



## Original research

## Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts



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## ARTICLE INFO

## Article history:

Received 11 November 2013

Received in revised form

14 February 2014

Accepted 26 March 2014

Available online 28 March 2014

## Keywords:

Cystic echinococcosis

Selenium nanoparticles

Protoscolices

*Echinococcus granulosus*

## ABSTRACT

Cystic echinococcosis (hydatid cyst, CE) as a zoonotic parasitic infection caused by the larval stage of the dog tapeworm *Echinococcus granulosus* is still an important economic and public health concern in the world. One of the treatment options for CE is surgical removal of the cysts combined with chemotherapy using albendazole and/or mebendazole before and after surgery. Currently, many scolical agents, which have some complications, have been used for inactivation of the cyst contents. Therefore the development of new scolical agents with low side effects and more efficacies is an urgent need for surgeons. The present study was aimed to investigate the *in vitro* scolical effect of selenium nanoparticles biosynthesized by a newly isolated marine bacterial strain *Bacillus* sp. MSh-1 against protoscolices of *E. granulosus*. Protoscolices were aseptically aspirated from sheep livers having hydatid cysts. Various concentrations (50–500 µg/ml) of Se NPs (in size range of about 80–220 nm) were used for 10–60 min. Viability of protoscolices was confirmed by 0.1% eosin staining. The results indicated that biogenic Se NPs at all concentrations have potent scolical effects especially at concentrations 500 and 250 µg/ml after 10 and 20 min of application, respectively. In conclusion, the findings of present study proven that Se NPs have potent scolical effects, therefore may be used in CE surgery. However, the *in vivo* efficacy of these NPs remains to be explored.

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## 1. Introduction

Cystic echinococcosis (CE) or hydatid disease as a zoonotic parasitic infection caused by the larval stage of the dog tapeworm *Echinococcus granulosus* is still an important economic and public health concern in many countries of the world, such as Iran. The disease affects humans as well as domestic livestock including cattle, sheep, camels, pigs, horses and others [1,2]. The final host is the dog, in which adult tapeworms attached to the intestinal epithelium undergo sexual reproduction, leading to the development of eggs. These eggs are shed into the environment with the feces. The eggs contain an oncosphere are ingested by a suitable intermediate host. Oncospheres released from the eggs penetrate the intestinal mucosa and via the portal system, are disseminated

in the liver, lungs, muscle or other organs, where the hydatid cysts grow up [3].

Surgery is the preferred treatment for particular WHO stage disease. Chemotherapy with benzimidazoles and PAIR (puncture, aspiration, injection and reaspiration) are recommended as alternative treatments to surgery, especially for the patients who cannot tolerate surgery [4]. However, albendazole and mebendazole used in treatment of hydatid cysts showed different adverse effects such as hepatotoxicity, severe leucopenia, thrombocytopenia and alopecia [5]. In the other hand to reduce the risk of intraoperative spillage of the cyst contents (scolecocytes) and subsequently recurrence of CE and secondary infection, which is observed in nearly 10% of the postoperative cases, the use of effective scolical agents are obligatory [5,6]. The common scolical agents, hypertonic saline, Ag-nitrate, cetrimide, and ethanol, which used for inactivation of the cyst contents present different dangerous side effects such as sclerosane colangitis (biliary tract fibrosis), liver necrosis and methaemoglobinaemia [7,8]. Therefore the development of new

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scolicidal agents with low side effects and more efficacies is an urgent need for surgeons [9].

Selenium (Se) is an essential micronutrient element with fundamental roles in human health [10]. So far, it has been used in different medical therapies such as cancer prevention including lung, esophagus, prostate and gastric-cardiac cancers, antiviral activities and antioxidant effects [11–13]. In addition, Se has additional important health activities related to the immune response and successful reproduction [13,14]. It has currently been demonstrated that nanoparticles (NPs) due to their large surface-volume ratio showed various unique properties. NPs are also able to enter cells more frequently than other particles [15]. Few studies have shown that NPs particularly Se NPs can effectively inhibit the growth of some bacteria such as *Staphylococcus aureus* and pathogenic *Escherichia coli* [15,16]. The mechanism of Se against microorganism remains unclear but there is some studies showed that the inorganic forms of selenium can react with membrane peroxidases to generate oxygen free radicals, such as superoxide anion ( $O_2^-$ ) [17]. Furthermore, the ability of biogenic Se NPs to induce apoptosis in another form of eukaryotic cell, the *Leishmania major* promastigotes, has been previously reported [18].

However, to the best of our knowledge and according to a survey of the literature, the effect of biogenic Se NPs on *E. granulosus* protoscoleces remains mainly unexplored. The aim of this *in vitro* study was to evaluate the scolicidal effects of biogenic Se NPs against protoscoleces of *E. granulosus*.

## 2. Materials and methods

### 2.1. Chemicals

Eosin powder was purchased from Sigma–Aldrich, St Louis, MO, USA. Selenium dioxide ( $SeO_2$ ), nutrient broth, nutrient agar, *n*-octyl alcohol, sodium dodecyl sulfate (SDS) and Tris base were purchased from Merck Chemicals (Germany). All other chemicals and solvents were of analytical grade. The micro-organism used in this study was identified as *Bacillus* sp. MSh-1 by the methods described earlier [10]. The organism was continuously conserved on nutrient agar plates complemented with 1.26 mM  $SeO_2$  using continuous sub-culturing every 14 days.

### 2.2. Biosynthesis and characterization of the Se NPs

Se NPs were prepared according to the method described elsewhere [10]. Briefly, a sterile nutrient broth (NB) medium was supplemented with the  $Se^{+4}$  ions (100 mg/L; equal to 1.26 mM  $SeO_2$  solution) and 100 ml of this medium was transferring to a 500-ml Erlenmeyer flask. The medium was inoculated with 1 ml of the fresh inoculums ( $OD_{600}$ , 0.1) of *Bacillus* sp. MSh-1 and was incubated aerobically at 30 °C in a shaker incubator (150 rpm). After 14 h, the bacterial cells and Se NPs were removed from the culture medium by using centrifugation at 4000 g (10 min). The pellets were washed with 0.9% NaCl solution using centrifugation, transferred to a mortar and then it was frozen by adding liquid nitrogen and was then disrupted by a pestle. The resulting slurry was ultrasonicated at 100 W for 5 min and washed three times by sequential centrifugation (10,000 g, 5 min), with a 1.5 M Tris–HCl buffer (pH 8.3) containing 1% SDS and deionized water. The next step involved extracting and purifying the Se NPs through an organic-aqueous partitioning system (*n*-octyl alcohol–water). For transmission electron microscopy, an aqueous suspension containing the Se NPs was dispersed ultrasonically, and a drop of the suspension was placed on carbon-coated copper TEM (transmission electron microscope) grids and dried under an IR lamp. The crystalline structure of the Se NPs was evaluated by the X-ray

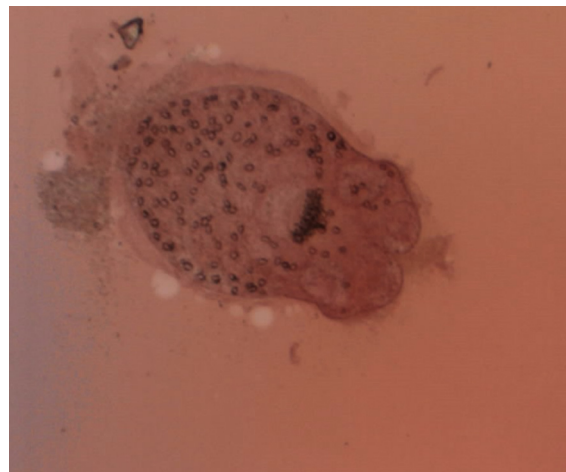


Fig. 1. Dead protoscolice after exposure to biogenic Se NPs and staining with 0.1% eosin.

diffraction (XRD) technique using an X-ray diffractometer (Philips PW1710) with  $CuK\alpha$  radiation ( $\lambda = 1.5405 \text{ \AA}$ ) over a scanning range of Bragg angles from 20° to 80 °C.

### 2.3. Collection of protoscoleces

Protoscoleces of *E. granulosus* were collected from the naturally infected livers of sheep and goats slaughtered at Kerman abattoir, southeastern Iran and carried to the Parasitology Laboratory at the Department of Parasitology and Mycology, Kerman University of Medical Sciences, Kerman, Iran. The hydatid fluid aspirated by a 20 ml syringe and aseptically transferred into an Erlen Meyer flask was left to set for 30 min for protoscoleces to settle down. The supernatant was discarded and the protoscoleces were washed two times with PBS (pH 7.2) solution. The viability of the protoscoleces was confirmed by their flame cell motility and impermeability to eosin solution (0.1%) under a light microscope. For further use, the concentration of protoscoleces was confirmed as  $2 \times 10^3$  protoscoleces in 1 ml of saline solution (0.9%) with more than 90% viability.

### 2.4. Scolicidal effects of Se NPs

To investigate the scolicidal effects of Se NPs against protoscoleces of hydatid cysts, four concentrations of the Se NPs (50, 125, 250 and 500  $\mu\text{g/ml}$ ) were used with different exposure times (10, 20, 30 and 60 min). Initially, 0.5 ml of the protoscoleces ( $2 \times 10^3/\text{ml}$ ) solution was placed in test tubes. Then 0.5 ml of various concentrations of Se NPs was added to each test tube. The contents of the tubes were gently mixed and then incubated at 37 °C for 10, 20, 30 and 60 min. At the end of each incubation time the upper phase was carefully removed so as not to interrupt the protoscoleces. Fifty  $\mu\text{l}$  of 0.1% eosin stain was then added to the remaining settled protoscoleces and mixed gently again. The upper portion of the solution was discarded after 10 min of incubation. The remaining pellet of protoscoleces was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead protoscoleces were determined by counting 300 protoscoleces. In addition, normal saline were used as control group and all experiments were performed in three replicates.

### 2.5. Viability test

In order to evaluate the viability of protoscoleces, eosin solution with a concentration of 0.1% (1 g of eosin powder in 1000 ml

distilled water) was used. After exposure to the stain, dead protozoa absorbed eosin and colored red (Fig. 1), but alive protozoa remained colorless and showed characteristic muscular movements and flame cell activity (Fig. 2). Mortality rate of protozoa was determined, as the percent of dead protozoa to the total protozoa.

### 2.6. Statistical analysis

SPSS Software 17 for windows (SPSS Inc., Chicago) was used for statistical analysis. The differences between groups were determined using *t*-test and *P*-values less than 0.05 were considered to be significant.

## 3. Results

### 3.1. Characterization of Se NPs

The TEM micrograph clearly illustrates individual Se NPs with a small amount of aggregation and the NPs have a spherical shape (Fig. 3). Size distribution measured from manual counting of 400 individual particles from different TEM images showed that the size of nanoparticles was 80–220 nm and NPs with the size of 105–130 nm had the most frequency. The XRD pattern of Se NPs showed the presence of broad peaks without any clear lattice parameters (results not shown). Thus the obtained Se NPs are amorphous.

### 3.2. Scolicidal effects of Se NPs

Fig. 4 shows scolicidal effects of various concentrations of the Se NPs (50–500 µg/ml) for 10, 20, 30 and 60 min against protozoa of *E. granulosus*. It could be observed that the Se NPs in all concentrations exhibited significant scolicidal effects in compared with control group ( $P < 0.05$ ). All protozoa were killed after 10 min of exposure to concentration of 500 µg/ml of Se NPs. In addition, after 20 min exposure time, the scolicidal activity of Se NPs at concentration of 250 µg/ml was 100%. In contrast, Se NPs at concentration 125 µg/ml killed 41.4%, 73.4%, 86.6% and 100% of the protozoa and at the concentration 50 µg/ml killed 16.2%, 27.8%, 41.6% and 56.5% of the protozoa after 10, 20, 30 and 60 min application, respectively. By increasing the exposure time with Se NPs in all concentrations the mortality rate was significantly increased ( $P < 0.05$ ). Furthermore, it seems that in three exposure times (20 min, 30 min, 60 min) by increasing in Se NPs



Fig. 2. Live protozoa after staining with 0.1% eosin.

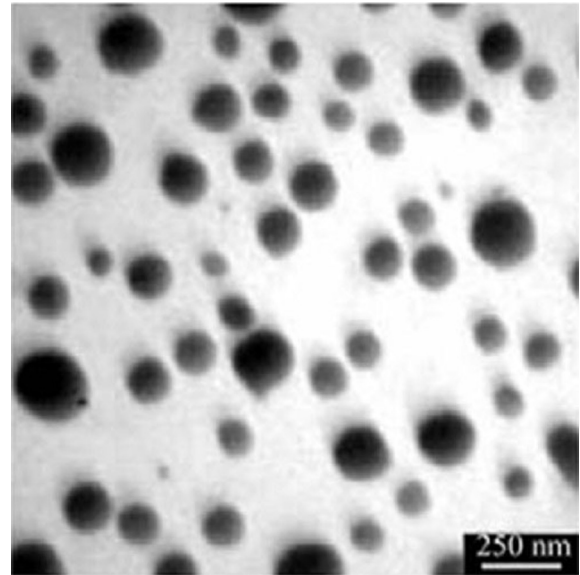


Fig. 3. Transmission electron micrograph of the purified selenium nanoparticles (Se NPs) synthesized by *Bacillus* sp. MSh-1.

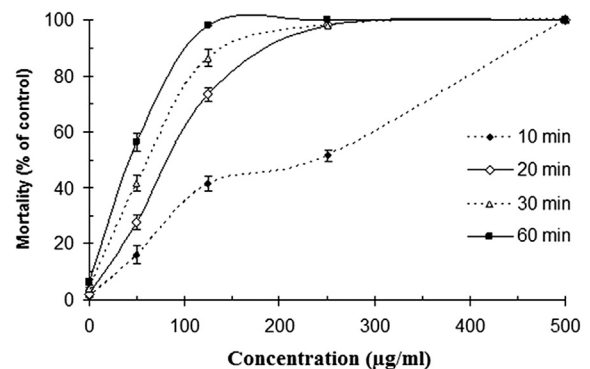


Fig. 4. Scolicidal effects of biogenic Se NPs against protozoa of *Echinococcus granulosus* at the various concentrations following various exposure times.

concentration to above 250 µg/ml the mortality rate wasn't significantly increased ( $P > 0.05$ ). Therefore, these results showed potent *in vitro* scolicidal activity for biogenic Se NPs.

## 4. Discussion

Surgery is still the first choice treatment for complicated cases of CE. However, it has been associated with local recurrence or secondary dissemination [4]. As regards, spillage of the cyst contents is a main cause of recurrence. Therefore inactivation of the scolex with a scolicidal agent prior to opening or removing a cyst is strongly recommended [18].

Up to now, in various studies scolicidal effects of hypertonic saline, silver nitrate, cetrimide, ethyl alcohol (95%),  $H_2O_2$  and povidone iodine (10%), mannitol, albendazole, chlorhexidine gluconate, honey and some plant extracts have been proven [19–28]. However, majority of these scolicidal agents may lead to undesirable complications that limit their use in treatment of CE [7,8]. For these reasons, finding a rapid and complete scolicidal effect with no local or systemic side effects are some properties of a proper scolicidal agent, which the surgeons require for surgical success of hydatid cyst [9].



Selenium is the main component of selenoenzymes, which are found to protect animal cells from oxidative damage [29]. Currently, stimulation of immune responses and reduction of overall cancer mortality are the main advantages of Se intake. However, dose and chemical form of selenium derivatives play an important role in both their bioavailability and biological activities [30]. At present, due to higher biological activity, higher anti-oxidant effects and lower cytotoxicity of Se NPs compared to those of Se ions, trend of research is now being directed towards synthesis and biological application of Se NPs [18].

This work for the first time describes the scolicidal effects of biogenic Se NPs against protoscoleces of hydatid cysts on *in vitro* model. Our finding showed that Se NPs have a potent scolicidal activity especially at concentrations 500 and 250 µg/ml (100% mortality rate) after 10 and 20 min of application, respectively. Thus, these results revealed that scolicidal effects of Se NPs at concentration 500 µg/ml was comparable with scolicidal effects of 20% hypertonic saline (15 min), 20% silver nitrate (20 min), 0.5–1% cetrimide (10 min), H<sub>2</sub>O<sub>2</sub> 3% (15 min) and 95% ethyl alcohol (15 min) as previously described [9,19–25].

It has previously been indicated that different living parasites including *Trypanosoma* and *Leishmania* and also other higher microorganisms, need trace amounts of selenium ions [31]. However, we showed higher *in vitro* concentrations of Se NPs were toxic for protoscoleces of *E. granulosus*. Similar to these results were found by Beheshti et al. [18] that proven high *in vitro* concentrations of this vital compound were toxic to the both stages of *L. major* and caused some biochemical hallmarks, such as DNA fragmentation, in the parasite. Moreover, in the case of cytotoxicity effects of Se NPs, recently Shakibaie et al. [32] showed that no biochemical changes were observed from the orally administration of 2.5, 5 and 10 mg/kg of Se NPs to male mice for two weeks, but a dose of 20 mg/kg of Se NPs indicated signs of toxicity including lower body weight and changes in clinical chemistry and hematological parameters.

Despite the efficient *in vitro* scolicidal effects of biogenic Se NPs, the NPs preparation is time consuming and need more attention about the sterility process. In addition, the toxicity, *in vivo* efficacy and combination of these NPs with other method like PAIR treatment remain to be explored in further studies. In conclusion, the findings of present study proved that the biogenic Se NPs have potent scolicidal effects against protoscoleces of *E. granulosus* and therefore may be used in treatment of hydatid cyst.

#### Ethical approval

None.

#### Funding

This study was supported by the Research Center for Tropical and Infectious diseases and Vice Chancellor for Research, Kerman University of Medical Sciences (project no.91/360) (Kerman, Iran) and Iranian Nanotechnology Initiative Council.

#### Author contribution

Hossein Mahmoudvand: **study design**  
Majid Fasihi Harandi: **writing**  
Mojtaba Shakibaie: **study design**  
Mohammad Reza Aflatoonian: **data collections**  
Naser ZiaAli: **writing**  
Mahsa Sadat Makki: **data analysis**  
Sareh Jahanbakhsh: **data collections**

#### Conflicts of interest

None.

#### Acknowledgments

This study was supported by the Research Center for Tropical and Infectious diseases and Vice Chancellor for Research, Kerman University of Medical Sciences (project no.91/360) (Kerman, Iran) and Iranian Nanotechnology Initiative Council. We would like to thank Dr. Shokohi for data analysis and Dr. Saedi Dezaki for collection of infected livers of animals. The authors declare that there is no conflict of interest in this study.

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