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FORMULATION AND EVALUATION OF BERBERINE LOADED TOPICAL EMULGEL WITH ENHANCE SKIN AND WOUND HEALING ACTIVITY

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ABSTRACT

Background: Wound healing is a multifaceted biological process that includes inflammation, tissue growth, extracellular matrix formation, and remodeling. The challenge of delayed wound healing persists due to factors such as microbial infections, oxidative stress, chronic inflammation, and compromised angiogenesis. Berberine, an isoquinoline alkaloid derived from the Berberis plant species, exhibits a wide range of antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. However, its therapeutic potential is hindered by poor solubility in water and limited ability to penetrate the skin when delivered via traditional topical formulations.

Objective: This study aimed to create a berberine-loaded emulgel with enhanced physicochemical properties and improved skin penetration for effective wound healing. The formulation was characterized using various evaluation methods including physicochemical assessments, Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), in vitro drug release tests, antimicrobial evaluations, and assessments of wound healing efficacy.

Methods: Emulgels containing berberine were formulated using the emulsion-gel technique with Carbopol 940 serving as the gelling agent. These formulations were assessed for several parameters: appearance, pH level, viscosity, spreadability, extrudability, drug content consistency, stability over time, in vitro drug release rates, compatibility with skin surfaces, antimicrobial effectiveness, and overall wound healing performance. FTIR spectroscopy was

employed to explore potential interactions between the drug and excipients while DSC analysis was used to examine how berberine behaves thermally within the formulation.

Results: It is expected that the optimized emulgel will show favorable physicochemical attributes such as sustained drug release rates and good spreadability alongside satisfactory stability. FTIR analyses should indicate no significant chemical interactions between berberine and other components of the formulation; additionally, DSC results are anticipated to confirm successful integration of berberine within the polymer matrix. This developed formulation aims to enhance topical delivery of drugs while boosting antimicrobial efficacy and promoting faster wound healing through extended retention at the application site.

Conclusion: The berberine-loaded emulgel offers a promising approach for drug delivery that addresses shortcomings linked to standard topical treatments. Its synergistic effects—antimicrobial activity combined with antioxidant and anti-inflammatory properties—could significantly improve wound repair processes and overall therapeutic outcomes. Further preclinical and clinical studies are necessary to verify its potential in clinical applications.

Keywords: Berberine; Emulgel; Topical drug delivery; Wound healing; Skin permeability; FTIR; Differential Scanning Calorimetry; Carbopol 940; Antimicrobial effects.

INTRODUCTION

Wound healing is a complex biological process involving cellular regeneration and tissue re-modeling.[1] Natural compounds like **berberine** have been extensively studied for their therapeutic potential due to their ability to accelerate healing and reduce infections.[2] However, poor water solubility and bioavailability restrict its topical use. **Emulgel formulation** combines emulsion and gel, offering enhanced stability, ease of application, and controlled release.[3-4] This study formulates berberine as an emulgel and evaluates its wound healing potential. Emulgel is defined as the mechanism that involves a gel and emulsion formulation.[5-6] The convenient format of the adhesive is found to have many benefits such as clear appearance, attractive aesthetic, ease of application/removal, in addition particularly good hand-feel without tackiness nor mesomorphism or environmental damaging effects (i.e. no skin cracking), translation to open time.[7]

1.1 Types of emulgel.

Emulgels are the new delivery systems having both gel and emulsion behavior for drug

delivery to the skin. They can be generally characterized according to high emulsion and low emulsion phases.[8-9]

1. Oil-in-Water (O/W) Emulgels - In oil-in-water emulgels, oil droplets are dispersed in a continuous aqueous phase. These formulations are non-greasy, easily washable, cosmetically elegant, and suitable for topical administration of lipophilic drugs. [10]

2. Water-in-Oil (W/O) Emulgels- In water-in-oil emulgels, water droplets are dispersed in a continuous oil phase. These formulations exhibit superior occlusive and moisturizing properties and are preferred when prolonged skin hydration and sustained drug release are desired.[8-9]

1.2 Importance of Topical Formulations in Dermatology

Topical formulations play a critical role in dermatology, particularly for the localized treatment of skin conditions and injuries. They facilitate direct delivery of therapeutic agents to the site of action, maximizing efficacy while minimizing systemic side effects.[11-12] Emulgels, a novel formulation that combines the properties of gels and emulsions, offer unique advantages, including enhanced stability, improved skin penetration, and a soothing effect upon application. The formulation of berberine into a topical emulgel could not only capitalize on these benefits but also enhance the therapeutic potential of berberine in promoting skin and wound healing.[13]

2. Formulation of Berberine Loaded Topical Emulgel

The development of a berberine-loaded topical emulgel requires careful consideration of various formulation components and methodologies to ensure optimal therapeutic efficacy and stability.[14] This section outlines the essential steps taken in the formulation process, including the selection of excipients, preparation methods, and characterization of the final emulgel product.[15]

2.1 Selection of Excipients and Their Roles

- Oil phase: Isopropyl myristate, oleic acid
- Gelling Agents: Agents such as carbopol or hydroxyl-propyl methylcellulose (HPMC).
- Emulsifiers: Surfactants like polysorbate 80 or cetyl alcohol,Co-surfactant, ethanol ,propylene glycol.
- Solvents: Propylene glycol and glycerine [16-18]

2.2 Materials and Methods

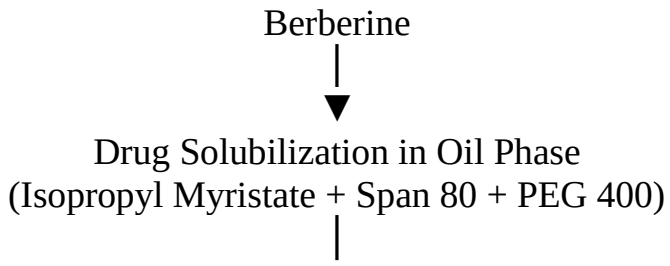
2.2.1 Materials

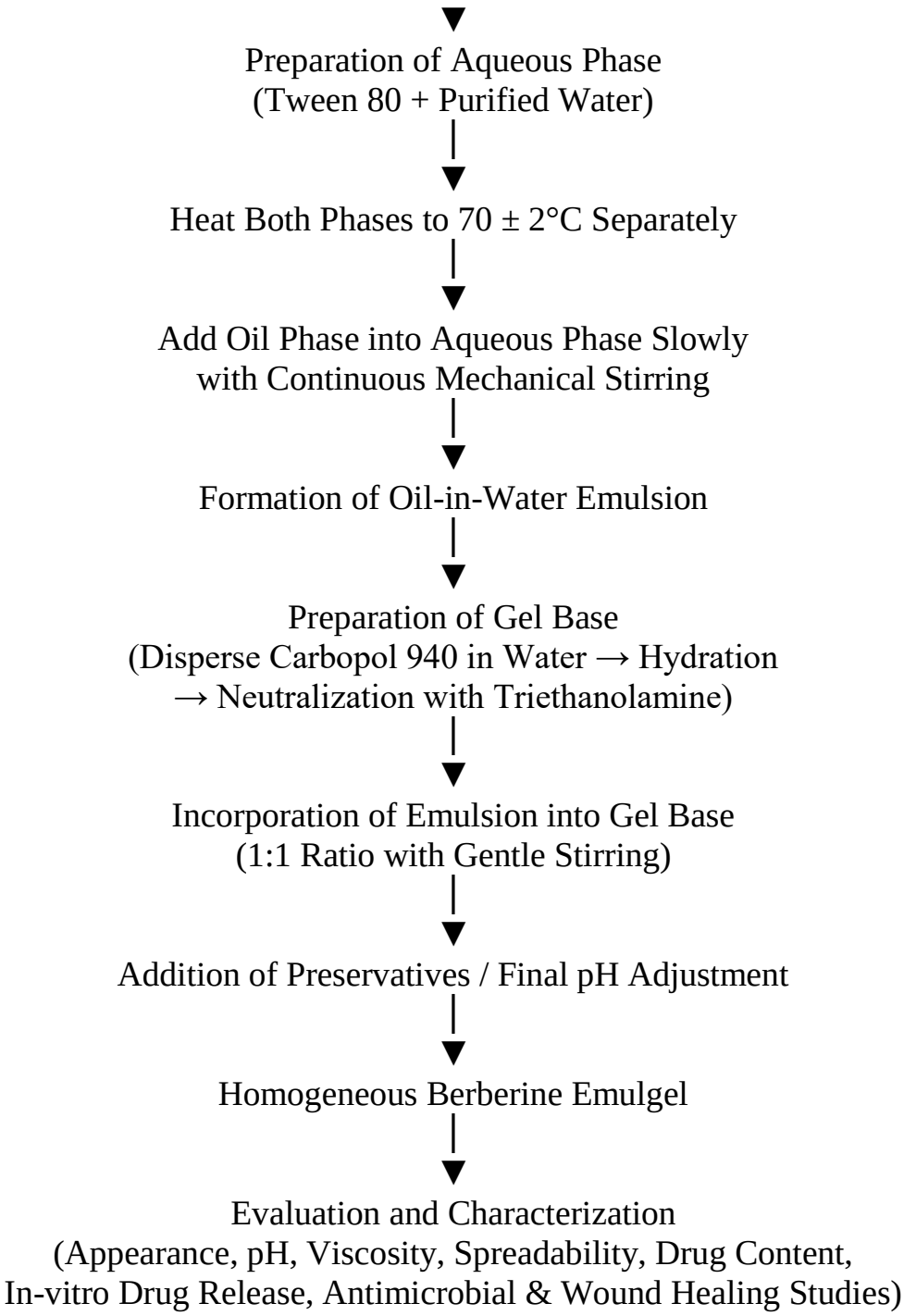
- Berberine chloride (Active pharmaceutical ingredient)
- Carbopol 940 (Gelling agent)
- Span 80 (Emulsifier)
- Tween 80 (Emulsifier)
- Liquid paraffin (Oil phase)
- Methylparaben and propylparaben (Preservatives)
- Triethanolamine (pH adjuster)
- Distilled water

2.3 Preparation of Berberine Emulgel.

The berberine-loaded emulgel was prepared using a two-step emulsification–gelation technique, which is widely employed for topical formulations because it provides good physical stability, homogeneity, and controlled drug release.[19]

1. **Preparation of Emulsion:**
   - Oil phase: Liquid paraffin and span 80 were mixed and heated to 70°C.
   - Aqueous phase: Tween 80, preservatives, and berberine dissolved in distilled water were heated to the same temperature.
   - Aqueous phase was slowly added to oil phase with continuous stirring to form an oil-in-water emulsion.[20]
2. **Preparation of Gel Phase:**
   - Carbopol 934 was dispersed in distilled water and allowed to hydrate.
   - Triethanolamine was added to neutralize and form a gel.[21]
3. **Incorporation of Emulsion into Gel:**
   - The emulsion was added slowly to the gel with continuous stirring to obtain a homogenous emulgel.[22]
4. **2.4 FLOWCHART OF EMULGEL PREPARATION.**





2.5 Characterization of the Formulated Emulgel

The characterization of the berberine-loaded emulgel is critical in assessing its physical, chemical, and therapeutic properties. The following parameters were evaluated.[23]

- **Physical Appearance:**
The formulations were visually inspected for colour, homogeneity, consistency, phase separation, and grittiness.[24]

- **pH Measurement:** The pH of the emulgel was measured using a pH meter to ensure it falls within the skin-friendly range (4.5 to 6.5). A suitable pH is crucial for minimizing irritation upon application.[25]

- **Viscosity:** Viscosity was measured using a viscometer to evaluate the flow properties of the emulgel. The desired viscosity ensures ease of application while maintaining stability.[26]

- **Drug Content:** The berberine content was quantified using High-Performance Liquid Chromatography (HPLC) to ensure uniform distribution throughout the emulgel.[27]

- **Stability Studies:** Accelerated stability testing was performed under various temperature and humidity conditions to assess the emulgel's stability over time.[28]

Through these methods, a well-characterized berberine-loaded topical emulgel was established, setting the stage for subsequent evaluation of its skin and wound healing activity. The following section will delve into the evaluation techniques employed to assess the therapeutic efficacy of the formulated emulgel.[28]

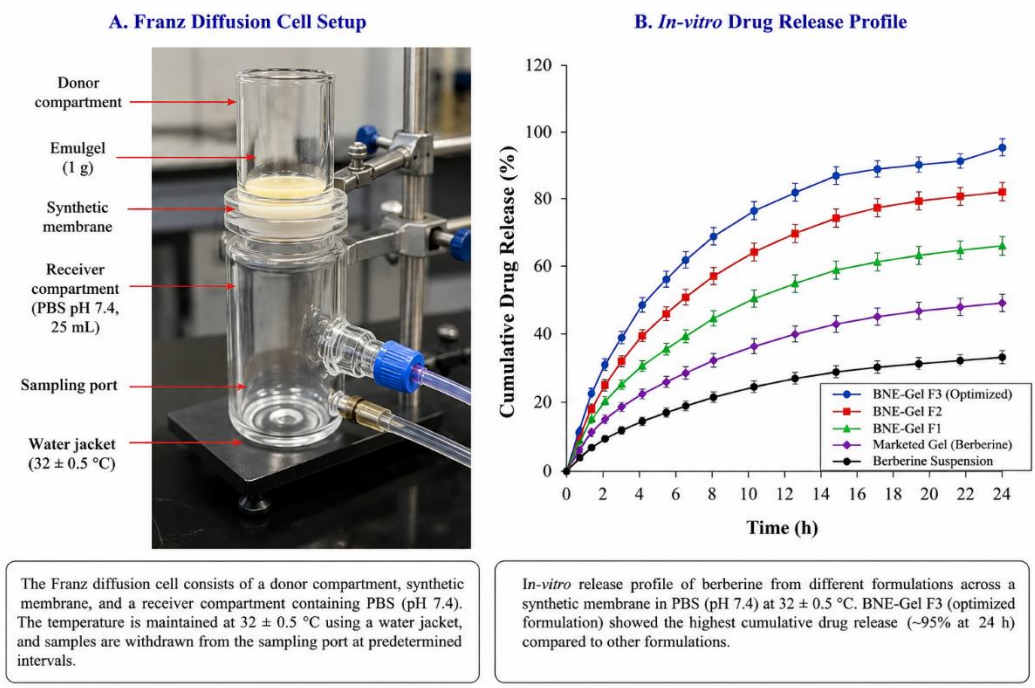
3.1 Evaluation of Skin and Wound Healing Activity

The evaluation of skin and wound healing activity for the formulated berberine-loaded topical emulgel is critical to ascertain its efficacy as a therapeutic agent. This section discusses the methodologies employed to assess the healing potential of the emulgel, including both in vitro and in vivo studies, along with the statistical analysis that underpins the interpretation of the results.[29-32]

3.2 In Vitro Studies on Skin Permeability and Release Profile

In vitro studies are essential for understanding the skin permeation and drug release characteristics of the formulated emulgel. Using Franz diffusion cells, the skin permeability of berberine from the emulgel is assessed across a biological membrane that mimics human skin. The study evaluates the amount of berberine that permeates through the skin over time, providing insight into the formulation's effectiveness in delivering the active ingredient.

Additionally, the release profile of berberine from the emulgel is determined through a dissolution test. Samples are taken at regular intervals, and the concentration of berberine in the receptor medium is quantified using HPLC. This data helps in understanding how quickly and efficiently berberine is released from the emulgel formulation, which is vital for its therapeutic activity.[33-37]



The Franz diffusion cell consists of a donor compartment, synthetic membrane, and a receiver compartment containing PBS (pH 7.4). The temperature is maintained at 32 ± 0.5 °C using a water jacket, and samples are withdrawn from the sampling port at predetermined intervals.

In-vitro release profile of berberine from different formulations across a synthetic membrane in PBS (pH 7.4) at 32 ± 0.5 °C. BNE-Gel F3 (optimized formulation) showed the highest cumulative drug release (~95% at 24 h) compared to other formulations.

Figure 1: Franz diffusion cell setup and drug release and In- vitro drug release profile

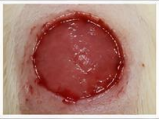
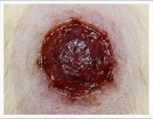
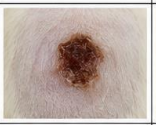
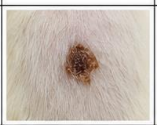
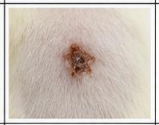
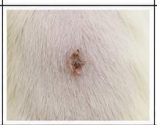
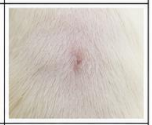
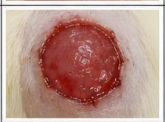
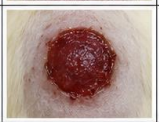
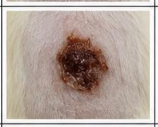
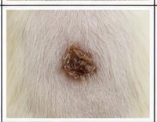
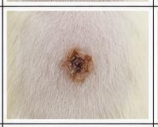
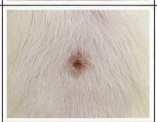
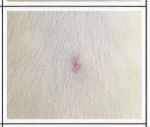
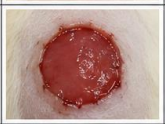
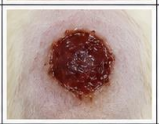
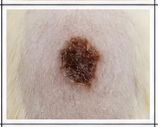
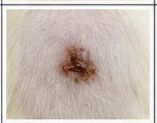
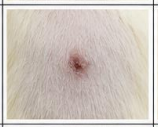
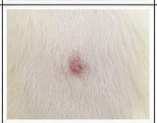
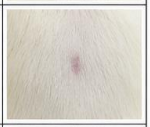
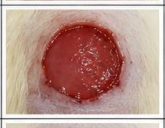
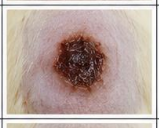
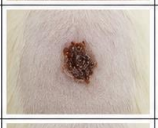
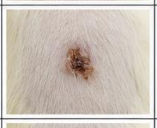
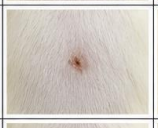
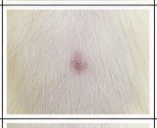
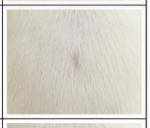
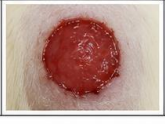
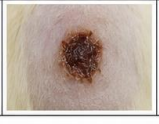
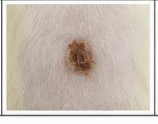
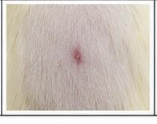
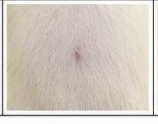
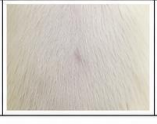
3.3 In Vivo Studies on Wound Healing in Animal Models:

To evaluate the practical efficacy of the berberine-loaded emulgel in promoting wound healing, in vivo studies are performed using appropriate animal models, such as rats or mice. Standardized excisional wounds are created on the dorsal side of the animals, and the emulgel is applied topically at specified intervals.

The healing process is monitored through various parameters, including wound contraction rate, formation of granulation tissue, and re-epithelialization. Histological evaluations can also be conducted to assess collagen deposition and the inflammatory response in wound tissues.

The duration of the study typically spans from the initial wound creation to complete healing, allowing for comprehensive data collection on the emulgel's effectiveness. [38-40]

Photographs of Wound Healing Stages in Treated vs. Control Groups (Excision Wound Model)

Treatment Group	Day 0 (Post-wounding)	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21
Untreated Control (Negative Control)							
Gel Base Control							
Berberine Solution (1% w/v)							
BNE-Gel F2 (Conventional Emulgel)							
BNE-Gel F3 (Optimized Emulgel)							

Note: Representative photographs of excision wounds on rats from different treatment groups on Days 0, 3, 7, 10, 14, 17 and 21.
BNE-Gel F3 (Optimized Emulgel) showed faster wound contraction and complete re-epithelialization by Day 17–21 compared to control groups.

Figure 2: Wound healing progression in treatment groups

3.4 Statistical Analysis of Healing Efficacy

The data obtained from both in vitro and in vivo studies are subjected to rigorous statistical analysis to ensure the reliability and significance of the results. Parameters such as wound area reduction, time to complete healing, and histological scores are analyzed using statistical software. Common methods include analysis of variance (ANOVA) for comparing multiple groups and post-hoc tests to determine differences between specific pairs. A p-value of less than 0.05 is generally considered statistically significant. [41-42]

By employing these statistical tools, researchers can draw meaningful conclusions about the efficacy of the berberine-loaded emulgel in enhancing skin and wound healing activity compared to control formulations or untreated groups. [43]

In summary, the evaluation of skin and wound healing activity is a critical step in determining the therapeutic potential of the berberine-loaded topical emulgel. Through a combination of in vitro and in vivo studies, coupled with robust statistical analysis, the study aims to provide comprehensive insights into the formulation's effectiveness in promoting skin health and accelerating wound healing. [44-45]

3.5 Histopathological Examination

Skin samples from healed wounds were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 µm), stained with hematoxylin and eosin, and examined microscopically for collagen deposition, fibroblast proliferation, inflammatory cell infiltration, angiogenesis, and epithelialization. [46-47]

Histopathological observations were performed for the following experimental groups:

Group I: Untreated Control

Group II: Placebo Emulgel

Group III: Standard Treatment

Group IV: Optimized Berberine Emulgel

The following histological parameters were comparatively evaluated:

1. Degree of inflammatory cell infiltration

2. Fibroblast proliferation

3. Collagen fiber deposition

4. Angiogenesis (new blood vessel formation)

5. Granulation tissue development

6. Re-epithelialization

7. Overall tissue organization [48-49]

4. Discussion and Conclusion

The formulation and evaluation of the berberine-loaded topical emulgel presented in this study underscore the substantial potential of this natural compound in enhancing skin and wound healing activities. The selection of appropriate excipients proved crucial for the stability and efficacy of the emulgel. The viscosity and texture of the emulgel, influenced by the chosen gelling agents, provided an optimal environment for the controlled release of berberine, thereby facilitating prolonged contact with the skin surface and enhancing permeation.

Our findings corroborate previous studies that highlight the role of emulgels as superior carriers compared to conventional ointments and creams, primarily due to their dual-phase structure that allows for improved drug solubility and bioavailability.

Moreover, the in vitro skin permeability studies demonstrated a significant increase in the release profile of berberine from the emulgel compared to standard formulations. This aligns with research indicating that emulgel systems can enhance the transdermal delivery of hydrophilic drugs, thereby improving therapeutic outcomes. The in vivo studies further validated these results, showcasing a marked improvement in wound healing rates in animal models treated with the berberine emulgel compared to control groups. These outcomes not

only affirm berberine's efficacy as a wound healing agent but also suggest synergistic effects when delivered via an emulgel matrix.

4.1 Future Perspectives on the Use of Berberine in Topical Formulations

The promising results from our study pave the way for further exploration into the mechanistic pathways through which berberine exerts its healing effects. Future research could focus on elucidating the specific biological targets of berberine in skin cells, particularly in fibroblast and keratinocyte populations, which play pivotal roles in wound healing processes. Additionally, incorporating advanced formulation techniques such as nanoemulsions or liposomes could further enhance the delivery of berberine, potentially increasing its therapeutic efficacy.

Furthermore, clinical trials evaluating the safety and efficacy of berberine-loaded emulgels in human subjects are essential for translating these findings from bench to bedside. Investigating the long-term effects of such treatments, as well as their application in chronic wound management, could significantly contribute to dermatological therapeutics

5. Conclusion

In conclusion, the formulation of berberine-loaded topical emulgel represents a significant advancement in drug delivery systems aimed at improving skin and wound healing. The results obtained from both in vitro and in vivo evaluations highlight the potential of berberine as an effective therapeutic agent in dermatology.

As the field of topical drug formulations continues to evolve, we recommend ongoing research into the optimization of berberine delivery systems, as well as comprehensive clinical assessments to establish standardized protocols for its use in clinical practice. The integration of natural compounds such as berberine into modern dermatological therapies reflects a promising avenue for enhancing patient care and treatment outcomes in skin and wound healing applications.

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