcohol, glycerin, and sodium hydroxide were purchased from Merck (India). Methyl paraben and propyl paraben were obtained from SD Fine Chem Limited (India). Double-distilled water was used throughout the study. All materials were analytical or pharmaceutical grade.

Preparation of Calendula Extract

Calendula officinalis flowers (100 g) were extracted using 70% hydroethanolic solvent through maceration at room temperature for 14 days with periodic stirring. The extract was filtered through Whatman filter paper No. 41 and concentrated using a rotary evaporator under vacuum at 45°C. The yield was approximately 18% w/w. The extract was standardized to contain 5% w/w flavonoids (as quercetin equivalent) using high performance liquid chromatography (HPLC) [13].

Formulation of Transdermal Gels

Five different transdermal gel formulations were prepared with varying gelling agents and excipients. All formulations contained standardized *Calendula officinalis* extract (5% w/w). The formulations were prepared using the hot and cold method. Briefly, gelling agents were dispersed in cold distilled water, followed by the addition of other components. The mixture was gently heated (40-45°C) while stirring continuously until a homogeneous gel was formed. The pH was adjusted to 6.5-6.8 using sodium hydroxide. Preservatives (methyl paraben 0.18% and propyl paraben 0.02%) were added prior to cooling. The detailed composition of each formulation is presented in Table 1.

Table 1: Composition of *Calendula officinalis* Transdermal Gel Formulations (F1-F5)

Ingredients	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)	F4 (%w/w)	F5 (%w/w)
Calendula extract	5.0	5.0	5.0	5.0	5.0
Carbopol-940	1.5	0	1.0	0	0
Carbopol-934	0	1.0	0	0	0

HPMC K4M	0	0	0	1.5	1.0
Propylene glycol	5.0	5.0	5.0	5.0	5.0
Glycerin	5.0	5.0	5.0	5.0	5.0
Isopropyl alcohol	3.0	3.0	3.0	3.0	3.0
Methyl paraben	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.

Physicochemical Evaluation

Organoleptic Properties

The prepared gels were assessed for appearance, color, odor, and consistency under standardized conditions.

pH Determination

The pH of each formulation was determined using a calibrated digital pH meter by immersing the electrode directly in the gel. Measurements were performed in triplicate at room temperature.

Viscosity and Spread ability

Viscosity was determined using a Brookfield viscometer (DV-II Pro) at 25°C using spindle #62 at 50 rpm. Spread ability was evaluated using the method of Hosmani and Vyas. One gram of gel was placed between two glass slides of standard dimension. A standard weight of 500 g was placed on the top slide, and the diameter of the spread gel was measured at 60 seconds [14]. Spread ability (S) was calculated using the formula:

$$S = (M \times L) / T$$

Where M is the mass (g),
L is the length traveled (cm
T is the time taken (seconds)

Drug Content Analysis

One gram of gel was dissolved in 50 mL of 70% ethanol and filtered. The filtrate was analyzed using HPLC to determine the content of Calendula extract (expressed as quercetin equivalents). The analysis was performed using a reversed phase C18 column with mobile phase comprising acetonitrile and 0.1% phosphoric acid (40:60 v/v) at a flow rate of 1 mL/min. Detection was performed at 360 nm.

***In Vitro* Permeation Studies**

Permeation studies were conducted using Franz diffusion cells with a 2 cm^2 effective diffusion area and 12 mL receptor compartment volume [15]. Freshly excised full thickness rat skin (Westar strain) was mounted between donor and receptor compartments. Each gel formulation (1 g) was placed in the donor compartment, and the receptor compartment was filled with phosphate buffer saline (PBS, pH 7.4) maintained at 37± 1°C with continuous magnetic stirring at 100 rpm. Samples (200 µL) were collected at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours) and analyzed using HPLC as described above. The cumulative amount permeated (Q) was calculated, and flux (J) and permeability coefficient (Kp) were determined from the slope of the Q vs. time plot.

Wound Healing Evaluation

Fibroblast Proliferation Assay

Human dermal fibroblasts (HDFs) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells were seeded in 96 well plates at a density of 5×10^3 cells/well. After 24 hours, cells were treated with various concentrations of gel extracts (0.5-10 µg/mL) for 48 hours. Cell proliferation was assessed using the MTT assay, with absorbance measured at 570 nm using a microplate reader.

Collagen Synthesis Assessment

Conditioned media from treated fibroblasts were collected and analyzed for soluble collagen content using the Sircol assay according to the manufacturer's protocol. Briefly, collagen was bound to Sircol dye reagent, precipitated, and redissolved in alkali reagent. The absorbance was measured at 540 nm.

Anti-inflammatory Evaluation

Cytokine Quantification

Human monocytes (THP-1 cells) were stimulated with lipopolysaccharide (LPS, 100 ng/mL) in the presence or absence of gel extracts (1-10 µg/mL) for 6 hours. Conditioned media were collected and analyzed for pro-inflammatory cytokines (TNF-α and IL-6) using enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Results were expressed as pg/mL.

Stability Studies

Stability studies were performed according to ICH guidelines [16]. Gel formulations were stored in airtight containers under accelerated storage conditions (40 ± 2°C / 75 ± 5% relative humidity) for three months. Samples were analyzed at 0, 30, 60, and 90 days for pH, viscosity, drug content, and microbial contamination. Results were compared to baseline values to assess degradation and formulation stability.

Results

Physicochemical Properties

All five formulations exhibited acceptable organoleptic properties with uniform, yellowish brown color, consistent gel appearance, and characteristic odor. The pH values ranged from 6.4 to 6.8, which is acceptable for topical application and non-irritating to the skin. Viscosity measurements showed that F3 (containing Carbopol 940 at 1% concentration) demonstrated the highest viscosity (18,500 cP), followed by F1 (15,200 cP) and F4 (14,800 cP). Spreadability values indicated that F3 possessed optimal spreadability (26.4 g.cm/s), facilitating ease of application. Drug content analysis confirmed uniform distribution of Calendula extract in all formulations, ranging from 4.8% to 5.2% w/w.

In Vitro Permeation Profile

Permeation studies revealed that F3 demonstrated the optimal release profile with a flux of 4.8 ± 0.3 µg/cm²/h and a permeability coefficient of 0.24 ± 0.02 cm/h. Cumulative permeation at 24 hours was 115 ± 8 µg/cm² for F3, representing sustained and controlled release of the active constituents. F1 and F2 exhibited higher initial release but faster depletion, while F4 and F5 showed prolonged but lower permeation rates, suggesting potential accumulation in the skin layers.

Wound Healing Properties

Fibroblast proliferation assays demonstrated dose dependent increases in cell viability with all gel formulations. At 10 µg/mL concentration, F3 induced the highest fibroblast proliferation (185% of control, $p<0.01$), followed by F4 (172% of control) and F2 (165% of control). Collagen synthesis assessment showed that F3 treated fibroblasts produced significantly higher collagen content (2.8 ± 0.15 µg/mL) compared to untreated controls (0.8 ± 0.10 µg/mL), indicating enhanced extracellular matrix deposition essential for wound healing.

Anti inflammatory Activity

Cytokine quantification studies revealed significant suppression of LPS induced TNF-α and IL-6 production by the gel formulations. Treatment with F3 at 10 µg/mL reduced TNF-α levels to 145 ± 12 pg/mL from 890 ± 45 pg/mL in untreated LPS stimulated cells (83.7% inhibition). Similarly, IL-6 levels were reduced to 210 ± 18 pg/mL from 1350 ± 60 pg/mL (84.4% inhibition). These anti-inflammatory effects were comparable to dexamethasone (positive control) at equivalent concentrations.

Stability Studies

All formulations demonstrated good stability under accelerated storage conditions. After 90 days, pH variations were minimal (±0.2 units), viscosity remained consistent (within 10% of initial values), and drug content remained above 95% for all formulations. Notably, F3 showed the least variation in all parameters, confirming its superior formulation stability. No microbial contamination was detected in any formulation throughout the study period.

Discussion

The development of transdermal gel formulations containing botanical extracts requires careful optimization of formulation components to achieve therapeutic efficacy while maintaining acceptable

physicochemical properties [17]. In this study, five distinct formulations were developed using different gelling agents to investigate their influence on gel characteristics and biological activity. The selection of gelling agents was based on their established use in topical pharmaceuticals and their potential to modulate drug release profiles [18]. The pH range (6.4-6.8) achieved across all formulations is conducive to topical application, maintaining the integrity of skin physiology while preventing microorganism proliferation [19]. The viscosity variations among formulations reflected the properties of the individual gelling agents, with Carbopol derived formulations demonstrating superior rheological properties. The optimal viscosity of F3 facilitated spread ability without compromising skin contact, an essential criterion for topical formulations [20].

In vitro permeation studies confirmed that the gelling matrix significantly influenced transdermal delivery. F3 controlled and sustained release profile is particularly advantageous for prolonged therapeutic action while minimizing fluctuations in local drug concentrations [21]. The permeability coefficients obtained suggest that all formulations can effectively penetrate the stratum corneum and epidermis, reaching viable skin layers where therapeutic effects manifest. The robust wound healing properties demonstrated in fibroblast proliferation assays align with the traditional uses of Calendula and previously published data on plant derived wound healers [22]. The flavonoids rich composition of the Calendula extract is known to stimulate fibroblast chemotaxis and proliferation through activation of growth factor signaling pathways. Enhanced collagen synthesis observed with F3 treatment is crucial for establishing mechanical strength and structural integrity in healing wounds [23].

The anti-inflammatory efficacy demonstrated in this study is consistent with the reported inhibitory effects of Calendula on NF-κB-mediated inflammatory responses. The suppression of TNF-α and IL-6, which are critical mediators of inflammation, suggests that the gel formulations effectively modulate the inflammatory phase of wound healing, a key determinant of wound resolution rates and scar formation [24]. The comparable efficacy to dexamethasone is noteworthy, as it supports the potential of Calendula as a natural alternative to synthetic corticosteroids for managing inflammatory skin conditions. The excellent stability profile across all formulations under accelerated storage conditions indicates that the incorporation of Calendula extract does not compromise the chemical or physical integrity of the gel matrix. The minimal degradation observed suggests that the hydroethanolic extraction method and formulation excipients provide effective preservation of the bioactive constituents. These findings support the potential for commercial development and long term storage without significant loss of efficacy.

Among the five formulations evaluated, F3 emerged as the optimal formulation due to its superior performance across all evaluation parameters. The combination of appropriate viscosity, excellent spread ability, controlled drug release, enhanced biological activity, and superior stability makes F3 the most promising candidate for further development and clinical evaluation.

Conclusion

This study successfully formulated and comprehensively evaluated five transdermal gel formulations (F1-F5) containing standardized *Calendula officinalis* extract. Formulation F3, based on Carbopol 940 as the gelling agent, demonstrated optimal performance characteristics including ideal viscosity, superior spread ability, sustained and controlled release profile, enhanced wound healing properties, and significant anti-inflammatory activity. The robust stability profile under accelerated storage conditions further supports its commercial viability. The biological assays confirmed the traditional wound healing and anti-inflammatory uses of Calendula while providing mechanistic insights into its therapeutic effects. These findings establish a rational scientific foundation for the development of Calendula based topical therapeutics for wound management and inflammatory skin conditions. Future studies should encompass clinical efficacy evaluation, skin irritation and sensitization testing, and *in vivo* wound healing models to further validate the therapeutic potential of the optimized F3 formulation.

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