ORIGINAL ARTICLE

Veterinary Research Forum. 2026; 17 (2): 127 - 133
doi: 10.30466/vrf.2025.2047654.4572
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

In vitro evaluation of activatable melittin encapsulated in liposome and albumin nanoparticles against *Leishmania*

Soheila Akhzari[1], Sedigheh Nabian[2]*, Mohammad Taheri[3]

[1] Department of Animal Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran; [2] Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [3] Rastegar Reference Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Info

Article history:

Received: 28 December 2024
Accepted: 14 July 2025
Available online: 15 February 2026

Keywords:

Cytotoxicity
Drug delivery
Leishmania major
Melittin

Abstract

Leishmaniasis comprises a spectrum of clinical manifestations caused by protozoan parasites of the genus *Leishmania*, order Trypanosomatida. Cutaneous leishmaniasis remains a significant zoonotic disease prevalent in tropical and subtropical regions, particularly in developing countries. Despite ongoing research, a definitive cure for this parasitic infection is still needed. This study explored the potential of activatable melittin (AM) as a selective treatment for cutaneous leishmaniasis caused by *Leishmania major*. The AM was designed using PepFold and ExPASy servers, incorporating a matrix metalloproteinase -2/9 cleavable linker to target *L. major*-infected macrophages selectively. To enhance drug delivery and reduce potential toxicity, AM was encapsulated within albumin nanoparticles and liposomes. The anti-leishmanial efficacy of these formulations was evaluated at AM concentrations ranging from 25.00 to 100 µg mL^{-1} over 48 hr, with each experiment performed in 10 independent replicates (n = 10 *per* group). Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test revealed a significant reduction in the average number of intracellular amastigotes *per* macrophage in the liposome-treated and albumin nanoparticle-treated groups (7.00 ± 1.50 amastigotes *per* macrophage) compared to the untreated infected control group (35.00 ± 3.20 amastigotes *per* macrophage). Treatment with 25.00 µg mL^{-1} of AM encapsulated in non-toxic albumin nanoparticles and liposomes demonstrated the most promising anti-leishmanial effect, resulting in an approximately 80.00% reduction in intracellular *L. major* amastigotes (compared to control).

© 2026 Urmia University. All rights reserved.

Introduction

Leishmaniasis is caused by a protozoan parasite from over 20 *Leishmania* species. Different species of this parasite cause a wide range of diseases collectively known as leishmaniasis.[1] The clinical presentation of leishmaniasis is characterized by a spectrum of cutaneous manifestations, ranging from nodular and plaque-like lesions to ulcerative lesions. These lesions are typically painless and demonstrate a granulomatous pattern with a well-defined border.[2] Due to the epidemiological complexity of the transmission loop (various vectors and reservoirs), this disease has become increasingly important.[3] There are two primary types of cutaneous leishmaniasis in Iran: urban and rural. Urban leishmaniasis, also known as anthroponotic cutaneous leishmaniasis, is caused by *Leishmania tropica*. It is transmitted by *Phlebotomus sergenti* sand flies, with humans serving as the primary reservoir. In contrast, rural leishmaniasis, or zoonotic cutaneous Leishmaniasis, is caused by *Leishmania major*, transmitted by *Phlebotomus papatasi* sand flies. Rodents act as the primary reservoir for this form of the disease.[4,5] The treatment of leishmaniasis remains a significant challenge due to several factors, including the high cost and dosage of existing drugs, the emergence of drug resistance, potential side effects, and the lack of affordable, novel anti-leishmanial drugs. Despite numerous efforts to develop low-cost, minimally invasive treatments, the complications associated with leishmaniasis persist. Thus, the discovery of new drugs with reduced toxicity and enhanced therapeutic efficacy is imperative.[6,7] Melittin, a 26-amino acid cationic and amphipathic peptide primarily found in bee venom,[8] has demonstrated anti-protozoal properties in recent studies.[9-11] Given these findings, we employed melittin in this study to combat cutaneous leishmaniasis. Melittin exhibits a wide range of biological activities, including antimicrobial, antiviral, antiprotozoal, anti-inflammatory, and anti-cancer effects.[12] Specifically,

***Correspondence:**

Sedigheh Nabian. DVM, PhD
Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: nabian@ut.ac.ir

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

melittin can induce apoptosis in *Leishmania*, leading to cell death.[13] Previous research has indicated that acylated synthetic cecropin A-melittin hybrids may offer a safe and effective treatment for canine leishmaniasis.[14,15] Akhzari *et al.* demonstrated that melittin exhibited an inhibitory effect on the Chinese hamster ovary cell line. It has been reported that melittin induces inhibitory effects on the proliferation of various cancer cells through the induction of apoptosis, necrosis, or lysis. Furthermore, increasing the concentration and incubation time of the peptide can enhance its cytotoxicity. Based on flow cytometric analyses, melittin at concentrations of 1.00 and 1.80 µg μL^{-1} induced apoptosis in HeLa cells after 24 hr of incubation, while melittin at a concentration of 4.00 µg μL^{-1} induced late apoptosis and necrosis of the cells. In cells infected with the *Leishmania* parasite, the expression of matrix metalloproteinase (MMP) 2 and MMP9 occurs, which regulates the cellular response to *Leishmania* infection. The C-terminal portion of melittin is cationic, and the addition of a polyanionic peptide containing seven glutamic acid residues through a linker that can be cleaved by MMP2 and MMP9 can inhibit the activity of melittin.[16] Given the upregulated expression of MMP-2 and -9 in parasite-infected cells, these enzymes can be exploited to design peptide-based treatments using melittin. We hypothesize that MMP-mediated cleavage of a linker connecting a polyanionic peptide to melittin within infected cells will disrupt this complex, releasing the active form. To deliver this activatable melittin (AM), we encapsulated it within albumin nanoparticles and liposomes. To determine the optimal dosage, we evaluated the anti-leishmanial effects of various concentrations of encapsulated AM.

Materials and Methods

Design of AM peptide for selective function. Considering the increased expression of MMP-2 and MMP-9 in inflammatory and parasite-infected cells, we designed an AM peptide for selective function. To achieve this, we employed a cleavable peptide linker specific to MMP-2 and MMP-9, highlighted in red. The melittin sequence was obtained from the NCBI database (Accession No. NP_001131099.1). A linker cleavable by MMP-2 and MMP-9,[17] was incorporated, followed by the addition of seven glutamic acid (Ajinomoto, Tokyo, Japan) residues as an anionic inhibitory peptide. Peptide folding was assessed using the PepFold online server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) and various analyses, including isoelectric point calculation, were performed using the ExPASy server (https://www.expasy.org/). The designed peptide sequence was GIGAVLKVLT TGLPALISWIKRKRQQGGPVGLIGKEEEEEEE which was sent to Bio-company (Pepmic, Suzhou, China) for synthesis.

Preparation of AM-loaded liposome. In this study, liposomes encapsulating varying concentrations of AM peptide (25.00, 50.00, and 100 µg *per* 100 µL) were prepared to achieve optimal therapeutic efficacy. The AM was incorporated into liposomes using a film hydration method.[18] Briefly, lipids (distearoylphosphatidylcholine: cholesterol: distearoylphosphatidylethanolaminepoly) at a molar ratio of 55: 40: 5) and AM (10.00 µg μL^{-1} in phosphate-buffered saline) were dissolved in chloroform and mixed in glass tubes. The solvent was removed by rotary evaporation and subsequent freeze-drying. The lipid film was hydrated with 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer (10.00 mM; pH 7.20) containing 5.00% dextrose, followed by vortexing and brief sonication under argon to form multilamellar vesicles. Extrusion through 200 and 100 nm pore-size poly-carbonate filters yielded approximately 100 nm small uni-lamellar vesicles with a polydispersity index of less than 0.20, as measured by dynamic light scattering (Horiba Jobin, Jyovin, Japan). Liposomes were stored at 4.00 ˚C.

Preparation of AM-loaded albumin nanoparticle. For this study, AM, high-purity $ZnCl_2$ (Merck, Darmstadt, Germany); at least 99.00%), albumin (Sigma-Aldrich, St. Louis, USA) with a purity suitable for the intended application (with 98.00% purity or higher), and distilled water were used. First, considering the solubility of AM, a suitable solvent (water) was chosen. Then, AM solutions with different concentrations (25.00, 50.00, and 100 µg) were prepared with high accuracy. Subsequently, an albumin solution at a concentration of 0.20 g mL^{-1} was prepared in distilled water or a suitable buffer (phosphate buffer at pH of 7.40). To prevent albumin denaturation, the solution was prepared and stored at a low temperature (4.00 ˚C). The albumin solution was slowly added to the AM solution with gentle stirring. The mixture was incubated at 55.00 ˚C and pH of 7.00 using appropriate buffers (phosphate buffer) for a specific time (30 min). Then, the $ZnCl_2$ solution with a specific concentration (2.00 µg mL^{-1}) was slowly added to the AM-albumin mixture with constant stirring. The rate of $ZnCl_2$ addition is crucial and should be carried out drop by drop at a constant rate. Each portion of $ZnCl_2$ was added to the mixture with a specific time interval (every 10 min) and constant stirring. This gradual method was used to control the size and increase drug loading in the nanoparticles. After the completion of $ZnCl_2$ addition, the mixture was incubated for a specific time (1 hr) at room temperature with gentle stirring to complete the precipitation. The nanoparticles were subsequently collected using centrifugation at 15,000 rpm for 30 min at 4.00 ˚C. The resulting nanoparticles had an average size of approximately 150 nm (as measured by dynamic light scattering) with a polydispersity index of less than 0.30. The nanoparticle precipitate was washed three times with

distilled water or a suitable buffer to remove free AM and $ZnCl_2$. The drug loading and encapsulation efficiency were determined using ultraviolet-visible spectroscopy (Boeco, Hamburg, Germany). The encapsulation efficiency for AM in albumin nanoparticles was found to be 75.00%. All experiments were repeated at least three times to ensure the accuracy and reproducibility of the results.[1]

Parasite culture. We used *L. major* promastigotes of Iranian strain (MRHO/IR75/ER), obtained from the Parasitology Department of Health Faculty of Tehran University of Medical Sciences, in this research. For mass culture, we used Novy MacNeal Nicolle medium and Roswell Park Memorial Institute (RPMI)-1640 (Geniran, Tehran, Iran) complete medium supplemented with L-glutamine 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 IU mL^{-1} penicillin-streptomycin (Jahad-e-Daneshgahi, Tehran, Iran), and 10.00% heat-inactivated fetal bovine serum (Jahad-e-Daneshgahi). The cultures were incubated at 27.00 ˚C under sterile conditions.[17]

Macrophage culture. In this study, we utilized the RAW264/7 macrophage cell line, procured from the National Center for Biological and Genetic Resources (Tehran, Iran). This adherent cell line was cultured in RPMI 1640 medium under standard conditions (37.00 ˚C; 5.00% CO_2) until reaching confluence. Cells were passaged at approximately 80.00% confluency.

Evaluation of cytotoxicity of melittin on macrophages *in vitro*. To assess the cytotoxic effects of melittin on macrophages, 500 µL of a macrophage suspension (1.00 × 10^5 cells *per* mL) was seeded into each well of a six-well plate. Cells were incubated at 37.00 ˚C with 5.00% CO_2 for 24 hr. After removing the supernatant, 100 µL of melittin at various concentrations (0.50, 1.80, and 4.00 µg mL^{-1}) was added to the wells. The plates were further incubated at 37.00 ˚C with 5.00% CO_2 for 48 hr. At 4, 24, and 48 hr post-treatment, the cells were washed, fixed with methanol, and stained with 10.00% Giemsa solution. The stained cells were then examined under a light microscope at 1,000 × magnification. The morphological changes observed in the melittin-treated macrophages were compared to a control group of macrophages infected with *L. major* amastigotes; however, not treated with melittin (the control group in this study received no therapeutic medication). This design allowed for comparison with the treatment group to determine whether observed changes are attributable to the medication itself or other factors).

Evaluation of cytotoxicity of albumin nanoparticle and liposome on macrophages. To assess the cytotoxicity of albumin nanoparticles and AM-loaded liposomes on macrophages, 500 µL of cultured macrophages (1.00 × 10^5 cells *per* mL) were seeded into each well of a six-well plate. After a 24-hr incubation at 37.00 ˚C with 5.00% CO_2, the supernatant was removed. Subsequently, 100 µL of albumin nanoparticles or AM- loaded liposomes at various concentrations (25.00, 50.00, and 100 µg mL^{-1}) were added to the wells, followed by an additional 48-hr incubation at 37.00 ˚C. At 4, 24, and 48 hr post-treatment, cells were washed, fixed with methanol, and stained with 10.00% Giemsa stain. Macrophages were then visualized under a light microscope at 1,000 × magnification and compared to a control group.

Evaluation of inhibitory function of nanoparticle and liposome on amastigote forms of parasites in macrophages. To assess the efficacy of albumin nano-particles and liposomes encapsulating AM against *L. major* amastigotes within macrophages, *L. major* promastigotes were cultured in RPMI-1640 medium supplemented with 10.00% fetal bovine serum. Cultures were incubated at 27.00 ˚C for 5 days to reach the stationary phase. Subsequently, 500 µL of RPMI-1640 medium containing 10.00% serum and 2.00 × 10^4 macrophages was seeded into each well of a six-well plate and incubated for 6 - 10 hr to allow macrophage adherence. Macrophages were seeded onto plates in a 5.00% CO_2 incubator at 37.00 ˚C to allow attachment. After incubation, the supernatant was aseptically removed, and the cells were washed with culture medium. Stationary-phase promastigotes were added at a 1 : 10 ratio (1 macrophage : 10 promastigotes). After 24 hr, non-adherent pro-mastigotes were removed by washing with RPMI culture medium. Liposomal and albumin nanoparticles containing 25.00, 50.00, or 100 µg of AM, or empty liposomes and empty albumin nanoparticles as negative controls, were added to each well. A positive control group treated with a standard anti-leishmanial drug was not included in this particular experiment. At 4, 24, and 48 hr post-treatment, cells were fixed with methanol, stained with 10.00% Giemsa, and examined under a light microscope at 1,000 × magnification. Results were compared to the untreated infected macrophage control group and the negative control groups (empty carriers). The severity of infection was quantified by counting the number of amastigotes *per* macrophage in multiple fields of view (at least 100 macrophages *per* well). The variability in these counts was assessed using the Root Mean Squared Error as a measure of the consistency of the treatment effect.

Statistical analysis. Statistical analysis was performed using a one-way ANOVA followed by Tukey's post-hoc test, using IBM SPSS statistics (version 29.0; IBM Corp., Armonk, USA; $F([2.00], [27.00]) = [12.50]$, $p < 0.001$). The sample size (n) for each group in the statistical analysis was 10 independent experimental replicates.

Results

In this research, we engineered an AM peptide with selective targeting capabilities (Fig. 1). This peptide was designed to have selective targeting capabilities, meaning it can potentially target *Leishmania*-infected macrophages.

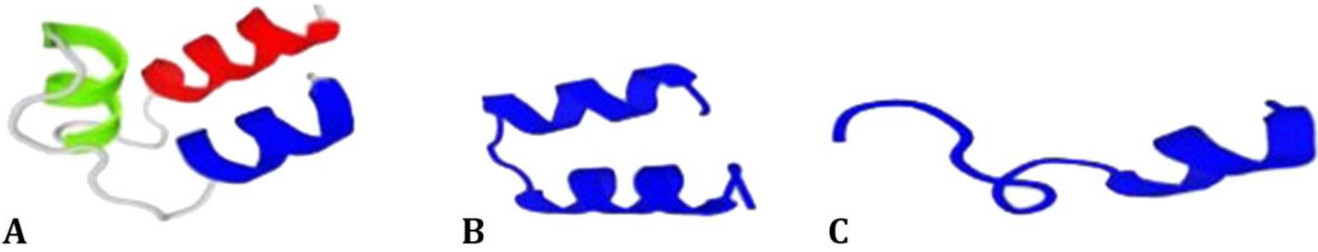

Fig. 1. The peptide folding prediction. **A)** Melittin plus matrix metalloproteinases (MMP) linker and polyanionic peptide; **B)** Melittin; **C)** The MMP linker and polyanionic peptide.

To understand the inherent toxicity of native melittin, macrophages were exposed to various concentrations of native melittin (0.50, 1.80, and 4.00 µg mL^{-1}) for 48 hr. The results clearly demonstrated dose-dependent cytotoxicity, meaning that as the concentration of melittin increased, the damage to the macrophages also increased. The observed damage included cell membrane damage, leading to the disruption of the cell outer layer integrity, cell shrinkage, loss of the cell normal shape resulting in irregular morphology, and detachment of the cells from the surface they were adhered. In contrast, the uninfected and untreated macrophage control group showed no changes, confirming that the observed effects were due to melittin (Fig. 2).

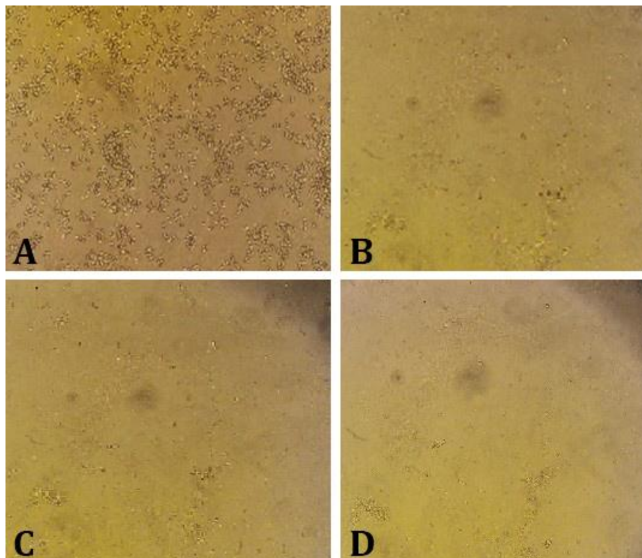

Fig. 2. Treatments of macrophages with melittin for 24 hr (10 ×). **A)** Control; **B)** The cells treated with melittin at concentration of 0.50 µg mL^{-1}; **C)** The cells treated with melittin at concentration of 1.80 µg mL^{-1}; and **D)** The cells treated with melittin at concentration of 4.00 µg mL^{-1}.

Following the examination of native melittin toxicity, macrophages were treated with AM peptide encapsulated in albumin nanoparticles and liposomes at concentrations of 50.00 and 100 µg mL^{-1} for 48 hr. The purpose of this encapsulation was to improve selective targeting and reduce potential side effects of the AM peptide. The results showed that at these high concentrations, the encapsulated formulations, similar to native melittin, induced significant cytotoxicity characterized by cell membrane damage, cell shrinkage, and detachment from the culture surface. However, when macrophages were treated with a lower concentration of 25.00 µg mL^{-1} of AM-loaded nanoparticles and liposomes, no significant cytotoxic effects were observed. This finding suggests that the cytotoxicity induced by the encapsulated AM formulations is dose-dependent and that encapsulation at lower concentrations can mitigate these effects (Fig. 3).

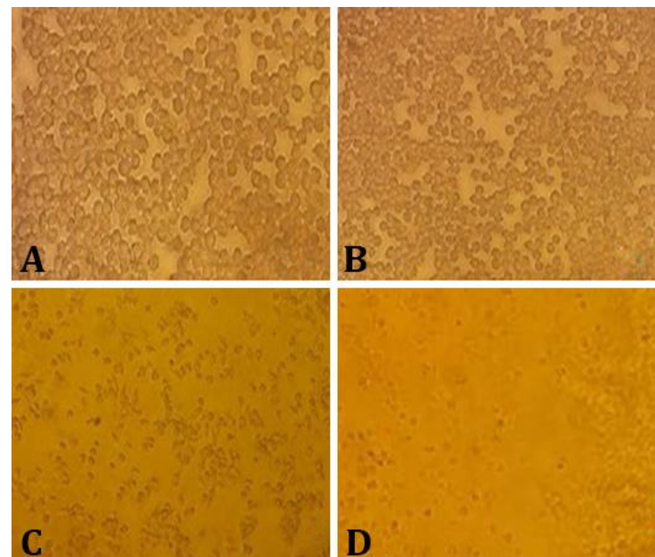

Fig. 3. Treatments of macrophages with activatable melittin (AM) for 48 hr (40 ×). **A)** Control; **B)** Cells treated with AM at concentration of 25.00 µg mL^{-1}; **C)** Cells treated with AM at concentration of 50.00 µg mL^{-1}; and **D)** Cells treated with AM at concentration of 100 µg mL^{-1}.

In addition to examining cytotoxic effects, the next step evaluated the therapeutic effect of AM peptide-loaded liposomes and albumin nanoparticles on *Leishmania* parasites. Statistical analysis revealed a significant reduction in the number of intracellular amastigotes. The average number of amastigotes *per* macrophage in the untreated *L. major*-infected control group was 35.00 ± 2.10. Treatment with liposome-encapsulated AM (25.00 µg mL^{-1}) resulted in the 80.00 ± 1.50% reduction in

amastigotes, while treatment with albumin nanoparticle-encapsulated AM (25.00 µg mL^{-1}) resulted in the 78.00 ± 1.70% reduction in amastigotes. These reductions were statistically highly significant compared to the control group (p < 0.001). These findings demonstrated that treatment with both liposome and albumin nanoparticle-encapsulated AM significantly reduced the intracellular parasite load (Fig. 4). These findings, which indicated the high efficacy of these treatments in reducing parasite load, might have had important implications for the development of new therapeutic approaches for leishmaniasis (Fig. 5).

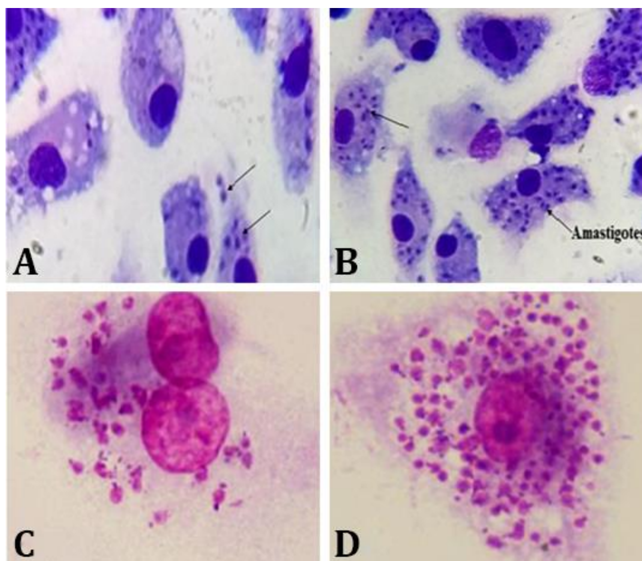

Fig. 4. Images comparing macrophages infected with *Leishmania major* amastigotes (black arrows) to macrophages treated with peptide-loaded albumin nanoparticles and liposomes, both at a dose of 25.00 µg mL^{-1} for 48 hr (Giemsa staining; 100 ×). **A)** Macrophages treated with albumin nanoparticles; **B)** Control macrophages; **C)** Macrophages treated with liposomes; and **D)** Control macrophages. The results indicate a significant reduction in amastigotes in the treated macrophages compared to the controls.

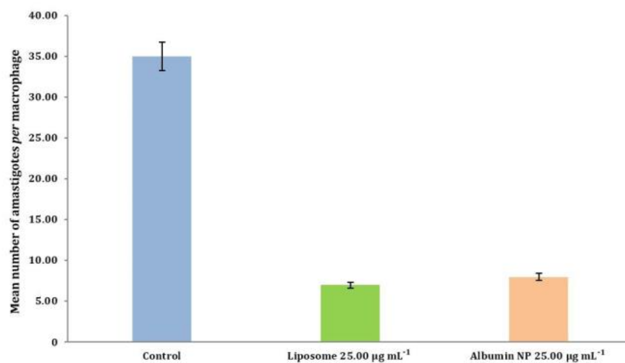

Fig. 5. Reduction of *Leishmania major* amastigotes in macrophages following treatment with liposomes and albumin nanoparticles (25.00 µg mL^{-1}) compared to untreated control. Error bars are standard deviations. NP: Nanoparticle.

Discussion

Despite advancements in newer, more effective, and less complex treatment methods, cutaneous leishmaniasis remains a significant health issue, particularly in countries with low socioeconomic levels. While systemic treatment with pentavalent antimony compounds is still the standard first-line therapy, insufficient treatment response, emerging drug resistance, and side effects are major concerns, often necessitating careful selection of the most appropriate treatment option.[19] The inhibitory effect of melittin on cell lines has been investigated by Olson *et al.*[12] This study demonstrated that melittin, in a dose-dependent manner, damaged macrophage cell lines, which aligned with our findings showing significant cytotoxicity of native melittin at concentrations of 1.80 µg mL^{-1} and above (Fig. 2). Previous research has shown melittin ability to inhibit cell proliferation through apoptosis, necrosis, or lysis. Increasing melittin concentration and incubation time enhances its cytotoxicity. To achieve selective targeting, we designed the AM structure incorporating the MMP-2/9 cleavable linker. To further support the proposed MMP-mediated activation mechanism, future studies will include *in vitro* protease cleavage assays using recombinant MMP-2 and MMP-9 to directly demonstrate the cleavage of the AM peptide linker. Additionally, experiments employing specific MMP inhibitors in our macrophage infection model could provide further evidence for the role of these proteases in AM activation and anti-leishmanial efficacy. By incorporating this AM into liposome nanoparticles or albumin carriers, researchers have demonstrated its targeted delivery to *Leishmania*-infected macrophages. This strategy significantly reduces the drug toxicity compared to free melittin, as supported by our *in vitro* cytotoxicity assays which showed no significant cytotoxic effects of encapsulated AM at 25.00 µg mL^{-1}, while native melittin exhibited significant cytotoxicity at concentrations as low as 1.80 µg mL^{-1} (Fig. 3). While we did not directly calculate half-maximal inhibitory concentration values in this study, the observed lack of significant cytotoxicity at 25.00 µg mL^{-1} encapsulated AM suggests a considerably higher half-maximal inhibitory concentration compared to the reported half-maximal inhibitory concentration values for free melittin in macrophage cell lines. The elevated MMP expression in the infected micro-environment facilitates the release of active melittin, leading to a substantial reduction in *Leishmania* amastigotes. While melittin exhibits minimal hemolytic activity at concentrations below 0.25 µg mL^{-1}, concentrations exceeding 1.00 µg mL^{-1} can induce 90.00% hemolysis of human erythrocytes.[20] Liposomal and albumin nanoparticle formulations were independently employed to deliver AM into *Leishmania*-infected macrophages. An *in vitro* comparison was conducted to evaluate their

differential effects on these infected cells. Our results demonstrated that both liposomal and albumin nanoparticle formulations containing 25.00 μg mL^{-1} of AM significantly reduced the number of amastigotes within *L. major*-infected macrophages by approximately 80.00% compared to untreated cells (Fig. 4). These findings underscored the efficacy of AM encapsulated in liposomes and albumin nanoparticles against intracellular amastigotes.[21] Leishmaniasis, a disease caused by obligate intracellular parasites, targets macrophages, crucial components of the body immune system. Upon phagocytosis, these parasites reside within phagolysosomal vacuoles, limiting drug accessibility and necessitating high drug dosages to control the infection. Consequently, adverse side effects are often observed.[22]

Our results showed that albumin nanoparticles and liposomes could effectively deliver drugs to macrophages, which was consistent with the findings of Vuarchey *et al*.[23] Liposome-based delivery systems offer a multitude of advantages, particularly in the context of targeted and precise drug delivery, which significantly contributes to the reduction of systemic drug toxicity.[24] These advantages stem from their unique structural characteristics, including their ability to encapsulate both hydrophilic and hydrophobic drugs within their bilayer membrane, thereby enhancing drug solubility and stability.[25] In our study, while both carriers demonstrated significant anti-leishmanial activity, potential functional distinctions existed. Liposomes, with their lipid bilayer structure, might offer a different drug release profile compared to albumin nanoparticles, possibly leading to a more sustained release of AM within the macrophages. Albumin nanoparticles, on the other hand, might exhibit different cellular uptake mechanisms, potentially through albumin-specific receptors on macrophages, which could influence the intracellular concentration of AM. Further investigation into the drug release kinetics from both carriers and a direct comparison of their cellular uptake efficiency and specific targeting in *Leishmania*-infected macrophages would be valuable to elucidate these functional differences. According to the results of this study, albumin nanoparticles and liposomes containing AM could be engineered with specific surface modifications, such as incorporation of targeting ligands or antibodies, enabling them to selectively bind to target cells or tissues, such as infected macrophages in the case of leishmaniasis. This targeted delivery minimizes off-target drug distribution, thereby reducing systemic toxicity and improving therapeutic efficacy. The controlled release of encapsulated drugs from liposomes also contributes to sustained drug levels at the target site, reducing the frequency of administration and further minimizing potential side effects. Our findings aligned with results reported by Cui *et al.*,[26] Malma *et al.*,[27] and Gour *et al.*,[28] reinforced the utility of drug delivery systems targeting macrophages, the primary site of *Leishmania* infection. Indeed, liposomes and nanoparticles have emerged as promising carriers in recent years.[27] These nanostructures offer significant advantages, including controlled drug release, protection of drug payloads, and enhanced cellular uptake, due to their nanoscale size. Consequently, they can effectively traverse biological barriers, delivering drugs to target sites and potentially prolonging systemic circulation.[28] Consistent with this, our study demonstrated that encapsulating AM within albumin-liposome carriers at a concentration of 25.00 μg mL^{-1} effectively enhanced anti-leishmanial efficacy while minimizing toxicity. A significant limitation of this study was the absence of *in vivo* validation in animal models. While our *in vitro* results were promising, further studies are necessary to evaluate the efficacy and safety of AM-loaded nanoparticles and liposomes in a living system, considering factors, such as pharmacokinetics, biodistribution, and potential systemic toxicities. Future research will focus on these crucial *in vivo* assessments to translate our findings towards potential clinical applications.

This study demonstrated the successful encapsulation and delivery of AM using both albumin nanoparticles and liposomes, effectively targeting *Leishmania*-infected macrophages. The significant reduction in amastigote burden, achieved with a concentration of 25.00 μg mL^{-1} AM, highlights the potential of these nanocarriers as a viable therapeutic strategy for cutaneous leishmaniasis. This approach addressed the limitations of conventional treatments, such as systemic toxicity and drug resistance, by leveraging the targeted delivery capabilities of nanoparticles and liposomes. The ability to engineer these nanostructures with specific surface modifications, coupled with the controlled release of AM, underscored their potential to enhance drug efficacy while minimizing off-target effects. Furthermore, the alignment of our findings with previous research reinforced the significance of macrophage-targeted drug delivery in combating intracellular parasitic infections. Ultimately, this research provided a promising avenue for developing more effective and less toxic treatments for cutaneous leishmaniasis, particularly in resource-limited settings where the disease burden is highest.

Conflict of interest

The authors declare no conflict of interest.

References

1. Akhzari S, Nabian S, Taheri M, et al. Therapeutic evaluation of activatable melittin for selective treatment of leishmaniasis *in vitro* and *in vivo*. Bulg J Vet Med 2023; 27(1): 0019. doi: 10.15547/bjvm.2022-0019.

2. Akhzari S, Rezvan H, Zolhavarieh SM, et al. Comparison of proinflammatory gene expression in lesions caused by either burn injuries or cutaneous leishmaniasis. Gene Cell Tissue 2017; 4(1): e42887. doi: 10.17795/gct-42887.
3. Akhzari S, Rezvan H, Nourian A, et al. Evaluation of pro-inflammatory genes expression in the spleen and wounds of BALB/c mice infected with *Leishmania major* [Persian]. J Ilam Univ Med Sci 2018; 26(2): 77-86.
4. Razavi MR, Shirzadi MR, Mohebali M, et al. Human cutaneous leishmaniasis in Iran, up to date-2019. J Arthropod Borne Dis 2021; 15(2): 143-151.
5. Rezvan H, Hamoon Navard S, Akhzari S. Comparison of pro-inflammatory gene expression profile in spleen and peripheral blood neutrophils of BALB/C mice infected with *Leishmania major* [Persian]. JSSU 2022; 29(11): 4268-4280.
6. Salari S, Bamorovat M, Sharifi I, et al. Global distribution of treatment resistance gene markers for leishmaniasis. J Clin Lab Anal 2022; 36(8): e24599. doi: 10.1002/jcla.24599.
7. Sangenito LS, da Silva Santos V, d'Avila-Levy CM, et al. Leishmaniasis and Chagas disease - neglected tropical diseases: treatment updates. Curr Top Med Chem 2019; 19(3): 174-177.
8. Tichy J, Novak J. Detection of antimicrobials in bee products with activity against viridans streptococci. J Altern Complement Med 2000; 6(5): 383-389.
9. Wade D, Boman A, Wåhlin B, et al. All-D amino acid-containing channel-forming antibiotic peptides. Proc Natl Acad Sci U S A 1990; 87(12): 4761-4765.
10. Bazzo R, Tappin MJ, Pastore A, et al. The structure of melittin. A 1H-NMR study in methanol. Eur J Biochem 1988; 173(1): 139-146.
11. Adade CM, Oliveira IR, Pais JA, et al. Melittin peptide kills *Trypanosoma cruzi* parasites by inducing different cell death pathways. Toxicon 2013; 69: 227-239.
12. Olson ES, Aguilera TA, Jiang T, et al. *In vivo* characterization of activatable cell penetrating peptides for targeting protease activity in cancer. Integr Biol (Camb) 2009; 1(5-6): 382-393.
13. Kulkarni MM, McMaster WR, Kamysz W, et al. Antimicrobial peptide-induced apoptotic death of Leishmania results from calcium-dependent, caspase-independent mitochondrial toxicity. J Biol Chem 2009; 284(23): 15496-15504.
14. Alberola J, Rodríguez A, Francino O, et al. Safety and efficacy of antimicrobial peptides against naturally acquired leishmaniasis. Antimicrob Agents Chemother 2004; 48(2): 641-643.
15. Zarrinnahad H, Mahmoodzadeh A, Hamidi MP, et al. Apoptotic effect of melittin purified from Iranian honey bee venom on human cervical cancer HeLa cell line. Int J Pept Res Ther 2018; 24(4): 563-570.
16. Akhzari S, Nabian S, Shayan P, et al. Designing of RNA molecule translating for activitable melittin as selective targeting of *Leishmania* infected cells. Iran J Parasitol 2021; 16(3): 443-453.
17. Tempone AG, da Silva AC, Brandt CA, et al. Synthesis and antileishmanial activities of novel 3-substituted quinolines. Antimicrob Agents Chemother 2005; 49(3): 1076-1080.
18. Ghanbarzadeh S, Valizadeh H, Zakeri-Milani P. Application of response surface methodology in development of sirolimus liposomes prepared by thin film hydration technique. Bioimpacts 2013; 3(2): 75-81.
19. Pacheco-Fernandez T, Markle H, Verma C, et al. Field-deployable treatments for leishmaniasis: intrinsic challenges, recent developments and next steps. Res Rep Trop Med 2023; 14: 61-85.
20. Vartak DG, Gemeinhart RA. Matrix metalloproteases: underutilized targets for drug delivery. J Drug Target 2007; 15(1): 1-20.
21. Moghimipour E, Aghel N, Zarei Mahmoudabadi A, et al. Preparation and characterization of liposomes containing essential oil of *Eucalyptus camaldulensis* leaf. Jundishapur J Nat Pharm Prod 2012; 7(3): 117-122.
22. Mahmoodzadeh A, Zarrinnahad H, Bagheri KP, et al. First report on the isolation of melittin from Iranian honey bee venom and evaluation of its toxicity on gastric cancer AGS cells. J Chin Med Assoc 2015; 78(10): 574-583.
23. Vuarchey C, Kumar S, Schwendener RA. Albumin coated liposomes: a novel platform for macrophage specific drug delivery. Nanotechnol Devt 2011; 1(1): e2. doi: 10.4081/nd.2011.e2
24. Veerareddy PR, Vobalaboina V, Nahid A. Formulation and evaluation of oil-in-water emulsions of piperine in visceral leishmaniasis. Pharmazie 2004; 59(3): 194-197.
25. Su CW, Chiang CS, Li WM, et al. Multifunctional nanocarriers for simultaneous encapsulation of hydrophobic and hydrophilic drugs in cancer treatment. Nanomedicine (Lond) 2014; 9(10): 1499-1515.
26. Cui Z, Zhou Z, Sun Z, et al. Melittin and phospholipase A2: promising anti-cancer candidates from bee venom. Biomed Pharmacother 2024; 179: 117385. doi: 10.1016/j.biopha.2024.117385.
27. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 2009; 30(11): 592-599.
28. Gour JK, Srivastava A, Kumar V, et al. Nanomedicine and leishmaniasis: future prospects. Dig J Nanomater Biostructures 2009; 4(3): 495-499.