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ORIGINAL ARTICLE

Lethal effects of gold nanoparticles on protoscolices of hydatid cyst: in vitro study

Sara Napooni[1] · Mohsen Arbabi[1] · Mahdi Delavari[1] · Hossein Hooshyar[1] · Sima Rasti[1]

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Abstract

Recurrence of hydatidosis may results from cyst fluid spillage during surgery. To inactivate the cyst content, different scolicidal agents have been introduced. Nevertheless, novel and more effective treatments are needed due to the associated complications. In the current study, we examined the scolicidal effects of gold nanoparticles on *Echinococcus granulosus* protoscoleces. Hydatid cysts of sheep liver were collected in this study. The cyst fluid containing protoscoleces was aspirated aseptically. The protoscoleces were exposed to gold nanoparticles at different concentrations (250, 500, 1000, 2000, and 4000 μg/mL). Eosin staining method was applied to determine the viability of protoscoleces at 5, 10, 20, 30, and 60 min. Moreover, cytotoxicity analysis, scanning electron microscopy (SEM) of ultrastructural changes, DNA fragmentation, were performed, and protoscoleces were studied following treatment. The significant scolicidal effects of gold nanoparticles were observed at all concentrations compared to the control group. A total of 4000 μg/mL of gold nanoparticles could destroy 76% of protoscoleces in 60 min. Cytotoxic effects of gold nanoparticle on J774 macrophage cell line, in minimum and maximum concentration (500 and 4000 μg/mL), were 1 and 11%, respectively. After treatment of protoscoleces with gold nanoparticles in different times and concentrations, considerable alteration in size, ultrastructure changes in tegument and shape of sucker, as well as DNA fragmentation of parasite were seen. Based on the results, gold nanoparticles could exert scolicidal effects on hydatid cyst protoscoleces; therefore, they can be applied in hydatid cyst treatment.

Keywords Cystic hydatid disease · Gold nanoparticles · Scolicidal · Cytotoxicity · Ultrastructure

Introduction

Hydatid cyst or cystic echinococcosis (CE) is a parasitic disease, caused by the larval stage of *Echinococcus granulosus*. It is recognized as a public health issue, associated with major economic problems in developing countries, such as Iran (FasihiHarandi et al. 2012). The *Canidae* family, as the definitive host, consists of adult tapeworms bound to the intestinal epithelium. According to previous reports, humans, besides endemic livestock, may be intermediate hosts for worms forming hydatid cysts in different organs (Gholami et al. 2013).

✉ Mohsen Arbabi
arbabi4.mohsen@yahoo.com

1 Department of Medical Parasitology, Faculty of Medicine, Kashan University of Medical Sciences, P.O.Box: 8715988141, Kashan, Iran

Today, surgery is recognized as the best option for hydatid cyst treatment. Alternative strategies include the PAIR procedure and chemotherapy (with benzimidazoles), particularly for surgery-intolerant patients (Moro and Schantz 2006). It is necessary to use effective scolicidal agents to decrease the risk of subsequent infection, recurrence of CE, and intraoperative cyst fluid spillage (Moro and Schantz 2009; Kilicoglu et al. 2006).

To inactivate protoscoleces intraoperatively, various scolicidal agents, including silver nitrate, hypertonic saline, and ethanol, have been suggested. Nevertheless, most of these agents cause serious side effects, e.g., sclerosing cholangitis, methemoglobinemia, and liver necrosis (Wang et al. 2016; Adas et al. 2009). Therefore, a suitable scolicidal solution for hydatid cyst surgery should be cost-effective, causing rapid and complete scolicidal effects, without any systemic side effects (Spicher et al. 2008). In the past centuries, plant combinations and extracts have been used for the treatment of diseases, such as infectious diseases, considering the fewer side effects, high

accessibility, and cost-effectiveness (Sugimoto et al. 2007). Recently, major developments have been made in the function of gold nanoparticles in different areas (Want et al. 2017; Barabadi et al. 2017), including the health domain (Naseri et al. 2016; Berners-Price and Filipovska 2011). As demonstrated in several studies, gold can play a particularly important role in the detection and treatment of tropical diseases.

In previous studies on parasitic diseases of animals (Alvarez et al. 2010), direct use of gold nanoparticles could be helpful in areas beyond classical diagnosis, based on parasite detection through microscopy of blood or lymph fluid. Considering their surface oxidation resistance and chemical inertness, these nanoparticles have different applications in nanoscale technologies and devices (Faa et al. 2017). For instance, they have been applied as antimicrobial compounds to prevent waterborne pathogens, including *Salmonella typhi* and *Escherichia coli*, which may develop resistance to common bactericides (Lima et al. 2013). With this background in mind, we examined the in vitro antiprotoscolice effects of gold nanoparticles on *E. granulosus* infections.

Methods

Collection of protoscoleces and viability test

Hydatid cysts, infecting the sheep livers, were collected from a slaughterhouse in Kashan, North of Isfahan, Iran. The cysts were rapidly transferred in an icebox to the laboratory of Parasitology Department, Kashan University of Medical Sciences, Kashan, Iran. The hydatid cyst fluid, besides protoscoleces, was collected, based on a method proposed by Smyth and Barret (1980).

The hydatid fluid with protoscoleces was aspirated in a syringe (20 mL) and transferred aseptically to an Erlenmeyer flask; then, it was left for 40 min to allow protoscoleces to settle. After discarding the supernatant, PBS (pH, 7.2) was used to wash protoscoleces at least 3 times. First, flame cell motility with 0.1% eosin stain solution, containing eosin powder (1 g) in distilled water (1000 mL), was used to confirm the protoscolece viability under a light microscope (Fig. 1).

In the next step, unstained protoscoleces were considered viable at 10 min after staining, while stained protoscoleces were considered nonviable; therefore, tonality distinguished the protoscoleces (alive or dead) (Abdel-Baki et al. 2016). At the beginning of the study, the protoscolece count (per milliliter) was adjusted as 2000 parasites in NaCl solution (0.9%) with 99% viability; these parasites were considered suitable for further examination.

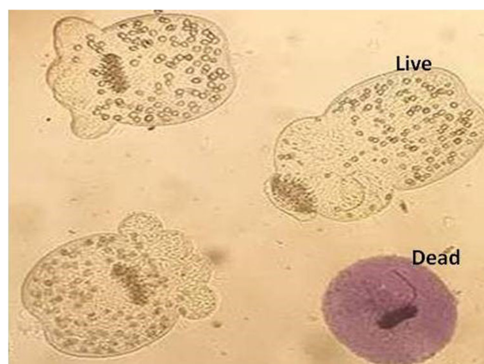

Fig. 1 Protoscoleces without staining and dead protoscoleces after treatment with gold nanoparticles and 0.1% eosin staining. Alive protoscoleces remained colorless and exhibited flame cell activity and muscular movements, while dead protoscoleces were red and absorbed eosin

Cytotoxicity assay by MTT method

Cell culture

After purchasing mouse macrophage cells (J774A.1 cell line) from Pasteur Institute, they were cultured in DMEM medium containing 10% FBS at 37 °C in a 5% CO_2 atmosphere. The cells were grown in cell culture flasks (Lam et al. 2009). Then, 105 macrophages (J774) were cultured in each well for 24 h on a 48-well plate. The wells were filled with various nanoparticle concentrations and incubated for 1 day at a temperature of 37 °C in a 5% CO_2 atmosphere. Afterwards, 20 μL of MTT solution (final density, 5 mg/mL) was added. The plates containing macrophages and drugs were incubated for 3–5 h at 37 °C, and then, the top liquid was removed from the wells. Next, 100 μL of DMSO was added for each sink. After 15 min, optical absorption was read using an ELISA reader (Model 680, BIORAD Co.) at a wavelength of 570 nm. The cell survival was measured as follows:

Survival rate = (AT-AB)/(AC-AB) × 100.

where AB is the optical absorption of the blank sink, AC is the optical absorption of the control sink, and AT is the optical absorption of the treated cell.

Analysis of scolicidal effects

The rinsed protoscoleces (0.5 mL; 2000/mL) were first placed in each test well. Then, 0.5 mL of each nanoparticle concentration (250, 500, 1000, 2000, and 4000 μg/mL) was added to the wells. The content of the tubes was mixed slowly and incubated for 5, 10, 20, 30, and 60 min at 37 °C. After incubation, the number of protoscoleces was determined using 0.1% eosin staining and a light microscope. Nontreated parasites in RPMI 1640 medium were considered as the control group. The protoscoleces fatality rate (PFR) was determined according to the formula:

$$PFR = \frac{\text{Number of Deaths}}{\text{Number of Lives (control)}} \times 100$$

Morphometric study

The length and width of protoscoleces were measured using a ruler in optical microscopy. As soon as the drug showed effectiveness, all indications for protoscoleces were re-measured, and the obtained values were documented.

Ultrastructural assay

To perform topographic studies and to assess changes in protoscoleces, scanning electron microscopy (SEM) was employed. Next, the images were used to assess structural changes in the morphological properties of protoscoleces (including the sucker, hook, and neck). Protoscoleces were rinsed in sodium cacodylate buffer (pH, 7.2) and kept for 1 h in a primary fixative, containing 2.5% glutaraldehyde and sodium cacodylate buffer (100 mM). Next, 2% osmium tetroxide was used to fix the samples in cacodylate buffer, and then, they were rinsed with distilled water. The samples were placed in 50–70% ethanol and dipped in hexamethyldisilazane. Finally, after covering with gold nanoparticles, images were acquired using JEOL JSM-840 microscope.

DNA fragmentation assay

After treatment of protoscoleces with nanoparticles based on the noncolumnar DNA extraction kit, protoscoleces were combined with protease and proteinase K at 56 °C for 1.5 h. Then, they were mixed with lyses buffer and rinsed with special buffers. After continuous centrifugation and vortexing, as outlined by the manufacturer, DNA extraction liquid was obtained. By performing 1.5% agarose gel electrophoresis, the extracted DNA was assessed in terms of DNA defragmentation.

Statistical analysis

The experiments in this study were performed in triplicate. ANOVA and *t* tests were applied to determine the differences among the groups. Moreover, differences between the groups were examined using *t* test. For analyzing the data, SPSS v. 17(SPSS Inc., Chicago, IL, USA) was used at a significance level of 0.05.

Results

Cytotoxicity assay

The present study showed that gold nanoparticles had dose-dependent scolicidal effects on protoscoleces of hydatid cysts. Based on the toxicity analysis, minimum viability was 99% at a concentration of 500 μg/mL, while toxicity level was 89% at 4000 μg/mL (Table 1). Therefore, toxicity of nanoparticles was negligible.

Morphometric assessment of protoscoleces

The results of protoscolece treatment at various nanoparticle concentrations and intervals are presented in Table 2. The length and width of protoscoleces after treatment with gold nanoparticles were assessed. Based on the findings, the length and width of protoscoleces decreased after 1 h at a concentration of 4000 μg/mL and maximum effect was observed.

Assessment of fatality on nanoparticle assays

Table 3 indicates the scolicidal effects of gold nanoparticles. The scolicidal activity of 250 μg/mL of nanoparticles after 5, 10, 30, and 60 min was 1.1, 1.1, 3.1, 7.7, and 15.2%, respectively. Additionally, the scolicidal effect of gold nanoparticles at 2.2, 6.3, 7.2, 13.2, and 18.1%, respectively. The results clearly showed that maximum fatality effect was nearly 76.7% for 4000 μg/mL of nanoparticles after 60 min. Moreover, the scolicidal effects of gold nanoparticle concentrations were significant in comparison with the controls in different exposure times ($p < 0.05$). Therefore, scolicidal activities of gold nanoparticles at concentrations of 2000 and 4000 μg/mL were effective in shorter exposures.

SEM assay

After treatment of protoscoleces with gold nanoparticles for 5, 10, 20, 30, and 60 min at concentrations of 250, 500, 1000,

Table 1 Toxicity level of gold nanoparticles

Gold nanoparticle concentrations (μg/mL)	Cell viability
4000	89%
2000	92%
1000	97%
500	99%

Table 2 Morphometric analysis of hydatid cyst protoscolecses after adding different concentrations of gold nanoparticles

Concentration of gold na noparticles (μg/mL)	5 min		10 min		20 min		40 min		60 min		Comparison groups
	Length	width	Length	width	Length	width	Length	width	Length	width	
250	329 ± 5.2	257 ± 5	329 ± 4.9	256 ± 5	327 ± 5.1	254 ± 5	325 ± 5	253 ± 5.1	324 ± 5	250 ± 4.8	$p > 0.05$
500	328 ± 5	254 ± 5.2	327 ± 5	255 ± 4.8	325 ± 4.8	253 ± 5.2	323 ± 4.9	251 ± 5	323 ± 5.1	247 ± 4.9	$p > 0.05$
1000	327 ± 4.7	254 ± 4.9	326 ± 5.1	254 ± 4.8	324 ± 4.9	251 ± 4.9	322 ± 5.1	249 ± 4.8	321 ± 4.9	245 ± 5	$p > 0.05$
2000	323 ± 4.9	254 ± 4.7	325 ± 4.7	253 ± 5.1	322 ± 5.1	250 ± 5	320 ± 4.8	246 ± 4.9	319 ± 4.9	242 ± 4.8	$p > 0.05$
4000	321 ± 5	253 ± 4.7	322 ± 5	250 ± 5	320 ± 5.2	248 ± 4.9	319 ± 4.9	244 ± 4.9	318 ± 5	241 ± 4.6	$p > 0.05$
Control	330	261	330	260	330	259	329	259	329	259	$p < 0.05$

2000, and 4000 μg/mL, some changes were observed at 4000 μg/mL in 60 min, as demonstrated in Fig. 2.

Detection of DNA fragmentation

After adding various concentrations of gold nanoparticles in different intervals, DNA was extracted and electrophoresed. The highest fragmentation rate was reported at a concentration of 4000 μg/mL after 60 min. In contrast, no fragmentation was detected in DNA samples from the negative control (Fig. 3).

Discussion

Hydatid cyst, as a zoonotic disease, is considered a major clinical problem in animals and humans worldwide, mainly in regions where subsistence farming and animal husbandry constitute essential parts of the community (Moro and Schantz 2009; da Silva 2010).

Overall, 75% of hydatid cysts are detected in the liver (Malik et al. 2010), which are treated via medicinal approaches or surgical operation (Bari et al. 2011). Surgical operation is the first choice of treatment for complicated cases of hydatidosis. However, spillage of protoscolece-rich fluids is possible during surgery, causing local reoccurrence, cyst formation or relapse, and secondary dissemination of echinococcosis, which may result in death (Abdel-Baki et al. 2016). Therefore, removal of cyst content can lead to recurrence, and use of scolicidal agents before opening or removing cysts is strongly suggested (Malik et al. 2010). To overcome this problem, some chemical substances with scolicidal effects have been applied, while many of them have limited applications, considering their side effects (Moazeni and Nazer 2010; Shahnazi et al. 2014).

Infertilization and inactivation of protoscolices with scolicidal agents, associated with high efficacy and minimum side effects, have been suggested as an alternative to opening or removing cysts (Adas et al. 2009). 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) (Bari et al. 2011; Budke et al. 2017).

Drugs, which are currently used in the treatment of hydatidosis, have shown different levels of effectiveness (Barzinji et al. 2009). Albendazole has been used as the standard treatment against echinococcosis. It is commonly used pre- and postoperatively in echinococcosis treatment, although no definite treatment duration has been proposed. In the late 1970s, mebendazole and albendazole were used in humans for the first time. Chemotherapy was ineffective in almost 20–40% of patients. Albendazole has been shown to be significantly more effective than mebendazole in the treatment of hydatid and liver cysts (Tamarozzi et al. 2014; Shahnazi et al. 2016; Zhang et al. 2017). Nevertheless, the

Table 3 Fatality of hydatid cyst protoscoleces after adding different concentrations of gold nanoparticles

Concentration of gold nanoparticles (μg/mL)	Fatality rate					
	5 min	10 min	20 min	30 min	60 min	Comparison groups
250	1.1%	1.1%	3.1%	7.7%	15.2%	$p > 0.05$
500	2.2%	6.3%	7.2%	13.2%	18.1%	$p > 0.05$
1000	8.3%	10.2%	22.2%	30.3%	33.3%	$p > 0.05$
2000	21.3%	33.4%	36.1%	55.2%	60.6%	$p > 0.05$
4000	50.2%	57.3%	66.2%	74.4%	76.7%	$p < 0.05$

*ANOVA and *t* test

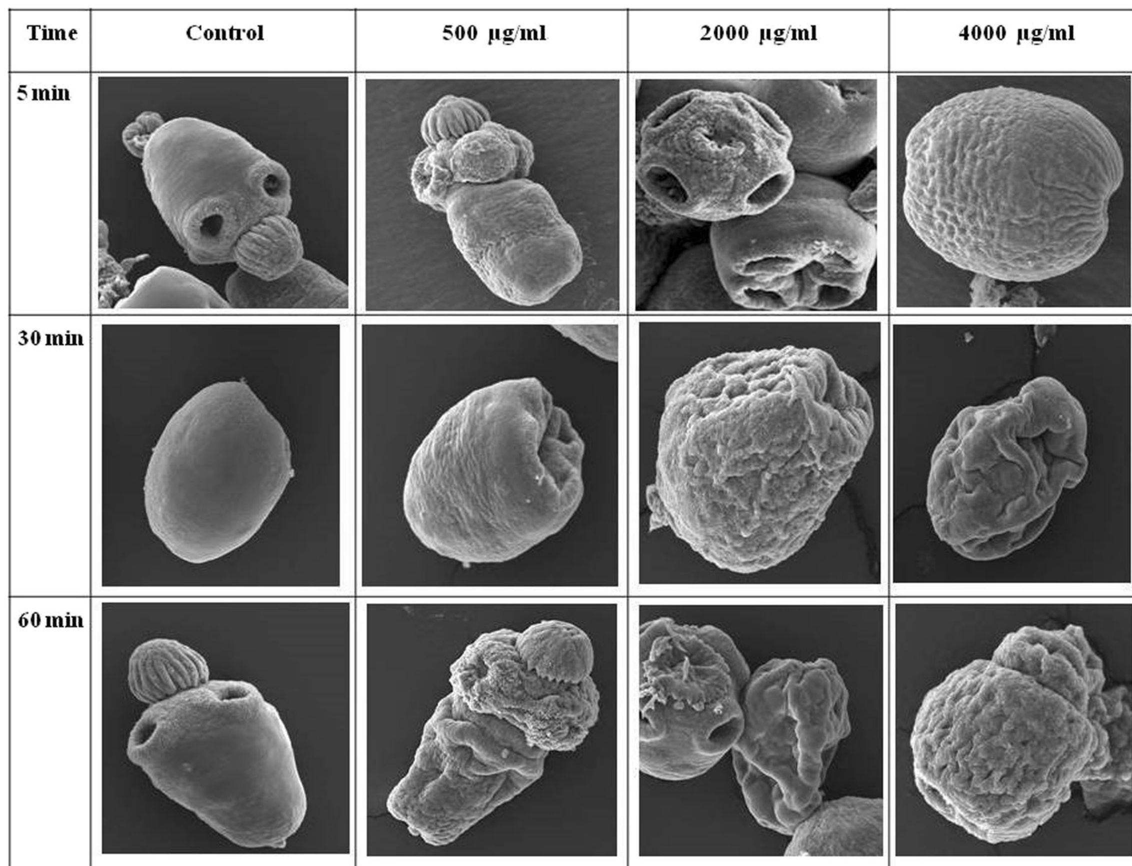

Fig. 2 SEM of treated protoscolices of *Echinococcus granulosus.* SEM view showing the denatured and changed tegumental surface and hooks as well as shape of suckers

metabolites of agents, such as mebendazole, albendazole, albendazole sulfoxide, and benzimidazole, may be toxic in some cases and cause severe hepatobiliary complications (Topcu et al. 2006).

In several studies, different agents, including mannitol, chlorhexidine gluconate, hypertonic saline, fungal chitosan, Huaier aqueous extract, *Sambucus ebulus*, *Allium sativum*, and *Berberis vulgaris* have been used to inactivate hydatid cysts (Gholami et al. 2013; Fakhar et al. 2015; Lv et al. 2013). According to the literature, 1.5% cetrimide, considering its scolicidal activity, can eliminate 86.9 and 92.6% of protoscolices within, respectively, 5 and 10 min of exposure (Khan et al. 2010).

In another study, silver nitrate (20%) and ethyl alcohol (95%) could entirely remove protoscolices within 20 and 15 min (Caglar et al. 2008). However, in several studies, scolicidal activities of hypertonic saline (Mahmoudvand et al. 2014a), silver nitrate, cetrimide, mannitol, hydrogen peroxide, ethyl alcohol (95%), povidone iodine, albendazole, chlorhexidine gluconate (Mahmoudvand et al. 2014b), and AmB-coated silver nanoparticles (82.3 and 71.6%, respectively) (Lashkarizadeh et al. 2015) have been confirmed.

Researchers have started to globally examine the actual effect of different medicinal plants, which have remained understudied in scientific experiments. In recent years, scolicidal activities of many plants, such as *Allium cepa*, *Ocimum bacilicum*, *Allium sativum*, *Sambucus ebulus*, *Trachyspermum ammi*, *Zataria multiflora*, *Zingiber officinale* (Gholami et al. 2013; Moazeni and Nazer 2010; Haghani et al. 2014; Moazeni et al. 2012; Moazeni and Nazer 2011; Moazeni and Roozitalab. 2012), and *Salvadora persica* (Abdel-Baki et al. 2016), have been screened in vitro.

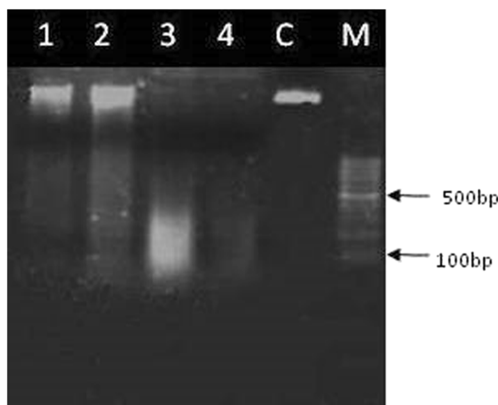

Fig. 3 Analysis of DNA fragmentation via agarose gel electrophoresis (lines 1–4, DNA of protoscoleces treated with 500, 1000, 2000, and 4000 µg of gold nanoparticles after 1 h of incubation; C, control or untreated; M, DNA marker)

In recent years, several studies have investigated the scolicidal effects of metal nanoparticles such as selenium (Mahmoudvand et al. 2014a) and silver (Rahimi et al. 2015) on hydatid cysts and showed their antiparasitic properties.

Overall, use of natural scolicidal agents, with no harmful side effects, is a safe treatment option (Moazeni and Nazer 2011). Today, in biomedical sciences, application of nano metal products has highlighted the necessity of effective methods in the management of parasites. Several studies have exhibited the antiparasitic and inhibitory effects of gold, silver, chitosan, and oxidized metals on various parasites, such as *Giardia*, *Malaria*, *Toxoplasma gondii*, *Leishmania*, and larvae of insects (Said et al. 2012; Kumar et al. 2015; Santhoshkumar et al. 2011; Das et al. 2013; Allahverdiyev et al. 2011; Mayelifar et al. 2015; Beheshti et al. 2013).

Recent studies have evaluated the application of gold nanoparticles as inorganic drugs against different diseases (e.g., rheumatoid arthritis and tumors) (Tsai et al. 2007). Therefore, review of the preventive effects of gold nanoparticles on protoscoleces of hydatid cyst can be effective in identifying therapies with less toxicity. The in vitro properties of metals on hydatid cyst protoscoleces have been studied. The present study showed the major impact of gold nanoparticles on protoscoleces. These nanoparticles showed more significant effects at all concentrations, compared to the control group. By increasing the exposure time, the mean mortality of protoscoleces increased from 1 to 76% (from minute 5 to minute 60), using gold nanoparticles. Overall, various studies have revealed the positive effect of gold nanoparticles. Moreover, cytotoxic and ultrastructural analyses via SEM, DNA fragmentation, and morphometric analysis have been performed after treatment with gold nanoparticles at different concentrations and intervals (Mohamed et al. 2017; Vinoj et al. 2015; Shamaila et al. 2016). The main mechanisms identified of gold nanoparticle in the biological response are the production of ROS and oxidative stress and DNA damage induction, cell cycle effects, and potential interference (Mikami et al. 2013). In addition, this biological metal to affecting the tegument of parasite and inhibit the synthesis of proteins (Kumar et al. 2014; Barabadi et al. 2017). Ultrastructural changes in the tegument are linked to a possible action of the nanoparticle as an inhibitor of protein synthesis.

Conclusion

The results demonstrated the significant scolicidal activity of gold nanoparticles against hydatid cyst protoscoleces. These nanoparticles can be used as an alternative in hydatid cyst treatment to overcome the disadvantages of chemical drugs, particularly their side effects. Effective concentrations of metal nanoparticles, such as gold, can be used in future studies on hydatid cyst treatment in laboratory animals.

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Compliance with ethical standards

Ethical considerations Ethical issues have been completely observed by the authors. All procedures performed in this study were done according to the local ethics review committee of Kashan University of Medical Sciences that approved this work.

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

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