

# Formulation and Evaluation of Miltefosine–Curcumin Transdermal Gel for Topical Treatment of Cutaneous Leishmaniasis

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## ABSTRACT

Cutaneous leishmaniasis (CL) is a neglected tropical disease affecting millions globally, characterized by disfiguring skin lesions. Oral miltefosine, though effective, causes systemic toxicity and teratogenicity, while curcumin, a natural polyphenol, offers anti-inflammatory and wound-healing properties with poor dermal bioavailability. In this study, we report the formulation and evaluation of a Carbopol-based hydrogel co-loaded with miltefosine (MTF) and curcumin (CUR) for topical delivery. The gels were optimized using varying polymer concentrations and permeation enhancers (propylene glycol and oleic acid). Physicochemical properties (pH, viscosity, spreadability, drug content), in vitro release, ex vivo permeation (porcine ear skin), skin retention, and biological activity (promastigote inhibition, amastigote reduction, keratinocyte cytotoxicity, skin irritation) were systematically investigated. The optimized gel (1% MTF + 1% CUR in Carbopol 940, with 10% PG and 5% OA) showed a pH of 5.8, pseudoplastic rheology, uniform drug content, sustained release over 24 h, and enhanced dermal retention while minimizing systemic permeation. Biological assays revealed synergistic anti-leishmanial efficacy, with significant intracellular amastigote clearance compared to single-drug formulations. Cytotoxicity studies confirmed biocompatibility, and no irritation was observed ex vivo. These findings suggest that MTF–CUR transdermal gel is a promising candidate for localized CL therapy.

**Keywords:** Cutaneous leishmaniasis, Miltefosine, Curcumin, Transdermal gel, Carbopol hydrogel, Franz diffusion, Topical therapy

## I. INTRODUCTION

### 1.1 Global burden of cutaneous leishmaniasis

Leishmaniasis, caused by protozoa of the genus *Leishmania* and transmitted via infected

female phlebotomine sandflies, remains one of the most neglected tropical diseases. Cutaneous leishmaniasis (CL) accounts for approximately 600,000–1,000,000 new cases annually, concentrated in the Middle East, South America, Africa, and South Asia [1,2]. CL manifests as ulcerative, chronic skin lesions that may persist for months to years, often leading to scarring, social stigma, and psychological distress [3]. Despite its non-lethal nature, CL has a high disability-adjusted life years (DALYs) burden, particularly among children and women in endemic zones.

### 1.2 Current treatment challenges

Systemic therapies such as pentavalent antimonials, amphotericin B, and oral miltefosine are commonly used for CL management. However, these treatments have significant drawbacks including parenteral administration (antimonials), systemic toxicity (hepatic, renal, and cardiac), teratogenicity (miltefosine), long treatment durations, and poor patient compliance [4,5]. Emergence of resistant *Leishmania* strains further complicates disease control [6].

Local therapies, such as intralesional antimonial injection, cryotherapy, thermotherapy, and topical formulations, provide site-specific benefits but have variable efficacy. A rationally designed topical formulation that combines direct anti-leishmanial activity with host tissue repair and minimal systemic toxicity would significantly improve CL management [7].

### 1.3 Rationale for miltefosine and curcumin combination

Miltefosine (hexadecylphosphocholine), initially developed as an anticancer agent, is the first oral drug approved for leishmaniasis. Its mechanisms include disruption of parasite lipid metabolism, mitochondrial dysfunction, induction of apoptosis-like death, and immunomodulation

[8,9]. However, oral miltefosine is limited by gastrointestinal side effects, teratogenicity, and risk of resistance due to its long half-life [10]. Delivering miltefosine topically can localize its action while reducing systemic exposure.

Curcumin, derived from *Curcuma longa*, is a hydrophobic polyphenol with reported antioxidant, anti-inflammatory, antimicrobial, and wound-healing activities [11]. It also exhibits direct antileishmanial activity via generation of reactive oxygen species and interference with parasite survival pathways [12]. Curcumin's poor water solubility and bioavailability restrict therapeutic use; however, its incorporation in topical nanocarriers and hydrogels has shown enhanced dermal delivery [13].

The combination of miltefosine and curcumin in a topical formulation is hypothesized to:

1. Provide synergistic antiparasitic effects.
2. Enhance lesion healing through curcumin's anti-inflammatory and wound-healing actions.
3. Reduce required miltefosine dose, mitigating systemic risks.

#### 1.4 Aim of the study

The present research aimed to formulate and evaluate a Carbopol-based transdermal gel co-loaded with miltefosine and curcumin for topical treatment of CL. The specific objectives were:

- Develop stable hydrogel formulations with optimal drug loading.
- Characterize physicochemical properties including rheology, pH, spreadability, and drug content.
- Evaluate *in vitro* drug release and *ex vivo* skin permeation/retention using Franz diffusion cells.
- Assess *in vitro* biological activity against *Leishmania* promastigotes and intracellular amastigotes.
- Determine cytotoxicity to mammalian keratinocytes and potential skin irritation.

## II. MATERIALS AND METHODS

### 2.1 Materials

Miltefosine ( $\geq 99\%$  purity) and curcumin ( $\geq 95\%$ ) were procured from Sigma-Aldrich. Carbopol 940 (polyacrylic acid crosslinked polymer), propylene glycol (PG), oleic acid (OA), triethanolamine (TEA), and other analytical reagents were purchased from Merck India. Porcine ear skin was obtained fresh from a local abattoir. *Leishmania* major promastigotes and murine

macrophage RAW 264.7 cell line were used for biological assays. All solvents were HPLC grade.

### 2.2 Formulation of gels

Carbopol hydrogels were prepared by dispersing Carbopol 940 (0.6–1.2% w/w) in distilled water, allowing hydration overnight. Miltefosine was solubilized in a small volume of ethanol:PG (1:1), while curcumin was dissolved in ethanol and homogenized into a fine suspension. Both solutions were added gradually into hydrated Carbopol under magnetic stirring. PG (5–15%) and OA (1–5%) were incorporated as permeation enhancers. The gels were neutralized with TEA until pH 5.5–6.5 was achieved.

Formulation codes (F1–F9) varied by drug concentration (MTF 0.5–2%, CUR 0.5–2%) and enhancer content (PG 5–15%, OA 1–5%). A placebo gel (without drugs) was also prepared.

### 2.3 Physicochemical characterization

- **Appearance:** Visual examination for color, clarity, homogeneity, and phase separation.
- **pH:** Measured at 25 °C using a calibrated pH meter.
- **Viscosity & rheology:** Brookfield cone-plate viscometer at varying shear rates (0.1–100  $s^{-1}$ ).
- **Spreadability:** Glass slide–plate method; diameter of spread under 500 g load in 5 min recorded.
- **Drug content uniformity:** 1 g gel dissolved in methanol:water (70:30), sonicated, filtered, and analyzed via HPLC.

### 2.4 Stability studies

Optimized formulations were stored at 4 °C, 25 °C, and 40 °C for 3 months. Periodic evaluation of appearance, pH, viscosity, and drug content was conducted.

### 2.5 *In vitro* drug release

Franz diffusion cells (1.77  $cm^2$  surface area, 12 mL receptor) were used with cellulose acetate membrane. Receptor medium was PBS (pH 7.4) with 20% ethanol to maintain sink conditions. Gel equivalent to 10 mg MTF + 10 mg CUR was applied to the donor compartment. Aliquots (1 mL) were withdrawn at 1, 2, 4, 6, 8, 12, and 24 h, replaced with fresh medium, and analyzed by HPLC.

### 2.6 *Ex vivo* skin permeation and retention

Porcine ear skin was prepared by removing subcutaneous fat and mounted on Franz

cells. Gels were applied to the donor compartment. Receptor medium was PBS:ethanol (80:20). After 24 h, receptor fluid samples were analyzed. The skin was tape-stripped to separate stratum corneum, epidermis, and dermis, and drug content was extracted using methanol for quantification.

### 2.7 In vitro anti-leishmanial activity

- **Promastigote assay:** L. major promastigotes ( $1 \times 10^6$  /mL) were incubated with serial dilutions of gel extracts (MTF 0.1–10  $\mu$ M, CUR 1–20  $\mu$ M) for 72 h. Viability assessed by MTT assay.
- **Intracellular amastigote assay:** RAW 264.7 macrophages infected with promastigotes (MOI 10:1) for 6 h, then exposed to gel extracts for 48 h. Giemsa staining quantified intracellular parasite burden.

### 2.8 Cytotoxicity and skin irritation

- **Keratinocyte cytotoxicity:** HaCaT cells exposed to gel extracts; cell viability determined by MTT assay after 24 h.
- **Ex vivo skin irritation:** Porcine skin treated with optimized gel for 72 h, evaluated visually for erythema/edema and histologically (H&E staining).

### 2.9 Data analysis

Drug release kinetics fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. Statistical significance assessed using ANOVA with Tukey's post-hoc test ( $p < 0.05$  considered significant).

## III. RESULTS

### 3.1 Formulation and appearance

All formulations were homogenous, smooth, and free from grittiness. Curcumin imparted a yellow-orange color. Optimized formulations showed no phase separation.

### 3.2 Physicochemical properties

- **pH:** 5.5–6.3, compatible with skin physiology.
- **Viscosity:** 5–15 Pa·s at low shear; pseudoplastic flow observed.
- **Spreadability:** 3.5–4.5 cm, ensuring ease of topical application.
- **Drug content:** 95–103% of theoretical; RSD <3%.

### 3.3 Stability

Optimized gel (F7) retained homogeneity and >95% drug content at 25 °C for 3 months.

Slight pH decrease at 40 °C observed but within acceptable limits.

### 3.4 In vitro release

Cumulative release after 24 h:

- MTF:  $72 \pm 3\%$
- CUR:  $65 \pm 4\%$

Release followed Higuchi model ( $R^2 > 0.98$ ), indicating diffusion-controlled kinetics. Enhancers (PG + OA) increased release rate significantly ( $p < 0.05$ ).

### 3.5 Ex vivo permeation and retention

After 24 h, receptor fluid contained <5% of total applied drug, indicating minimal systemic permeation. Retention in epidermis and dermis was high:

- MTF:  $28 \pm 2 \mu\text{g}/\text{cm}^2$
- CUR:  $22 \pm 3 \mu\text{g}/\text{cm}^2$

OA and PG increased dermal retention by 2-fold compared to formulations without enhancers.

### 3.6 Anti-leishmanial activity

- **Promastigotes:** MTF  $IC_{50} = 2.8 \mu\text{M}$ ; CUR  $IC_{50} = 15.2 \mu\text{M}$ ; combination gel  $IC_{50}$  significantly lower ( $1.2 \mu\text{M}$  MTF +  $8.1 \mu\text{M}$  CUR equivalent).
- **Amastigotes:** Combination gel reduced intracellular parasite load by  $78\% \pm 4$  compared to 55% with MTF alone and 38% with CUR alone ( $p < 0.01$ ).

### 3.7 Cytotoxicity and skin irritation

HaCaT viability >85% at therapeutic concentrations. Ex vivo skin treated with optimized gel showed no visible erythema or edema, and histology confirmed intact epidermal layers.

## IV. DISCUSSION

This study demonstrates the feasibility of a Carbopol-based hydrogel delivering both miltefosine and curcumin for topical CL therapy. The optimized gel combined desirable physicochemical properties with effective local drug retention and significant antiparasitic efficacy.

### 4.1 Formulation considerations

Carbopol 940 was selected for its favorable gelling capacity, biocompatibility, and shear-thinning behavior that aids spreadability and residence on lesion surfaces [14]. The inclusion of PG and OA enhanced drug solubility and dermal

penetration, consistent with reports of their synergistic enhancer action [15].

#### 4.2 Release and permeation

Sustained release profiles align with diffusion-controlled mechanisms typical of hydrophilic gels [16]. Limited transdermal permeation with high dermal retention is ideal for CL, where parasites reside within dermal macrophages [17].

#### 4.3 Biological efficacy

The combination of MTF and CUR produced synergistic antiparasitic effects, reducing  $IC_{50}$  values significantly. Curcumin's anti-inflammatory and wound-healing effects complement miltefosine's direct antiparasitic action [18,19]. Such synergy could enable dose reduction of MTF, lowering toxicity risk.

#### 4.4 Safety profile

Cytocompatibility with keratinocytes and lack of irritation in ex vivo models suggest good tolerability. This is critical for CL patients, where lesions often occur on sensitive, exposed skin.

#### 4.5 Clinical implications and limitations

Topical delivery of MTF-CUR could offer a non-invasive, patient-friendly alternative to systemic drugs and intralesional injections. However, in vivo efficacy and safety studies in animal models (e.g., BALB/c mice infected with *L. major*) are necessary to confirm therapeutic benefit. Long-term stability and clinical pharmacokinetics must also be established.

### V. CONCLUSION

A novel transdermal gel co-loaded with miltefosine and curcumin was successfully formulated and characterized. The optimized formulation demonstrated sustained drug release, enhanced dermal retention, synergistic antiparasitic activity, and favorable safety. These findings support further in vivo and clinical development of this topical therapy for cutaneous leishmaniasis.

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