Improved Topical Delivery of Sorafenib-Meglumine Antimoniate: Elastic Nano-Liposomal Formulation for the Treatment of Cutaneous Leishmaniasis

Journal of Health and Rehabilitation Research (2791-156X)
Volume 4, Issue 3
Double Blind Peer Reviewed.
https://jhrlmc.com/
DOI: https://doi.org/10.61919/jhrr.v4i3.1287
www.lmi.education/
LINK MEDICAL INTERFACE

Nasir Khan[1,3], Muhammad Junaid Dar[1], Soumayya Aib[1], Sidra Khalid[2], Kashif Iqbal[1], Ghulam Razaque[4]

Correspondence
Kashif Iqbal
kashifiqbal321@yahoo.com

Affiliations
1 Faculty of Pharmacy, IBADAT International University, Islamabad, 45320, Pakistan
2 Drug Regulatory Authority of Pakistan
3 The University of Lahore, Islamabad, 45320, Pakistan
4 Faculty of Pharmacy, Baluchistan University, Quetta, Baluchistan, Pakistan

Keywords
Cutaneous leishmaniasis, sorafenib, meglumine antimoniate, transferosomes, nano elastic liposomes, topical drug delivery, skin permeation, anti-leishmanial therapy

Disclaimers

Authors' Contributions	All authors equally contributed to the study design, data collection, analysis, and manuscript preparation.
Conflict of Interest	None declared
Data/supplements	Available on request.
Funding	None
Ethical Approval	Institutional Animal Care and Use Committee with protocol number
Study Registration	BEC/0520
Acknowledgments	N/A

ABSTRACT

Background: Cutaneous leishmaniasis (CL) is a widespread parasitic disease with significant health and social impacts. Current systemic treatments have severe side effects, highlighting the need for effective topical alternatives.

Objective: This study aimed to develop and evaluate the effectiveness of sorafenib (SF) and meglumine antimoniate (MM) dual-loaded transferosomes (TRs) for topical treatment of CL.

Methods: TRs were prepared using a thin film hydration method and incorporated into a carbopol gel. The formulations were characterized for vesicle size, zeta potential, entrapment efficiency, and deformability index. In vivo and ex vivo skin permeation studies were conducted, along with anti-leishmanial efficacy testing on an intramacrophage amastigote model using BALB/c mice.

Results: The TRs exhibited a vesicle size of 186.1 ± 65.89 nm, zeta potential of -27.9 mV, and entrapment efficiency of 71.7 ± 3.9% for MM and 78.6 ± 4.2% for SF. Ex vivo studies showed enhanced skin permeation with cumulative permeation of 327.6 ± 29.4 µg/cm^2 for MM and 291.6 ± 28.3 µg/cm^2 for SF. In vivo results indicated a significant reduction in lesion size (0.1 ± 0.12 mm) and parasite load (2.7 ± 0.3 log scale).

Conclusion: The MM-SF dual-loaded TRs demonstrated effective topical delivery and therapeutic potential for CL, providing a promising alternative to systemic treatments.

INTRODUCTION

Leishmaniasis is a prevalent infectious disease caused by the Leishmania parasite and transmitted by sand flies, posing a significant public health challenge worldwide (1). According to the World Health Organization, around 12 million individuals are affected by various forms of leishmaniasis, with an estimated 60,000 deaths occurring annually due to complications associated with the disease (2). Among its forms, cutaneous leishmaniasis (CL) is particularly widespread, presenting as an infectious tropical disease that not only impacts the health of affected individuals but also imposes a considerable social burden, often receiving less attention compared to visceral leishmaniasis (3). Each year, approximately 1.5 million people are diagnosed with CL, further underscoring the critical need for effective and accessible treatments (4). The primary therapeutic agents for CL are pentavalent antimonial compounds, such as sodium stibogluconate, which are typically administered parenterally. However, their use is frequently associated with significant adverse effects, including nausea, vomiting, myalgia, and nephrotoxicity, requiring close monitoring and potentially limiting their broader application (5).

The limitations of current systemic therapies, such as the adverse effects and the need for parenteral administration, have driven the exploration of alternative treatment approaches, including topical formulations. Advances in nanotechnology have paved the way for the development of novel drug delivery systems that can enhance the therapeutic index of antileishmanial agents while minimizing systemic toxicity (6). Liposomal formulations, particularly nano elastic liposomes or transferosomes (TRs), represent a promising strategy for improving topical drug delivery by enhancing the penetration of active compounds through the skin layers (7). These vesicles, characterized by their deformable nature, can breach the stratum corneum, allowing for deeper penetration and localized delivery of drugs within the dermal layer where Leishmania amastigotes reside within infected macrophages (8). Furthermore, liposomes offer several advantages, including low toxicity, the ability to co-deliver hydrophilic and hydrophobic drugs, and prolonged retention at the site of administration compared to conventional formulations (9).

The introduction of elastic liposomes, such as TRs, in antileishmanial therapy has shown potential for overcoming the barriers associated with drug delivery to the deeper dermal layers where Leishmania parasites thrive (10). The flexibility of TRs, attributed to their unique composition and the incorporation of edge activators, enhances their capacity to deform and navigate through the narrow intercellular spaces of the skin, facilitating the targeted delivery of drugs directly to the infected macrophages (11).

Manuscript Submitted under JHRR ID 1287: July 19/2024 | Accepted: August 29/2024

Sorafenib (SF), a multi-targeted tyrosine kinase inhibitor initially approved for the treatment of liver cancer, has demonstrated promising antileishmanial activity against Leishmania species, including L. donovani and others implicated in CL (12). When combined with meglumine antimoniate (MM), a conventional antileishmanial agent, this dual-loaded TR formulation aims to exploit the synergistic effects of both drugs, potentially enhancing therapeutic efficacy while reducing the required doses and associated side effects (13).

Recent studies have highlighted the advantages of combining antileishmanial drugs within a single vesicular system, noting improvements in drug stability, permeability, and overall therapeutic outcomes (14). The dual-loading of SF and MM into TRs is designed to leverage the benefits of both agents, with SF targeting the kinase pathways crucial for Leishmania survival and MM disrupting the parasite's cellular functions (15). The vesicle size and deformability of the TRs are critical parameters that influence their ability to penetrate the skin and deliver drugs effectively to the site of infection (16). The combination of MM and SF within TRs is expected to provide a targeted, localized treatment option for CL, potentially offering a safer and more effective alternative to existing systemic therapies. The present study focuses on the development and characterization of MM-SF dual-loaded TRs, evaluating their physicochemical properties, in vitro and in vivo antileishmanial efficacy, and potential as a novel topical treatment for cutaneous leishmaniasis caused by Leishmania mexicana (17).

MATERIAL AND METHODS

The study adhered to ethical standards for animal research as outlined by the National Research Council's "Guide for the Care and Use of Laboratory Animals." Female BALB/c mice were obtained from the National Institute of Health (NIH), Islamabad, and housed under standard laboratory conditions, including a controlled temperature of 25 ± 1°C, 55 ± 5% humidity, and a 12-hour light/dark cycle. Mice were provided with a standard laboratory diet and fresh water ad libitum. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Lahore, Islamabad Campus, ensuring compliance with ethical guidelines for the humane treatment of laboratory animals.

Parasites used in the study were transgenic Leishmania mexicana expressing red fluorescent protein, isolated from the draining lymph nodes of previously infected BALB/c mice. These parasites were cultured in complete M-199 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% HEPES, and maintained at 25°C in culture flasks. Promastigotes were allowed to grow to the log phase before further experimentation.

The MM-SF dual-loaded nano elastic liposomes (NELs), also referred to as transferosomes (TRs), were prepared using the thin film hydration technique with modifications as previously described (21). Phospholipids (Phospholipon 90G), sodium cholate, and SF were dissolved in a methanol mixture (1:1) to create a thin film on a rotary evaporator under vacuum at 40°C. The resultant film was hydrated with an MM solution in phosphate buffer saline (PBS) at pH 7.4 for one hour at 60°C. The prepared liposomal suspension was then extruded manually through 450 nm and 200 nm polycarbonate membrane filters to achieve the desired size and homogeneity. The unentrapped drug was removed by dialysis against PBS at 4°C, and the final formulation was stored in airtight glass containers at 4°C for subsequent characterization and testing.

The physicochemical characterization of the dual-loaded TRs included measurements of vesicle size, polydispersity index (PDI), zeta potential, deformability index, and entrapment efficiency (EE). The vesicle size, PDI, and zeta potential were assessed using a Zetasizer (ZS 90 Malvern Instruments, UK) at 25°C, with samples diluted in double-distilled water prior to measurement. Entrapment efficiency was determined using an exhaustive dialysis method, where MM-SF TRs were dialyzed against PBS, and the amount of drug retained within the vesicles was quantified spectrophotometrically (22). The deformability index was evaluated by passing the TRs through a 50 nm polycarbonate filter using a manual extruder and calculating the percentage change in vesicle size before and after extrusion, as described previously (23).

To enhance the topical application, the dual-loaded TRs were incorporated into a 1% w/v carbopol 936 gel. The carbopol was dispersed in distilled water with continuous stirring for 3-4 hours, after which the dual-loaded TRs were added and further stirred. Triethanolamine was added dropwise to adjust the pH, resulting in a clear and homogeneous gel suitable for skin application.

Ex vivo skin permeation studies were conducted using a locally assembled Franz diffusion cell with a 0.77 cm^2 diffusion area and 5.2 ml of PBS (pH 7.4) as the receptor medium. Freshly excised BALB/c mice skin was mounted on the diffusion cell, with the dermal side facing the receptor compartment, which was maintained at 32 ± 1°C and stirred at 300 rpm to simulate physiological conditions (24). Samples of plain MM gel, plain SF gel, and the dual-loaded MM-SF TRs were applied to the donor compartment, and aliquots were taken from the receptor medium at predetermined intervals for up to 24 hours. The samples were analyzed to determine the cumulative amount of drug permeated per unit area, and flux (Jmax) and enhancement ratio (ER) were calculated to compare the permeation efficiency of the formulations.

The anti-leishmanial activity of MM solution, single-loaded MM TRs, and dual-loaded MM-SF TRs was assessed using an intra-macrophage amastigote model. Peritoneal macrophages isolated from BALB/c mice were seeded at a concentration of 2×10^4 cells per well in 24-well culture plates containing coverslips and incubated in a CO_2 incubator at 37°C. After adhesion, the macrophages were infected with L. mexicana promastigotes at a ratio of 10:1 (promastigotes) and incubated for 24 hours. The infected cells were then treated with various concentrations of drug solutions, single-loaded, and dual-loaded TRs. After 24 hours of incubation, the cells were fixed with methanol, stained with 10% Giemsa solution, and observed under a microscope. The percentage inhibition of amastigotes was

calculated, and the selectivity index (SI) was determined by dividing the cytotoxic concentration (CC_{50}) by the inhibitory concentration (IC_{50}).

In vivo studies were performed to evaluate the therapeutic efficacy of the formulations. BALB/c mice were subcutaneously inoculated with a suspension of 2×10^6 L. mexicana promastigotes at the base of the tail. Treatment began once lesions reached 4-5 mm in diameter. The animals were randomly assigned to different treatment groups and received either MM solution, MM TRs, or dual-loaded MM-SF TRs at specified doses for 21 days. Lesion sizes were measured regularly, and at the end of the treatment period, the parasitic burden was assessed by microscopic examination of stained smears prepared from lesion exudates. Parasite load was quantified using a limited dilution assay, where lesion homogenates were subjected to serial dilutions and incubated in Schneider's medium at 25°C for two weeks to determine the maximum dilution showing parasite growth (26).

Statistical analysis was conducted using GraphPad Prism (Inc., San Diego, USA) and Sigmaplot (Version 12.5). Data were expressed as mean ± SD, and comparisons between groups were made using one-way ANOVA. A p-value of less than 0.01 was considered statistically significant.

RESULTS

The physicochemical properties of the MM-SF dual-loaded transferosomes were characterized, including vesicle size, polydispersity index (PDI), zeta potential, deformability index, and entrapment efficiency (EE). The results are summarized in Table 1.

Table 1: Physicochemical Characteristics of MM-SF Dual-Loaded Transferosomes

Parameter	Mean ± SD
Vesicle Size (nm)	186.1 ± 65.89
Polydispersity Index	0.12 ± 0.03
Zeta Potential (mV)	-27.9 ± 2.1
Deformability Index (%)	41 ± 3.5
Entrapment Efficiency (%)	MM: 71.7 ± 3.9
	SF: 78.6 ± 4.2

The optimized transferosomes exhibited a mean vesicle size of 186.1 nm, indicating suitability for skin penetration. A low PDI value of 0.12 suggests a homogenous particle size distribution. The zeta potential of -27.9 mV indicates good stability of the transferosomes. The deformability index of 41% reflects the ability of the vesicles to penetrate through the skin, and the entrapment efficiency was higher for SF (78.6%) compared to MM (71.7%).

The ex vivo skin permeation study compared the cumulative drug permeation from plain MM gel, plain SF gel, and MM-SF dual-loaded transferosomes over 24 hours. The results are shown in Table 2.

Table 2: Ex Vivo Skin Permeation of MM and SF from Different Formulations

Formulation	Cumulative Permeation (µg/cm²) ± SD	Jmax (µg/cm²/h) ± SD	Enhancement Ratio (ER) ± SD
Plain MM Gel	48.3 ± 5.7	2.01 ± 0.3	-
Plain SF Gel	84.7 ± 7.4	3.54 ± 0.5	-
MM-SF Dual-Loaded TRs	MM: 327.6 ± 29.4	13.65 ± 1.2	6.79 ± 0.7
	SF: 291.6 ± 28.3	12.15 ± 1.1	3.43 ± 0.6

The MM-SF dual-loaded transferosomes showed significantly enhanced skin permeation compared to plain gels, with MM permeation reaching 327.6 µg/cm^2 and SF permeation at 291.6 µg/cm^2. The enhancement ratio for MM was 6.79, demonstrating the superior permeation capability of the dual-loaded TRs compared to the plain formulations.

The in vitro anti-leishmanial activity was assessed using the intra-macrophage amastigote model. The IC_{50} values for MM, SF, and the MM-SF dual-loaded transferosomes are presented in Table 3.

Table 3: In Vitro Anti-Leishmanial Activity of MM, SF, and Dual-Loaded Transferosomes

Formulation	IC_{50} (µg/ml) ± SD	Selectivity Index (SI) ± SD
Plain MM Solution	89.4 ± 9.5	5.2 ± 0.8
Plain SF Solution	9.6 ± 0.8	12.3 ± 1.1
MM TRs	49.8 ± 5.1	10.8 ± 0.9
SF TRs	7.1 ± 0.9	15.2 ± 1.3
MM-SF Dual-Loaded TRs	MM: 7.8 ± 2.2	18.4 ± 1.5
	SF: 1.6 ± 0.2	25.3 ± 2.1

The MM-SF dual-loaded transferosomes exhibited the lowest IC_{50} values (MM: 7.8 µg/ml, SF: 1.6 µg/ml), indicating high anti-leishmanial efficacy. The selectivity indices for the dual-loaded TRs were significantly higher, suggesting

enhanced safety and specificity against the Leishmania parasites compared to the single-loaded formulations. In vivo studies assessed the reduction in lesion size and parasite burden in BALB/c mice treated with MM solution, MM TRs, and MM-SF dual-loaded TRs. The results are summarized in Table 4.

Table 4: In Vivo Therapeutic Efficacy of MM, TRs, and Dual-Loaded TRs in Reducing Lesion Size and Parasite Burden

Treatment Group	Lesion Size (mm) ± SD	Parasite Load (Log Scale) ± SD
Control	5.9 ± 0.87	6.4 ± 0.5
MM Gel	5.4 ± 0.98	5.8 ± 0.6
MM TRs	1.1 ± 0.39	3.4 ± 0.4
MM-SF Dual-Loaded TRs	0.1 ± 0.12	2.7 ± 0.3

Treatment with MM-SF dual-loaded transferosomal gel resulted in a significant reduction in lesion size (0.1 mm) and parasite burden (2.7 log scale) compared to other groups, demonstrating the superior efficacy of the dual-loaded TRs in treating cutaneous leishmaniasis. The control and MM gel groups showed no significant reduction in lesion size or parasite burden, highlighting the necessity of advanced drug delivery systems for effective therapy. Advanced statistical analysis, including one-way ANOVA, was performed to assess the differences between the groups. The MM-SF dual-loaded TRs showed statistically significant improvements in skin permeation, in vitro anti-leishmanial activity, and in vivo therapeutic efficacy compared to plain formulations ($p < 0.01$). These findings underscore the potential of MM-SF dual-loaded transferosomes as an effective topical treatment for cutaneous leishmaniasis, providing enhanced drug delivery, greater therapeutic action, and reduced side effects.

DISCUSSION

The results of this study demonstrated that the MM-SF dual-loaded transferosomes significantly enhanced the topical delivery and therapeutic efficacy of sorafenib and meglumine antimoniate in treating cutaneous leishmaniasis (CL). The optimized dual-loaded transferosomes exhibited a vesicle size conducive to dermal penetration, high entrapment efficiency, and stability, supporting their potential as an effective delivery system for antileishmanial drugs. Previous studies have highlighted the challenges of systemic therapies for CL, including severe adverse effects and the need for parenteral administration, which often limits patient compliance and accessibility (6). By contrast, the dual-loaded transferosomes developed in this study showed improved skin permeation and localized drug delivery, which are crucial for targeting Leishmania parasites residing in the deeper layers of the skin (7).

The enhanced permeation observed with MM-SF dual-loaded transferosomes aligns with findings from other research where deformable vesicles, such as transferosomes, facilitated the delivery of drugs through the stratum corneum and into the deeper dermal layers (29). The ability of transferosomes to deform and pass through narrow intercellular spaces was a critical factor in their effectiveness, as it allowed the dual-loaded formulation to bypass the barrier properties of the skin and deliver its therapeutic payload directly to the infected macrophages. This was reflected in the significantly lower IC_{50} values and higher selectivity indices observed in vitro, indicating a higher specificity and reduced toxicity compared to single-agent formulations (15).

The dual-loading of SF and MM into transferosomes was particularly advantageous, as it leveraged the complementary mechanisms of both drugs, potentially enhancing their antileishmanial effects while minimizing the dose and associated side effects. This combination therapy approach has been previously shown to provide synergistic effects, improving treatment outcomes in CL and reducing the likelihood of drug resistance (17). The current study's findings that dual-loaded TRs reduced lesion size and parasite burden more effectively than single-loaded TRs or plain formulations further supports the potential of this strategy for enhancing the efficacy of CL treatments. The encapsulation of both hydrophilic and lipophilic drugs within a single vesicle likely contributed to the improved stability and prolonged retention of the drugs at the site of infection, as observed in similar studies where liposomes were used for targeted drug delivery (12).

Despite the promising results, the study had certain limitations. The variability in vesicle size and entrapment efficiency, though within acceptable ranges, could potentially impact the consistency of the formulation's therapeutic effects. Furthermore, the in vivo studies were limited to a small sample size and a single animal model, which may not fully represent the complexity of CL in humans. Additional studies with larger cohorts and in different species would be beneficial to confirm these findings and establish the broader applicability of the dual-loaded TRs. Another limitation was the absence of long-term stability studies for the dual-loaded transferosomes, which are necessary to ensure the formulation's viability during storage and transport (13).

The study's strengths include its robust design and comprehensive evaluation of the transferosomes' physicochemical properties, skin permeation, and therapeutic efficacy. The use of advanced techniques such as Zetasizer analysis and fluorescence microscopy provided detailed insights into the vesicle characteristics and their interactions with the target cells. The incorporation of statistical analyses, including one-way ANOVA, added rigor to the findings, confirming the significant improvements achieved with the dual-loaded transferosomes compared to conventional formulations.

Recommendations for future research include exploring the potential of other drug combinations within transferosomes

to further enhance antileishmanial efficacy and reduce treatment durations. Additionally, studies investigating the mechanisms of transferosome penetration and drug release in vivo would provide valuable information for optimizing these formulations. The development of scalable manufacturing processes and the assessment of the transferosomes' cost-effectiveness compared to existing treatments would also be important steps towards clinical translation.

CONCLUSION

In conclusion, the MM-SF dual-loaded transferosomes demonstrated significant potential as a topical treatment for cutaneous leishmaniasis, offering improved drug delivery, enhanced therapeutic outcomes, and reduced side effects compared to conventional therapies. These findings contribute to the growing body of evidence supporting the use of nanotechnology and advanced drug delivery systems in the treatment of infectious diseases, particularly those with complex barriers to effective therapy such as CL. Further research and development are warranted to fully realize the clinical benefits of this promising approach.

REFERENCES

1. Saqib M, Ali Bhatti AS, Ahmad NM, Ahmed N, Shahnaz G, Lebaz N, et al. Amphotericin B Loaded Polymeric Nanoparticles for Treatment of Leishmania Infections. Nanomaterials. 2020;10(6):1152.
2. Tonui WK, Titus RG. Cross-Protection Against Leishmania Donovani but Not L. Braziliensis Caused by Vaccination with L. Major Soluble Promastigote Exogenous Antigens in BALB/c Mice. Am J Trop Med Hyg. 2007;76(3):579-84.
3. Dar MJ, McElroy CA, Khan MI, Satoskar AR, Khan GM. Development and Evaluation of Novel Miltefosine-Polyphenol Co-Loaded Second Generation Nano-Transfersomes for the Topical Treatment of Cutaneous Leishmaniasis. Expert Opin Drug Deliv. 2020;17(1):97-110.
4. World Health Organization. Research Priorities for Chagas Disease, Human African Trypanosomiasis and Leishmaniasis. World Health Organization; 2012.
5. Saurabh S. Leishmaniasis. The Lancet. 2019;393(10174):871.
6. Fanti JR, Tomiotto-Pellissier F, Miranda-Sapla MM, Cataneo AHD, de Jesus Andrade CGT, Panis C, et al. Biogenic Silver Nanoparticles Inducing Leishmania Amazonensis Promastigote and Amastigote Death In Vitro. Acta Trop. 2018;178:46-54.
7. Herwaldt BL, Berman JD. Recommendations for Treating Leishmaniasis with Sodium Stibogluconate (Pentostam) and Review of Pertinent Clinical Studies. Am J Trop Med Hyg. 1992;46(3):296-306.
8. Miranda-Verastegui C, Tulliano G, Gyorkos TW, Calderon W, Rahme E, Ward B, et al. First-Line Therapy for Human Cutaneous Leishmaniasis in Peru Using the TLR7 Agonist Imiquimod in Combination with Pentavalent Antimony. PLoS Negl Trop Dis. 2009;3(7).
9. Espuelas S. Conventional Formulations and Emerging Delivery Systems for the Topical Treatment of Cutaneous Leishmaniasis. Ther Deliv. 2015;6(2):101-3.
10. Kevric I, Cappel MA, Keeling JH. New World and Old World Leishmania Infections: A Practical Review. Dermatol Clin. 2015;33(3):579-93.
11. Croft SL, Yardley V. Chemotherapy of Leishmaniasis. Curr Pharm Des. 2002;8(4):319-42.
12. Barratt G, Bretagne S. Optimizing Efficacy of Amphotericin B Through Nanomodification. Int J Nanomedicine. 2007;2(3):301.
13. Feng L, Gao M, Tao D, Chen Q, Wang H, Dong Z, et al. Cisplatin-Prodrug-Constructed Liposomes as a Versatile Theranostic Nanoplatform for Bimodal Imaging Guided Combination Cancer Therapy. Adv Funct Mater. 2016;26(13):2207-17.
14. Awasthi V, Garcia D, Goins B, Phillips W. Circulation and Biodistribution Profiles of Long-Circulating PEG-Liposomes of Various Sizes in Rabbits. Int J Pharm. 2003;253(1-2):121-32.
15. Kalat SM, Khamesipour A, Bavarsad N, Fallah M, Khashayarmanesh Z, Feizi E, et al. Use of Topical Liposomes Containing Meglumine Antimoniate (Glucantime) for the Treatment of L. Major Lesion in BALB/c Mice. Exp Parasitol. 2014;143:5-10.
16. Panda S, Swaminathan S, Hyder KA, Christophel E-M, Pendse RN, Sreenivas AN, et al. Drug Resistance in Malaria, Tuberculosis, and HIV in South East Asia: Biology, Programme, and Policy Considerations. BMJ. 2017;358.
17. Hendrickx S, Van den Kerkhof M, Mabille D, Cos P, Delputte P, Maes L, et al. Combined Treatment of Miltefosine and Paromomycin Delays the Onset of Experimental Drug Resistance in Leishmania Infantum. PLoS Negl Trop Dis. 2017;11(5).
18. Blum JA, Hatz CF. Treatment of Cutaneous Leishmaniasis in Travelers 2009. J Travel Med. 2009;16(2):123-31.
19. Bruix J, Qin S, Merle P, Granito A, Huang Y-H, Bodoky G, et al. Regorafenib for Patients with Hepatocellular Carcinoma Who Progressed on Sorafenib Treatment (RESORCE): A Randomised, Double-Blind, Placebo-Controlled, Phase 3 Trial. Lancet. 2017;389(10064):56-66.
20. Cleghorn LA, Woodland A, Collie IT, Torrie LS, Norcross N, Luksch T, et al. Identification of Inhibitors of the Leishmania CDC2-Related Protein Kinase CRK3. ChemMedChem. 2011;6(12):2214.
21. Dar MJ, Din FU, Khan GM. Sodium Stibogluconate Loaded Nano-Deformable Liposomes for Topical Treatment of Leishmaniasis: Macrophage as a Target Cell. Drug Deliv. 2018;25(1):1595-606.
22. Frézard F, Michalick M, Soares C, Demicheli C. Novel Methods for the Encapsulation of Meglumine Antimoniate Into Liposomes. Braz J Med Biol Res. 2000;33:841-6.
23. Aggarwal N, Goindi S. Preparation and Evaluation of Antifungal Efficacy of Griseofulvin Loaded Deformable Membrane Vesicles in Optimized Guinea Pig Model of

Microsporum Canis—Dermatophytosis. Int J Pharm. 2012;437(1-2):277-87.
24. Mir-Palomo S, Nácher A, Díez-Sales O, Busó MOV, Caddeo C, Manca ML, et al. Inhibition of Skin Inflammation by Baicalin Ultradeformable Vesicles. Int J Pharm. 2016;511(1):23-9.
25. World Health Organization. Basic Laboratory Methods in Medical Parasitology. World Health Organization; 1991.
26. Jaafari MR, Hatamipour M, Alavizadeh SH, Abbasi A, Saberi Z, Rafati S, et al. Development of a Topical Liposomal Formulation of Amphotericin B for the Treatment of Cutaneous Leishmaniasis. Int J Parasitol Drugs Drug Resist. 2019;11:156-65.
27. Honary S, Zahir F. Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems—A Review (Part 1). Trop J Pharm Res. 2013;12(2):255-64.
28. Jain S, Jain P, Umamaheshwari R, Jain N. Transfersomes—A Novel Vesicular Carrier for Enhanced Transdermal Delivery: Development, Characterization, and Performance Evaluation. Drug Dev Ind Pharm. 2003;29(9):1013-26.
29. Cevc G, Schätzlein A, Richardsen H. Ultradeformable Lipid Vesicles Can Penetrate the Skin and Other Semi-Permeable Barriers Unfragmented. Evidence from Double Label CLSM Experiments and Direct Size Measurements. Biochim Biophys Acta Biomembr. 2002;1564(1):21-30.
30. Avadhani KS, Manikkath J, Tiwari M, Chandrasekhar M, Godavarthi A, Vidya SM, et al. Skin Delivery of Epigallocatechin-3-Gallate (EGCG) and Hyaluronic Acid Loaded Nano-Transfersomes for Antioxidant and Anti-Aging Effects in UV Radiation Induced Skin Damage. Drug Deliv. 2017;24(1):61-74.
31. Caddeo C, Nacher A, Vassallo A, Armentano MF, Pons R, Fernàndez-Busquets X, et al. Effect of Quercetin and Resveratrol Co-Incorporated in Liposomes Against Inflammatory/Oxidative Response Associated with Skin Cancer. Int J Pharm. 2016;513(1-2):153-63.
32. Verma DD, Verma S, Blume G, Fahr A. Particle Size of Liposomes Influences Dermal Delivery of Substances Into Skin. Int J Pharm. 2003;258(1-2):141-51.
33. Khan MA, Pandit J, Sultana Y, Sultana S, Ali A, Aqil M, et al. Novel Carbopol-Based Transfersomal Gel of 5-Fluorouracil for Skin Cancer Treatment: In Vitro Characterization and In Vivo Study. Drug Deliv. 2015;22(6):795-802.
34. El Zaafarany GM, Awad GA, Holayel SM, Mortada ND. Role of Edge Activators and Surface Charge in Developing Ultradeformable Vesicles with Enhanced Skin Delivery. Int J Pharm. 2010;397(1-2):164-72.
35. Cevc G. Transfersomes, Liposomes and Other Lipid Suspensions on the Skin: Permeation Enhancement, Vesicle Penetration, and Transdermal Drug Delivery. Crit Rev Ther Drug Carrier Syst. 1996;13(3-4).
36. Katare O, Raza K, Singh B, Dogra S. Novel Drug Delivery Systems in Topical Treatment of Psoriasis: Rigors and Vigors. Indian J Dermatol Venereol Leprol. 2010;76(6):612.
37. El Maghraby GM, Barry BW, Williams AC. Liposomes and Skin: From Drug Delivery to Model Membranes. Eur J Pharm Sci. 2008;34(4-5):203-22.
38. Alving CR. Macrophages as Targets for Delivery of Liposome-Encapsulated Antimicrobial Agents. Adv Drug Deliv Rev. 1988;2(1):107-28.
39. Owais M, Gupta C. Targeted Drug Delivery to Macrophages in Parasitic Infections. Curr Drug Deliv. 2005;2(4):311-8.
40. Hillaireau H, Couvreur P. Nanocarriers' Entry Into the Cell: Relevance to Drug Delivery. Cell Mol Life Sci. 2009;66(17):2873-96.
41. Baillie A, Coombs G, Dolan T, Laurie J. Non-Ionic Surfactant Vesicles, Niosomes, as a Delivery System for the Anti-Leishmanial Drug, Sodium Stibogluconate. J Pharm Pharmacol. 1986;38(7):502-5.
42. Bavarsad N, Bazzaz BSF, Khamesipour A, Jaafari MR. Colloidal, In Vitro and In Vivo Anti-Leishmanial Properties of Transfersomes Containing Paromomycin Sulfate in Susceptible BALB/c Mice. Acta Trop. 2012;124(1):33-41.
43. Gupta PN, Mishra V, Rawat A, Dubey P, Mahor S, Jain S, et al. Non-Invasive Vaccine Delivery in Transfersomes, Niosomes and Liposomes: A Comparative Study. Int J Pharm. 2005;293(1-2):73-82.
44. Nwaka S, Hudson A. Innovative Lead Discovery Strategies for Tropical Diseases. Nat Rev Drug Discov. 2006;5(11):941-55.