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ORIGINAL RESEARCH

Scolicidal Effects of Nanoparticles Against Hydatid Cyst Protoscolices in vitro

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Background: *Echinococcus granulosus* is causative agent of cystic echinococcosis (CE), which has a cosmopolitan distribution. The current methods for the treatment of human CE include surgery. Therefore, the development of new scolicidal agents with low side effects and more efficacies is an urgent need.

Purpose: The present study aimed to compare the scolicidal efficacies of silver, iron, copper, silica and zinc oxide nanoparticles (NPs) against hydatid cyst protoscolices in vitro.

Methods: Hydatid cysts of sheep liver and lung were collected. The cyst fluid containing protoscolices was aspirated aseptically. The scolicidal activities of the silver, iron, copper, silica and zinc nanoparticles (Ag-NP, Fe-NP, Cu-NP, Si-NP and Zn-NP) were tested at different concentrations of 0.25, 0.5 and 1 mg/mL following 10, 30 and 60 min of incubation in triplicate. Viability of protoscolices was confirmed by 0.1% eosin staining.

Results: Results showed that Ag-NPs at all concentrations tested had the highest scolicidal effect. Ag-NPs at 1 mg/mL concentration after 60 min of exposure time showed 80% mortality rate. Si-NPs had the high scolicidal activity at 1 mg/mL concentration (52.33%), Cu-NPs at 0.5 mg/mL concentration (41%), Fe-NPs at 1mg/mL concentration (28%) and Zn-NPs at concentration of 1mg/mL after 60 mins (15.67%).

Conclusion: The findings of the present study showed that Ag-NPs, Fe-NPs, Cu-NPs, Si-NPs and Zn-NPs had potent scolicidal effects and that Ag-NPs are recommended as effective scolicidal agents. However, further in vivo studies are required to evaluate the efficacy of these nanoparticles.

Keywords: scolicidal, hydatid cyst, protoscolices, silver nanoparticle, iron nanoparticle, copper nanoparticle, silica nanoparticle, in vitro

Introduction

Cystic echinococcosis (CE) is one of the most important worldwide parasitic and zoonotic diseases, in humans, wild animals as well as domestic livestock including cattle, sheep, camels, pigs, horses and others. Hydatid disease is the result of tissue invasion with the intermediate stage of a dog tapeworm, *Echinococcus granulosus*. Hydatid cysts (larval stages) develop in the different organs of the host such as liver, lung even in heart, brain, bone, spleen and kidneys which may lead to death.[1] The annual incidence rate of cystic hydatid disease can vary from 1 to 200 per 100,000 inhabitants in various endemic regions.[2] Iran is considered as an endemic (Southern parts) and hyperendemic (Northern parts) area of cystic hydatid disease.[2]

Currently, there are two treatment options for CE: surgery (consisting of conservative and laparoscopic, percutaneous drainage consisting of puncture, aspiration, injection and re-aspiration (PAIR) and chemotherapy.[3] Besides, the surgery

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becomes impractical when cysts are formed either in multiple organs or in high risk locations including brain and spinal tissues.[4] A lot of efforts have been conducted to prepare an efficient vaccine. However, there is not yet an effective vaccine against chronic hydatid disease (CHD) for human.[5]

The current chemotherapeutic agents used for the treatment of hydatidosis are benzimidazole derivatives, such as mebendazole and albendazole. However, albendazole and mebendazole used in the treatment of hydatid cysts showed different adverse effects such as abnormalities in liver function, abdominal pain, diarrhea, nausea, dizziness, a headache hepatotoxicity, severe leucopenia, thrombocytopenia and alopecia.[6] In addition, albendazole was shown to be teratogenic in rats, therefore its use is limited.[7,8]

To date, many chemical scolicidal agents have been used for the inactivation of the hydatid cyst protoscolices. A perfect scolicidal agent is defined as being effective in low concentrations in a shorter exposure time, stable after by dilution with the cyst fluid, non-toxic, eliminator of cyst protoscolices, more efficient, less harmful for tissue host, low cost and easily available.[9]

There is a high tendency among researchers to evaluate and present nanoparticles as an alternative option due to being easily available, low side effects and cost. Use of effective scolicidal agents during hydatid cyst surgery is essential to prevent the secondary infection.[10]

Although the production of nanosized particles had occurred in several ways in ancient times and hundred years ago, nanomedicine as a modern science was first confirmed in the 1990s of the last century only.[11]

This study was conducted for the first time to determine the scolicidal effect of silver, iron, copper, silicon and zinc oxides nanoparticles in vitro.

Methods

Protoscolices Collection

Hydatid cysts from livers and lungs of sheep infected with hydatid cyst were collected from an industrial slaughterhouse and transferred to the parasitology laboratory of Veterinary Medicine Faculty. Then, the surface of cysts was disinfected using 70% ethyl a and 25 mL of cyst fluid were aspirated by sterile syringe and transferred into glass cylinders and left to set for 30 min. The protoscolices settled down at the bottom of the cylinders. The supernatant was removed and the yielded protoscolices were washed three times with PBS and tested by 0.1% eosin to assess the viability of protoscolices. The samples of protoscolices which were over 90% viability were selected for further testing.

Evaluation of the Scolicidal Effect of the AgO, Fe2O3, CuO, SiO2 and ZnO Nanoparticle in vitro

For evaluation of the scolicidal effect of the iron oxide nanoparticles, concentrations of 0.25, 0.5 and 1 mg/mL were suspended in distilled sterile water and added to the microtubes, to which a drop of protoscolex-rich sediment was added. The contents of the tubes were gently mixed. The tubes were incubated at 37 °C for 10, 30 and 60 min. At the end of each incubation time, the upper phase was carefully removed so as not to disturb the protoscolices. One milliliter of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. The upper portion of the solution was discarded after 15 min of incubation. The remaining pellet of protoscolices was then smeared on a manually scaled glass slide, covered with a cover glass (24 × 50 mm), and examined under a light microscope. The percentages of dead protoscolices were determined by counting a minimum of 500 protoscolices. The experiments were performed with three samples (triplicate).

Viability Test

In order to evaluate the viability of protoscolices, an 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 protoscolices absorbed eosin and colored red, but alive protoscolices remained colorless.

Nanoparticles Characterization

The nano metal powder (99%, 20 nm–40 nm) was purchased from the Iranian Nanomaterials Pioneers Co. The metal oxide nanoparticles used in this experiment were analytical grade with the highest purity. Figure 1. represents Electron Microscope images (TEM & SEM) of nanoparticles.

Preparation of Nanoparticles

For evaluation of the scolicidal effect of the NPs, concentrations of 0.25, 0.5 and 1 mg/mL of nanoparticles were suspended in distilled sterile water.

Scanning Electron Microscopy

The protoscolices of *E. granulosus* were washed three times in PBS. Then, protoscolices were dried in room

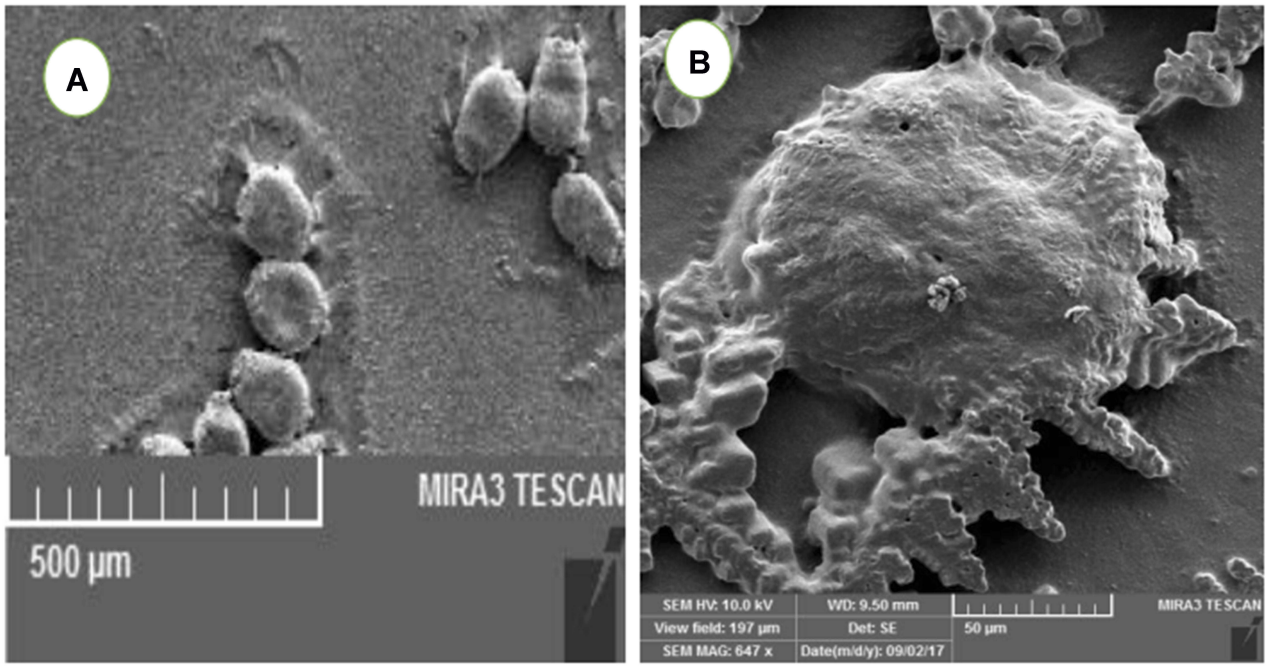

Figure 1 Scanning electron microscopy (SEM) micrograph of protoscolices of *E. granulosus*. Protoscolices without nanoparticles (**A**), 1 mg/mL concentrations of Ag-NPs that covered protoscolex (**B**).

temperature. Following drying, the protoscolices were carried out in ascending concentrations of ethanol. Finally, processed samples were sputter-coated with gold and examined by SEM (MIRA3 FEG-SEM, Tescan co.).

Statistical Analysis

Data were analyzed by GraphPad software version 5.

Results

The results showed that the Ag-NPs at all concentrations had the highest scolicidal effect. The Ag-NPs at 1 mg/mL concentration after 60 min of exposure time showed a 80% mortality rate. The Si-NPs had the highest scolicidal activity at 1 mg/mL concentration (52.33%), while the highest scolicidal activity of Cu-NPs at 0.5 mg/mL concentration (41%), Fe-NPs at concentration of 1 mL (18%) and ZnO-NPs at concentration of 1mg/mL after 60 mins (15.67%). The scolicidal effect of ZnO NPs at the concentration of 0.25 mg/mL after 30 mins of the application was lower than that in other concentration (12%). However, all concentrations of Ag-NPs showed statistically significant difference in the protoscolicidal activity with different dilutions ($p>0.05$). The mortality rate of hydatid cyst protoscolices after different exposure times and concentrations of the five nanoparticles following various exposure times are presented in Table 1. The Scanning electron

Table 1 Scolicidal Effect Percentage (%) of the Nanoparticles at Different Concentrations and Various Exposure Times

Concentrations	Times	Positive Control	Ag-NPs	Fe-NPs	Cu-NPs	Si-NPs	Zn-NPs	Negative Control
0.25 mg/mL	10 min	100	37.33	21	13	18	19.67	4.66
	30 min	100	45	20.67	16.67	25	12	6
	60 min	100	51	16.67	22.33	28.67	13	4.33
0. 5 mg/mL	10 min	100	52.33	28	21	45.33	13.67	4.66
	30 min	100	58.33	20.33	32	45	13	6
	60 min	100	55.33	20.67	41	47	14.33	4.33
1 mg/mL	10 min	100	65.67	27	22.33	43.67	14.67	4.66
	30 min	100	66.67	24.33	32	49.67	13.67	6
	60 min	100	80	23	34	52.33	15.67	4.33

microscopy (SEM) micrograph of protoscolices with and without the nanoparticles is shown in Figure 1. The scolicidal effects of different concentrations of Ag-NPs, Fe-NPs, Cu-NPs, Si-NPs and Zn-NPs after various exposure times are shown in Figure 2.

Discussion

Surgery is still the first choice treatment for complicated cases of cystic echinococcosis. However, it has been associated with local recurrence or secondary dissemination.[3] Up to date, many chemical scolicidal agents have been used for the inactivation of the hydatid cyst protoscolices. Many of these scolicidal agents may cause undesirable complications that limit their use. For example, adverse side effects have been reported for 20% hypertonic saline, 20% silver nitrate, 0.5–1% cetrimide, ethyl alcohol and 20 mg/mL albendazole sulfoxide.[10] The elimination of protoscolices with scolicidal agents, associated with high efficacy and minimum side effects, have been suggested as an alternative to opening or removing cysts.[13] Moreover, chemotherapy can be applied for patients who are not proper candidates for surgery; it can be also applied as a complementary treatment to surgery (before surgery, after surgery or both).[14]

The findings indicated that scolicidal effects of 20% silver nitrate (20 min), 0.5-1% cetrimide (10 min), 20% hypertonic saline (15 min), 95% ethyl alcohol (15 min) and 3% hydrogen peroxide (15 min), as previously described.[12,15–18]

Today, application of nanometal products has highlighted the necessity of effective methods in the management of parasites but, the nanoparticles will likely contaminate the environment[19] and safe use practices and toxicity thresholds need to be established to minimize the impact on beneficial bacteria, animals and the food chain.[20,21]

Several studies have exhibited the antiparasitic and inhibitory effects of gold, silver, chitosan, and oxidized metals on protoscolices, such as Mahmoudvand et al (2014) used various concentrations (50-500 mg/mL) of selenium nanoparticles (in size range of about 80-220 nm) were used for 10-60 min. The results indicated that biogenic Se-NPs at all concentrations have potent scolicidal effects, especially at concentrations 500 and 250 mg/mL after 10 and 20 min of application, respectively.[22] In conclusion, the findings of the present study

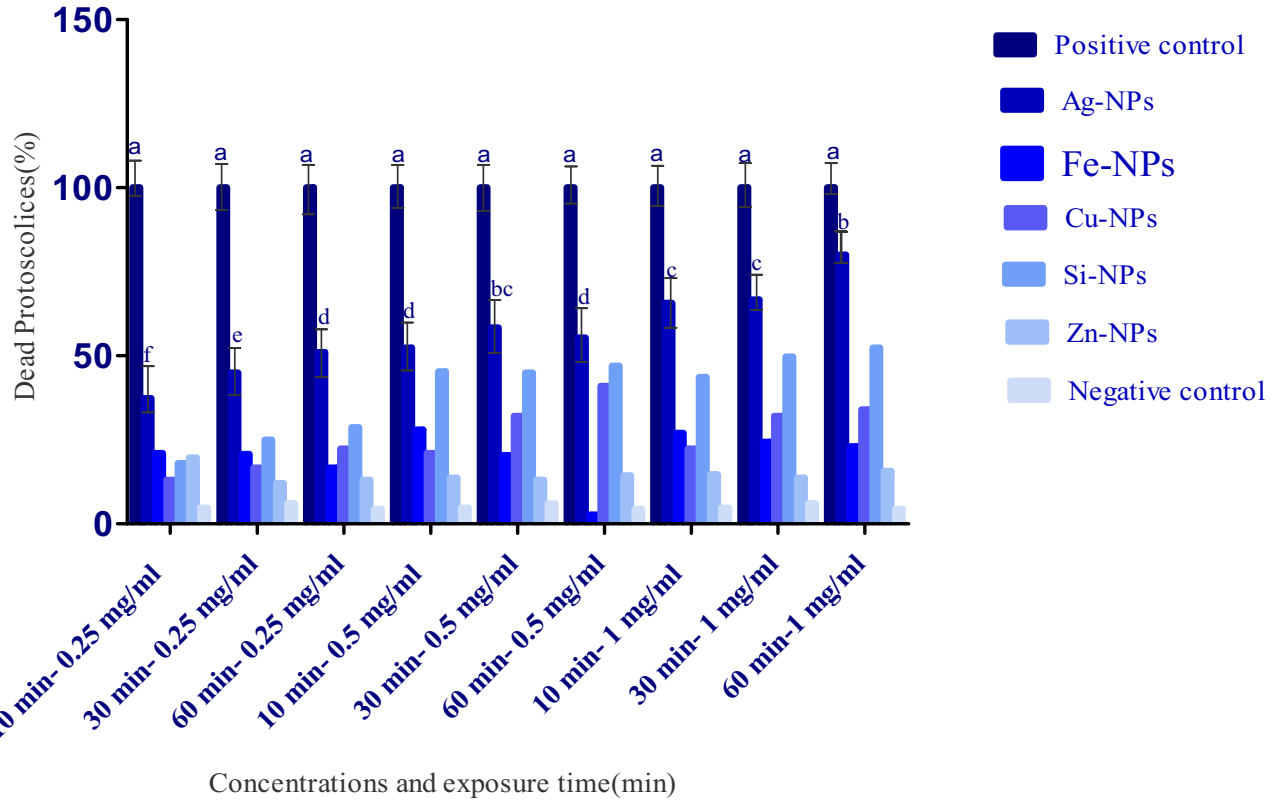

Figure 2 Scolicidal effects of different concentrations of the nanoparticles after various exposure times (The letters a to f indicate the difference between the scolicidal effects of different concentrations of nanoparticles, positive control and negative control). The different letters a to f with the bars indicate significant differences as determined using the Duncan test.

have proven that Ag-NPs have the highest potent scolicidal effects and therefore may be used in CE surgery.[22]

Malekifard et al (2017) investigated the efficacy of gold nanoparticles on scolices of hydatid cyst and indicated gold nanoparticles of all concentrations had significant scolicidal effects. Gold nanoparticles at the concentration of 1 mg/mL led to killing all protoscoleces at 60 mins.[23]

The scolicidal effect of green synthesized silver NPs at different concentrations (0.025, 0.05, 0.1 and 0.15 mg/mL) and different exposure times (10, 30, 60 and 120 min) against protoscolices of CHD were previously investigated.[24] The findings showed that the Ag-NPs at all concentrations had high scolicidal effects.[24] The concentrations 0.1 and 0.15 mg/mL after 120 min of exposure times showed 83% and 90% mortality rate, respectively.[24] The least scolicidal activity of biosynthesized Ag-NPs was 40% (0.025 mg/mL and 10 min). It was reported that biogenic Ag-NPs may be considered as a potential scolicidal agent for CHD surgery due to being economical, safer and non-toxic compared to the used chemical materials.[24]

In the present study we observed a highest scolicidal effect (80%) of Ag-NPs at 1 mg/mL concentration after 60 min exposure time but, this time long for surgical operations. The best time in clinical conclusion is 10 mins; therefore Ag-NPs have a 65.67% scolicidal effect in 10 mins. Silver nanoparticles are used in very small quantities, so they are not more expensive than silver nitrate. Metal oxide nanoparticles as well as Ag-NPs due to a change in the surface properties leading to a drastic increase in the bandgap that influences the adsorption and penetration of the nanoparticles.[25] The Ag-NPs have a smaller size and high dispersion than silver nitrate, thus shows high scolicidal activity.[26]

According to the results, zinc oxide nanoparticles have a low scolicidal activity (12%) at the concentrations of 0.25 mg/mL after 30 min of application. It seems that the differences in the result of different studies are due to the differences in nanoparticles, concentration and exposure time.

Generally, the findings of this study indicated that all concentration of Ag-NPs have statistically significant difference in the protoscolicidal activity with different dilutions ($p>0.05$) and these NPs are recommended as powerful scolicidal agents in surgery. Further studies are required to evaluate the efficacy of Ag-NPs, Fe-NPs, Cu-NPs, Si-NPs and Zn-NPs in vivo.

Conclusion

Our results suggest that Ag-NPs, Fe-NPs, Cu-NPs, Si-NPs and Zn-NPs have potent scolicidal effects, and among these nanoparticles, silver nanoparticles have the highest scoliosidial activities. However, further studies are required to evaluate the efficacy of these nanoparticles in vivo and their clinical applications.

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Disclosure

The authors report no conflicts of interest in this work.

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