RESEARCH ARTICLE

Scolicidal effect of zinc oxide nanoparticles against hydatid cyst protoscolices *in vitro*

Roghayeh Norouzi[1,*], Marzie Hejazy[2], Amin Ataei[1]

[1] Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[2] Department of Basic science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

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ABSTRACT

Objective(s): Nanoparticles (NPs) are particles with the size range from 1 to 100 nanometers that are made in different shapes. Nanotechnology is an emerging technology that look forward to open some new chances in order to destroy and control of microorganisms using of materials and systems at the scale of the atom. *Echinococcus granulosus* is the cause of cystic echinococcosis (CE) that has a cosmopolitan distribution. This parasite produces hydatid cysts that cause suffering various parts of mammalian host including liver, heart, brain, lung, and bone which may be fatal. The current methods for treatment of human CE involve surgery.

Methods: This study was undertaken for the first time to evaluate the scolicidal effect *of zinc oxide* nanoparticles (ZnO- NPs) against hydatid cyst protoscolices *in vitro*. The scolicidal activities of the *zinc oxide* nanoparticles were tested in concentrations of 50,100 and 150 mg/ml following 10, 30 and 60 minutes of incubation and were repeated three times. Results were analyzed by SAS software.

Results: The results showed that at the concentration of 50 mg/ml of nano Zno mortality rate is about 19.6% protoscolices at 10 minutes. In 150 mg/ml concentration, the black ZnO particles were covered entire of all protoscolices, and they could not be seen or counted.

Conclusions: This investigation showed statistically significant differences in the protoscolicidal activity with different dilutions of that ZnO NPs but, is not recommended as a powerful scolicidal agent.

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INTRODUCTION

Cystic echinococcosis (CE) is one of the most important worldwide parasitic diseases, in domestic, wild animals and humans. Hydatid disease is the consequence of organ invasion with the tapeworm *Echinococcus granulosus* intermediate stage, that the dog is major host of it. Hydatid cysts (larval stages) develop in the different tissues of the infected host such as lung, liver, brain, bone, spleen, heart and kidneys which may be fatal [1].

There are two treatment protocols for cystic echinococcosis: surgery (comprises of conservative drainage including of puncture, aspiration, injection and re-aspiration and pharmacotherapy [2].

* Corresponding Author Email: *roghayehnorouzi123@gmail.com*

The current pharmacotherapy drugs against hydatidosis are Benzimidazole family derivatives e.g. Albendazole and Mebendazole.These drugs reported to have some adverse effects includings, increase in the resistance of protoscolices, abnormalities in liver function, gastrointestinal problems such as diarrhea, abdominal pain, nausea, dizziness, headache and some teratogenic effects. The offer of new drugs can solve such adverse effects [3, 4].

Scolicidal drugs used during hydatid cyst surgery play a critical role in surgical success. Scolicidal drugs reduce the hazard of spillage of live protoscolices. A perfect drug against cystic

echinococcosis must be effective in low doses and shorter exposure duration. It must be stable after dilution with the cyst fluid, non-toxic, more efficient, less harmful for host tissue, low cost and easy available [5].

Till now, many efforts have been performed for the application of alternative scolicidal drugs such as nanoparticles. Use of effective scolicidal drugs during hydatid cyst surgery is necessary to prevent the secondary infection [6, 7].

New functionalities and properties of matter are observed in a wide range of applications. Nanotechnology provides important new tools expected to have most impact on many areas in medical sciences. Polymer coated metal NPs have recently appeared as an active and novel field of advanced researches. For example, silver is an important accessible metal, and its NPs are superior to other nanosized metal particles for their antimicrobial effects. However, their stability is a serious problem with polar terminal groups like hydroxyl groups or amine are usually used for their stabilization [8].

Zinc nanoparticles are used in different field of industry and health. It was used as antifungal antibacterial, anti-corrosive, UV protection agent, catalyst and also used in nanosensors, electronic/ nano-optical devices, sunscreens, cosmetic products, food additive and etc [9].

During recent decay the usage of nanoparticles has attracted the attention of researchers, in the world and Iran. This study was undertaken for the first time to evaluate the scolicidal effects of zinc oxide nanoparticles *in vitro*.

MATERIAL AND METHODS

Protoscolex preparation

Lungs and livers of sheep that were infected to hydatid cyst were gathered from industrial slaughterhouse of Tabriz and then transferred to the laboratory of parasitology, veterinary Medicine Faculty. The excision performed to bring out hydatid cyst from liver.

The superficial parts of cysts were sterilized by 70% Alcohol, after disinfection 25 ml of hydatic cyst fluid was extracted by syringe and poured to cylinders. 30 minutes later, after removing supernatant the protoscolices deposits at the bottom of the cylinders were washed with PBS three times and tested by 0.1% eosin for assessment of viability of protoscolices. The samples of protoscolices that have over 90 percents livability, were selected for testing.

ZnO NPs synthesis and characterization

The nano-zinc is metal grey powder. ZnO NPs average particle size was 15 nm (10-15 nm) and bulk density was about 0.20- 0.40 g/m^3. ZnO NPs was purchased from the Nanoshel LLC, Wilmington, DE, USA. Nanoshel ZnO NPs particle is produced by evaporation process. ZnO NPs were characterized by transmission electron microscopy (TEM, Leo 906, Zeiss 100 KV, Germany). The BET specific surface area was reported about 30-50 m^2/g. The zinc oxide nanoparticles used in our experiment possessed analytical grade and the highest purity.

Evaluation of the scolicidal effect of ZnO NPs in vitro

For evaluation of the scolicidal effect of the ZnO NPs, concentrations of 50 and 100 mg/ml of nanoparticles were disposed in distilled sterile water and added to the microtubes, then, a drop of protoscolex rich deposit was purred into microtubes. The contents of the microtubes were mixed. The microtubes were incubated at 37 °C for 10, 30 and 60 minutes. The upper phase was thrown away so as not to distress the protoscolices. One ml of 0.1% eosin stain was purred into the remaining deposit of protoscolices and then mixed softly. The upper part of the solution was thrown away after 15 minutes of incubation. The remaining protoscolices in the pellet was then smeared on a scaled glass slide, covered with a cover glass (50×24 mm), and examined under a light microscope. The percentages (%) of dead protoscolices were determined by counting a minimum of 500 protoscolices. The experiments were performeded in triplicate.

Statistical Analysis

Data were analyzed by SAS software version 9.4.

RESULTS AND DISCUSSION

Fig. 1 illustrates the TEM image of ZnO nano particles. The results were showed that zinc oxide nanoparticles at the dose of 50 mg/ml killed 19.6% of protoscolices after 10 minutes of application. Scolicidal effects of nano ZnO at the dose of 50 mg/ml after 30 minutes of application was lower than others concentrations (12%). However, all concentrations showed statistically significant difference in the protoscolicidal activity with different dilutions (50 and 100mg/ml) ($p > 0.05$). In 150 mg/ml concentration, the black ZnO particles were covered on all protoscolices, and they could

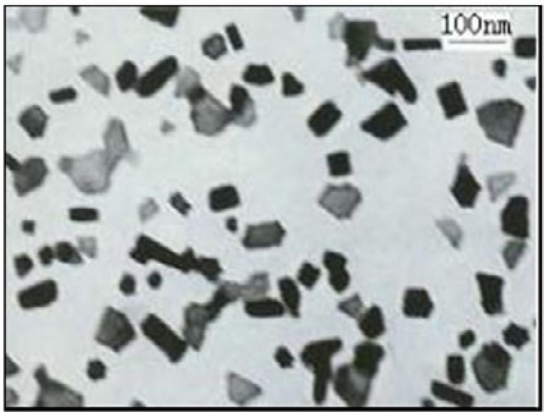

Fig. 1. The TEM image of 15 nm ZnO NPs

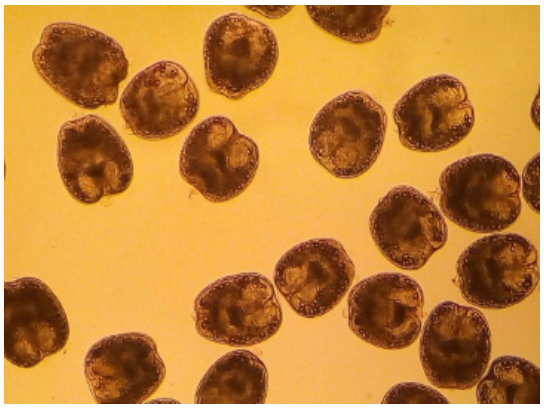

Fig. 2. Image of live protoscolices

Table 1. Scolicidal effect of ZnO NPs at the 50 mg/ml concentration following various exposure times

Exposure time(min)	Experiments	Dead protoscolices	Mortality rate (%)
10	1	18	18
	2	20	20
	3	21	21
	Negative control	8	8
	Total	59	19.6
30	1	10	10
	2	12	12
	3	14	14
	Negative control	7	7
	Total	36	12
60	1	12	12
	2	14	14
	3	13	13
	Negative control	10	10
	Total	39	13

not be seen or counted.

The mortality rate of protoscolices after exposure to different dose of the ZnO NPs in different exposure durations are presented in Tables 1-3 and Figs. 4. 0.1% eosin staining was confirmed viability of protoscolices. After 5 minutes exposure times with eosin stain, the protoscolices that had not absorption of dye with the motion of the flame cells were counted as viable.

Image of live and dead protoscolices after exposure to ZnO nano particles showed in Figs. 2 and 3. The results showed that ZnO nano particles was no recommended to use as a scolicidal agent.

Up to date, a lot of chemical drugs have been applied for treatment of the hydatid cyst protoscolices. Some of these drugs may have adverse side effects which limit their usage. It has been reported adverse effects for 20% hypertonic saline, 0.5% - 1% cetrimide, ethanol, 20% silver nitrate and 20 mg/mL albendazole [10].

Inactivation of protoscolices with scolicidals, associated with great efficacy and at least side effects, have been proposed as a superseded of removing or opening cysts [11]. Moreover, chemotherapy could be applied for patients whom are not candidates for surgery operations. Also, it can be also used as a complementary treatment of surgery (after or before of surgury) [12].

Today, application of nano metal agents in management of parasites as antiparasitic was reported in some studies. Inhibitory effects of chitosan, silver, gold, and oxidized metals on various parasites, such as *Giardia*, *Plasmodium*, *Toxoplasma gondii*, *Leishmania* and larvae of insects were reported [13-19].

Some studies showe the antimicrobial and antifungal properties of zinc oxide nanoparticles. In the present study the effect of ZnO NPs on the protoscolices of hydatid cyst on in vitro model investigated. In this study we reported a higher scolicidal effect (19.6%) of ZnO NPs at a lower dose (50 mg/mL) after exposure time (10 min). According to the results, zinc oxide nanoparticles have a low scolicidal activity (12%) at the dose of

Table 2. Scolicidal effect of ZnO NPs at the 100 mg/ml concentration following various exposure times

Exposure time(min)	Experiments	Dead protoscolices	Mortality rate (%)
10	1	14	14
	2	12	12
	3	15	15
	Negative control	8	8
	Total	41	13.6
30	1	11	11
	2	13	13
	3	15	15
	Negative control	10	10
	Total	39	13
60	1	12	12
	2	15	15
	3	16	16
	Negative control	10	10
	Total	43	14.33

Table 3. Scolicidal effect percentage (%) of ZnO NPs in different concentrations and various exposure times

Dose	Time		
Concentrations of ZnO NPs	10 min	30 min	60 min
50 mg/ml	19.6	12	13
100 mg/ml	13.6	13	14.33

Fig. 3. Image of dead protoscolices

50 mg/mL after 30 min of application.

Malekifard *et al.* (2017) investigated the scolicidal efficacy of gold nanoparticle and indicated gold nanoparticle of all concentrations had significant scolicidal effects. Gold nanoparticle at the concentration of 1 mg/ml led to kill all protoscoleces at 60 minutes [20].

Mahmoudvand *et al.* (2014) used various doses (50-500 mg/ml) of selenium nanoparticles (80- 220 nm) for 10- 60 minutes. The results indicated that biogenic Se nano particles at all concentrations (especially at 500 and 250 mg/ml; for 10- 20 minutes) have potent scolicidal effects [21].

Rahimi *et al.* (2015) showed silver NPs with different concentrations (0.025, 0.05, 0.1 and 0.15 mg/mL) and different exposure times (10, 30, 60 and 120 minutes) have great scolicidal effects. The doses 0.1 and 0.15 mg/mL after 2 hours exposure revealed 83% and 90% mortality rate, respectively. The lowest scolicidal activity was 40% (0.025 mg/mL at 10 minutes). This investigation suggested that biogenic silver nano particles may have a scolicidal potential for CHD surgery because of being safety and efficacy compared to the other chemical drugs[22].

It seems that the differences in result of different studies are due to the differences in nanoparticles, concentration and times exposure.

The findings of this study indicated a low scolicidal activity of ZnO NPs and it's not potential as a component of a new scolicidal agent in hydatid cyst surgery.

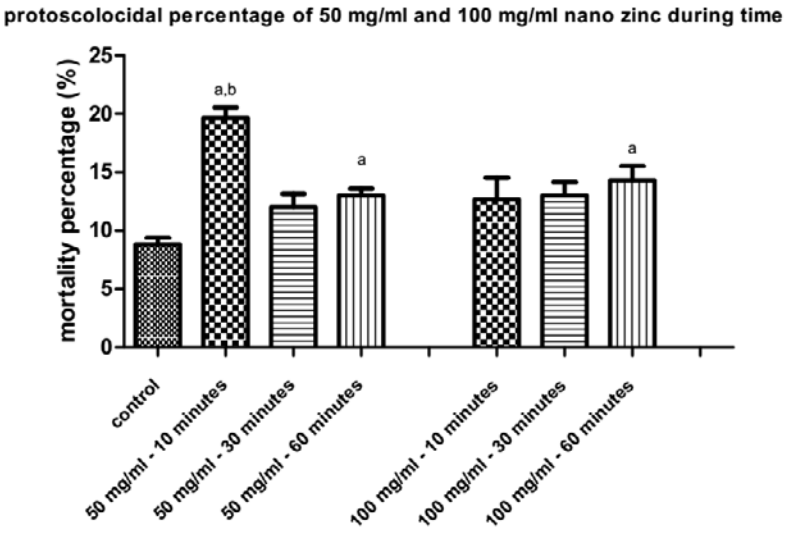

Fig. 4. Scolicidal effects of different doses of nano ZnO after various exposure times

CONCLUSIONS

Our results suggest that ZnO NPs at the concentration of 50 mg/ml killed 19.6% of protoscolices after 10 minutes of application and is not suitable for cyst surgery as a scolicidal agent.

CONFLICTS OF INTEREST

None of authors had conflict of interests.

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