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ORIGINAL ARTICLE

Leishmanicidal effects of amphotericin B in combination with selenium loaded on niosome against *Leishmania tropica*

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Abstract The strategy for improving the treatment of leishmaniasis by the World Health Organization, is the development of new drugs and combination therapy. The aim of this survey was to investigate the effect of amphotericin B (AmB) in combination with selenium, in a simple or niosomal form, on *Leishmania tropica* (*L. tropica*) by in vitro advanced assays. In this study, a niosomal formulation of AmB with selenium was prepared and characterized based on size and morphology. Using MTT assay, macrophage model, flow cytometry, and qPCR, the cytotoxicity and efficiency of the niosomal formulation and simple form of combination were evaluated. No toxicity was reported for both the niosomal and simple form of the combination. The niosomal formulation significantly showed higher inhibitory effect on the promastigote and amastigote forms of *L. tropica* than simple combination form. Interleukin (IL)-10 significantly decreased while the level of IL-12 and metacasoase as Th-1 activator significantly increased ($P < 0.001$). The findings of this study indicated that niosomes are the stable carriers for this combination, easy to produce and provide promising results as an effective formulation in the inhibition of extracellular and intracellular forms of *L. tropica* in compared with simple combination form.

Keywords Amphotericin B · Selenium · Niosome · Gene expression · *Leishmania tropica*

Introduction

Leishmaniasis is a vector-borne disease caused by the protozoan parasite, *Leishmania.* About 1.6 million cases of infections with visceral (VL) and cutaneous (CL) forms of leishmaniasis (Alvar et al. 2012) are reported annually. Presently, there is no vaccine available for the prevention of leishmaniasis (Kedzierski et al. 2006).

Leishmaniasis is among the neglected diseases which have limited therapeutic options. Due to toxicity, parasite resistance and high cost (Croft et al. 2006b), there have been limitations to the use of currently available drugs such as antimonial compounds as the first line of treatment and liposomal amphotericin B (AmB), miltefosine, terbinafine, pentamidine and paromomycin (Croft et al. 2006a; Moosavian Kalat et al. 2014; Farajzadeh et al. 2015a, 2016) as the second line of treatment (Freitas-Junior et al. 2012).

Consequently, the high prevalence of CL and the onset of resistance to conventional drugs requires the innovation and development of novel, nontoxic and more efficient drugs (Santos et al. 2008). Nowadays, the use of novel drug delivery systems such as niosomes is an attractive alternative treatment for leishmanisis (Frézard et al. 2009).

The strategy of niosomes is targeted delivery to tissues in order to improve the efficacy and reduce the cytotoxicity of drugs such as anticancer, antibiotics and antifungal

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drugs (Donowitz 1994; Owais and Gupta 2005). Niosomes are non-immunogenic, non-toxic and highly biocompatible vesicles (Wagh and Deshmukh 2015).

AmB, as the second line of leishmaniasis treatment binds to ergosterol as the main sterol in *Leishmania* species (Perez et al. 2016). Previous studies showed that AmB was effective against *L. amazonensis*, *L. infantum,* and *L. braziliensis* (Cunha et al. 2015; Duarte et al. 2016). However, liposomal formulations of AmB such as Abelcet, AmBisome (Gilead Sciences, USA), and Amphocil are more efficacious, but are more expensive and unaffordable (Agrawal et al. 2005).

Selenium, as a trace element is used in different parts of medicine such as anticancer treatment, antioxidant effects and antiviral activities (Whanger 2004; Rayman 2005, 2012). Selenium nanoparticles would easily enter into cells and inhibit bacterial growth (Yang et al. 2009; Tran and Webster 2011). In addition, selenium nanoparticles are also effective against *L. major*, *L. tropica* and *L. infantum* (Beheshti et al. 2013; Mahmoudvand et al. 2014; Soflaei et al. 2014).

The strategies of the World Health Organization (WHO) for improving the treatment of leishmaniasis include the development of new drugs and combination therapy (WHO 2012). The aim of drug combinations is to prevent or delay the development of resistance, shorten the duration of treatment and also increase the efficacy of drugs in the treatment of parasites, viral and bacterial infections (White 1999; Olliaro and Taylor 2003). Combination therapy are vital for the treatment of some diseases such as tuberculosis, AIDS, malaria and leishmaniasis (Kremsner and Krishna 2004; Yazdanpanah et al. 2004; Mitchison and Davies 2012).

Some studies have shown that drug combinations have been used in the treatment of leishmaniasis such as investigation of in vitro and in vivo effect and clinical trial of combining miltefosine with other standard leishmanicidal drugs (Seifert and Croft 2006; Aguiar et al. 2010; Omollo et al. 2011). The antileishmanial effect of sodium stibogluconate (SbV) combined with paromomycin, allopurinol or verapamil have been clinically and experimentally investigated (Thakur et al. 2000; Shokri et al. 2012; Riabi et al. 2013).

In order to prevent the development of resistance, shorten the duration of treatment and also reduce the side effects of conventional drugs in the treatment of leishmaniasis, this study aimed to investigate the effect of AmB combined with selenium, in the simple or niosomal form on *L. tropica* by an in vitro MTT assay, macrophage model, flow cytometry, and qPCR.

Materials and methods

Drug preparation

Glucantime (Sanofi-Aventis, Paris, France), AmB (India, B. No. GI50253), and selenium dioxide 99.9% (Sigma-Aldrich/Lot 079K368021) were obtained from the Provincial Health system. AmB and selenium were dissolved in sterile distilled water, according to the manufacturer's instructions. Prior to each assay, final combination concentrations of 12.5 + 12.5, 25 + 25, 50 + 50, 100 + 100, and 200 + 200 μg/ml (Selenium + AmB) were prepared in Rosewell Park Memorial Institute (RPMI-1640) medium (Riabi et al. 2013).

Niosomal formulation

Film hydration method was used to achieve the niosomal combination of AmB plus selenium (Rogerson et al. 1987). To this end, non-ionic surfactants (Span40 and Twin40 with ratio 6/4) and cholesterol were dissolved in chloroform in a round-bottom flask and evaporated using a rotary evaporator (Buchi, Switzerland) at 180 rpm and 70 °C for 15 min. The obtained thin layer of film was hydrated with 5 ml of deionized water, which included 25 mg of AmB and 25 mg of selenium with the same potency (1%) at 70 °C for 30 min. In order to complete the hydration, niosome was kept at room temperature for 24 h. The prepared niosome was stored at 4 °C for further studies.

Characterization of niosomal formulation

The shape of noisome was determined by optical microscopy (Zeiss, Germany) and micrographs were captured. In addition, niosomal size was determined using dynamic light scattering with Master Sizer 2000 E (Malvern, UK). The tests were done in triplicate.

Cytotoxicity assay

For the evaluation of drug cytotoxicity, murine macrophages (J774-A1 cells, ECACC no.91051511) were obtained from the Pasteur Institute of Iran (Tehran, Iran) and cultured in complete RPMI-1640 medium, treated with different concentrations of drugs and incubated at 37 °C in 5% CO_2 for 72 h. After the incubation period, 10% MTT powder (product No. M 5655, purchased from Sigma-Aldrich®, USA) following the manufacture's instruction was added and incubated for 3 h. Optical density was determined at 490 nm by the ELISA-reader (Bio Tek-ELX800) after the addition of isopropanol alcohol (Ganguly et al. 2006). The CC_{50} (cytotoxicity concentration for

50% of cells) was calculated by the probit test. Furthermore, the selectivity index (SI) = $CC_{50}/IC_{50} \geq 10$ (Escudero-Martínez et al. 2017) was used to check drug safety; non-toxic was assessed.

Anti-promastigote assay

Parasite growth was assessed by counting live promastigotes in a Neubauer chamber. Then 2×10^6 logarithmic-phase of *L. tropica* promastigotes (MHOM/IR/75/Mash2) cultured in RPMI-1640 with 100 µg streptomycin/mL and 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Sigma, Aldrich, France) were treated with different concentrations of combination and niosomal form. After 72 h incubation at 24 ± 1 °C, MTT assay was performed three times and OD (optical density) was determined by spectrophotometer at 490 nm as previously mentioned. The probit test was used to calculate the IC_{50} value (50% inhibitory concentration).

Anti- amastigote assay

The stationary phase promastigotes of *L. tropica were* added to the murine macrophage cell-line. The combination adhered to the slides after 2 h incubation at 37 °C and 5% CO_2, at a ratio of 10:1 promastigote: macrophage, respectively. After incubation for 24 h at 37 °C and 5% CO_2, intramacrophage amastigotes were treated with different concentrations of drugs. Then, the treated cells were fixed and stained while amastigotes in 100 macrophages were counted. The infected macrophages without drugs and macrophages with no parasite and no drugs were considered as untreated and negative control, respectively. All experiments were repeated in triplicates and the IC_{50}values were determined using the probit test.

Flow cytometry assay

An Apoptosis Detection Kit with FITC annexin V and 7AAD (BD Pharnigen™) was used for the evaluation of apoptotic and necrotic values of treated promasigotes to indicate the externalization of phospholipid classes as an index of anti-leishmanial potential for drugs. The treated promastigotes cells with AmB plus selenium, AmB plus selenium niosome (with different concentrations of 12.5 + 12.5, 50 + 50, and 200 + 200 µg/ml), and glucantime (with concentrations of 12.5, 50, and 200 µg/ml) were incubated at 24 ± 1 °C for 72 h then the assessment of apoptotic values was carried out according to the FITC kit manufacture's instruction. The sample containing the medium and parasite with no drugs were considered as untreated control.

Quantitative real-time PCR (qPCR) assay

RNA isolation and analysis

According to the manufacturer's instructions, EZ-10 Spin Column Total RNA Miniprep Kit (Bio Basic Inc., Canada) was used for RNA extraction of extracellular promastigotes and intra murine macrophages amastigotes.Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to determine the quantity and purity of RNA.

qPCR

For the detection of relative expression (IL-12, IL-10 in mouse macrophages and metacaspase in *L. tropica* promastigotes), quantitative real-time PCR (qPCR) assay was carried out. According to the RT reagent kit (Takara, Clontech) manufacture's instruction, cDNA was synthesized with a total of 500 ng RNA at 37 °C for 15 min using the Revert Aid M-MuLV reverse transcriptase with a random hexamer primer (Fermentas, Vilnius, Lithuania).

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)(Chandra et al. 2008; Koutsoni et al. 2014) was used as reference gene for gene expression of IL-12 and IL-10 in murine macrophage cells (J-774 A) and *RPS18* Ribosomal protein (S18) (Zhong et al. 2013; Kumar and Engwerda 2014) for gene expression of metacaspase in *Leishmania* (Table 1). qPCR was performed using 10 µl SYBR premix Taq™ (Takara, Clontech), 250 n/mol of the forward and reverse primers for both, and 1 µL of cDNA diluted in RNase-free water on a real-time PCR cycler (Qiagen, Chatsworth, CA) at 95 °C for 1 min; 40 cycles at 94 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. The qPCR reactions for each sample were performed two times. Each run was performed using a positive and negative control.

ΔCT was calculated using the formula:

$$[\Delta CT = CT(\text{target})\_CT(\text{reference})],$$

However, gene expression levels of IL-10, IL-12 and metacaspase were determined by $2^{-\Delta Ct}$ method. In addition, Fold increase (FI) was determined by the comparative threshold assay ($2^{-\Delta\Delta CT}$).

Statistical analysis

SPSS version 22 (SPSS Inc., Chicago, IL, USA) was used for data analysis, while probit test was used to determine IC_{50} and CC_{50} values. Analysis of variance (ANOVA) and independent *t* test were used to evaluate the differences between the two formulations in comparison with glucantime and the untreated control. Mean $2^{-\Delta Ct}$ for treatment

Table 1 Primers used for the real-time PCR

Primers	Gene	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Product size (bp)
Macrophages murine cells	IL-12 P40	CTGGAGCACTCCCCATTCCTA	GCAGACATTCCCGCCTTTG	160
	IL-10	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGC	101
	GAPDH	AGCTTCGGCACATATTTCATCTG	CGTTCACTCCCATGACAAACA	89
Promastigotes of *L. i tropica*	Metacaspase	CAGCAACAATTCCTGGCGATA	AAGTTTGAAGTAAAAGGAGACAATTTGG	140
	RPS18 Ribosomal protein (S18)	GTTGAGGTGCGTGGTCTGTC	TGCAGGTTGCTCAGGAGCTT	166

and control for each cytokine were compared using GRAPHPAD PRISM 6 (GraphPad Software Inc, San Diego, CA, USA).Level of statistical significance was determined at $P \leq 0.05$.

Results

Characterization of niosome

The size and morphology of selenium and AmB noisomal formulation in combination was assessed. Niosomal formulation procedure has spherical multi-layer vesicles of 50.2 ± 0.48 μm size (Fig. 1). The microscopic picture was determined in triplicates (Fig. 2).

Cytotoxicity effects on macrophage cells

After cytotoxic analysis of the macrophage cell-line, no toxic effect was observed for the combination and its niosomal formulation at various concentrations (Fig. 3). The ratio between CC_{50} on macrophages murine cells and IC_{50} against *L. tropica* amastigotes (SI) was calculated. The niosomal formulation had the highest SI (Table 2).

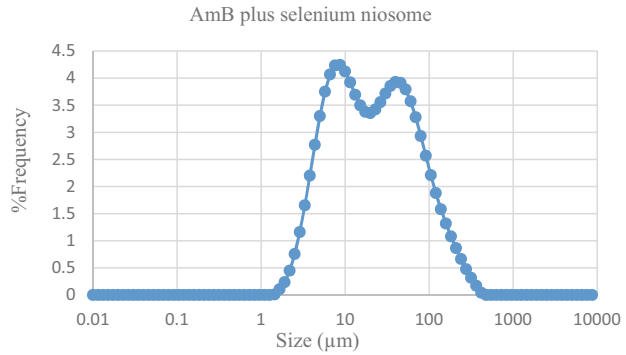


Fig. 1 The size distribution graph in terms of the frequency of Span 40/Tween 40 (molar ratio = 6/4) of AmB plus selenium niosome

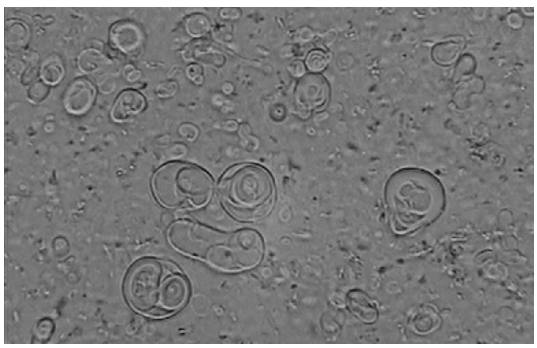


Fig. 2 Microscopy pictures of the AmB plus selenium niosome (Span 40/Tween 40 with molar ratio: 6/4)

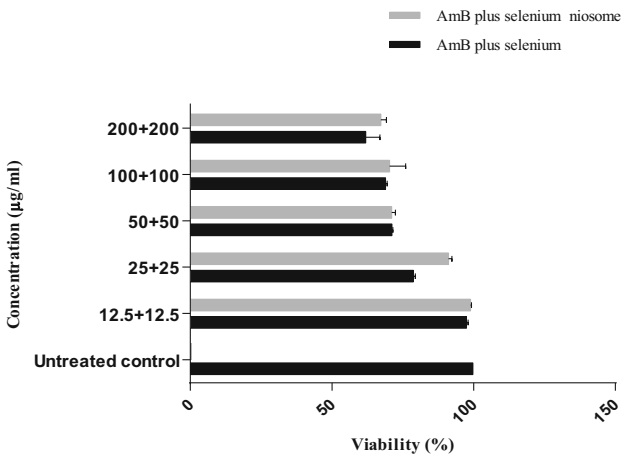


Fig. 3 The cytotoxicity analysis of the different concentrations AmB plus selenium, AmB plus selenium niosome, and untreated control on the J774 macrophage cells

Anti-leishmnial effects on promastigote form of *L. tropica*

Although, both combinations showed inhibitory effects against *L. tropica* promastigotes, the niosomal formulation was significantly more effective ($P \leq 0.05$). The IC_{50} values of AmB plus selenium, niosomal formulation and glucantime were 6.7 ± 1.9 μg/ml, 18.2 ± 0.9 μg/ml and 1445 ± 97.3 μg/ml, respectively (Table 2). In addition, the

Table 2 Comparison of the IC_{50} values of glucantime, AmB plus selenium and AmB plus selenium niosome on *L. tropica* promastigote and amastigote and CC_{50} values of drugs on macrophage and selectivity index (SI)

Drug	Amastigote		Promastigote		Macrophage	*SI (selectivity index)
	IC_{50} ± SD (μg/ml)	*P* value	IC_{50} ± SD (μg/ml)	*P* value	CC_{50} ± SD (μg/ml)	
Glucantime	222.31 ± 28.04	$P < 0.001$	1445 ± 97.3	$P < 0.001$	1634 ± 29.61	7.35
AmB plus selenuim	8.2 ± 2.2	$P < 0.001$	18.22 ± 0.98	$P < 0.001$	354.5 ± 48.7	42.13
AmB plus selenuim niosome	1.73 ± 0.4	$P < 0.001$	6.7 ± 1.9	$P < 0.001$	499 ± 90	288.43

IC_{50} of promastigotes: concentration of drug that caused 50% of growth inhibition of promastigotes

IC_{50} of amastigotes: concentration of drug that caused 50% of growth inhibition of amastigotes

CC_{50}: concentration of drug that caused 50% of growth inhibition in macrophages

SI: selectivity index (CC_{50}/IC_{50})

effect of the niosomal combination or simple form exhibited a dose- response manner (Fig. 4).

Anti-leishmnial effects on amastigote form of *L. tropica*

The mean number of macrophages with adherent amastigotes treated with both formulations was calculated. Combinations of various concentrations were able to significantly inhibit the number of amastigotes per macrophages compared with glucantime and the untreated control ($P < 0.001$) (Table 3). With regards to the IC_{50} values, the niosomal formulation was significantly more effective than the simple combination (Table 2) (IC_{50} = 1.73 ± 0.4 vs. 8.2 ± 2.2 μg/ml).

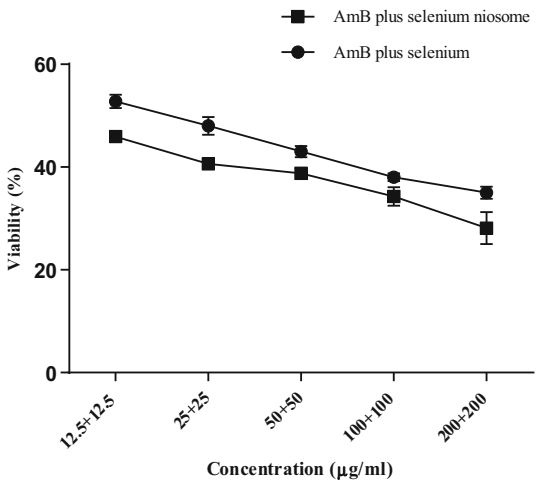


Fig. 4 Comparison of viability effect of AmB plus selenium niosome with simple form of combination on *L. tropica* promastigotes by MTT assay ($P < 0.05$)

Flow cytometric analysis

Apoptosis and necrosis in promastigotes treated with AmB plus selenium, niosomal formulation, glucantime or untreated control were assessed using double-stained flow cytometric assay (annexin V and PI). An increase in apoptotic value from 43.25, 68.29 (12.5 + 12.5 μg/ml) to 73.67, 89.55 (200 + 200 μg/ml) was observed for AmB plus selenium and niosomal form, respectively. Programmed cell death (PCD) exhibited a dose-dependent pattern, that is, the effect was more significant at the highest niosomal formulation concentrations (200 + 200 μg/ml) compared to the simple form of combination, glucantime and untreated control (Fig. 5).

Gene expression effects

The results of gene expression showed that IL-12 and metacaspase levels significantly increased ($P < 0.01$). In contrast however, IL-10 level significantly decreased relative to glucantime and untreated control ($P < 0.01$) from the least to highest concentrations (Fig. 6).

Discussion

The first line of treatment for leishmanisis consists of the application of pentavalent antimonials including meglumine antimoniate (MA) and sodium stibogluconate (SbV).Other treatments such as ketoconazole (Momeni et al. 2003), paramomycine, gentamycine (Tolouei et al. 2011), verapamil (Shokri et al. 2012), terbinafine (Farajzadeh et al. 2015a, b) and dapsone (Osorio et al. 1998) have been used for CL treatment but have limited efficacy. Presently, there have been intense researches on developing new strategies for leishmaniasis treatment (Sundar et al. 2014).

Table 3 Comparison of the overall mean effect of various concentrations of AmB plus selenium, AmB plus selenium niosome and glucantime on the mean number of amastigotes in each macrophage

Concentration	AmB plus selenui		AmB plus selenium niosome		Concentration	Glucantime	
(μg/ml)	Mean ± SD	*P* value	Mean ± SD	*P* value	(μg/ml)	Mean ± SD	*P* value
0 (Untreated control)	32 ± 0.46	NR	32 ± 0.46	NR	0 (Untreated control)	22 ± 1	NR
12.5 + 12.5	14.5 ± 0.35	$P \leq 0.001$	9.23 ± 0.28	$P \leq 0.001$	12.5	21 ± 0.26	$P \leq 0.001$
25 + 25	14 ± 0.53	$P \leq 0.001$	9.14 ± 1.16	$P \leq 0.001$	25	20 ± 0.75	$P \leq 0.001$
50 + 50	13.2 ± 0.26	$P \leq 0.001$	8.45 ± 0.08	$P \leq 0.001$	50	15 ± 0.17	$P \leq 0.001$
100 + 100	12.5 ± .46	$P \leq 0.001$	6.44 ± 0.72	$P \leq 0.001$	100	12 ± 0.26	$P \leq 0.001$
200 + 200	8.8 ± 0.5	$P \leq 0.001$	3.26 ± 0.17	$P \leq 0.001$	200	10 ± 0.62	$P \leq 0.001$

Co-administration of antileishmanial drugs with synergistic activity increased drug efficacy, prevented drug resistance, reduced dosage and decreased drug cytotoxicity (Yazdanpanah et al. 2004; White 1999; Farajzadeh et al. 2015a).

Niosomes as a drug carrier authorize the entrapment of a large number of drugs with a wide range of solubility, stability, long shelf life and enable drug delivery to target site in a controlled manner. Therefore, they can reduce drug toxicity and also enhance efficacy (Mahale et al. 2012; Bayindir et al. 2015; Duarte et al. 2016).

The current study presents an evaluation of the in vitro leishmanicidal activities of drug combinations including non-toxic concentrations of AmB plus selenium as a noisome or simple form. The results of the study showed a positive interaction between AmB and selenium. The niosomal combination of drugs decreased the proliferations of *L. tropica* extra-cellular promastigotes by about 4-fold in contrast to the use of simple form of combination. Consequently, this also resulted in inhibition of *L. tropica* amastigotes at between 54.7 and 72.5%. In addition, the niosomal combination caused programmed cell death and increased metacaspase in *L. tropica* promastigotes and decreased IL-10 expression for Th-2 inhibition and increased IL-12 for Th-1 activation to destroy the parasites.

However, AmB showed effective lishmanicidal activity against *Leishmania* species by bonding with the membrane sterols of the parasites (Torrado et al. 2008; Prajapati et al. 2011; Chattopadhyay and Jafurulla 2011), amidst high toxicity levels(Annaloro et al. 2009).Ambisome®, Abelcet®, SinaAmphoLeish®, Amphocil®are efficient against *Leishmania* species and are fairly safe, but highly unstable and expensive; thereby, limiting their use. Additionally, liposomal formulations of AmB are usually administered by an intravenous route for the treatment of leishmaniasis (Frankenburg et al. 1998; Yardley and Croft 2000; Sundar and Chakravarty 2010; Varikuti et al. 2017; Wijnant et al. 2018). All these are in agreement with the results of this study.

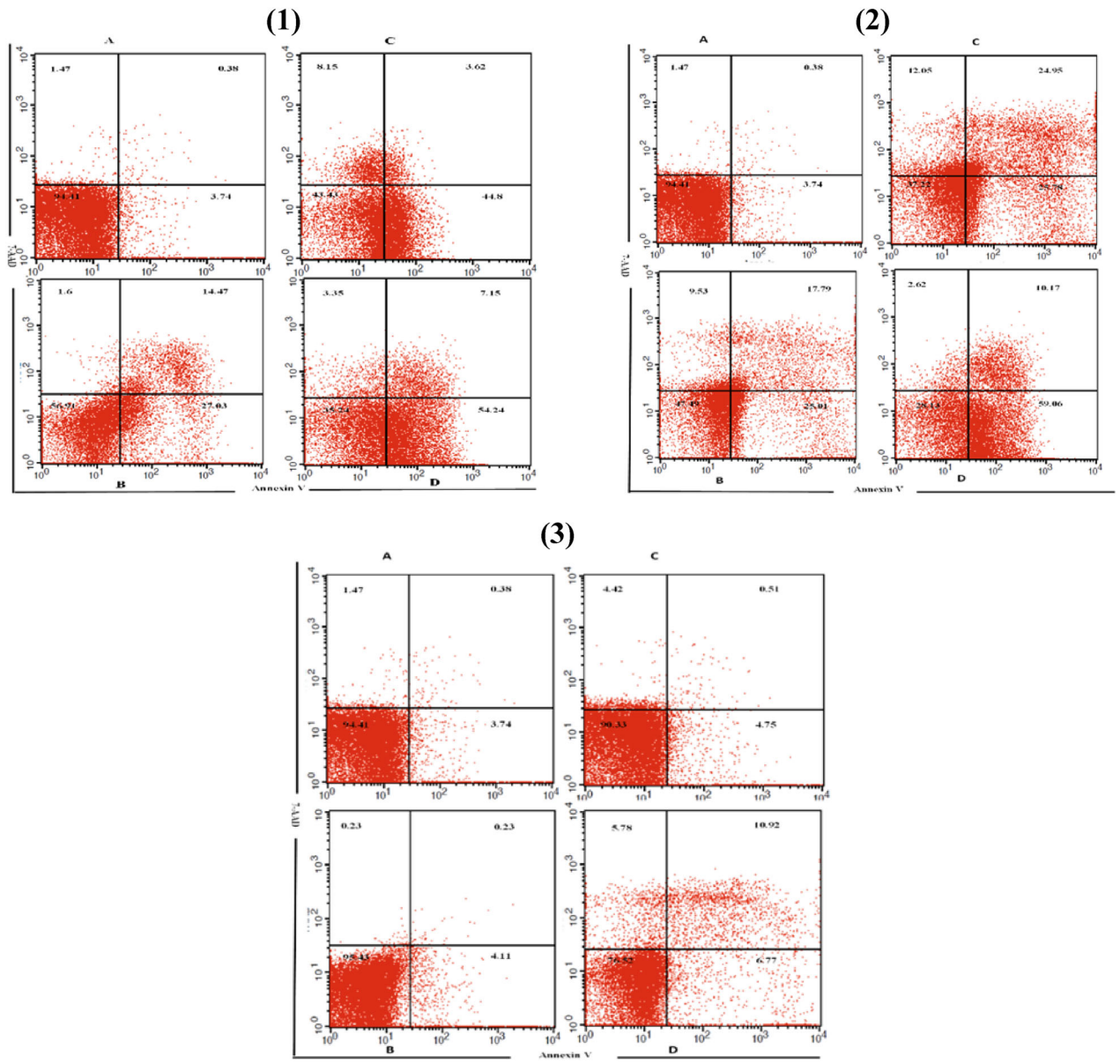
Previous studies showed a connection between selenium as an antitumor, antioxidant and antiparasitic agents, especially against *Tripanasoma*. Selenium has a potent and selective effect against some species of *Leishmania* (Lobanov et al. 2016; Cassago et al. 2006). Other studies have reported the antileishmanial activities of selenium and nano-selenium against *L. infantum, L. major, L. tropica* and *L. braziliensis* (Beheshti et al. 2013; Mahmoudvand et al. 2014; Martín-Montes et al. 2017).

The IC_{50} values showed that the AmB niosomal formulation plus selenium was more effective against *L. tropica* at various stages with a high safety index (SI = 261.57) and also, exhibited inhibitory effect in comparison to simple form of combination.

From the results of the current study, the IC_{50} values for both formulations on promastigotes were significantly higher than the amastigotes forms. These findings are corroborated by previous studies that showed that extra cellular form of *Leishmania* were 2-60 times more resistant in comparison with the intra cellular form due to biochemical and physiological differences in terms of their modes of action in response to the drugs (Lira et al. 1999).

Programmed cell death in *Leishmani* spp. was due to reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), cytochrome c release, phosphatidylethanolamine (PE), activation of peptidase (such as metacaspase) and mitochondrial depolarization when treated with drugs (Debrabant et al. 2003; Murray et al. 2005; Kaye and Scott 2011). The metacaspase, as a cysteine peptidases in *L. major* and *L. donovani* plays an important role in cell death (Lee et al. 2002; Ambit et al. 2008; Zalila et al. 2011; Raina and Kaur 2012).The results of flow cytometry of intra cellular form of *L. tropica* treated with combination of niosomal or simple form showed a high level of apoptosis especially in the niosomal form. In addition, metacaspase increased which is indicative of programmed cell death compared with glucantime as a standard drug.

Some cytokines, like tumor necrosis factor-α (TNF-α), IL-12, colony-stimulating factor (GM-CSF) and interferon-



(1) A: Untreated control, B: AmB plus selenium 12.5+12.5(µg/mL), C: AmB plus selenium 50+50(µg/mL), D: AmB plus selenium 200+200(µg/mL).
(2) A: Untreated control, B: AmB plus selenium niosome 12.5+12.5(µg/mL), C: AmB plus selenium niosome 50+50(µg/mL), D: AmB plus selenium niosome 200+200(µg/mL).
(3) A: Untreated control, B: Glucantime 12.5(µg/mL), C: Glucantime 50(µg/mL), D: Glucantime 200(µg/mL).

Fig. 5 The apoptotic and necrotic profiles of *Leishmania tropica* promastigote with annexin V/PI at various concentrations of AmB plus selenium (1), AmB plus selenium niosome (2) and glucntime (3)

γ (IFN-γ) are Th1-type immunity activation markers against *Leishmania* infection. On the other hand, other cytokines which are activators of Th2-type cell response such as IL-4, IL-10, IL-13, and the transforming growth factor-β (TGF-β) enables the development of the disease (Prajapati et al. 2011; Gannavaram et al. 2016). The results of this study showed that IL-12 levels increased, while IL-10 levels decreased significantly from a dose response effect of 12.5–200 µg/ml, as expected. Therefore, the niosomal combination of AmB plus selenium can modulate the immune system against the disease.

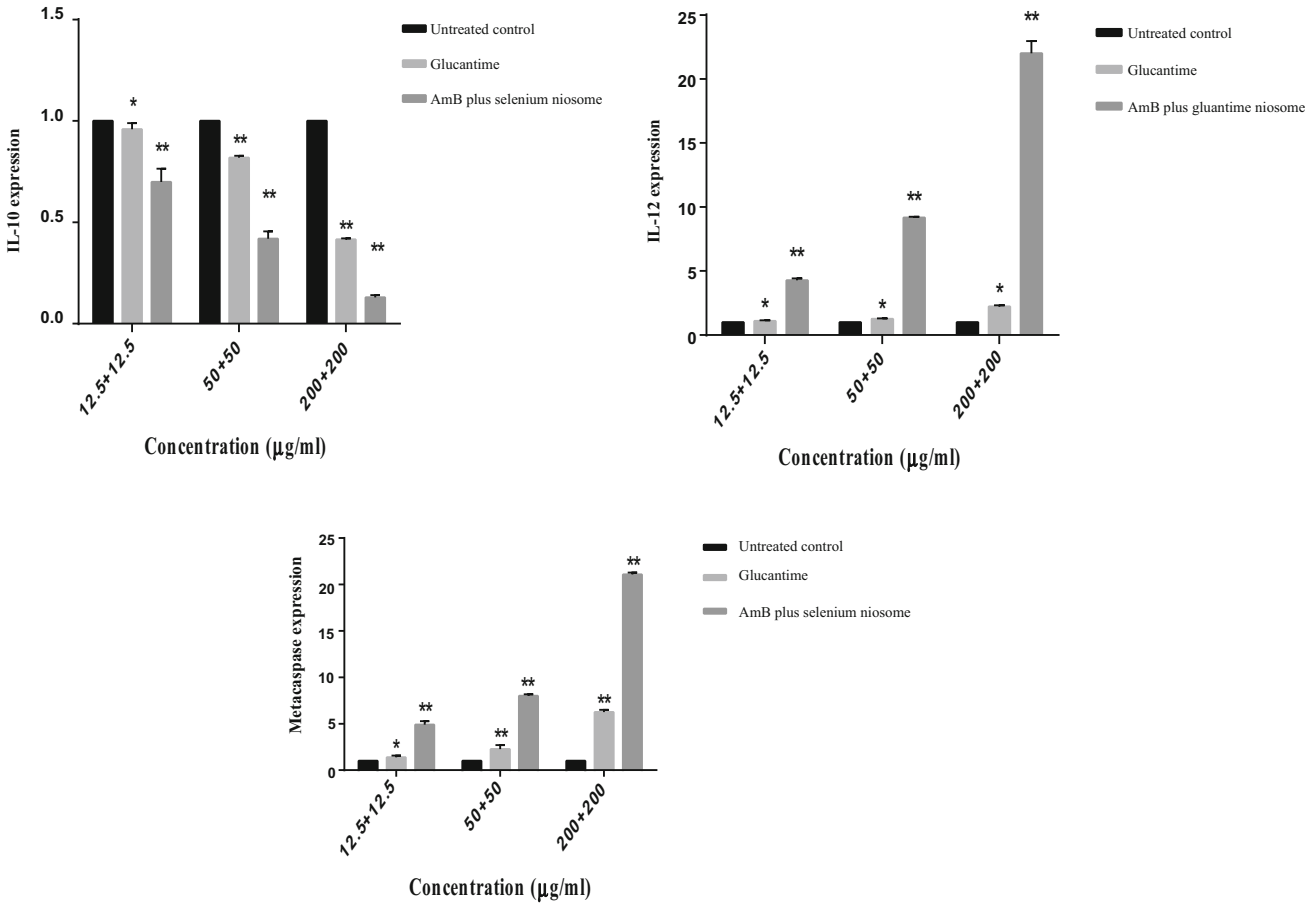


Fig. 6 The gene expression profiles of IL-12p40, metacaspase, and IL-10 on the *Leishmania.tropica* treated by the AmB plus selenium niosome and glucantime in comparison with untreated control (**$P < 0.001$, *$P < 0.01$), as measured by using real-time PCR

Conclusion

AmB and selenium are effective against *Leishmania* species. Our results showed that a combination of the niosomal formulation of these drugs showed superior efficacy in the treatment of CL due to *L. tropica* in an in vitro model. The niosomes prepared a suitable environment for better drug efficiency by acting as perfect carriers for the drug delivery. These findings suggest that niosomes are stable carrier's for this combination and are easy to produce and affordable. Consequently, they provide promising results for the effective formulation of CL treatment in clinical settings.

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Author Contributions SF and MM conceived and designed the study. PK contributed to preparation of niosomal formulation. HS analyzed the data. IS and MM contributed to study concept, data interpretation and manuscript preparation. All authors contributed in reviewed, revised, and confirmed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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