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Anti-leishmanial effect of itraconazole niosome on *in vitro* susceptibility of *Leishmania tropica*

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ABSTRACT

The novel niosomal system aimed to deliver the active drug entity to the target site. The objective of this study was to prepare and evaluate the effect of itraconazole niosome on the *in vitro* susceptibility of *Leishmania tropica* as compared to itraconazole alone or tartar emetic. The overall growth rate of promastigotes treated with various concentrations of itraconazole niosome was significantly lower than that of itraconazole alone (IC50 = 0.24 μg/ml *vs.* IC50 = 0.43 μg/ml, $P < 0.01$). In contrast, the mean multiplication rate of amastigotes inside the macrophages and also the mean number of amastigotes in each macrophage treated with itraconazole niosome (34.9 and 3.0) were significantly lower ($P < 0.01$) than those treated with itraconazole alone (62.0 and 3.8) or tartar emetic (63.9 and 4.2), respectively. These findings indicated that niosomes could be developed as a novel drug delivery for itraconazole in the *in vitro* model. Further studies are required to evaluate the effect of itraconazole niosome on volunteer human subjects.

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1. Introduction

Leishmaniasis constitutes an important public health problem in endemic countries. The incidence of this disease is more than 2 million cases per year and 350 million people in 98 countries and territories are at risk (WHO, 2010). Although cutaneous leishmaniasis (CL) is a self-healing disease, it can result in disfiguring scar and long-lasting stigmas, which may destroy underlying structures, like nose, ear, or the exposed sites of skin, which causes the psychological suffering of patients (Alvar et al., 2012).

Pentavalent antimony compounds (SbV) are still considered the first line of treatment for all forms of leishmaniasis; but, there are some reports of drug resistance and unresponsiveness to the treatment with these drugs (Pourmohammadi et al., 2011).

Treatment failure is common in many endemic areas (Croft et al., 2006). In Iran, CL has been significantly increased and resistance is emerging (Hadighi et al., 2006). Due to long-term administrations, partial effectiveness, and variable toxicity, tremendous efforts have been currently made for developing novel drugs and alternative therapies (Da Silva et al., 2012). Resistance may be natural or acquired and frequently associated with decreased cellular accumulation of the drug (Croft et al., 2006); however, other factors including therapy duration, suboptimal treatment, low compliance, pharmacological deficiencies, and host immune status have also led to resistance (Croft et al., 2006).

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Table 1 – Preparation of different formulations, vesicle morphology and size determination.

Formulation	Morphology	Amount of niosomes	Shape	Size (μm)
1	Large, tubular with crystal	Many	MLV	0.3 ± 11
2	Large, spherical	Few	MLV	0.15 ± 10.5
3	Large, tubular	Many	MLV	0.36 ± 5.4
4	Large, tubular	Few	MLV	0.15 ± 7.5
5	Large, spherical	Many	MLV	0.35 ± 9.5

MLV, multilayer vesicles.

Anti-fungal agents including azoles derivatives (fluconazole, econazole, miconazole, ketoconazole, and itraconazole) have had clinically acceptable leishmanicidal activities (De Beule and Cauwenberg, 1989). Itraconazole is a synthetic triazole dioxolane derivative, which can interfere with the sterol biosynthesis present in the leishmanial membranes (Croft et al., 2006). Moreover, as compared with meglumine antimoniate, itraconazole has several advantages. It is an oral treatment, which is more acceptable for patients, is a cheaper drug, and has fewer side-effects. However, treatment duration, to which efficacy is linked, is relatively long (Baroni et al., 2009; Wagh and Deshmukh, 2010). But, the disadvantage of itraconazole is its poor penetration into *Leishmania*, which could be a reason why itraconazole is not as effective as meglumine antimoniate (Glucantime). Itraconazole noisomes were having larger zone of inhabitation than marketed formulation when activity was evaluated against *Candida albicans* (Wagh and Deshmukh, 2012). But, there have been several reports on the conflicting effect of itraconazole on human and veterinary leishmaniasis (Srinivas et al., 2009). Some studies have demonstrated reasonable efficacy of itraconazole in the therapy of cutaneous leishmaniasis (Consigli et al., 2006). In contrast, other clinical studies have shown poor results or ineffective therapy with itraconazole (Nassiri-Kashani et al., 2005).

Niosome is a lipid vesicle made of non-ionic surfactant, cholesterol, and phospholipids that are similar to cellular membrane (Wagh and Deshmukh, 2010). In this study, the niosomal formulation of itraconazole was prepared and its efficacy was evaluated on *in vitro* MTT assay and macrophage model. Niosomal formulation was characterized for its size, morphology, entrapment efficiency, release study, and stability. The objective of this pre-clinical study was to improve the penetration and efficacy of itraconazole as coupled with niosomes, compared with itraconazole or tartar emetic alone on *in vitro* susceptibility assay as there is no animal model for *L. tropica*. The novel vesicular system aimed to deliver the active drug entity to the selective target action.

2. Materials and methods

2.1. Drug preparation

Tartar emetic (Merck Germany) and itraconazole were obtained from commercial sources in Iran. Itraconazole was dissolved in sterile distilled water according to the manufacturer's instructions and the aliquots were stored at −20 °C until use. The stock solutions were defrosted and diluted in medium just prior to assay. Potassium antimony (tartar emetic) solution (a trivalent antimony) was prepared just before the assay at room temperature and diluted in RPMI1640 medium to prepare the final concentrations of 0.5, 1, 1.5, 2, 2.5, and 5 μg/ml (Carrio et al., 2000). Tartar emetic was 10 times more effective for *Leishmania* species than pentavalent antimony (Jeddi et al., 2011). In the present study, this antimony was used as the control drug.

2.2. Niosome preparation

Itraconazole niosomes were prepared using film hydration method (Uchegbu and Florence, 1995). Briefly, in a round-bottom flask, the appropriate amounts of non-ionic surfactants (Span40, Span60) along with cholesterol and 10 mg itraconazole were dissolved in chloroform. The organic solvent was evaporated in a rotary evaporator at 60 °C. The thin layer of film was left to cover the inner walls of the flask. The obtained film was hydrated by 10 ml of phosphate buffer saline (PBS, pH = 7.4) for 1 h at 55 °C. The obtained niosomes were solicited for 30 min by bath sonication.

2.3. Determining vesicle size and morphology

The size distribution of the vesicles was determined using laser light diffraction method by Malvern apparatus (Malvern Master Sizer X, Malvern, UK). Also, niosomal formulations were morphologically investigated using the camera-attached

Table 2 – Percent of itraconazole entrapment efficiency in selected niosomal formulation.

Sample	Amount of unloaded itraconazole 1st (mg)	Amount of unloaded itraconazole 2nd (mg)	Amount of unloaded itraconazole 3rd (mg)	Mean (mg)	Encapsulation (%)
1	34.6	35	34.7	34.8	17.2
2	32.5	33.8	33.4	33.2	20.9
3	35.4	36.6	36.1	36	14.2

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