Parasitology Research (2019) 118:2669–2678
https://doi.org/10.1007/s00436-019-06382-y

PROTOZOOLOGY - ORIGINAL PAPER

In vitro and in vivo anti-parasitic activity of biogenic antimony sulfide nanoparticles on *Leishmania major* (MRHO/IR/75/ER)

Sina Mohtasebi[1,2] · Mehdi Mohebali[1,3] · Samira Elikaee[1] · Behnaz Akhoundi[1] · Abbas Rahimi Foroushani[4] · Aref Teimouri[1] · Hamed Yarizadeh[1]

Received: 7 March 2019 / Accepted: 21 June 2019 / Published online: 5 July 2019

Abstract

The aims of this study were to produce biogenic antimony sulfide nanoparticles (NPs) using *Serratia marcescens* (*S. marcescens*) and investigate the potential anti-leishmanial effects of these NPs on *Leishmania major* (*L. major*) (MRHO/IR/75/ER) in both in vitro and in vivo experiments. Biogenic antimony sulfide NPs were synthesized through intracellular biological methods using *S. marcescens*. The efficiency of various concentrations of antimony sulfide NPs was assessed using in vitro experiments on amastigotes of *L. major* at various times post-infection. In vivo experiments were carried out in BALB/c mice inoculated subcutaneously with 2×10^6 *L. major* promastigotes (MHROM/IR/75/ER) and treated with antimony sulfide NPs (70 μg/mL, tropically), meglumine antimoniate (glucantime) as positive control and sterile phosphate-buffered saline (PBS, pH 7.4) as vehicle control. Results of in vitro experiments revealed that the anti-leishmanial activity increased when the antimony sulfide NPs concentration increased. The IC50 (50% inhibitory concentration) of antimony sulfide NPs against amastigotes was calculated as 62.5 μg/mL. In in vivo experiments, the average size of lesions significantly decreased to 8.6 ± 2.7 mm^2 in mice inoculated with *L. major* promastigotes and treated with antimony sulfide NPs, compared with that in the negative control group ($P = 0.015$). Furthermore, results showed that antimony sulfide NPs significantly decreased the parasite load in the test group, compared with the negative control group ($P = 0.001$). Various concentrations of antimony sulfide NPs showed a great anti-leishmanial efficiency against *L. major* (MRHO/IR/75/ER), with the greatest efficiency shown by a concentration of 62.5 μg/mL in in vitro and in vivo experiments.

Keywords Anti-leishmanial activity · Biogenic antimony sulfide · Biological synthesis · *Leishmania major* · Nanoparticles · *Serratia marcescens*

Section Editor: Sarah Hendrickx.

✉ Mehdi Mohebali
mohebali@tums.ac.ir

Sina Mohtasebi
sina.mohtasebi@gmail.com

Samira Elikaee
elikaees@gmail.com

Behnaz Akhoundi
behnazakhoundi@yahoo.com

Abbas Rahimi Foroushani
rahimifo@tums.ac.ir

Aref Teimouri
aref_teimouri@yahoo.com

Hamed Yarizadeh
hy10101010@gmail.com

[1] Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2] Students Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran

[3] Research Center for Endemic Parasites, Tehran University of Medical Sciences, Tehran, Iran

[4] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Introduction

Leishmaniasis is a cosmopolitan infectious disease caused by members of the protozoan parasite *Leishmania*, which is transmitted to mammals by the bite of female infected sand flies (Grimaldi and Tesh 1993; den Boer et al. 2011). Cutaneous leishmaniasis (CL) is the most usual clinical manifestation of leishmaniasis, which is considered as a major public health problem in the world. More than 90% of the leishmaniasis cases occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, and Syria (Old World) and in Brazil and Peru (New World) (Desjeux 2004). In Iran, zoonotic CL caused by *L. major* is a common health problem, particularly in rural areas (Yaghoobi-Ershadi and Javadian 1996). The geographic distribution and prevalence of CL have increased globally during the past decade (Reithinger et al. 2007); however, an effective vaccine is not still available (Ghorbani and Farhoudi 2018). Therefore, accurate diagnosis schemes and ideal treatments of patients are critical for the control of the disease. Currently, the recommended therapy for the treatment of human leishmaniasis includes sodium stibogluconate (Pentostam) and meglumine antimoniate (glucantime) (Aronson et al. 2017), used alone or in combination with other compounds (Roberts et al. 1998; Veeken et al. 2000). Invasive and painful injections, frequent side effects, and high toxicity of these drugs have made the treatment procedure difficult. Furthermore, drug resistance in *Leishmania* spp. is reported during recent years (Croft et al. 2006). Moreover, pharmaceutical wastes contaminate the environment and threaten human and animal health (Zuccato et al. 2000). Therefore, studies have been carried out to find novel, effective, and safe alternative medicines for prophylaxis and treatment of leishmaniasis (Davidson 1998).

Nowadays, nanotechnology is widely used in various scientific fields. In particular, nanoparticles (NPs) are used for in vitro and in vivo studies on parasites (Khan et al. 2015). These studies are mainly motived by their use in the treatment of a variety of diseases. A new strategy in biotechnology has contributed to the biosynthesis of particular NPs using microorganisms as novel bio-factories. Biosynthetic processes have been developed to prepare applicable NPs such as gold (Au), silver (Ag), selenium (Se), cadmium sulfide, and magnetite iron oxide NPs (Mandal et al. 2006; Jha et al. 2009a; Vaidyanathan et al. 2009; Shakibaei et al. 2010; Thakkar et al. 2010; El-Khadragy et al. 2018). Biogenic synthesized NPs are environmentally friendly compounds due to their controlled toxicity and size characteristics and rapid synthesis (Rai et al. 2011). Biological antimony sulfide NPs have been synthesized successfully in *S. marcescens* and the antibacterial activity of these NPs has been reported against *Staphylococcus aureus* and *Escherichia coli* (Bahrami et al. 2012). Furthermore, cytotoxic and parasiticidal properties of antimony sulfide NPs have been demonstrated in protozoan parasites such as *L. infantum* (Soflaei et al. 2012). Although the chemical synthesis of antimony sulfide NPs has been reported in the literature, a few studies have been carried out to assess the biological synthesis of antimony sulfide NPs. In recent years, interests in the use of inorganic NPs in various healthcare materials and industrial products have significantly increased. Examples include antimicrobials, catalysts, lubricants, and microelectronics devices (Balaji et al. 2009; Jha et al. 2009b). To the best of the author's knowledge, no studies have been published on the effects of biogenic antimony sulfide NPs on *L. major* in vitro and in vivo. Therefore, the aims of the current study were to prepare biogenic antimony sulfide NPs through intracellular biological methods from *S. marcescens* and investigate the NPs potential anti-leishmanial effects against *L. major* (MRHO/IR/75/ER) using in vitro and in vivo experiments.

Materials and methods

Compounds

RPMI 1640 media and penicillin-streptomycin (pen/strep) were purchased from Sigma-Aldrich, USA. Heat-inactivated fetal bovine serum (FBS) and high-glucose Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibco, USA. The BALB/c mice-derived macrophage cell line RAW 264.7 was provided by the Iranian Biological Resource Center, Tehran, Iran. Cell culture slides were purchased from SPL Life Sciences, South Korea. Ethanol and methanol were purchased from Merck, Germany. Deionized distilled water (D.W.) was used in all experiments. The *L. major* promastigotes (MHROM/IR/75/ER) were provided by the Department of Parasitology, Tehran University of Medical Sciences, Tehran, Iran.

Animals

Forty male BALB/c mice (6–8 weeks old), weighing 20–25 g, were purchased from the Animal Breeding Stock Facility, Razi Vaccine and Serum Institute of Iran, Karaj, Iran. Mice were housed under standard laboratory conditions (light/dark cycles, controlled temperatures of 22 ± 2 °C) with free access to food and fresh drinking water. The current study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (Approval No. 94-03-160-30138) on April 25, 2017. Animal care and experiments were carried out according to Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health and approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran.

Biogenic antimony sulfide nanoparticles preparation

Biogenic antimony sulfide NPs were synthesized from antimony chloride through intracellular biological methods using *S. marcescens* isolated from the Caspian Sea through the solid-state fermentation method in the Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences (Bahrami et al. 2012). Briefly, antimony-supplemented nutrient agar (NA) plates (SbCl3, 1% *w*/*v*) were first inoculated with *S. marcescens* and incubated aerobically at 30 °C overnight. Bacterial cells were harvested from the surface of media using sterile loops, suspended in normal saline, and then washed with D.W. using centrifugation at 5000*g* for 10 min. Pellets were frozen by adding liquid nitrogen (LN) and then powdered using mortar and pestle. The resulting slurry containing antimony sulfide NPs and cell residues were suspended in a two-phase partitioning system of n-octyl alcohol and D.W. (1:2 ratio) and then the mixture was shaken vigorously. After centrifuging at 5000*g* for 15 min, the biogenic NPs were precipitated at the bottom of tubes. Supernatants were discarded and the precipitated NPs were resuspended in a mixed solvent system containing chloroform, ethyl alcohol, and water (3:1:4 ratio). Organic and aqueous phases were separated via centrifugation and the upper aqueous phase containing antimony NPs was collected and used in further assays.

Parasite culture

For the mass culture of *L. major* promastigotes (MHROM/IR/75/ER), promastigotes were cultured in RPMI 1640 media supplemented with 10% of heat-inactivated FBS and 1% of pen/strep. Culture was incubated at 24 °C for promastigote proliferation and the parasites were transferred weekly from previous cultures into fresh media.

In vitro experiments

The BALB/c mice-derived macrophage cell line RAW 264.7 was cultured (2×10^4 cells per well) in high-glucose DMEM with 10% (*v*/*v*) of heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere of 5% CO_2 in 8-well culture chamber slides. After 4 h, unattached macrophages were washed off with PBS and attached macrophages were infected with *L. major* promastigotes with the ratio of 10 promastigotes per macrophage. The cultures were incubated at 37 °C for 24 h in 5% CO_2 until promastigotes were phagocyte by macrophages. After incubation, media in culture chambers were discarded and each well of the slides was washed with 1–2 mL PBS to remove the extracellular promastigotes. Then, chambers were treated with fresh medium containing serial concentrations of antimony sulfide NPs (1000, 500, 250, 125, 62.5, 31.25, and 15.62 μg/mL). Chambers with uninfected macrophages and *L. major* infected macrophages were assessed as controls. Then, slides were incubated again for 24, 48, and 72 h. Based on the incubation time, slides were air-dried, fixed, and stained with Giemsa (1:10 dilution) (Elikaee et al. 2018). For each culture, at least 100 macrophages were counted and the percentage of the infected macrophages (the macrophages with amastigotes) and mean number of the amastigotes per 100 infected macrophages were calculated. The in vitro assays were carried out in three independent experiments and each experiment in each time point was done in three wells.

Cytotoxicity evaluation

Cytotoxic effect of antimony sulfide NPs on macrophage was assessed and compared with control cultures. The following formula was used for toxicity evaluation:

$$\frac{\text{Mean of macrophages in any NPs concentration}***}{\text{Mean of macrophages in well of macrophage control}***} \times 100$$

***In each time point (in 100 microscopic fields by direct counting)

IC50 (50% inhibitory concentration) evaluation

The IC50 was calculated using the percentage of infected macrophages and parasite load in treated infected macrophage compared with the control group (non-treated) according to the following formula, respectively:

$$\text{IC50} = \frac{\text{Mean percentage of infected macrophages in any NPs concentration}*}{\text{Mean percentage of infected macrophages in well of macrophage} + \text{amastigotes control}*} \times 100$$

*In each time point (in 100 microscopic fields by direct counting)

$$\mathrm{IC50} = \frac{\text{Mean percentage of amastigotes in any NPs concentration}^{**}}{\text{Mean percentage of amastigotes in well of macrophage + amastigotes control}^{**}} \times 100$$

**In each time point (in 300 infected macrophages)

In vivo experiments

Forty male BALB/c mice were infected using a subcutaneous injection of 2×10^6 infective *L. major* promastigotes (MHROM/IR/75/ER) into the base of mice tails. Following lesion development, mice were chosen according to the lesion size to ensure similar levels of infections. Chosen mice were then divided into four major groups ($n = 10$ per group) as follows: group 1 included mice treated topically with 70 μg/mL of antimony sulfide NPs diluted in sterile PBS twice a day for 28 consecutive days; group 2 included mice treated topically with 200 μL of sterile PBS without antimony sulfide NPs as vehicle control group twice a day for 28 consecutive days; group 3 included mice treated intramuscularly (IM) with 20 mg/kg of glucantime as positive control group daily for 14 consecutive days; and group 4 included infected untreated mice as a negative control group. Treatment started 4 weeks post-inoculation when lesions were established and appeared at injection sites. All mice in the treatment groups showed similar lesion sizes. Mice were monitored daily for 28 days and lesion sizes were documented for each group.

Treatment evaluation

Effects of the treatments were assessed based on the changes in lesion sizes and lesion parasite loads as demonstrated using Giemsa-stained skin smears.

Measurement of the lesion size

Size of the lesions was measured weekly using caliper tool (Mitutoyo, Taiwan). Size of the lesions was calculated using the mean values between the horizontal and vertical directions. The wound regions were reported in square millimeter.

Parasite load

To calculate the parasite load, smears were prepared from the margins of lesions on glass slides, fixed with absolute methanol, stained with Giemsa, and studied for the presence of amastigotes or infected macrophages using Zeiss light microscope (Carl Zeiss, Germany). The positive cases with *L. major* amastigotes and the parasite loads were analyzed based on the WHO guidelines of 4+ (1–10 parasites/1 field), 3+ (1–10 parasites/10 fields), 2+ (1–10 parasites/100 fields), and 1+ (1–10 parasites/1000 fields) (WHO 2014).

Statistical analysis

Statistical analysis was carried out using Student's *t* test and SPSS software v.21 (IBM Analytics, USA). Statistical significances in the mean lesion diameters within the groups were calculated using one-way analysis of variance (ANOVA) test and post hoc Tukey's test (GraphPad Prism, USA). In vitro anti-leishmanial activities (IC50) were calculated using linear regression analysis. *P* values < 0.05 were considered statistically significant (Fletcher et al. 1996).

Results

In vitro experiment

The results of cytotoxicity evaluation revealed that the *L. major* amastigotes and macrophages degenerated in three concentrations of 1000, 500, and 250 μg/mL of antimony sulfide NPs compared with controls of macrophage and

Table 1 In vitro anti-leishmanial effects of various concentrations of antimony sulfide nanoparticles on the mean number of amastigotes per 100 infected macrophages using Giemsa stain

Antimony sulfide nanoparticles concentrations	Time (h)			*P* value
	24 no. of amastigotes per 100 infected macrophages ± SD	48 no. of amastigotes per 100 infected macrophages ± SD	72 no. of amastigotes per 100 infected macrophages ± SD	
15.625 μg/mL	137 ± 4	291 ± 5	282 ± 3	0.06
31.25 μg/mL	121 ± 5	214 ± 4	204 ± 6	0.04*
62.5 μg/mL	116 ± 3	154 ± 2	147 ± 5	0.014*
NC	141 ± 6	321 ± 4	389 ± 3	

Values are represented as mean ± SD; *significant at $P < 0.05$ compared with negative control; *NC*, negative control; the cultures were carried out in three independent experiments and each experiment in each time point was done in three wells; counting of amastigotes was carried out in triplicate

Table 2 In vitro anti-leishmanial effects of various concentrations of antimony sulfide nanoparticles on infected macrophages rate using Giemsa stain

Antimony sulfide nanoparticles concentrations	Time (h)			*P* value
	24 (%)	48 (%)	72 (%)	
15.625 μg/mL	20	22	32	0.07
31.25 μg/mL	17	10	19	0.03*
62.5 μg/mL	10	3	8	0.001*
NC	20	24	37	

Values are represented as mean; *significant at $P < 0.05$ compared with negative control; *NC*, negative control; the cultures were carried out in three independent experiments and each experiment in each time point was done in three wells; counting of amastigotes was carried out in triplicate

macrophage plus amastigotes using a light microscope; hence, these concentrations were reported as cytotoxic. Furthermore, 70% of the cells were degenerated in 125 μg/mL concentration of antimony sulfide NPs compared with controls. These results obtained through at least 100 fields of microscopic examination. The IC50 of *L. major* amastigotes was calculated after 24, 48, and 72 h of exposure to various concentrations of antimony sulfide NPs. The IC50 of antimony sulfide NPs included 62.5 μg/mL based on the statistical analysis. Results of in vitro anti-leishmanial activity of various concentrations of antimony sulfide NPs are summarized in Tables 1 and 2. Results showed that the anti-leishmanial effect was amplified as the concentration of antimony sulfide NPs increased. The greatest anti-leishmanial effect was seen for 62.5 μg/mL concentration of antimony sulfide NPs after 72 h among non-cytotoxic concentrations of antimony sulfide NPs include 15.62, 31.25, and 62.5 μg/mL. The anti-leishmanial effect of antimony sulfide NPs with concentrations of 62.5 and 31.25 μg/mL were statistically significant in the test group ($P < 0.05$), compared with that in the negative control group (Table 1). The decrease in infected macrophage rate was statistically significant using antimony sulfide NPs with concentrations of 62.5 and 31.25 μg/mL, compared with that in the negative control group ($P < 0.05$) (Table 2). In the group receiving antimony sulfide NPs at a dose of 62.5 μg/mL, few parasites were observed per many studied fields (Fig. 1).

In vivo experiments

Lesion size

During the treatments, lesions grew normally in the vehicle and negative control mice with no treatments. Anti-leishmanial effects of antimony sulfide NPs on the lesion size (mm^2) of BALB/c mice shown are described in Table 3. In the group that received antimony sulfide NPs (70 μg/mL, local treatment), the size of lesions decreased within a 28-day treatment period (Table 3). A significant statistical difference was seen in decreased lesion sizes between the group receiving antimony sulfide NPs and control groups ($P = 0.015$). In mice with no treatments (negative control and vehicle control), lesions swelled at the site of infections. In glucantime-treated mice, as a positive control, the lesion size decreased during the treatment. The mean area of

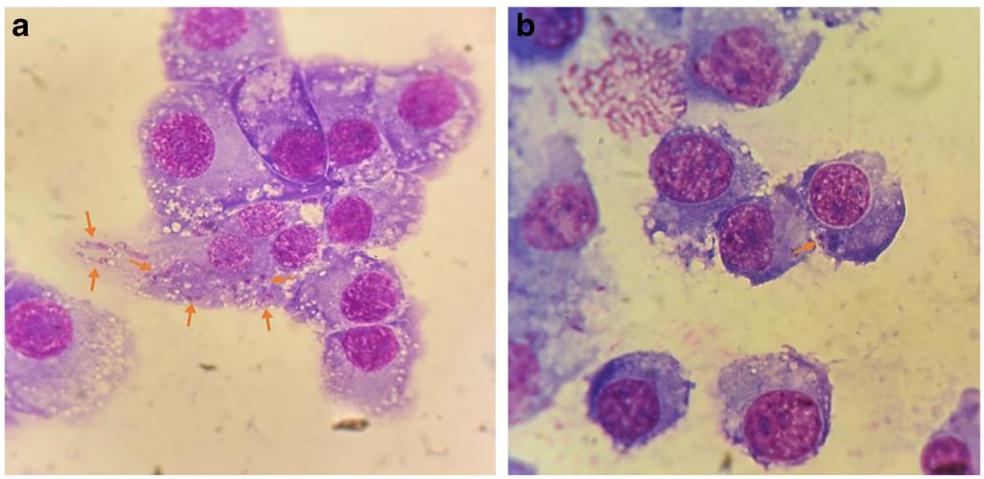

Fig. 1 Parasite load through the treatment. Smears were prepared from macrophage cell line RAW 264.7 in the experimental groups after 72 h of the treatment, stained with Giemsa, and examined under a light microscope: **a** control group; **b** 62.5 μg/mL of antimony sulfide nanoparticles. Arrows show *L. major* amastigotes

Table 3 Anti-leishmanial effects of antimony sulfide nanoparticles on the lesion size (mm^2) of BALB/c mice, compared with control groups

Group	Before treatment mean ± SD	1-week mean ± SD	2-week mean ± SD	3-week mean ± SD	4-week mean ± SD	*P* value
Antimony sulfide NPs	12.3 ± 2.1	11.9 ± 2	11.1 ± 2.4	10.25 ± 1.9	8.6 ± 2.7	0.01*
PBS without drug	12.7 ± 2.2	13.6 ± 2.4	14.1 ± 2.2	14.8 ± 2.8	15.6 ± 2	0.4
Glucantime®	12.5 ± 3.1	11.2 ± 3.9	9.6 ± 2.9	***	***	0.02*
NC	12.1 ± 2.7	12.7 ± 2.3	13.25 ± 3.6	14.8 ± 4.6	15.7 ± 3.3	

Values are represented as mean ± SD; *significant at $P < 0.05$ compared with negative control; ***the mice were treated daily intramuscularly injections of 20 mg/kg of Glucantime® for 2 weeks; *NC*, negative control; size of the lesions was measured in triplicate

lesions decreased in mice treated with antimony sulfide NPs, compared with that in untreated mice (Fig. 2).

Parasite load

Four weeks after the treatment, slides were prepared and studied carefully for the presence of amastigotes. Within a 28-day treatment period, mice in negative control and vehicle control groups presented a large number of amastigotes inside macrophages at the lesion sites. In mice treated with 70 μg/mL of antimony sulfide NPs, a number of amastigotes significantly decreased ($P = 0.001$), compared with that in the negative control group mice (Table 4). In a group that received antimony sulfide NPs at doses of 70 μg/mL, few parasites were observed per many searched fields (Fig. 3).

Discussion

For the first time, results from the current study proved the good potential of biogenic antimony sulfide NPs in treatment of CL using in vitro and in vivo experiments. Chemotherapy with pentavalent antimonial (Sb^V) compounds such as glucantime and pentostam has been the first line of treatment for all clinical forms of leishmaniasis since nearly 40 years ago (Aronson et al. 2017). The high toxicity, severe side effects, and microbial tolerance to these chemical agents urge further research to find safer and novel therapeutic agents. The main challenge in CL treatment is the fact that *Leishmania* spp. resides in the host macrophages. Hence, common available anti-leishmanial drugs have difficulties to penetrate the macrophage membrane (Prabhu et al. 2012). Therefore, studies to find novel medications with different mechanisms of action and innovative forms of delivery systems are important. Thus, nanotechnology becomes favorite as a promising approach for the treatment of CL (Gutiérrez et al. 2016). Nanotechnology can improve the available treatments because it reduces costs, increases bioavailability, and lowers toxicity and unwanted side effects of the current drugs. Generally, NPs such as liposomes, polymers, and nanospheres have been used widely as nanocarriers for the drug delivery to improve the therapeutic efficiency in previous studies (Debbage 2009; Wang et al. 2011). However, a few studies have been conducted to assess the effects of NPs on the parasite infection (Abaza 2016;

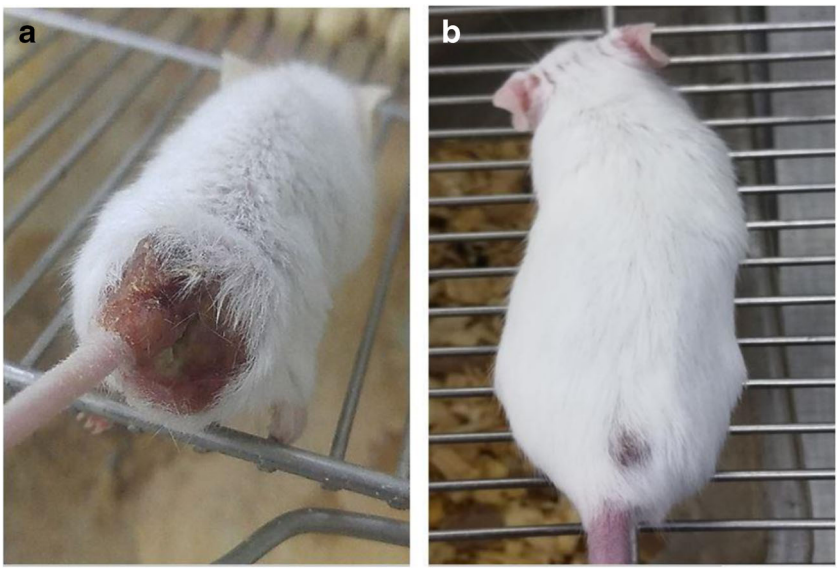

Fig. 2 Lesions of BALB/c mice infected with *L. major*. Photographs were taken on the last days of the treatment: **a** control group; **b** 70 μg/mL of antimony sulfide nanoparticles twice a day for 28 days

Table 4 Parasite load reduction in BALB/c mice before and after treatment with antimony sulfide nanoparticles compared with control groups

Parasite load			*P* value
Group	Before treatment	After treatment	
Antimony sulfide NPs	4^{+}	$*2^{+}$	0.001
PBS without drug	4^{+}	4^{+}	1
Glucantime	4^{+}	$*2^{+}$	0.001
NC	4^{+}	4^{+}	–

Values are represented as mean; *NPs*, nanoparticles; *significant at $P < 0.05$ compared with negative control; *NC*, negative control; amastigotes were counted in triplicate

Azami et al. 2018; Teimouri et al. 2018). In earlier years, studies have been published on the treatment of leishmaniasis using nanotechnology. For example, Mohebali et al. (2009) investigated the effects of Ag-NPs on *L. major* parasites. Results showed that Ag-NPs with a single dose of 40 ppm could reduce the size of the lesions, but the results were not statistically significant. However, secondary infections in Ag-NPs treated groups decreased significantly, compared with that in the control groups. Cytotoxicity effects of Ag-NPs on human cells must be addressed in future studies (Asha Rani et al. 2008). Similarly, Jebali and Kazemi (2013) studied effects of NPs such as Ag, Au, titanium dioxide (TiO_2), zinc oxide (ZnO), and magnesium oxide (MgO) NPs on *L. major* parasites under ultraviolet (UV), infrared (IR), and dark conditions. They showed that the highest activity of the NPs against *L. major* was seen for Ag-NPs, followed by Au-NPs, TiO2-NPs, ZnO-NPs, and MgO-NPs. However, they reported that the NPs included cytotoxicity on macrophages. Furthermore, they concluded that the use of metal oxide NPs for the treatment of CL might include positive or negative consequences (Jebali and Kazemi 2013). Other studies demonstrated that Au-NPs at concentrations of 0.4 and 40 μg/mL were able to significantly decrease the mean size of *L. major* cutaneous lesions in BALB/c mice after 8 weeks of treatment but with no effects on secondary infections (Torabi et al. 2011). Delavari et al. reported that ZnO-NPs at concentrations of 120 μg/mL included anti-leishmanial effects against *L. major* amastigotes in vitro. However, the efficiency could be different in animal models due to the absorption rate (Delavari et al. 2014). In the current study, in vitro experiments have revealed that various concentrations of antimony sulfide NPs are effective against *L. major* amastigotes. The anti-leishmanial effects of antimony sulfide NPs with 62.5 and 31.25 μg/mL concentrations were statistically significant in the test group ($P < 0.05$), compared with that in the negative control group. Moreover, results of this study have shown that decreased rate of infected macrophages was significant in antimony sulfide NPs with 62.5 and 31.25 μg/mL concentrations, compared with that in the negative control group ($P < 0.05$).

Local therapies with biogenic NP drugs are an ideal way to achieve ideal treatment results of CL due to the controllable size and fast absorption of these NPs (Mandal et al. 2006; Akbari et al. 2017). Former studies have been published on the biosynthesis of metal-based NPs using bacteria (Mandal et al. 2006; Thakkar et al. 2010). Synthesis of metal-based NPs through bacteria is considered a green technology and can be carried out using other organisms such as fungi and plants (Arwidsson and Allard 2010; Thakkar et al. 2010). Studies on the biosynthesis of Ag-NPs, magnetite iron NPs, Se-NPs, and Au-NPs have previously been carried out (Bharde et al. 2006; Husseiny et al. 2007; Balaji et al. 2009; Kathiresan et al. 2009; Shakibaei et al. 2010). In 2012, Rossi-Bergmann et al. studied the efficiency of biogenic Ag-NPs produced by *Fusarium oxysporum* against *L. amazonensis*, compared with chemical nanosilver particles and amphotericin B (AmB) in vitro and in vivo. Results showed similar leishmanicidal effects to those of AmB in BALB/c mice when biogenic and chemical nanosilver were used at

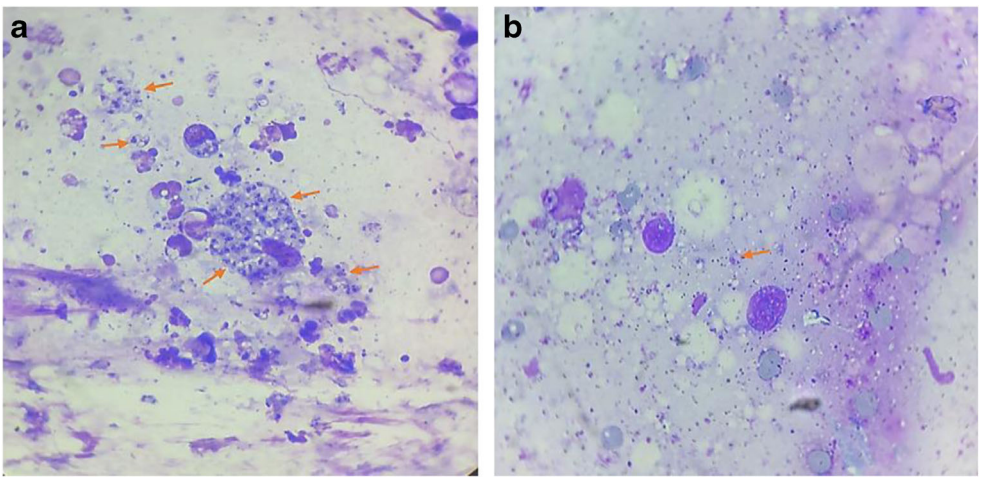

Fig. 3 Parasite load through the treatment; skin smears were prepared from the experimental groups, stained with Giemsa, and examined under a light microscope: **a** control group; **b** 70 μg/mL of antimony sulfide nanoparticles twice a day; arrows show *L. major* amastigotes

300- and 100-fold lower concentrations of AmB (Rossi-Bergmann et al. 2012). Furthermore, a study demonstrated that daily administration of biogenic Se-NPs (5 or 10 mg kg (− 1) day (− 1)) for 2 weeks delayed the development of cutaneous lesions of *L. major* (Beheshti et al. 2013). In 2012, Soflaei et al. studied the effects of biogenic antimony sulfide NPs from *S. marcescens* on *L. infantum* in vitro. Result demonstrated that biogenic antimony sulfide NPs included positive effects on promastigote proliferation of *L. infantum* and could induce apoptosis in promastigotes. They concluded that the particles could be used for the elimination of the parasite. Moreover, the efficiency of various antimony compounds in the treatment of various parasitic infections such as filariasis, leishmaniasis, schistosomiasis, trypanosomiasis, and lymphogranuloma have been proven (Berman 1997; Gutiérrez et al. 2016). Findings from in vivo experiments showed that antimony sulfide NPs significantly decreased the size of the *L. major* lesions after 4 weeks in BALB/c mice as susceptible animal models for CL, compared with negative control groups ($P = 0.015$). In mice treated with 70 μg/mL of antimony sulfide NPs, the number of amastigotes in lesions was significantly decreased in interventional groups, compared with that in negative control groups ($P = 0.001$). Based on the literature reviews, no studies have been published on effects of biogenic antimony sulfide NPs against *L. major* in vitro and in vivo despite several studies on antibacterial, antiviral, and antifungal activities of metal-based NPs. In contrast to clinical uses of pentavalent antimonials for more than half a century, the exact mechanism of action and the basis for the selective toxicity of these chemicals remain unclear; however, various mechanisms have already been suggested (Croft et al. 2006). Normally, Sb^V is not toxic to *Leishmania* spp., acting as a prodrug which is converted to highly toxic trivalent antimony (SbIII). This is supported by the evidence which shows that SbIII is much more toxic to stages of *Leishmania* spp. than the Sb^V is (Mottram and Coombs 1985; Roberts et al. 1995; Sereno and Lemesre 1997; Sereno et al. 1998; Ephros et al. 1999; Croft et al. 2006). Findings suggest that either *Leishmania* intracellularly changes Sb^V to SbIII and hence makes SbIII toxic to amastigotes directly or both SbIII and Sb^V are active against *Leishmania* amastigotes (Croft et al. 2006). Detoxification potentials of various bacterial strains for metal oxyanions have been described (Oremland et al. 2004). Antimony, as a toxic metal, is produced in the cytoplasm or other intracellular regions (e.g., cell walls) of the microorganisms (Feng et al. 2011). In the current study, bacterial potency of *S. marcescens* from the Caspian Sea for the preparation of antimony sulfide NPs was investigated. Current biogenic antimony sulfide NPs were composed of sulfur and antimony atoms at a ratio of 84/16 and these particles existed as Sb_2S_5 in the cytoplasm or other internal regions of the bacteria. Other characteristics of the antimony sulfide NPs are described in previous studies (Bahrami et al. 2012).

Conclusion

In the current study, antimony sulfide NPs were prepared and used alone against *L. major* (MRHO/IR/75/ER) in vitro and in vivo. Results have shown that various concentrations of antimony sulfide NPs include great anti-leishmanial effects against *L. major* (MRHO/IR/75/ER), with the greatest efficiency at concentrations of 62.5–70 μg/mL in in vitro and in vivo experiments. It seems that biological antimony sulfide NPs can be used as an alternative medicine for the elimination of *L. major*. However, use of biogenic antimony sulfide NPs is still at early stages. Therefore, further studies are necessary to investigate the pharmacologic and pharmacokinetics of antimony sulfide NPs. Hence, the authors should work further to present a significant outcome of the work.

Acknowledgments We would like to acknowledge all staff from the Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, for their helpful collaborations.

Availability of data and material The data that support the findings of this study are available on reasonable request to the corresponding author.

Funding information This study was supported by the Tehran University of Medical Sciences and Health Services (Project No. 94-03-160-30138), Tehran, Iran.

Compliance with ethical standards

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

Ethics approval Animal care and experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health and approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran. The study was approved by the Ethical Committee of Tehran University of Medical Sciences (Approval No. 94-03-160-30138).

Consent for publication Not applicable (no individual persons data)

References

Abaza SM (2016) Applications of nanomedicine in parasitic diseases. Parasitol United J 9(1):1–6

Akbari M, Oryan A, Hatam G (2017) Application of nanotechnology in treatment of leishmaniasis: a review. Acta Trop 172:86–90

Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, Carvalho E, Ephros M, Jeronimo S, Magill A (2017) Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Am J Trop Med Hyg 96:24–45

Arwidsson Z, Allard B (2010) Remediation of metal-contaminated soil by organic metabolites from fungi II—metal redistribution. Water Air Soil Pollut 207:5–18

Asha Rani P, Low Kah Mun G, Hande MP, Valiyaveettil S (2008) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3(2):279–290

Azami SJ, Teimouri A, Keshavarz H, Amani A, Esmaeili F, Hasanpour H, Elikaee S, Salehiniya H, Shojaee S (2018) Curcumin nanoemulsion as a novel chemical for the treatment of acute and chronic toxoplasmosis in mice. Int J Nanomedicine 13:7363–7374

Bahrami K, Nazari P, Sepehrizadeh Z, Zarea B, Shahverdi AR (2012) Microbial synthesis of antimony sulfide nanoparticles and their characterization. Ann Microbiol 62:1419–1425

Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A (2009) Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf B Biointerfaces 68:88–92

Beheshti N, Soflaei S, Shakibaie M, Yazdi MH, Ghaffarifar F, Dalimi A, Shahverdi AR (2013) Efficacy of biogenic selenium nanoparticles against *Leishmania major*: in vitro and in vivo studies. J Trace Elem Med Bio 27(3):203–207

Berman JD (1997) Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin Infect Dis 24: 684–703

Bharde A, Rautaray D, Bansal V, Ahmad A, Sarkar I, Yusuf SM, Sanyal M, Sastry M (2006) Extracellular biosynthesis of magnetite using fungi. Small 2:135–141

Croft SL, Sundar S, Fairlamb AH (2006) Drug resistance in leishmaniasis. Clin Microbiol Rev 19:111–126

Davidson RN (1998) Practical guide for the treatment of leishmaniasis. Drugs 56(6):1009–1018

Debbage P (2009) Targeted drugs and nanomedicine: present and future. Curr Pharm Des 15:153–172

Delavari M, Dalimi A, Ghaffarifar F, Sadraei J (2014) In vitro study on cytotoxic effects of ZnO nanoparticles on promastigote and amastigote forms of *Leishmania major* (MRHO/IR/75/ER). Iran J Parasitol 9(1):6–13

den Boer M, Argaw D, Jannin J, Alvar J (2011) Leishmaniasis impact and treatment access. Clin Microbiol Infect 17(10):1471–1477

Desjeux P (2004) Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 27(5):305–318

Elikaee S, Mohebali M, Rezaei S, Eslami H, Khamesipour A, Keshavarz H, Eshraghian MR (2018) Development of a new live attenuated *Leishmania major* p27 gene knockout: safety and immunogenicity evaluation in BALB/c mice. Cell Immunol 332:24–31

El-Khadragy M, Alolayan EM, Metwally DM, El-Din MFS, Alobud SS, Alsultan NI, Alsaif SS, Awad MA, Abdel Moneim AE (2018) Clinical efficacy associated with enhanced antioxidant enzyme activities of silver nanoparticles biosynthesized using *Moringa oleifera* leaf extract, against cutaneous leishmaniasis in a murine model of *Leishmania major*. Int J Environ Res Public Health 15(5):E1037

Ephros M, Bitnun A, Shaked P, Waldman E, Zilberstein D (1999) Stage-specific activity of pentavalent antimony against *Leishmania donovani* axenic amastigotes. Antimicrob Agents Chemother 43: 278–282

Feng R, Wei C, Tu S, Tang S, Wu F (2011) Simultaneous hyperaccumulation of arsenic and antimony in cretan brake fern: evidence of plant uptake and subcellular distributions. Microchem J 97:38–43

Fletcher RH, Fletcher SW, Wagner EH (1996) Clinical epidemiology: the essentials, 3rd edn. Williams and Wilkins, Baltimore

Ghorbani M, Farhoudi R (2018) Leishmaniasis in humans: drug or vaccine therapy? Drug Des Devel Ther 12:25–40

Grimaldi GJ, Tesh RB (1993) Leishmaniases of the New World: current concepts and implications for future research. Clin Microbial Rev 6: 230–250

Gutiérrez V, Seabra AB, Reguera RM, Khandare J, Calderón M (2016) New approaches from nanomedicine for treating leishmaniasis. Chem Soc Rev 45(1):152–168

Husseiny MI, Abd El-Aziz M, Badr Y, Mahmoud MA (2007) Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. Spectrochim Acta Part A 67:1003–1006

Jebali A, Kazemi B (2013) Nano-based antileishmanial agents: a toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis. Toxicol in Vitro 27:1896–1904

Jha AK, Prasad K, Kulkarni AR (2009a) Synthesis of TiO2 nanoparticles using microorganisms. Colloids Surf B Biointerfaces 71:226–229

Jha AK, Prasad K, Prasad K (2009b) A green low-cost biosynthesis of Sb2O3 nanoparticles. Biochem Eng J 43:303–306

Kathiresan K, Manivannan S, Nabeel AM, Dhivya B (2009) Studies on silver nanoparticles synthesized by a marine fungus *Penicillum fellutanum* isolated from coastal mangrove sediment. Colloids Surf B Biointerfaces 71:133–137

Khan I, Khan M, Umar MN, Oh DH (2015) Nanobiotechnology and its applications in drug delivery system: a review. IET Nanobiotechnol 9:396–400

Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G, Mukherjee P (2006) The use of microorganisms for the formation of metal nanoparticles and their application. Appl Microbiol Biotechnol 69:485–492

Mohebali M, Rezayat M, Gilani K, Sarkar S, Akhoundi B, Esmaeili J et al (2009) Nanosilver in the treatment of localized cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): an in vitro and in vivo study. Daru 17(4):285–289

Mottram JC, Coombs GH (1985) *Leishmania mexicana*: enzyme activities of amastigotes and promastigotes and their inhibition by antimonials and arsenicals. Exp Parasitol 59:151–160

Oremland RS, Herbel MJ, Blum JS, Langley S, Beveridge TJ, Ajayan PM, Sutto T, Ellis AV, Curran S (2004) Structural and spectral features of selenium nanospheres produced by Se-respiring bacteria. Appl Environ Microbiol 70:52–60

Prabhu P, Patravale V, Joshi M (2012) Nanocarriers for effective topical delivery of anti-infectives. Curr Nanosci 8(4):491–503

Rai M, Gade A, Yadav A (2011) Biogenic nanoparticles: an introduction to what they, how they are synthesized and their applications. In: Rai MK, Duran N (eds) In metal nanoparticles In Microbiology. Springer-Verlag, Berlin Heidelberg, Germany, pp 1–16

Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S (2007) Cutaneous leishmaniasis. Lancet Infect Dis 7:581–596

Roberts WL, Berman JD, Rainey PM (1995) In vitro antileishmanial properties of tri- and pentavalent antimonial preparations. Antimicrob Agents Chemother 39:1234–1239

Roberts WL, Mcmurray WJ, Rainey PM (1998) Characterization of the antimonial antileishmanial agent meglumine antimonate (glucantime). Antimicrob Agents Chemother 42(5):1076–1082

Rossi-Bergmann B, Pacienza-Lima W, Marcato PD, De Conti R, Durán N (2012) Therapeutic potential of biogenic silver nanoparticles in murine cutaneous leishmaniasis. J Nano Res 20:89–97

Sereno D, Lemesre JL (1997) Axenically cultured amastigote forms as an in vitro model for investigation of antileishmanial agents. Antimicrob Agents Chemother 41:972–976

Sereno D, Cavaleyra M, Zemzoumi K, Maquaire S, Ouaissi A, Lemesre JL (1998) Axenically grown amastigotes of *Leishmania infantum* used as an in vitro model to investigate the pentavalent antimony mode of action. Antimicrob Agents Chemother 42:3097–3102

Shakibaei M, Khorramizadeh MR, Faramarzi MA, Sabzevari O, Shahverdi AR (2010) Biosynthesis and recovery of selenium nanoparticles and the effects onmatrixmetalloproteinase-2 expression. Biotechnol Appl Biochem 56:7–15

Soflaei S, Dalimi A, Ghaffarifar F, Shakibaie M, Shahverdi AR, Shafiepour M (2012) In vitro antiparasitic and apoptotic effects of

antimony sulfide nanoparticles on *Leishmania infantum*. J Parasitol Res 2012:756568

Teimouri A, Azami SJ, Keshavarz H et al (2018) Anti-*Toxoplasma* activity of various molecular weights and concentrations of chitosan nanoparticles on tachyzoites of RH strain. Int J Nanomedicine 13: 1341–1351

Thakkar KN, Mhatre SS, Parikh RY (2010) Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol Biol Med 6:257–262

Torabi N, Mohebali M, Shahverdi AR, Rezayat SM, Edrissian GH, Esmaeili J et al (2011) Nanogold for the treatment of zoonotic cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): an animal trial with methanol extract of *Eucalyptus camaldulensis*. JPHS 1:13–16

Vaidyanathan R, Kalishwaralal K, Gopalram S, Gurunathan S (2009) Nanosilver—the burgeoning therapeutic molecule and its green synthesis. Biotechnol Adv 27:924–937

Veeken H, Ritmeijer K, Seaman J, Davidson R (2000) A randomized comparison of branded sodium stibogluconate and generic sodium stibogluconate for the treatment of visceral leishmaniasis under field conditions in Sudan. Tropical Med Int Health 5(5):312–317

Wang JJ, Zeng ZW, Xiao RZ et al (2011) Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomedicine 6:765–774

World Health Organization (2014) Manual for case management of cutaneous leishmaniasis in the WHO Eastern Mediterranean Region

Yaghoobi-Ershadi M, Javadian E (1996) Epidemiological study of reservoir hosts in an endemic area of zoonotic cutaneous leishmaniasis in Iran. Bull World Health Organ 74(6):587

Zuccato E, Calamari D, Natangelo M, Fanelli R (2000) Presence of therapeutic drugs in the environment. Lancet 355(9217):1789–1790