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# Use of topical liposomes containing meglumine antimoniate (Glucantime) for the treatment of *L. major* lesion in BALB/c mice

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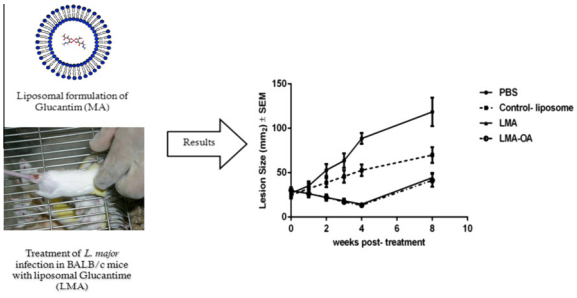
## HIGHLIGHTS

- Topical liposomal Glucantime reduced lesion sizes in BALB/c model of leishmaniasis.
- The spleen parasite burden was significantly lower in the groups treated with TLG.
- Oleic acid in liposomal formulation did not increase the effect of the treatment.
- TLG could be a good candidate for topical treatment of cutaneous leishmaniasis.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Treatment of cutaneous leishmaniasis (CL) is a dream for the patients, health care authorities and scientists. The aim of this study was to develop a topical liposomal meglumine antimoniate (MA, Glucantime™) (Lip-MA) formulation and evaluate the therapeutic effects of the preparation on lesion induced by *Leishmania major* in BALB/c mice. Liposomes containing 22.5% MA (6.4% $Sb^{+5}$) with and without oleic acid (LMA-OA and LMA) were formulated using fusion method plus homogenization and characterized for the size and encapsulation efficiency. The penetration of MA from the LMA-OA and LMA formulations through and into the skin was checked *in vitro* using Franz diffusion cells fitted with mouse skin at 37 °C for 8 h. The *in vitro* permeation data showed that almost 1.5% of formulations applied in the mouse skin were penetrated and the amount retained in the skin was about 65%. The 50% effective dose of LMA and LMA-OA against amastigotes of *L. major* was 46.36 and 41.01 μg/ml, respectively. LMA or LMA-OA was used topically twice a day for 4 weeks to treat the lesion induced by *L. major* in susceptible BALB/c mice. The results showed a significantly ($P < 0.001$) smaller lesion size in the treated groups of mice compared to the control groups which received either empty liposomes or phosphate-buffered saline (PBS). The spleen parasite burden was significantly ($P < 0.001$) lower in the treated groups compared to the control groups receiving either empty liposomes or PBS at the end of the treatment period. However, when the treatment was stopped, the lesion size progressed and spleen parasite burden increased in LMA and LMA-OA groups, but still was significantly less than the control groups ($P < 0.05$). There was no significant difference between the two formulations of LMA and LMA-OA. The results suggested that topical liposomes containing MA might be an appropriate choice for clinical trials for the treatment of CL.



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## 1. Introduction

Cutaneous leishmaniasis (CL) is a parasitic disease caused by different *Leishmania* species, CL is a self-limiting disease but therapy is necessary to shorten the duration of the lesion and diminish sequel of the disease such as smaller disfigure scar(s) (Asilian et al., 2010). Pentavalent antimonials remain a first-line treatment of CL with limitations such as side effects, needs multiple injections which not tolerated by the patients due to severe pain (Croft, 2001; Croft and Yardley, 2002; Markle and Makhoul, 2004). The tropical disease research (TDR) program of the WHO has emphasized on the development of alternative treatments to antimonials drugs on list of priorities in disease control strategies (Armijos et al., 2004).

Meglumine antimoniate (MA, Glucantime™) is an antimoniate derivative with molecular weight of 366.0 g which is highly soluble in water (Frezard et al., 2009).

Liposomes are colloidal particles which typically consist of phospholipid and cholesterol. The lipid molecules form bilayers enclose aqueous compartments and deliver drugs into the skin based on the similarity of the bilayers structure of lipid vesicles to natural membrane. The liposomal formulations are used successfully in the treatment of a number of dermatological disorders (Patel et al., 2010).

Even though several topical formulations were developed and reached to human clinical trials (Asilian et al., 1995; Shazad et al., 2005) and lipid-based formulations are developed and used in various types of studies (Cauchetier et al., 2003; Ferreira et al., 2004; Jaafari et al., 2009; Tempone et al., 2004), only a handful commercial topical formulation available for the treatment of CL. Antimonial derivatives were introduced in 1940s and still are used as primary therapy for the treatment of CL and visceral leishmaniasis (VL) (Croft and Yardley, 2002; Frezard et al., 2009; WHO, 2010).

The present study is focused on the development of liposomal formulation of Glucantime for dermal delivery and possible treatment of CL. For this purpose liposomal formulations containing Glucantime were developed using fusion method and characterized for the size, zeta potential and encapsulation efficacy. The penetration properties of the formulations were compared using cell diffusion study. The activity of prepared formulations was checked *in vitro* against *Leishmania major* promastigotes and amastigotes and *in vivo* on lesion caused by *L. major* in BALB/c mice.

## 2. Materials and methods

### 2.1. Animals and parasites

Female BALB/c mice 6–8 weeks old were purchased from Pasteur Institute (Tehran, Iran). The mice were maintained in animal house of Biotechnology Research Center, the animals were fed with tap water and standard laboratory diet (Khorassan Javane Co., Mashhad, Iran). Animals were housed in a colony room 12/12 h light/dark cycle at 21 °C with free access to water and food. The experiment was carried out according to Mashhad University of Medical Sciences, Ethical Committee Acts.

The virulence of *L. major* strain (MRHO/IR/75/ER) was maintained with passage in BALB/c mice. The amastigotes were isolated from spleen of infected mice and cultured in NNN medium and sub-cultured in RPMI 1640 (Sigma) containing 10%, v/v heat inactivated FCS, 2 mM glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin sulfate (RPMI-FCS). The mice were infected using *L. major* promastigotes harvested at stationary phase.

### 2.2. Chemicals

Soya phosphatidylcholine (SPC) and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Meglumine antimoniate (Glucantime®) was bought from Aventis (France), propyl paraben (PP), methyl paraben (MP), propylene glycol (PG), HEPES was purchased from Sigma (USA), and vitamin E from Merck (Darmstadt, Germany).

### 2.3. Preparation of liposomes containing MA

Liposomes containing MA were prepared by fusion method (Foldvari, 1998; Jaafari et al., 2009). Briefly, the lipid components consist of SPC (15%), Cholesterol (2%), propylene glycol (7%), vitamin E (0.3%), MP (0.1%) and PP (0.02%) were melted at about 75 °C (lipid melt). Aqueous phase (Glucantime solution 1.5 g/ml) was heated separately and added up to 100% to the previously heated lipid melted and vigorously stirred using vortex until cooled down to room temperature. Then, the final products were homogenized using a M110 L microfluidizer (Microfluidics, USA). To prepare the control empty liposomes, the same procedure as described for liposomal MA was followed except HEPES buffer was used instead of Glucantime solution.

### 2.4. Characterization of liposomes

The particle diameter and zeta potential of each sample were measured in triplicates using dynamic light scattering (Malvern, Nano-ZS).

Direct method was used to assess the percent of encapsulation, in this method, first each product was mixed well and then 1 ml of the sample was dialyzed against 500 ml phosphate buffer 10 mM for 24 h and every 12 h the medium was replaced with fresh buffer. After 24 h samples within dialysis bags were carefully weighed. Then, the samples inside the bags were dissolved in pure nitric acid and heated to dry completely and organic components of the samples were digested. Then, the ratio of 1:1 with hydrochloride acid:-water was added and boiled for 1 h, then mixed 1:1 with water and acid was added to reach 100 ml. Antimony content was determined using Atomic absorption Chemtech model CTA-3000, because antimony lamp generated wavelength 217.6 nm, the appropriate range was 10–40 μg/ml in which the sensitivity was equivalent to 0.29 μg/ml (Frezard et al., 2000).

### 2.5. Cell diffusion study

Jacketed Franz cell with a receiver volume of 25 ml was used at 37 °C and all the experiments were conducted in triplicate. Phosphate buffer of pH 7.4 was used as receiver medium. A suitable size of full-thickness dorsal skin of BALB/c mice was cut and mounted in the Franz cell, with the stratum corneum side facing upward. Hair on dorsal side of the mouse was removed using electrical clipper on the day before the experiment. The membranes were initially left in the Franz cells for 30 min in order to facilitate hydration. Subsequently, 1 g of the formulation was applied uniformly on to each membrane surface. A 5 ml aliquot of receiver solution was withdrawn from each receiver solution at 1, 2, 4, 8 and 24 h and replaced with the same volume of blank PBS solution to maintain the receptor phase volume at constant level. Aliquots of the collected samples were analyzed for MA content as explained before. The derived concentration values were corrected using the following equation:

$$\mathrm{Mt}(n) = \mathrm{Vr} \times \mathrm{Cn} + \mathrm{Vs} \times \sum \mathrm{Cm}$$

where Mt($n$) is the current cumulative mass of drug transport across the skin at time $t$, Cn the current concentration in the receiver medium, $\sum$Cm the summed total of the previous measured concentrations, Vr the volume of the receiver medium and Vs corresponds to the volume of the sample removed for analysis.