Annals of Parasitology 2021, 67(2), 203–212
doi: 10.17420/ap6702.330

Original paper

In vitro evaluation of albendazole nanocrystals against *Echinococcus granulosus* protoscolices

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ABSTRACT. *Echinococcus granulosus* is a zoonotic parasite causing hydatidosis in humans and animals. This study has been done in order to investigate the effect of albendazole nanocrystals on the viability of *E. granulosus* protoscolices. The average size and hydrodynamic diameter of albendazole nanocrystals were 976±218 and 1334±502 nm, respectively. Fertile hydatid cysts were isolated from the liver of slaughtered sheep. The isolated cysts were further identified using morphological and molecular techniques. The nucleotide sequence analysis indicated that the genotype of the protoscolices was *E. granulosus* sensu stricto with 100% similarity. The parasites were examined precisely for susceptibility to albendazole nanocrystals. The results revealed that albendazole nanocrystals are effective in removing protoscolices. It was observed that 1 µg/ml albendazole nanocrystals and albendazole completely inhibited the viability of the protoscolices within 17 and 23 days, respectively. The results suggested that albendazole nanocrystals can be used as an alternative effective treatment for *E. granulosus* infection.

Keywords: hydatid cyst, protoscolex, albendazole, nanocrystals, *Echinococcus granulosus*

Introduction

Cystic Hydatid Disease (CHD), known as human echinococcosis, is a zoonotic disease caused by the larval stage of the cestode *Echinococcus*. *Echinococcus granulosus* (*E. granulosus*) is the most common species causing cystic echinococcosis (CE) in humans [1,2]. Hydatid disease may lead to economic and public health concerns. In addition, CHD is endemic in several parts of the world including the Mediterranean countries, Central Asia, Northern and Eastern Africa, Australia, and South America [3,4]. *E. granulosus* is mostly found in dogs and wolves as definitive hosts while sheep, goat, swine, cattle, horses, and camels are intermediate host [5,6]. It has been reported that humans are accidental intermediate hosts [7]. *E. granulosus* is transmitted by the consumption of cyst-contaminated food or through faecal-oral route [8]. *E. granulosus* penetrates into the venous

circulation through the intestinal mucosa and the infection disseminates to multiple organs including liver and lung which are the most infected tissues [9].

Albendazole (ABZ) and mebendazole (MBZ) are benzimidazole drugs for the treatment of CHD [10–12]. In infected individuals, large cysts are eradicated by surgical treatment [13,14]. In addition, ABZ is also used for preventing the formation of secondary cysts [15]. ABZ do strong anti-parasitic activity against *E. granulosus*. However, the permeability and solubility of benzimidazole drugs are poor. In addition, the process of oral absorption could be limited, resulting in a consequent decrease in the bioavailability. Therefore, increasing oral bio-availability of the drug is clinically important in the treatment of systemic helminthiosis including echinococcosis [16].

Improvements of ABZ dissolution can be obtained by reduction in particle size to nanoscale and subsequently increase in the surface area. Increased systemic bioavailability of ABZ has also been reported when the drug was co-administered with fatty meal, fruit juice, co-solvent or surfactants [17]. Furthermore, several clinical studies showed that an enhanced systemic bioavailability of the parent drug or the active metabolite achieved by increased drug absorption corresponds to an improved anti-parasitic effect [18,19]. However, most of the aforementioned strategies have failed to achieve favorable outcomes in the improvement of ABZ bioavailability, with the methods proposed being generally difficult to scale up for industrial production. As a result, there is a need to search for a simple, efficient and superior method to produce new ABZ formulations with improved bio-availability. In order to achieve this goal, the formulation of ABZ nanocrystals (ABZ-NCs) seems to be a promising tool since it helps obtain particles with sizes below 1 μm and the ability to re-disperse it in aqueous media [20]. The most important key feature of ABZ-NCs is its very enlarged surface area, which results in a faster saturation in the dissolution layer around the particles with a consequent increase in the dissolution rate [21].

Therefore, the aim of this study is to synthesize ABZ nanocrystals using anti-solvent precipitation method. Characterization of the prepared ABZ-NCs is determined. The prepared ABZ-NCs are tested for their effectiveness against protoscolices isolated from sheep liver.

Materials and Methods

Preparation of ABZ nanocrystals

ABZ nanocrystals (ABZ-NCs) were prepared using the anti-solvent precipitation technique. ABZ was first dissolved in DMSO in concentration of 1.5 mg/ml. Then, 0.5 ml of the prepared drug solution was added dropwise into 10 ml water containing PVP K30 (0.5% w/v) and Tween 80 (0.25% v/v) under constant stirring at 1,200 rpm. PVP and Tween were used as suspension stabilizers. A milk-like suspension was formed and filtered to remove large particles. In order to remove DMSO from ABZ-NCs, the suspension was centrifuged at 10,000 rpm for 30 minutes. Then, the supernatant was removed and 10 ml water containing PVP K30 (0.5% w/v) and Tween 80 (0.25% v/v) were added to the sediment. The sediment was lyophilized, weighted and re-suspended in water with 0.5% w/v PVP K30 and 0.25% v/v Tween 80 to reach the final concentration of 75 μg/ml.

Scanning Electron Microscopy (SEM)

In order to observe SEM image of ABZ-NCs, a drop of final ABZ-NCs was placed on SEM aluminum studs and dried at 30°C. Then, the sample was sputter-coated with Au before examination using a Scanning Electron Microscope Zeiss 50 VP, TESCAN-Vega 3 (Joint Stock Company, Brno, Czech Republic).

Hydrodynamic particle size determination

The hydrodynamic particle size of ABZ-NCs was measured by a dynamic light scattering (DLS) technique. The particle size of ABZ-NCs in water (containing stabilizers) was measured using Microtrac Dynamic Light Scattering System (Nanotrac Flex, PA, USA).

Collection of protoscolices from hydatid cyst

Hydatid cysts were collected from the livers of naturally infected sheep that were slaughtered in Qom industrial slaughterhouse. After detecting the fertile and non-fertile cysts, the hydatid fluid of the fertile cyst was aspirated and transferred into clean and sterile glass cylinders. Protoscolices were washed several times using phosphate-buffered saline (PBS).

Viability test

The viability of protoscolices was determined using 0.01% eosin exclusion analysis. Then, the

viability of the protoscolices was examined under light microscopy [22,23]. It has been hypothesized that dead protoscolices appear stained while the viable ones remained colorless under microscopic examination. The red-purple stained protoscolices were considered dead while unstained ones were reported as alive. The viability of protoscolices samples higher than 95% was collected for further study.

Culture of protoscolices

Protoscolices were cultured in 1 ml of RPMI 1640 (ThermoFisher Scientific, Darmstadt, Germany) supplemented with pen-strep (penicillin100 µg/ml, streptomycin 100 µg/ml, (Thermo Fisher Scientific, Massachusetts, USA), then 1µg/ml albendazole nanocrystal and albendazole drug were added to culture, incubated at 37°C in 5% CO_2 [24,25]. Protoscolices were classified into four groups including A, B, C, D. Groups A, and B were treated with ABZ and ABZ-NC respectively. Group C has been culture by 1 ml culture medium containing 600 µl dimethyl sulfoxide (DMSO), and group D was considered as negative control without drug or DMSO. All cultures were assessed daily for protoscolices viability, using lightm icroscope and eosin staining method. The percentage of protoscolices viability was further estimated [26]. The viability was examined in a comparative table and the experiment continued until the number of parasites reached to zero.

Genotyping

Total DNA of the protoscolices was extracted using DNG-Plus kit (Cinna Clon, Tehran, Iran). PCR amplification was carried out by targeting the cytochrome oxidase subunit 1 gene (*Cox1*). The primary mixture included 12.5 µl of 2X Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark), 1 µl of extracted genomic DNA, 1 µl of forward primer (5′-TTTTTTGGGCATCCTGAGG TTTAT-3′), 1 µl of reverse primer (5′-TAAAGAA AGAACA TAATGAAAATG-3′) and 9.5 µl of add H_2O in a total volume of 25 µl. PCR was performed with an initial denaturation step for 5 minutes at 94°C, followed by 30 cycles including 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and final extension at 72°C for 5 minutes. The PCR amplicon was further subjected to sequencing using an ABI PRISM™ 3130 Genetic Analyzer automated sequencer (Applied Biosystems, California, USA).

Statistical analysis

For the purpose of determining the significant statistical difference between protoscoleces viability, Generalized Estimating Equation (GEE), were used. When data were measured repeatedly over times from the same individuals, one of the most important assumptions for many traditional statistical methods (e.g., ordinary least squares (OLS) regression) is not to analyze the data indecently [27]. Therefore, GEE models was preferred since it is a flexible regression-based which provides a powerful framework and reliable parameter estimates [27].

Ethics

The study project was approved by the ethics committee of Qom University of Medical Sciences approval number IR.MUQ.REC.1396.10 (Code Project: 95809). The permission was granted by Qom industrial slaughterhouse to collect liver tissue samples from the animals for this study.

Results

Isolation and identification of protoscolices

The protoscolices were isolated from the livers of naturally infected sheep. The morphological identification of the protoscolices was observed by eosin staining, as shown in Figure 1. The viable cells were showed an un-stained appearance with a clear hook and calcareous body and used for further

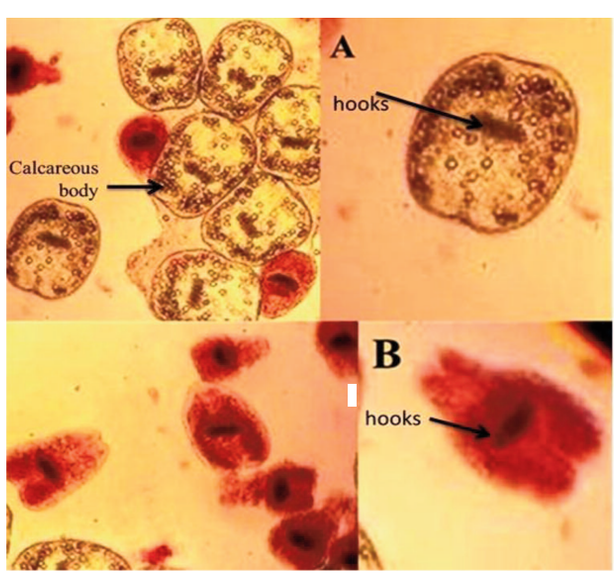

Figure 1. Morphological identification of the protoscolices using eosin staining. The viable cells appeared un-stained (A) while dead cells were red-stained (B).

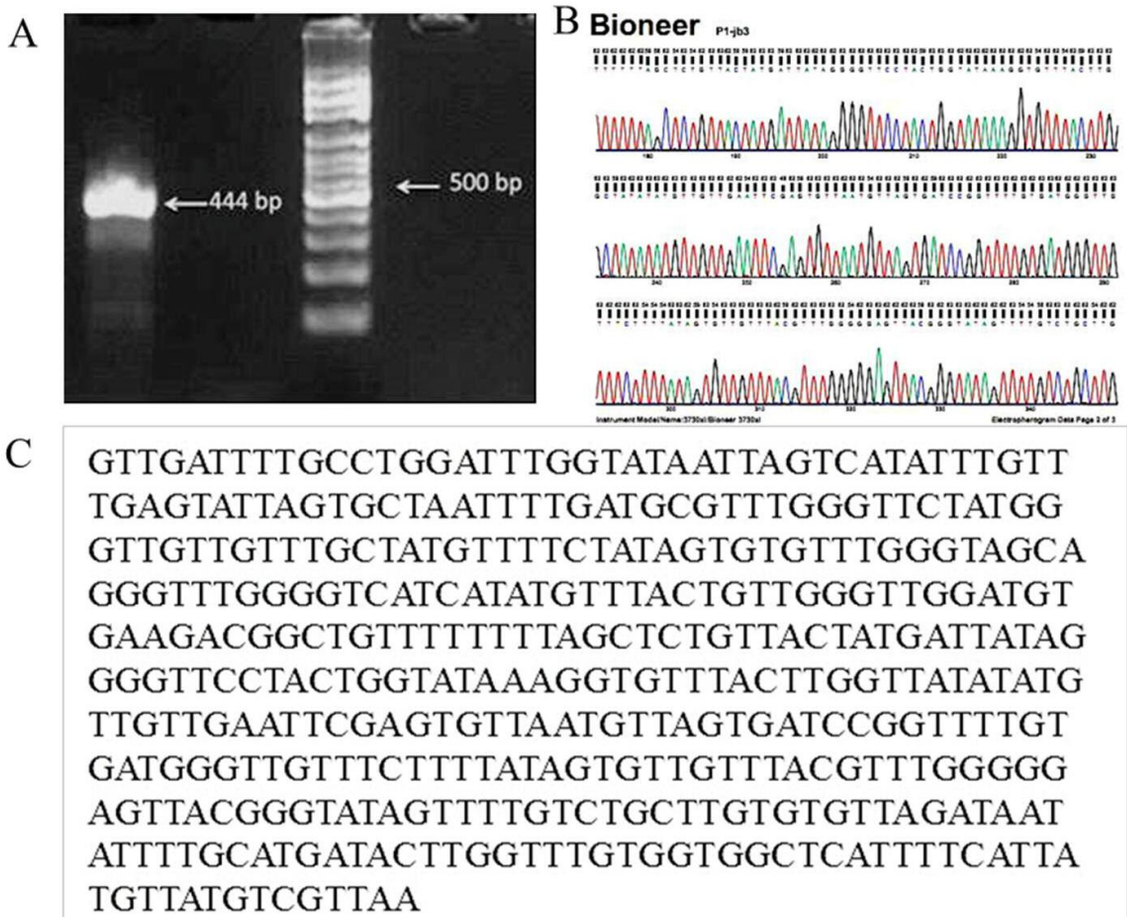

Figure 2. Molecular identification of the isolated protoscolices. PCR analysis of the isolate on an agarose gel (A), BLAST analysis of the sample, (C) nucleotide sequence of *Echinococcus granulosus* sensu stricto.

experimentations.

In order to identify the isolated protoscolices, the total DNA of them was extracted and amplified using a pair of primers designed for the cytochrome oxidase gene (Cox1). PCR analysis of the isolation was performed and a specific 444 bp band was expectable on an agarose gel (Figure 2A). It has been found that the isolate was positive for *Echinococcus* using genus-specific primers and cytochrome oxidase gene (Cox1) PCR. To identify the species, the 18S rRNA gene sequence of the protoscolices (Figure 2) was further compared to *Echinococcus* sequences in the GenBank database. It turned out that the isolated protoscoleces were belonged to *E. granulosus* sensu stricto with 100% similarity of 444 bps Figure (2B and 2C). The genome sequences of the isolation were submitted to the GenBank database under the accession numbers KT154000. In addition, the organism was identified as G1 genotype.

SEM images of ABZ-NCs

The size of the synthesized nanocrystals was determined by a scanning electron microscope (SEM), as shown in Figure 3. The nanoparticles were in spherical shape and the size of nanoparticles was further calculated using Image software. From SEM images, the average size of nanoparticles was 976±218 nm.

Hydrodynamic particle size

The hydrodynamic size distribution of obtained nanocrystals in water containing 0.5% w/v PVP K30 and 0.25% v/v TWEEN 80 was determined using DLS. The results of DLS analysis in number mode were shown in Figure 4. According to DLS results, it showed the nanocrystals of an average hydrodynamic diameter of 1,334±502 nm.

Activity of ABZ-NCs on the viability of E. granulosus

In order to investigate the activity of ABZ-NCs on *E. granulosus*, the parasites were incubated in

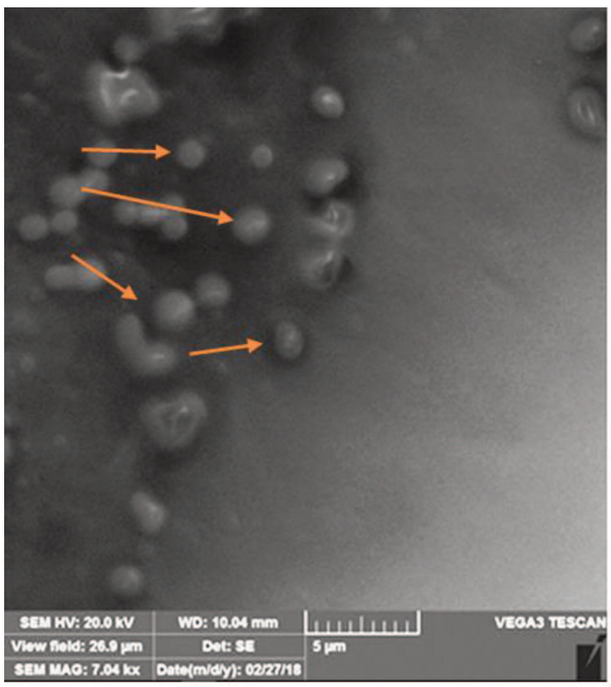

Figure 3. The SEM images of albendazole-nanocrystals. Nano-particles are shown with arrows. Scale bar is 5 µm.

RPMI 1640 medium containing the nanocrystals. ABZ-NCs inhibited 50% viability of *E. granulosus* within 9 days (Figure 5). It was observed that 1 µg/ml ABZ-NCs and albendazole completely inhibited the viability of the protoscolices within 17 and 23 days, respectively. While 100% inhibition of the negative control was detected when the parasite was treated with DMSO within 28 days.

Comparison of the mean number of protoscolices viability

As shown in Table 1, beta coefficient GEE analysis revealed that the change in protoscolices, response in DMSO group was 0.8 lower than the negative control (not significant in 1%). The average change in protoscolices, response in ABZ-NCs group was 37 which is lower than the control group (significant in 1%). The average change of protoscolices, response in ABZ group was 12 which is lower than the negative control (significant in 1%). In the negative control, the number of parasites decreased by an average of 3.8 per day.

Discussion

E. granulosus genotypes consist of G1–G3, G6–G9 and G10, while, G4 and G5 belong to *E. equines* and *E. ortleppi*, respectively. Three mitochondrial markers commonly used for genotyping of *E. granulosus* are Cox1, NAD1 and ATP6. Of these, Cox1 was selected for the genotyping of the protoscolices in this study as previously described [28]. Our results indicated that the DNA sequences of the amplified fragment were 100% identical to the *E. granulosus* G1 genotype (GenBank accession No: AB893250). So the genotype of *E. granulosus* protoscolices used in this study was confirmed as *E. granulosus* sensu stricto.

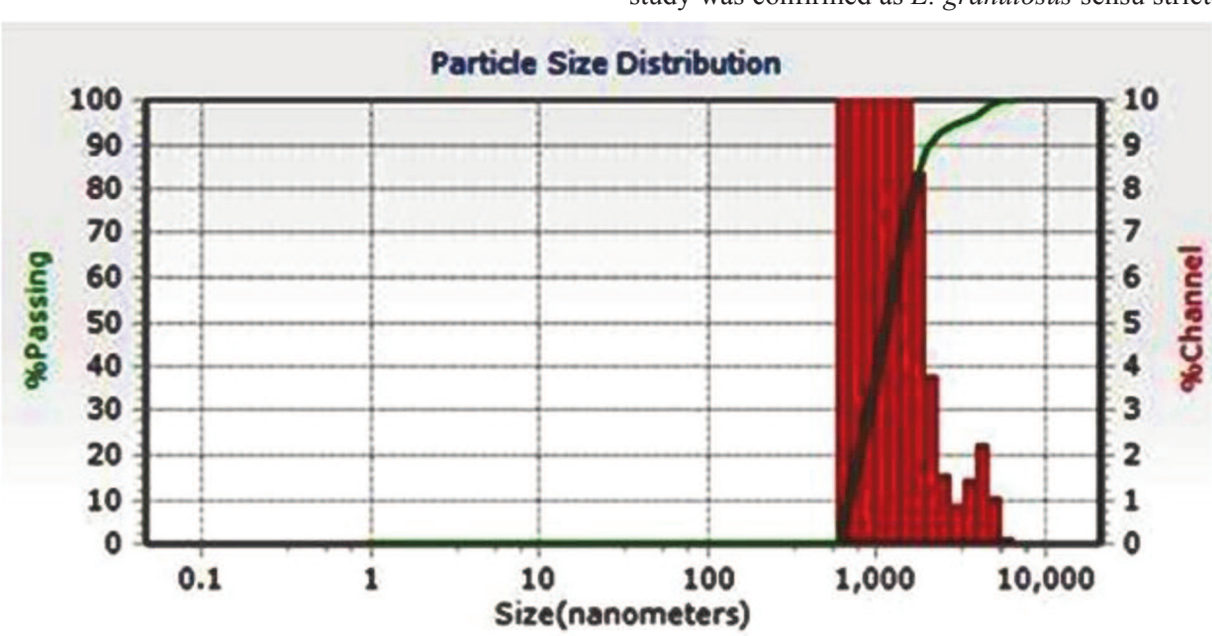

Figure 4. Particle size distribution of albendazole-nanocrystals using Dynamic Light Scattering (DLS)

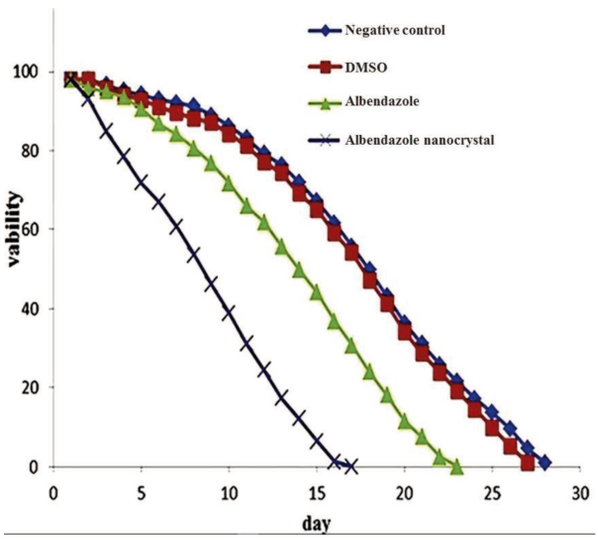

Figure 5. Effects of ABZ-NS on the viability of *Echinococcus granulosus*. The organism was treated with ABZ-NS, albendazole, and DMSO. The free RPMI 1640 medium was included as negative control. Viability of the parasite was determined daily.

Due to genetic changes and diversity of *E. granulosus* strains in different endemic regain of the disease, the presence of those strains can have a significant impact an epidemiology, pathology, effective treatment, vaccination against parasites, controlling and prevention if hydatid cyst disease in human [14,29,30].

Surgery is a prevalent treatment for complicated human cases of CHD. However, its success relies on the formation of new cysts, and relapse or secondary dissemination of CHD after surgical treatment which may lead to death because of the discharge of the cyst contents. In fact, the infertilization and inactivation of protoscolices with scolicidal agents are highly recommended due to minimal possible side effects and high efficacy as compared to opening or removing the cyst [31,32]. So far, numerous protoscolicidal agents including hypertonic saline, mannitol, chlorhexidine gluconate, huaier aqueous, *Allium sativum*, *Sambucus ebulus*, fungal chitosan, *Pistacia khinjuk* fruits, *Bunium persicum* (Boiss) and *Berberis vulgaris* have been applied for the inactivation of hydatid cyst contents [33–37]. Unfortunately, using the aforementioned protoscolicidal agents is restricted because of their poor efficacy, toxicity, and undesirable side effects. Hypertonic saline (20%) has been proved to be 100% effective on protoscolices of hydatid cysts. However acute hypernatremia can lead to drastic neurological symptoms such as necrosis, myelinolysis, convulsions, and intracranial bleeding [38]. Silver nitrate and ceramide have been shown to be 100% effective against protoscolices of the hydatid cyst; however, toxic reactions may also occur by the absorption of these ingredients. Although ABZ sulfoxide is the first drug to be choose for protoscolices, it causes hepatotoxicity by increasing the level of liver enzymes [39].

Pistacia khinjuk extract at the concentration of 100 mg/ml after 10 minutes of exposure removed 100% of protoscolices. Similarly, the mean lethal rate of protoscolices after 20 minutes of exposure to the concentration of 50 mg/ml was 100% [36]. It was previously reported that *Bunium persicum* essential oil at the concentrations of 25 and 50 µl/ml after 5 minutes of exposure removed 100% protoscolices. The mean mortality rate of protoscolices after 10

Table 1. Analysis of Generalized Estimating Equation (GEE) results for the comparison of the mean number of protoscolices viability parasites

Parameter	B	SD	95% Confidence Interval	P-value
(Intercept)	119.013	.4088	(118.212, 119.814)	.000*
DMSO	-.806	.3444	(-1.481, -.131)	.019**
ABZ-NC	-37.047	1.2626	(-39.522, -34.572)	.000*
ABZ	-12.521	.6278	(-13.752, -11.291)	.000*
Negative control	0[a]	.	.	.
Day	-3.794	.0928	(-3.976, -3.612)	.000*

Dependent Variable: Response; Model: (Intercept), Type, Day; B: beta coefficient GEE;
a. Set to zero because this parameter is redundant; *correlation is significant at 1% level;
**Correlation is significant at 5% level

minutes of exposure to a concentration of 12.5 µl/ml was 100% [37]. However, the difficult process of preparation and high cost are the main disadvantages of the mentioned protoscolicidal agents. Therefore, it cannot be considered as a promising scolicidal treatment.

During the last decades, the formulation of drugs as nanocrystals has rapidly evolved as a drug delivery strategy. The crucial feature of these systems is the quick dissolution rate, enhancing bioavailability after oral administration [40]. ABZ with low aqueous solubility leads to a variable oral bioavailability, however, because of the poor solubility and permeability of the crystalline phase of ABZ as well as its slow dissolution rate, oral usage of this drug in the treatment of infected patients has been limited [20]. Different methods could be used to improve its solubility such as nanonization, and formation of amorphous co-precipitates or preparation of inclusion complexes applying material such as cyclodextrins [41–42]. The main feature of ABZ-NCs is its greatly large surface area, which results in a faster saturation in the dissolution layer around the particles with the following increase in dissolution rate [21]. The formulation of ABZ-NCs appears to be a useful tool to achieve faster dissolution rate, since it is possible to obtain particles with sizes below 1µm and the ability to re-disperse the particles in aqueous media [20].

To the best of the authors' knowledge, this is the first study to report the scolicidal effect of ABZ-NCs against protoscolices of hydatid cysts *in vitro* model. Our results indicated that the removal of more protoscolices from the start of the treatment of protoscolices with ABZ (concentration 1 mg/ml) required at least 28 days, while, the treatment with ABZ-NCs required only 17 days. The Generalized Estimating Equation (GEE) analysis showed that all interventions were significant as compared to the control group and had a decreasing effect. The lowest reducing effect was reported by dimethyl sulfoxide (DMSO) with an average of 0.8% less than the control followed by ABZ which had a 12.5% reducing effect compared to the control and the highest reduction effect was reported by ABZ-NCs which was 37% lower than the negative control. This analysis also demonstrated that the time variable had a decreasing effect as well and with the passage of time, a decrease of 3.8% of infection was reported. The permeation behavior through the hydatid cyst membrane was assessed by albendazole-loaded nanoparticles (about 300 nm) which were prepared by the emulsion solvent evaporation method manifested an adequate entrapment efficacy (36.4±6.4%) to improve the apparent solubility of ABZ. Indeed, benzimidazole-loaded nanoparticles seem to be a promising formula concerning the treatment of hydatid cyst. The efficacy of praziquantel loaded hydrogenated castor oil solid lipid nanoparticle suspension in dogs naturally infected with *E. granulosus* was previously demonstrated [44].

The viability of protoscoleces isolated from mice treated with nano-ABZ was previously reported to be significantly lower than that treated with ABZ [45]. There are some significant features for an ideal scolicidal agent such as being effective in low concentrations in the shortest possible time, being stable in hydatid cyst, being able to remove the protoscolices, being non-toxic, and easily available at low cost. According to the findings, ABZ-NCs treatment of hydatid cyst could be a more effective alternative approach than ABZ. In relapse cases and secondary diffusions after surgical treatment, the use of ABZ-NCs could be influential as preventive measure.

The finding of the present study reported on the synthesis of ABZ-NCs as a scolicidal agent against hydatid cysts protoscolices *in vitro*. The evaluation of ABZ-NCs against hydatid cyst protoscolices revealed an effective scolicidal activity and results showed that the higher permeability and scolicidal rate of ABZ-NCs as compared to ABZ, therefore ABZ-NCs can be considered as a promising alternative nanopolymeric agent to improve the treatment of human CE. The present study demonstrated that the scolicidal activity of albendazole nanocrystals could be regarded as a scolicidal agent to reduce the risk of protoscolices release following CE surgery. However, further studies are required to investigate the anti-parasitic mechanism of albendazole nanocrystal against the parasite.

Acknowledgements

This study was supported by Qom University of Medical Sciences (Grant number 95809). We would like to express our gratitude to Qom Industrial slaughterhouse staff for their cooperation. Authors would like to thank Rouzdarou Pharmaceutical Company (Tehran, Tehran Province) for providing albendazole.

References

[1] Joshi U., Subedi R., Jayswal A., Agrawal V. 2020. Clinical characteristics and management of the hydatid cyst of the liver: a study from a Tertiary Care Center in Nepal. *Journal of Parasitology Research* 2020: 1-7. doi.org/10.1155/2020/8867744

[2] Sharafi S.M., Rostami-Nejad M., Moazeni M., Yousefi M., Saneie B., Hosseini-Safa A., Yousofi-Darani H. 2014. *Echinococcus granulosus* genotypes in Iran. *Gastroenterology and Hepatology From Bed to Bench* 7: 82-88.

[3] Craig P.S., McManus D.P., Lightowlers M.W., Chabalgoity J.A., Garcia H.H., Gavidia C.M., Gilman R.H., Gonzalez A.E., Lorca M., Naquira C. 2007. Prevention and control of cystic echinococcosis. *The Lancet Infectious Diseases* 7: 385-394. doi.org/10.1016/S1473-3099(07)70134-2

[4] Wahlers K., Menezes C.N., Wong M.L., Zeyhle E., Ahmed M.E., Ocaido M., Stijnis C., Romig T., Kern P., Grobusch M.P. 2012. Cystic echinococcosis in sub-Saharan Africa. *The Lancet Infectious Diseases* 12: 871-880. doi.org/10.1016/S1473-3099(12)70155-X

[5] Moro P.L., Budke C.M., Schantz P.M., Vasquez J., Santivanez S.J., Villavicencio J. 2011. Economic impact of cystic echinococcosis in peru. *PLoS Neglected Tropical Diseases* 5: e1179. doi:10.1371/journal.pntd.0001179

[6] Santivanez S., Garcia, H.H. 2010. Pulmonary cystic echinococcosis. *Current Opinion in Pulmonary Medicine* 16: 257. doi:10.1097/MCP.0b013e3283386282

[7] Moro P., Schantz P.M. 2009. Echinococcosis: a review. *International Journal of Infectious Disease* 13: 125-133. doi.org/10.1016/j.ijid.2008.03.037

[8] Ahmadpour E., Godrati-Azar Z., Spotin A., Norouzi R., Hamishehkar H., Nami S. 2019. Nanostructured lipid carriers of ivermectin as a novel drug delivery system in hydatidosis. *Parasites & Vectors* 12: 469. doi.org/10.1186/s13071-019-3719-x

[9] Khanfar N. 2004. Hydatid disease: a review and update. *Current Anaesthesia and Critical Care* 15: 173-183. doi.org/10.1016/j.cacc.2004.06.002

[10] Keshmiri M., Baharvahdat H., Fattahi S., Davachi B., Dabiri R., Baradaran H., Rajabzadeh F. 2001. Albendazole versus placebo in treatment of echinococcosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 95: 190-194. doi.org/10.1016/S0035-9203(01)90162-2

[11] Adnani Sadati S., Farahnak A., Molaei Rad M.B., Golestani A., Eshraghiyan M.R. 2016. A comparison between the effects of albendazole and mebendazole on the enzymatic activity of excretory/secretory products of *Echinococcus granulosus* protoscoleces *in vitro*. *Iranian Journal of Public Health* 45: 223.

[12] Yasawy M.I., Alkarawi M.A., Mohammed A.R. 2001. Prospects in medical management of *Echinoco - ccus granulosus*. *Hepatogastroenterology* 48: 1467-1470.

[13] Eshraghi M., Shahmoradi L., Ghoddoosi M., Sadati S.J.A. 2019. Diagnosis of primary hydatid cyst of thyroid gland: a case report. *Biomolecular Concepts* 10: 106-110. doi.org 10.1515/bmc-2019-0013

[14] McManus D., Thompson R. 2003. Molecular epidemiology of cystic echinococcosis. *Parasitology* 127: S37-S51. doi.org/10.1017/S0031182003003524

[15] Joseph El-On. 2002. Benzimidazole treatment of cystic Echinococcosis. *Acta Tropica* 58: 243-252. doi.org/10.1016/S0001-706X(02)00217-6

[16] Norouzi R., Hejazy M., Aziz D., Ataei A. 2020. The effect of *Taxus baccata L*. extract on hydatid cyst protoscolices in vitro. *Archives Razi of Institute* 75: 473-80. doi.org/10.22092/ari.2019.125573.1310

[17] Nagy J., Schipper H., Koopmans R., Butter J., Van Boxtel C., Kager P. 2002. Effect of grapefruit juice or cimetidine coadministration on albendazole bioavailability. *The American Journal of Tropical Medicine and Hygiene* 66: 260-263. doi.org/10.4269/ajtmh.2002.66.260

[18] Liu C.S., Zhang H.b., Jiang B., Yao J.M., Tao Y., Xue J., Wen A.D. 2012. Enhanced bioavailability and cysticidal effect of three mebendazole-oil preparations in mice infected with secondary cysts of *Echinococcus granulosus*. *Parasitology Research* 111: 1205-1211. doi.org/10.1007/s00436-012-2954-2

[19] Shuhua X., Jiqing Y., Mingjie W., Pieying J., Fanghua G., Junjie C., Wei J., Hotez P. 2002. Augmented bioavailability and cysticidal activity of albendazole reformulated in soybean emulsion in mice infected with *Echinococcus granulosus* or *Echinococcus multilocularis*. *Acta Tropica* 82: 77-84. doi.org/10.1016/S0001-706X(02)00027-X

[20] Paredes A.J., Llabot J.M., Sánchez Bruni S., Allemandi D., Palma S.D. 2016. Self-dispersible nanocrystals of albendazole produced by high pressure homogenization and spray-drying. *Drug Development and Industrial Pharmacy* 42: 1564-1570. doi.org/10.3109/03639045.2016.1151036

[21] Keck C.M., Müller R.H. 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. *European Journal of Pharmaceutics and Biopharmaceutics* 62: 3-16. doi.org/10.1016/j.ejpb.2005.05.009

[22] Almalki E., Al-Shaebi E.M., Al-Quarishy S., El-Matbouli M., Abdel-Baki A.A.S. 2017. In vitro effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on *Echinococcus protoscoleces. Saudi Journal of Biological Sciences* 24: 90-94. doi.org/10.1016/j.sjbs.2016.05.007

[23] Carmena D., Martínez J., Benito A., Guisates J.A. 2004. Characterization of excretory–secretory products from protoscoleces of *Echinococcus granu - losus* and evaluation of their potential for immuno-

diagnosis of human cystic echinococcosis. *Parasitology* 129: 371-378. doi.org/10.1017/S003118200400567

[24] M. van der Ree A., Mutapi F. 2015. The helminth parasite proteome at the host–parasite interface–informing diagnosis and control. *Experimental Parasitology* 157: 48-58. doi.org/10.1016/j.exppara.2015.06.007

[25] Walker M.J.F., Rossignol O., Torgerson P., Hemphill A. 2004. In vitro effects of nitazoxanide on *Echinococcus granulosus* protoscoleces and metacestodes. *Journal of Antimicrobial Chemotherapy* 54: 609-616. doi.org/10.1093/jac/dkh386

[26] Polat E., Aslan M., Cakan H., Saribas S., Ipek T., Kocazeybek B. 2009. The effects of albendazole and povidone ıodine for hydatid cysts protoscoleces, ın-vitro and vivo. *African Journal of Microbiology Research* 3: 743-746.

[27] Homish G.G., Edwards E.P., Eiden R.D., Leonard K.E. 2010. Analyzing family data: a GEE approach for substance use researchers. *Addictive Behaviors* 35: 558-563. doi.org/10.1016/j.addbeh.2010.01.002

[28] Cui S.J., Xu L.L., Zhang T., Xu M., Yao J., Fang C.Y., Feng Z., Yang P.Y., Hu W., Liu F. 2013. Proteomic characterization of larval and adult developmental stages in *Echinococcus granulosus* reveals novel insight into host–parasite interactions. *Journal of Proteomics* 84: 158-175. doi.org/10.1016/j.jprot.2013.04.013

[29] Moks E., Jõgisalu I., Valdmann H., Saarma U. 2008. First report of *Echinococcus granulosus* G8 in Eurasia and a reappraisal of the phylogenetic relationships of'genotypes' G5-G10. *Parasitology* 135: 647.

[30] Nakao M., McManus D., Schantz P., Craig P., Ito A. 2007. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology Cambridge* 134: 713. doi.org/10.1017/S0031182006001934

[31] Adas G., Arikan S., Kemik O., Oner A., Sahip N., Karatepe O. 2009. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (in vitro study). *World Journal of Gastroenterology* 15: 112.

[32] Rouhani S., Parviz, P., Spotin A. 2013. Using specific synthetic peptide (p176) derived AgB 8/1-kDa accompanied by modified patient's sera: a novel hypothesis to follow-up of cystic echinococcosis after surgery. *Medical Hypotheses* 81: 557-560. doi.org/10.1016/j.mehy.2013.07.003

[33] Gholami S., Rahimi-Esboei B., Ebrahimzadeh M., Pourhajibagher M. 2013. In vitro effect of *Sambucus ebulus* on scolices of hydatid cysts. *European Review for Medical and Pharmacological Sciences* 17: 1760-1765.

[34] Lv H., Jiang Y., Liao M., Sun H., Zhang S., Peng, X. 2013. In vitro and in vivo treatments of *Echinococcus granulosus* with Huaier aqueous extract and albendazole liposome. *Parasitology Research* 112: 193-198. doi.org/10.1007/s00436-012-3125-1

[35] Mahmoudvand H., Harandi M.F., Shakibaie M., Aflatoonian M.R., ZiaAli N., Makki M.S., Jahanbakhsh S. 2014. Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. *International Journal of Surgery* 12: 399-403. doi.org/10.1016/j.ijsu.2014.03.017

[36] Mahmoudvand H., Mirbadie S.R., Kia M.G., Badparva E., Lori S.S., Harandi M.F. 2016. Efficacy of *Pistacia khinjuk* fruits on viability of hydatid cyst protoscoleces and its acute toxicity in mice model. *Iranian Journal of Parasitology* 11: 383.

[37] Mahmoudvand H., Tavakoli Oliaei R., Mirbadie S.R., Kheirandish F., Tavakoli Kareshk A., Ezatpour B., Mahmoudvand H. 2016. Efficacy and safety of *Bunium persicum* (Boiss) to inactivate protoscoleces during hydatid cyst operations. *Surgical Infections* 17: 713-719. doi.org/10.1089/sur.2016.010

[38] Tappeh K.H., Einshaei A., Mahmudloo R., Mohammadzadeh H., Tahermaram M., Mousavi S.J. 2011. Effect of different concentrations of hypertonic saline at different times on protoscoleces of hydatid cyst isolated from liver and lung. *Türkiye Parazitolojii Dergisi* 35: 148. doi.org/10.5152/tpd.2011.37

[39] Yetim I., Erzurumlu K., Hokelek M., Baris S., Dervisoglu A., Polat C., Belet U., Buyukkarabacak Y., Guvenli A. 2005. Results of alcohol and albendazole injections in hepatic hydatidosis: experimental study. *Journal of Gastroenterology and Hepatology* 20: 1442-1447. doi.org/10.1111/j.1440-1746.2005.03843.x

[40] Ravichandran R. 2010. In vivo pharmacokinetic studies of albendazole nanoparticulate oral formulations for improved bioavailability. *International Journal of Green Nanotechnology: Biomedicine* 2: B46-B53.

[41] Loftsson T., Duchêne D. 2007. Cyclodextrins and their pharmaceutical applications. *International Journal of Pharmaceutics* 329: 1-11. doi.org/10.1016/j.ijpharm.2006.10.044

[42] Thakur A.K., Nirmala N., Harikumar S.L. 2013. Various techniques enhancing bioavailabilty of poorly water soluble drugs. *Journal of Drug Delivery and Therapeutics* 3: 215-221. doi.org/10.22270/jddt.v3i2.464

[43] Patel V.R., Agrawal Y. 2011. Nanosuspension: An approach to enhance solubility of drugs. *Journal of Advanced Pharmaceutical Technology & Research* 2: 81. doi.org/10.4103/2231-4040.82950

[44] Xie S., Pan B., Shi B., Zhang Z., Zhang X., Wang M., Zhou W. 2011. Solid lipid nanoparticle suspension enhanced the therapeutic efficacy of praziquantel against tapeworm. *International Journal of Nanomedicine* 6: 2367.

doi.org/10.2147/IJN.S24919
[45] Pensel P., Paredes A., Albani C.M., Allemandi D., Bruni S.S., Palma S.D., Elissondo M.C. 2018. Albendazole nanocrystals in experimental alveolar echinococcosis: enhanced chemoprophylactic and clinical efficacy in infected mice. *Veterinary Parasitology* 251: 78-84. doi.org/10.1016/j.vetpar.2017.12.022

Received 25 March 2021
Accepted 30 April 2021