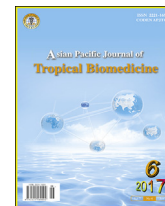




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Efficacy of combined albendazol and praziquantel and their loaded solid lipid nanoparticles components in chemoprophylaxis of experimental hydatidosis



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ABSTRACT

Objective: To evaluate the efficacy of combined ABZ and PZQ and their solid lipid nanoparticles in chemoprophylaxis of cystic echinococcosis (CE).

Methods: ABZ and PZQ loaded solid lipid nanoparticles (SLNs) were prepared by high shear homogenization and microemulsion congealing techniques with some minor modification. Nanoparticles average size, polydispersity index (PDI), and particle size distribution were determined by scanning electron microscopy (SEM) and photon correlation spectroscopy. Forty females BALB/c were experimentally infected by proto-scolec (PSC) and randomly divided into four equal groups of 10 mice. After the end of the 3 months treatment period and 2 months rest, mice were sacrificed and the peritoneal cavity was opened for removal, counting, measuring, and histological analysis of hydatid cyst.

Results: The results indicated that ABZ and PZQ chemoprophylaxis treatment reduced the wet weight and size of developed cysts 77.3% and 79%, respectively. The corresponding result for the ABZ and PZQ loaded SLNs was 83% and 85%, respectively.

Conclusions: This study for the first time demonstrated that ABZ and PZQ loaded SLNs is superior to free ABZ and PZQ for the chemoprophylaxis of CE in mice.

1. Introduction

Hydatidosis is still an important zoonotic parasitic disease and major public health problem in many countries of the world that can occur as a result of infection with the *Echinococcus granulosus* [1]. Human becomes infected following accidental ingestion of ova through direct contact with dog or indirect contact with contaminated food, soil or water [2]. Although, still surgery is the main treatment for CE, due to high

recurrence rate and inoperable cases, chemotherapy cannot be omitted to complete cure [3,4].

Moreover, pre-postoperative and combined with PAIR, drug therapy with benzimidazole such as ABZ or mebendazole (MBZ) is always recommended for prophylaxis of secondary CE. ABZ indicated more effectiveness and is the first drug choice in the chemotherapy of echinococcosis [5]. A recent study has shown that a combined chemotherapy with ABZ and PZQ in treatment and preventing recurrence of CE is more effective than ABZ alone [4,6–9]. ABZ is drug with limitation such as low water solubility, scarce gastrointestinal absorption and low drug level in plasma and hydatid cysts fluid. It seems that one of the achievable methods of overcoming this problem is to change the physical properties of the drug by producing an SLNs derivative form [10,11]. The main objective of current study was to evaluate the efficacy of combined ABZ and PZQ and their solid lipid nanoparticles in chemoprophylaxis of CE.

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2. Material and methods

2.1. Preparation of ABZ and PZQ loaded SLNs

ABZ and PZQ loaded SLNs were prepared by high shear homogenization and microemulsion-emulsion congealing techniques with some minor modification [12–14]. Briefly, 5 g Lipid phase [Compritol® 888 ATO (glyceryl dibehenate/behenate) were gifts from Gattefossé-France] were added in a 250 ml beaker and put in a water bath at 75 °C until they were completely melted. Afterward, 0.5 g ABZ (kindly donated by Damloran Razak Pharmaceutical Co., Ltd.) was added slowly into the melt and mixed well to fully dissolve the drug in lipid(s). The hot aqueous-surfactants solution consisting of 100 ml deionized water, 1 g Polysorbate 80 (Tween 80) and 1 g Polyvinyl alcohol (PVA) that previously heated at the same temperature under magnetic stirring (IKA®-Werke GmbH & Co. KG- Germany) was added gradually into the ABZ loaded lipid with stirring. The resulting primary hot nanoemulsion was sonicated for 5 min using a bath sonicator (Elma Hans Schmidbauer GmbH & Co. KG- Germany). The hot nanoemulsion was slowly dispersed in 100 ml of cold deionized water (4 °C) and homogenized at 12000 rpm for 15 min by Ultra Turrax (IKA®-Werke GmbH & Co. KG- Germany) to obtain a nanoparticle suspension. The suspension was further homogenized by high pressure homogenizer (AVESTIN-Canada) under a pressure of 500 bar and two homogenization cycles. Followed by twice centrifugations at 30000 g (Vision Scientific Co., Ltd.-South Korea) for 30 min, the sediments in presence of sucrose 5% as cryoprotectant were lyophilized using a freeze-drier (Operon Co., Ltd.-South Korea).

The obtained products were kept in refrigerator until further use. PZQ loaded SLNs were prepared in the same way but with minor modification and the control nanoparticles without drugs.

2.2. Experimental design, sampling and in vivo chemoprophylaxis

Protoscolices were prepared as described by Hailong Lv *et al.* [15]. Briefly, protoscolices were obtained aseptically from hydatid cyst in naturally infected sheep's liver with *E. granulosus* at municipal abattoir in Ahvaz, Southwestern part of Iran. The PSC were washed several times with sterile 0.9% sodium chloride injectable solution (Infusion) and without antibiotics or antifungal drugs stored in RPMI 1640 medium (BIO-IDEA, USA) overnight at 4 °C. The viability of PSC was assessed by their motility characteristics, microscopic examination for flame cell activity after 0.1% eosin staining and was found between 90 and 95%.

Forty female BALB/c mice (aged 6 to 8 weeks, body weight 25 g ± 5) were experimentally infected by injection of 2000 viable PSC suspended in 0.5 ml RPMI 1640 medium with a 19 gauge needle into the peritoneal cavity of each mouse and randomly divided into four equal groups of 10 mice as follows: Group 1: first control group, received only deionized water, group 2: second control group, received SLNs without drug, group 3: ABZ and PZQ group, treated with the ABZ (50 mg/kg) and PZQ (600 mg/kg) [6] and group 4: SLNs loaded ABZ and PZQ group, treated with the SLNs loaded ABZ and PZQ, respectively. Two days after infection the free drugs and drugs

loaded SLNs were suspended in deionized water and administered by gavage in a single daily dose for three months (5 consecutive days and two days break every week). The mice were housed in a controlled temperature (22–28 °C) in stainless steel cases in groups of 10, light-cycle (12 h light/12 h dark) and had free access to water and hard palette food. After 5 months (3 months treatment period and 2 months rest), animals were sacrificed by cervical dislocation under ether anesthesia and the peritoneal cavity was opened for removal, counting, measuring, and histological analysis of hydatid cysts. In the current study animal manipulations were in accordance with “Principles of Laboratory Animal Care”. That was approved by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences.

2.3. Nanoparticles characterization

2.3.1. Particle size analysis

Nanoparticles average size, polydispersity index (PDI), and particle size distribution were determined by photon correlation spectroscopy (PCS) using a scatteroscope (Qudix-South Korea). 1 ml of each samples were diluted (1:100) with deionized water and analyzed. All analyses were done in triplicate with mean values and standard deviations being reported.

2.3.2. Scanning electron microscopy

SEM of the nanoparticles was obtained using a Leo 1455 VP (Carl Zeiss, Germany). Briefly, 1 mg freeze-dried SLNs loaded with both drugs were separately re-dispersed in 1 ml deionized water under mechanical stirring and sonicated for 15 min with bath sonicator. 2 ml of the suspension was finely spread on a cover slip and dried at ambient temperature. After drying, the samples were coated with gold and the morphologies of the nanoparticles were imaged by SEM.

2.3.3. Drug loading (DL %) and drug entrapment efficiency (EE %) measurement

For the determination of entrapment efficiency and drug loading of ABZ and PZQ loaded SLNs, both direct and indirect methods were used. The contents of drugs in the samples were determined by UV-spectrophotometry by measuring the absorbance at 296 nm for ABZ and 263 nm for PZQ. The control sample was prepared in the same way but without drug and used as blank. The concentration was determined using the calibration curve of ABZ and PZQ. The equations for EE% and DL% of both drugs in SLNs were calculated follow [16]:

$$EE\% = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

$W_{\text{initial drug}}$: Total weight of drug.

$W_{\text{free drug}}$: Weight of free drug in supernatant.

$$DL\% = \frac{W_{\text{drug loaded}}}{W_{\text{drug loaded}} + W_{\text{lipid}}} \times 100$$

$W_{\text{drug loaded}}$: Amount of drug loaded.

W_{lipid} : Amount of lipid matrix.

2.4. Histological process

The specimens were placed in 10% formalin. Following fixation, the cysts were blocked in paraffin, then were sectioned using a microtome at 4–6 μm. Finally, the mounted sections were stained with H&E stain.

2.5. Statistical analysis of the data

The clinical efficacy study was compared using a one-way analysis of variance (ANOVA) test. The analysis was assessed using SPSS software version 22 for windows and a value of $P < 0.001$ was considered significant.

3. Results

3.1. Nanoparticles average size and polydispersity index (PDI)

The mean particle size of the ABZ and PZQ loaded SLNs ranges were approximately 122 ± 52.6 and 95.25 ± 44.85 nm, respectively. The polydispersity index (PDI) was calculated as 0.88 ± 0.01 and 0.82 ± 0.09 for ABZ and PZQ loaded SLNs, respectively.

3.2. Scanning electron microscopy investigation

The investigation of SLNs formulations under SEM showed spherical particles with the size below 1 nm, and some particle aggregates were obtained. The size measured by PCS was confirmed with this pictures. The particle aggregation might be caused by sample preparation (re-dispersed freeze-dried sample) and remain cryoprotectant in lyophilisation process.

3.3. Drug encapsulation efficiency and drug loading

The drug EE% and DL% of ABZ and PZQ loaded SLNs formulation were assessed as 51%, 3.4% and 59%, 3.1%, respectively.

3.4. Animal studies

The results of ABZ and PZQ and SLNs loaded ABZ and PZQ chemoprophylactic treatment in each group are shown in Table 1. All mice in deionized water and SLNs control groups infected and developed hydatid cysts in the peritoneal cavity (Figures 1–2).

The number of infected mice that developed cysts in the ABZ and PZQ and ABZ and PZQ loaded SLNs groups were 4 (40%)

and 3 (30%), respectively. Most of the cysts which developed in control groups were hyaline. While the percent of developed hyaline cysts were 95.7% and 93.6% in deionized water and SLNs control groups compared with 23.8.3% and 14.3% in ABZ and PZQ and SLNs loaded ABZ and PZQ regimen, respectively. The cysts sizes were measured by a caliper with 1 mm accuracy and were 6.65 ± 1.44 mm and 6.5 ± 1.45 mm in the ABZ and PZQ and SLNs loaded ABZ and PZQ groups, however, chemoprophylactic



Figure 1. Image from the mouse with secondary hydatid cyst produced 5 months after inoculation of PSC.



Figure 2. Image from the cysts of mice with secondary CE produced 5 months after inoculation of PSC.

Table 1

The results of ABZ and PZQ and SLN loaded ABZ and PZQ chemoprophylactic treatment in each group five months after infected with PSC.

Title	Groups			
	Deionized water control	SLNs control	ABZ and PZQ	ABZ and PZQ loaded SLNs
No. of mice (Total/Infected) % of Efficacy*	9/9	10/10	4/10 60%	3/10 70%
No. of developed cysts (Hyaline/Non hyaline)	67/3 95.7%	73/5 93.6%	5/16 23.8%	3/18 14.3%
% Hyaline cysts				
Size (mm) % of Efficacy	6.65 ± 1.44	6.5 ± 15.57	1.4 ± 1.18	
79%	0.98 ± 0.11 85%			
wet weight (mg) % of Efficacy	67.47 ± 1.45	65.15 ± 14.4	15.3 ± 11.48 77%	10.8 ± 0.18 83%

*The percent of efficacy was calculated according to the following equation: % of efficacy = $\frac{\text{Mean cyst weight or size of control group} - \text{Mean cyst weight or size of treated group}}{\text{Mean cyst weight or size of control group}} \times 100$

treatment with ABZ and PZQ and SLNs loaded ABZ and PZQ reduced size 1.4 ± 1.18 mm and 0.98 ± 0.11 mm, respectively.

The wet weight of cysts was measured using an electronic balance with 0.001 mg accuracy and were 67.47 ± 1.45 mg and 65.15 ± 14.4 mg in the ABZ and PZQ and SLNs loaded ABZ and PZQ groups. On the other hand, this figure was reduced by 15.3 ± 11.48 mg and 10.8 ± 0.18 in the ABZ and PZQ and SLNs loaded ABZ and PZQ groups, respectively.

3.5. Histological analysis

The typical structure of the germinal layer (GL) and laminated layer (LL) can be clearly seen in untreated mice cysts and two layers are shown well-formed and continuous (Figure 3A).

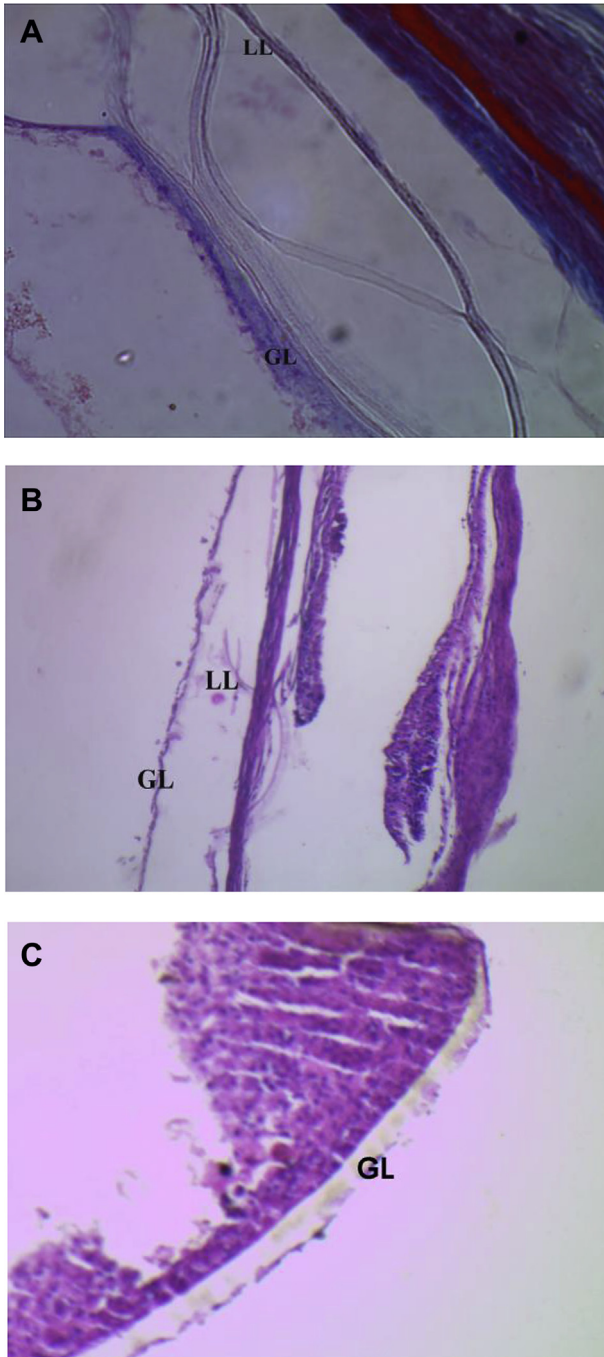


Figure 3. Images from cyst layers in untreated and treated groups. A. Untreated cyst, B. chemoprophylactic treatment with ABZ and PZQ, C. chemoprophylactic treatment with SLNs loaded ABZ and PZQ.

Also cells of the GL are equally distributed. But in the treated group of ABZ and PZQ structure of the LL is thin, breakage and discontinuous, loose, and there are reduced cell numbers in the GL (Figure 3B). In the treated group of ABZ and PZQ loaded SLNs, the GL is damaged and degenerated and cells numbers are very low. On the other, in this group LL is absent (Figure 3C). The microscopic evidence did not show PSC or brood capsule proliferation from the GL of cysts in treated and untreated groups.

4. Discussion

Appropriate therapy of CE should be considered regarding different factors of patient situation such as: cyst location and size, structural characterization, age and sometimes more additional points [17].

However, a safe and effective treatment for CE is a research priority. Until now it has been demonstrated that two benzimidazoles carbamate, ABZ and MBZ were shown to be effective against CE. Additionally PZQ has also been reported as the most active scolicidal agent against *E. granulosus* PSC [18,19]. The increasing of aqueous solubility and bioavailability of poorly soluble drugs has been studied extensively and different strategies have been developed. Some colloidal drugs carriers have been investigated to overcome this problem. The drugs loaded nanoparticles have been widely used in a diversity of dosage forms for various purposes, such as enhancing the bioavailability of drugs by utilizing improving solubility and dissolution rate of nanoparticles [20]. Recently, the data produced by researchers lead to development of some colloidal drug carriers. SLNs have emerged as able to be drug carrier for this aim [21]. The loading of ABZ into nanoparticles provides an fascinating alternative to enhance the drug dissolution rate [22].

Our data demonstrated that in control animals, all mice showed developed hydatid cyst in the peritoneal cavity, but 40% treated mice with free ABZ and PZQ were infected. The efficacy of ABZ and PZQ was 60% and number of infected mice was significantly lower in treated group than in control group ($P < 0.001$). Treatment with ABZ and PZQ improved in treated group and the efficacy rates of reduction in the size and wet weight of the cysts. Significantly clear reduction was detected on cyst wet weight and size in treated groups than in the control group ($P < 0.001$). However, more of the cysts which developed in untreated group of mice were hyaline when compared with the ABZ and PZQ group. On the other hand, the normal structural appearance of the LL and GL in control group and badly damaged cyst walls was clearly visible in the combination treatment group. The results of the present study confirm that ABZ and PZQ are of more effectiveness which is in agreement with all previous same studies [4,6–9].

In the current study, ABZ and PZQ loaded SLNs have been produced and used for CE treatment in mice for the first time and efficacy was evaluated. Results indicated that, all animals received SLNs alone developed hydatid cyst in the peritoneal cavity, but 30% treated mice with ABZ and PZQ loaded SLNs were infected. Present study demonstrated that, efficacy rates of ABZ and PZQ loaded SLNs were significantly higher in the control group compared to treated group ($P < 0.001$). On the other hand, the number of developed cysts in SLNs without drug group clearly was higher, than in the ABZ and PZQ loaded SLNs group.

As our best search, we can't find any study on the *in vitro* and *in vivo* medical treatment of CE with combination of ABZ and PZQ loaded SLNs and only a few study was available and published on the *in vivo* medical treatment of CE with drug loaded nanoparticles.

The *in vivo* therapeutic efficacy of PZQ-loaded hydrogenated castor oil SLNs (PZQ–HCO–SLNs) against cestodes was evaluated by Shuyu Xie *et al.* (2011). The SLNs significantly improve the pharmaceutical activity and efficacy of PZQ [23]. The results of Dvornoznakova *et al.* (2004) study showed that therapeutic efficacy of ABZ could be enhanced after loading into liposomes [24]. In the study the goal was to determine the ability of the drug dispersion through the cyst membrane. The dispersion of ABZ-loaded nanoparticles from the nanoparticles across the hydatid cyst layers was also enhanced than in free ABZ [25]. Leonardi *et al.* (2008) reported that the ABZ incorporation into biodegradable polymeric microparticles provides an fascinating alternative to enhance the drug dissolution rate [26].

In agreement with the above findings, in the current study significantly clear reduction was detected on cyst size and wet weight in ABZ and PZQ loaded SLNs group compared with their control group ($P < 0.001$). On the other hand, more cysts which developed in the SLNs without drug group of mice were hyaline than in treated group.

Additionally, the normal structural appearance of the layers of cysts in SLNs alone group and damaged cyst walls was clearly visible in the combination treatment group. In the combination treatment with ABZ and PZQ loaded SLNs group GL showed badly damage and LL was absent. Ahmadnia S. *et al.* reported that treatment of mice with free ABZ sulfoxide or ricobendazole and two different regimens of ABZ sulfoxide loaded SLNs revealed that the cysts reduced size and weight in the animals which received ABZ sulfoxide loaded SLNs compared to those of the control groups and the cysts showed more ultrastructural modification. The tegument disappeared, the GL completely distorted and the microfibrillar pattern of the LL was no more available [27]. In the *in vivo* study, it was reported that ABZ formulated as solid dispersion had greater chemoprophylactic and clinical efficacy than ABZ alone. Ultra structural changes (SEM and TEM analysis) in the GL of cysts recovered from ABZ formulated as solid dispersion and ABZ treated mice in both efficacy studies were markedly altered. Nevertheless, the damage extension appears to be greater after the treatment with ABZ formulated as solid dispersion [22].

In the third part of this study, ABZ and PZQ loaded SLNs group was compared with free ABZ and PZQ group. The results indicated that the effectiveness in ABZ and PZQ loaded SLNs group was higher than free ABZ and PZQ group. However, the number of developed cysts was equal in two treated group but only 14.3% in ABZ and PZQ loaded SLNs was hyaline cyst compared with free ABZ and PZQ group with 23.8%. Additionally, clear reduction was detected on cyst wet weight and size in ABZ and PZQ loaded SLNs groups compared with free ABZ and PZQ, no significant differences between the two formulations were detected ($P < 0.001$). However treatment with ABZ and PZQ loaded SLNs compared with free ABZ and PZQ, enhance the efficacy from 79% to 85% in size and 77%–83% in wet weight. The current results suggest that chemotherapy with ABZ and PZQ loaded nanoparticles enhance the effect of ABZ and PZQ and has higher efficacy than free ABZ and PZQ.

In conclusion, in this study, ABZ and PZQ loaded SLNs have been produced and used for CE treatment in mice for the first time. This study clearly demonstrated that SLNs are suitable carriers for the incorporation of ABZ and PZQ and ABZ and PZQ loaded SLNs were more effective than free ABZ and PZQ for the chemoprophylaxis treatment of CE in mice. It could be considered as an option for further investigations in clinical practice. It is recommended to design further study to evaluate the efficacy of ABZ and PZQ loaded SLNs on protoscolicidal effect, other drug administration, penetration in to cyst, different doses and treatment periods and compare that with free drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Moro P, Schantz PM. Echinococcosis: a review. *Int J Infect Dis* 2009; **13**(2): 125-33.
- [2] WHO. *First WHO report on neglected tropical diseases. Working to overcome the global impact of neglected tropical diseases*. Geneva: WHO; 2010. [Online] Available from: http://www.who.int/neglected_diseases/2010report/en/.
- [3] Eckert J. *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern*. Geneva: WHO; 2001. [Online] Available from: <http://whqlibdoc.who.int/publications/2001/929044522X.pdf>.
- [4] Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010; **114**(1): 1-16.
- [5] Smego RA, Sebanego P. Treatment options for hepatic cystic echinococcosis. *Int J Infect Dis* 2005; **9**(2): 69-76.
- [6] Moreno M, Urrea-Paris M, Casado N, Rodriguez-Caabeiro F. Praziquantel and albendazole in the combined treatment of experimental hydatid disease. *Parasitol Res* 2001; **87**(3): 235-8.
- [7] Jamshidi M, Mohraz M, Zangeneh M, Jamshidi A. The effect of combination therapy with albendazole and praziquantel on hydatid cyst treatment. *Parasitol Res* 2008; **103**(1): 195-9.
- [8] Rafiei A, Pipelzadeh MH, Jahanshahi A, Salim MRE. Comparing the effectiveness of albendazole and combination of albendazole and praziquantel in experimental hydatidosis. *Arch Clin Infect Dis* 2009; **4**(1): 9-12.
- [9] El-On J. Benzimidazole treatment of cystic echinococcosis. *Acta Trop* 2003; **85**(2): 243-52.
- [10] Pensel PE, Gamboa GU, Fabbri J, Ceballos L, Bruni SS, Alvarez LI, et al. Cystic echinococcosis therapy: albendazole-loaded lipid nanocapsules enhance the oral bioavailability and efficacy in experimentally infected mice. *Acta Trop* 2015; **152**: 185-94.
- [11] Vo CL-N, Park C, Lee B-J. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *Eur J Pharm Biopharm* 2013; **85**(3): 799-813.
- [12] Silva A, González-Mira E, García M, Egea M, Fonseca J, Silva R, et al. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound. *Colloids Surf B Biointerfaces* 2011; **86**(1): 158-65.

- [13] Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. *Adv Drug Deliv Rev* 2012; **64**: 83-101.
- [14] Keck CM, Kovacevic A, Muller RH, Savic S, Vuleta G, Milic J. Formulation of solid lipid nanoparticles (SLN): the value of different alkyl polyglucoside surfactants. *Int J Pharm* 2014; **474**(1–2): 33-41.
- [15] Lv H, Jiang Y, Liao M, Sun H, Zhang S, Peng X. In vitro and in vivo treatments of *Echinococcus granulosus* with Huaier aqueous extract and albendazole liposome. *Parasitol Res* 2013; **112**(1): 193-8.
- [16] Souto EB, Wissing SA, Barbosa CM, Muller RH. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *Int J Pharm* 2004; **278**(1): 71-7.
- [17] Gocan H, Surd A, Dobrescu I, Pop E. The role of ultrasonography in Albendazole treatment of hydatid liver cyst monitoring in children-three case reports. *Med Ultrason* 2010; **12**(4): 340-4.
- [18] Stamatakos M, Sargedi C, Stefanaki C, Safioleas C, Matthaopoulou I, Safioleas M. Anthelmintic treatment: an adjuvant therapeutic strategy against *Echinococcus granulosus*. *Parasitol Int* 2009; **58**(2): 115-20.
- [19] Bygott JM, Chiodini PL. Praziquantel: neglected drug? Ineffective treatment? Or therapeutic choice in cystic hydatid disease? *Acta Trop* 2009; **111**(2): 95-101.
- [20] Wu L, Zhang J, Watanabe W. Physical and chemical stability of drug nanoparticles. *Adv Drug Deliv Rev* 2011; **63**(6): 456-69.
- [21] Das S, Ng WK, Kanaujia P, Kim S, Tan RB. Formulation design, preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: effects of process variables. *Colloids Surf B Biointerfaces* 2011; **88**(1): 483-9.
- [22] Pensel PE, Castro S, Allemandi D, Bruni SS, Palma SD, Elissondo MC. Enhanced chemoprophylactic and clinical efficacy of albendazole formulated as solid dispersions in experimental cystic echinococcosis. *Vet Parasitol* 2014; **203**(1): 80-6.
- [23] Xie S, Pan B, Shi B, Zhang Z, Zhang X, Wang M, et al. Solid lipid nanoparticle suspension enhanced the therapeutic efficacy of praziquantel against tapeworm. *Int J Nanomedicine* 2011; **6**: 2367-74.
- [24] Dvoroznakova E, Hrcckova G, Boroskova Z, Velebny S, Dubinsky P. Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with *Echinococcus multilocularis*. *Parasitol Int* 2004; **53**(4): 315-25.
- [25] Truong Cong T, Faivre V, Nguyen TT, Heras H, Pirot F, Walchshofer N, et al. Study on the hydatid cyst membrane: permeation of model molecules and interactions with drug-loaded nanoparticles. *Int J Pharm* 2008; **353**(1–2): 223-32.
- [26] Leonardi D, Lamas MC, Olivieri AC. Multiresponse optimization of the properties of albendazole-chitosan microparticles. *J Pharm Biomed Anal* 2008; **48**(3): 802-7.
- [27] Ahmadnia S, Moazeni M, Mohammadi-Samani S, Oryan A. In vivo evaluation of the efficacy of albendazole sulfoxide and albendazole sulfoxide loaded solid lipid nanoparticles against hydatid cyst. *Exp Parasitol* 2013; **135**(2): 314-9.