Parasitology International 64 (2015) 435–440

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Chemoprophylactic and therapeutic efficacy of thymol in murine cystic echinococcosis

M. Maggiore [a], P.E. Pensel [a,b], G. Denegri [a,b], M.C. Elissondo [a,b,*]

[a] Laboratorio de Zoonosis Parasitarias, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), Funes 3350, 7600 Mar del Plata, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 15 June 2015
Accepted 17 June 2015
Available online 19 June 2015

Keywords:
Cystic echinococcosis
Echinococcus granulosus
Essential oils
Thymol
Albendazole

ABSTRACT

Cystic echinococcosis is a zoonotic disease caused by the larval stage of the cestode *Echinococcus granulosus*. The drugs commonly used against cystic echinococcosis are benzimidazoles. Unfortunately, 20%–40% of cases do not respond favorably to such chemotherapy. Consequently, the search of new therapeutic alternatives such as the use of traditional medicinal plants has been increased. The aim of the current experimental work was to investigate the chemoprophylactic and clinical efficacy of thymol on mice infected with *E. granulosus* metacestodes. Thymol (40 mg/kg) was administered under two different therapeutic schemes: dosing every 24 h over 20 days and treatment every 12 h for 10 days. Thymol demonstrated efficacy against experimental murine cystic echinococcosis. The chemoprophylactic and therapeutic effects of thymol were comparable to that of albendazole. Due to the lack of toxicity observed in mice at the tested doses; we consider that thymol is a potential alternative to be applied for the treatment of human hydatid disease.

1. Introduction

Cystic echinococcosis, an infection with the larval form of the dog tapeworm *Echinococcus granulosus*, is characterized by cystic lesions, most commonly in the liver and lungs [1]. This parasitic infection is a chronic, complex, and still neglected disease [2].

Currently four treatment approaches are in use: surgery, PAIR (puncture, aspiration, injection of protoscolicidal agent, reaspiration), chemotherapy, and watch and wait for inactive, clinically silent cysts [3]. The drugs commonly used against cystic echinococcosis are benzimidazoles, such as albendazole (ABZ) and mebendazole [4]. Unfortunately, 20%–40% of cases do not respond favorably to such chemotherapy and these drugs produce stabilization, rather than cure in the majority of patients [5]. With regard to these difficulties, novel therapeutical tools are needed to optimize treatment of cystic echinococcosis.

The control of helminthosis, and generally of all parasitic diseases, is usually made with synthetic anthelmintics. These anthelmintic drugs have some important disadvantages, such as cost and improper use leading to drug resistance, environmental pollution, and food residues [6]. Consequently, the search of new therapeutic alternatives such as the use of traditional medicinal plants has been increased.

Aromatic plants have pharmaceutical properties that are in part attributed to essential oils [7]. For their hydrophobic character, the essential oils and their components have a considerable potential of pharmacological applications as antimicrobial agents [7,8]. The role of essential oils against parasitic helminths has been studied by several authors [9–12]. However, there are few publications about the effect of these substances on *E. granulosus*. The in vitro effect of the essential oils of *Rosmarinus officinalis* (rosemary), *Mentha pulegium*, *Mentha piperita*, *Pistacia khinjuk* (pistachio) and *Trachyspermum ammi* (ajowan) has been demonstrated against protoscoleces of *E. granulosus* [13–16].

Thymol (2-isopropyl-5-methylphenol) is one of the major components of the essential oils of *Thymus vulgaris* and *Origanum vulgare* and is a widely known anti-microbial agent [17]. The in vitro and in vivo activities of thymol against *Leishmania panamensis* [18] and *Anisakis simplex* larvae [19] were proven. Moreover, several encouraging findings have been reported using thymol in vitro on *E. granulosus* protoscoleces, microcysts and metacestodes [20,21]. Thymol had anthelmintic effect against the larval stage, altering the viability, structure and ultrastructure of the cestode.

The aim of the current experimental work was to investigate the chemoprophylactic and clinical efficacy of thymol on mice infected with *E. granulosus* metacestodes.

2. Materials and methods

2.1. Chemicals

ABZ suspension (2.1 mg/ml) was prepared by dissolution of ABZ pure standard (Pharmaceutical grade, Parafarm, Buenos Aires, Argentina) in

* Corresponding author at: Laboratorio de Zoonosis Parasitarias, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), Funes 3250, 7600 Mar del Plata, Argentina.
E-mail address: c.elissondo@gmail.com (M.C. Elissondo).

http://dx.doi.org/10.1016/j.parint.2015.06.005
1383-5769/

deionized water (pH = 7.0) under shaking (12 h). ABZ suspension was vigorously shaken before its intragastric administration to mice.

Thymol was obtained from Sigma-Aldrich (USA). Thymol solution (2.4 mg/ml) was prepared by dissolution of the drug in olive oil and maintained under refrigeration (3 to 5 °C).

2.2. Protoscolex collection

Protoscoleces of *E. granulosus* were collected aseptically from liver and lung hydatid cysts of infected cattle slaughtered in an abattoir located in the southeast of Buenos Aires province, Argentina. The area where the cattle came from is known to include only the G1 strain of *E. granulosus* [22]. Viability was assessed as previously described [23].

2.3. Experimental animals and infection

Animal procedures and management protocols were approved by the Institutional Animal Care and Use Committee (act 2555-07-14) of the Faculty of Exact and Natural Sciences, National University of Mar del Plata, Mar del Plata, Argentina and carried out in accordance with the 2011 revised form of *The Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. Unnecessary animal suffering was avoided throughout the study. Female CF-1 mice (n = 100; body weight 25 g ± 5) were infected by intraperitoneal inoculation with 1500 *E. granulosus* protoscoleces/animal, suspended in 0.5 ml of medium 199 (Gibco). The animals were housed in a temperature controlled (22 ± 1 °C), light-cycled (12-h light/dark 174 cycle) room. Food and water were given ad libitum.

2.4. Experimental design

Two different experimental designs were conducted: a chemoprophylactic efficacy study (which simulates a cyst rupture during surgical practice and the concomitant drug treatment) and, a clinical efficacy study (simulating an experimental secondary hydatidosis).

2.4.1. Chemoprophylactic efficacy study

At the time point of infection, the animals were allocated into 5 experimental groups (n = 10) and treated as follows: a) distilled water control group, animals receiving distilled water as a placebo; b) oil control group, animals receiving olive oil as a placebo; c) ABZ group, animals were treated with ABZ suspension at the dose rates of 25 mg/kg every 24 h for 30 days; d) thymol group I, animals were treated with thymol at the dose rates of 40 mg/kg every 24 h for 20 days; and e) thymol group II, animals were treated with thymol (40 mg/kg) at 12-h intervals over 10 days. Treatments were performed by intragastric administration. Six months after infection, mice were euthanized, and necropsy was carried out immediately thereafter.

2.4.2. Clinical efficacy study

Protoscoleces of *E. granulosus* can differentiate to hydatid cysts if released within (or passaged into) an intermediate host. Cystic differentiation is completed after 2–3 months [24]. At 6 months post-infection, mice were allocated into the following experimental groups (10 animals/group) and treated as follows: a) distilled water control group, animals receiving distilled water as a placebo; b) oil control group, animals receiving olive oil as a placebo; c) ABZ group, animals were treated with ABZ suspension at the dose rates of 25 mg/kg every 24 h for 30 days; d) thymol group I, animals were treated with thymol at the dose rates of 40 mg/kg every 24 h for 20 days; and e) thymol group II, animals were treated with thymol (40 mg/kg) at 12-h intervals over 10 days. Treatments were performed by intragastric administration. At the end of the treatment period, animals were euthanized, and necropsy was carried out immediately thereafter.

2.5. Determination of parasite weight and morphologic study

At necropsy in both the prophylactic and efficacy studies, the peritoneal cavity was opened, and the hydatid cysts were carefully removed. The weight of the cysts collected from each individual animal was recorded using an analytical scale. Samples of cysts recovered from each mouse were processed for scanning and transmission electron microscopy (SEM and TEM) as described by Elissondo et al. [25].

2.6. Statistical analysis

Weight of cysts (reported as mean ± SD) were compared by means of Kruskal–Wallis (non-parametric ANOVA) followed by Dunn's multiple comparison test. A value of $P < 0.05$ was considered statistically significant. The statistical analysis was performed using the Instat 3.0 software program (GraphPad Software, San Diego, CA).

3. Results

3.1. Chemoprophylactic efficacy study

All of the infected mice (10/10) from the untreated control groups, ABZ-treated group and thymol-treated group I developed metacestodes in the abdominal cavity. On the contrary, in 1 out of the 10 thymol-treated group II mice, the infection did not progress. Statistically significant differences (ABZ group: $P < 0.001$; thymol group I: $P < 0.01$; thymol group II: $P < 0.05$) were observed in the weights of cysts recovered from untreated mice compared with those from the treated groups. On the other hand, no statistically significant differences were found between the weights of cysts recovered from the two thymol-treated groups and ABZ-treated group ($P > 0.05$) (Table 1).

All cysts removed from control mice appeared turgid, showing no observable collapse of the germinal layer, and no changes in ultrastructure were detected by SEM (Fig. 1a). TEM analysis of cysts recovered from the untreated control group revealed the typical features of *E. granulosus* metacestodes, with a distinct acellular outer laminated layer and a germinal layer without alterations (Fig. 1b). In contrast, the ultrastructural study of cysts developed in mice treated with ABZ and thymol revealed a normal laminated layer, but some alterations on the germinal layer were detected (Fig. 2). Studies by SEM revealed that the germinal layer of treated cysts lost the feature multicellular structure (Fig. 2a, c and e). TEM analysis of cysts from treated mice revealed the presence of some signs of degeneration as an increment in the number of vacuoles and lipid droplets (Fig. 2b, d and f).

3.2. Clinical efficacy study

Table 2 summarizes the cyst weights (mean ± SD) recorded after treatments on the different experimental groups (unmedicated control and treated groups) involved in the clinical efficacy study. Both thymol

Table 1
Chemoprophylactic efficacy study. Mean (±SD) weights (g) of hydatid cysts recovered at six months post-infection from artificially infected mice from the unmedicated control and treated groups. Treatments were given at 40 mg/kg, every 24 h over 20 days or every 12 h over 10 days.

	Chemoprophylactic efficacy study
	Wet weight (g) of cysts Mean ± SD
Unmedicated control group (olive oil)	12.3 ± 4.2
Unmedicated control group (distilled water)	10.6 ± 2.9
ABZ suspension	1.3 ± 0.9***
Thymol group I	2.9 ± 2.3**
Thymol group II	3.7 ± 2*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; statistically significant differences between treated group vs control group.

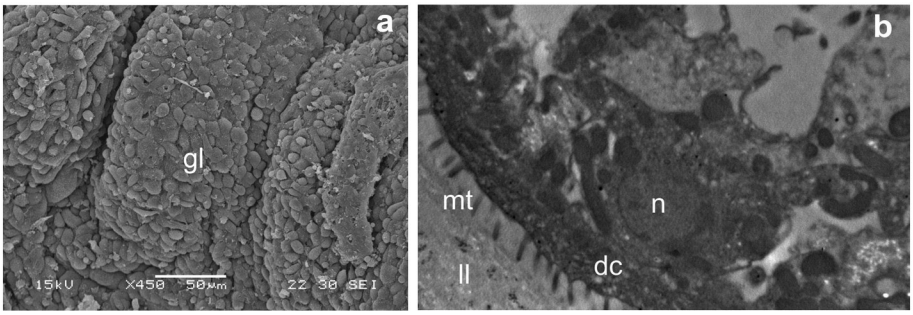

Fig. 1. Scanning and transmission electron microscopy (SEM and TEM, respectively) of hydatid cysts recovered from untreated control mice. (a) SEM (450×) image of hydatid cysts (gl = germinal layer). (b) TEM (12,000×) image of hydatid cysts (*ll* laminar layer; *mt* microtriches; *dc* distal citoplasm, *n* nucleus).

treatments and ABZ treatment resulted in a statistically significant reduction ($P < 0.01$) of the cysts weight compared to those obtained for unmedicated mice. No difference was found in mean cyst weight between treated groups ($P > 0.05$). However, differences in the ultrastructural changes observed in the germinal layer of cysts recovered from both thymol-treated groups were detected.

No changes in ultrastructure were detected by SEM and TEM from cysts removed from unmedicated mice (Fig. 1). Conversely, the TEM and SEM analyses of the cysts recovered from mice treated with thymol and ABZ showed changes in the germinal layer (Fig. 3). Ultrastructural damages induced by the shorter (12 h) dosing interval (Thymol group II) were greater than those induced by the 24 h dosing interval (Thymol

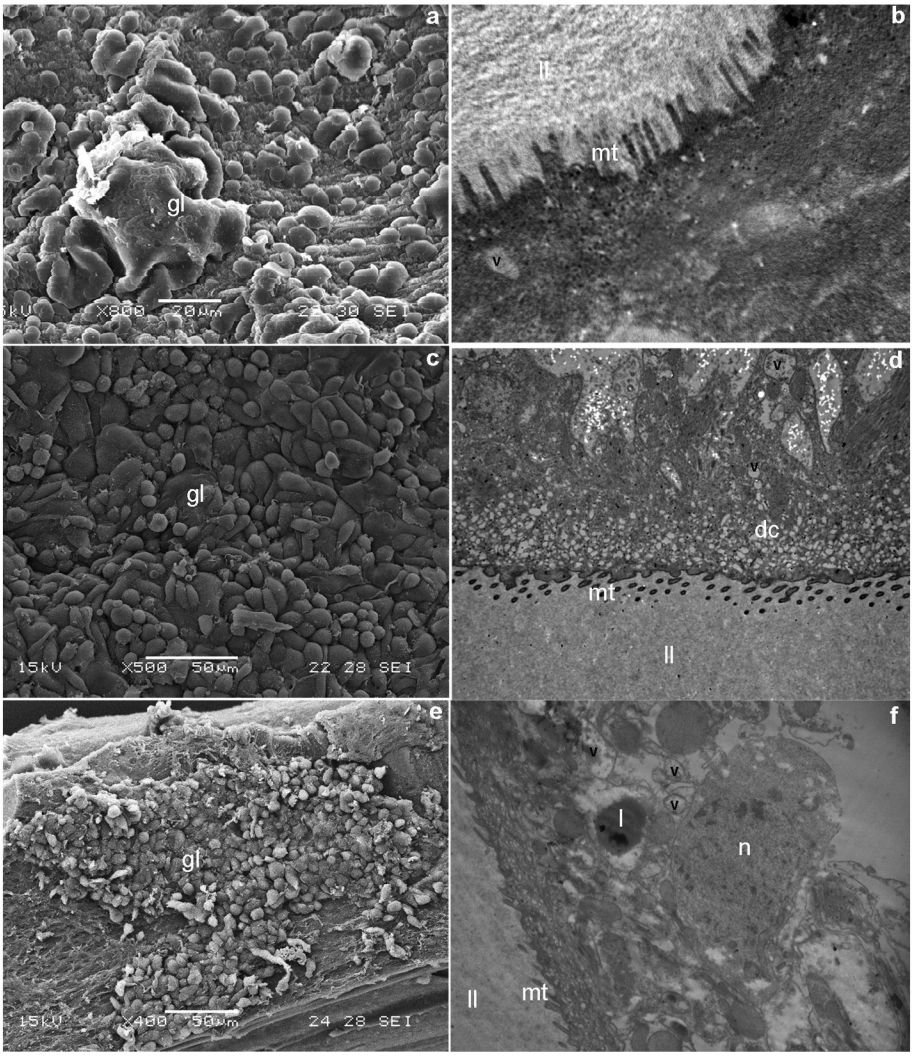

Fig. 2. Representative images of SEM and TEM of hydatid cysts recovered from infected mice treated with ABZ suspension or thymol during chemoprophylactic efficacy study. (a–b) Cysts recovered from mice treated with ABZ suspension every 24 h for 30 days (25 mg/kg). (a) SEM image of hydatid cysts. The germinal layer is altered (800×). (b) TEM of hydatid cysts. Note the presence of vacuoles (18,000×). (c–d) Cysts recovered from mice treated with thymol every 24 h for 20 days (40 mg/kg). (c) SEM image of hydatid cysts. Alterations in the germinal layer can be observed (500×). (d) TEM Scanning image of hydatid cysts. Note the presence of vacuoles (10,000×). (e–f) Cysts recovered from mice treated with thymol every 12 h for 10 days (40 mg/kg). (e) SEM image of hydatid cysts. Germinal layer is markedly altered. Note the presence of areas with total cell loss (400×). (f) TEM scanning image of hydatid cysts. Note the presence of vacuoles and lipid droplets (10,000×). (*gl* germinal layer; *ll* laminar layer; *dc* distal cytoplasm; *n* nucleus; *mt* microtriches; *v* vacuoles; *l* lipid droplets).

Table 2
Clinical efficacy study. Mean (±SD) weights (g) of the hydatid cysts recovered from artificially infected mice from the unmedicated control and treated groups. Treatments were performed after 6 months of inoculation at the dose rates of 40 mg/kg, every 24 h over 20 days or every 12 h over 10 days.

	Clinical efficacy study
	Wet weight (g) of cysts Mean ± SD
Unmedicated control group (olive oil)	15.4 ± 3.8
Unmedicated control group (distilled water)	13 ± 4.3
ABZ suspension	2.8 ± 2.9**
Thymol group I	4.5 ± 3.7**
Thymol group II	5.9 ± 2.4**

** $P < 0.01$, statistically significant differences between treated group vs control group.

group I) and ABZ. Regarding the ultrastructural study at SEM, only few groups of cells with an intact morphology could be observed after the 12 h interval (Fig. 3e). Moreover, TEM analysis of cysts from treated mice of this treatment group revealed the internal tissue completely distorted. Only debris of tissue of the germinal layer could be observed in all samples (Fig. 3f).

4. Discussion

In previous studies [20,21], the in vitro protoscolicidal effect of thymol on cultured *E. granulosus* was established. This work describes its efficacy on mice experimentally infected with protoscoleces of *E. granulosus*. Two different experimental designs were conducted: a chemoprophylactic efficacy study and, a clinical efficacy study. Moreover, both studies were performed under two different therapeutic schemes: thymol was administered every 24 h during 20 days or at 12-h intervals over 10 days.

The efficacy of thymol was compared with ABZ at an oral dose of 25 mg/kg. Other authors have compared the effect of different new drugs versus ABZ at doses up to 200 mg/kg [26]. ABZ is barely absorbed from the gastrointestinal tract since it is a poorly water-soluble drug. It was demonstrated that for monogastric species, an increment in the dose is not correlated to an increment in the AUC [27]. Moreover we demonstrated that concentrations of ABZ as low as 0.5 mg/kg exert a deleterious effect on hydatid cysts on the murine model of cystic echinococcosis [28].

After oral administration of 40 mg/kg of thymol, both therapeutic schemes demonstrated a chemoprophylactic effect. This preventive

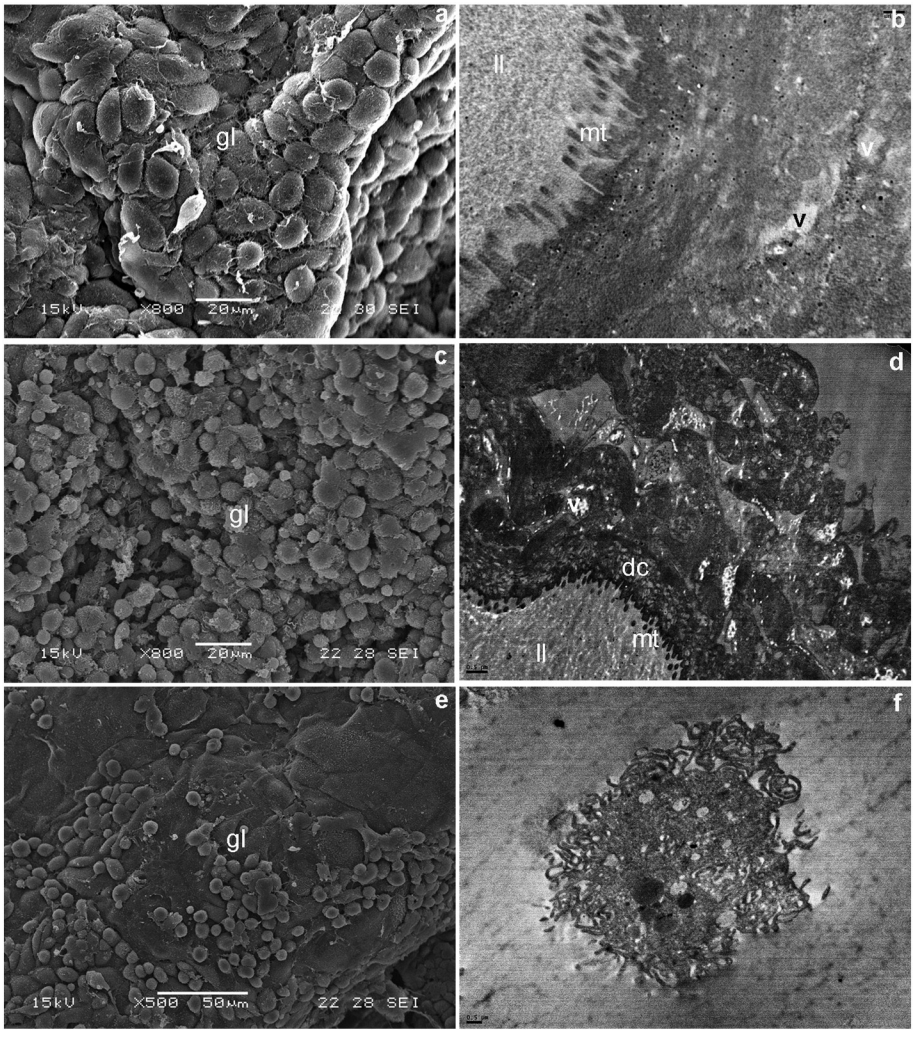

Fig. 3. Representative images of SEM and TEM of hydatid cysts recovered from infected mice treated with ABZ suspension or thymol during clinical efficacy study. (a–b) Cysts recovered from mice treated with ABZ suspension every 24 h for 30 days (25 mg/kg). (a) SEM image of hydatid cysts. The germinal layer is altered (800×). (b) TEM scanning image of hydatid cysts. Note the presence of vacuoles (18,000×). (c–d) Cysts recovered from mice treated with thymol every 24 h for 20 days (40 mg/kg). (c) SEM image of hydatid cysts. The germinal layer is slightly altered (800×). (d) TEM scanning image of hydatid cysts. Note the vacuolation of the internal tissue (10,000×). (e–f) Cysts recovered from mice treated with thymol every 12 h for 10 days (40 mg/kg). (e) SEM image of hydatid cysts. Note the extensive damage of the germinal layer (500×). (f) TEM scanning image of hydatid cysts. Only debris of tissue of the germinal layer could be observed (10,000×). (*gl* germinal layer; *ll* laminar layer; *dc* distal cytoplasm; *mt* microtriches).

effect was comparable with the preventive effect of ABZ suspension (25 mg/kg day for 30 days). In agreement with previous chemoprophylactic studies on the murine model of cystic echinococcosis [29–32], some alterations in the germinal layer were observed in those cysts that developed between the end of the experimental drug treatment and the euthanasia of mice. We speculate that a possible explanation could be that a deleterious drug effect on protoscoleces at the time of infection affected some protoscoleces but did not kill them. Those surviving protoscoleces had an aberrant or anomalous development with the presence of some ultrastructural alterations.

Hydatid cysts developed in all the infected animals involved in the clinical efficacy studies. Oral application of thymol in infected mice has a therapeutic effect on the hydatid cyst. Thymol led to a significantly reduced parasite weight comparable with ABZ suspension. No statistically significant differences were found between the weights of cysts recovered from the two thymol-treated groups. The ultrastructural changes induced in vivo by thymol were similar to those described in vitro by Elissondo et al. (2013) [21]. These similarities could be indicating the entrance of the drug into the cysts in treated mice.

However, ultrastructural damages induced by the 12 h dosing interval were greater than those induced by the 24 h dosing interval. Cysts recovered from treated mice with the shorter dosing interval revealed the internal tissue completely distorted. At TEM, only debris of tissue of the germinal layer could be observed in all samples. This stronger efficacy of the drug when it is administered twice a day coincides with the results reported by Ceballos et al. [28]. The authors demonstrated a marked effect of albendazole after its administration at a 12 h interval compared to a 48 h dosing interval. It is, therefore, clear that the sustained drug–parasite contact occurring after a shorter therapeutic interval induced a higher drug effect. The exposure of the hydatid cysts to enhanced drug concentrations obtained would correlate with increased efficacy in mice.

On the other hand, our results are not consistent with those reported by Kammerer and Pérez-Esandi (1975) [33]. The authors evaluated the effect of thymol at the doses of 4, 16 and 64 mg/kg. They administered the drug intramuscularly, 4 weekly doses for 2 weeks. They found no significant differences in the weights or histology of cysts recovered from treated mice in relation to controls. A possible explanation could be the differences in the route of administration, duration of treatment and total dose of thymol reached at the end of the trial.

Essential oil compounds such as those found in thyme extract are frequently used for the therapy of chronic and acute bronchitis. For these indications, several clinical trials have been carried out [34,35]. Systemic availability and the pharmacokinetics after oral administration of 1.08 mg of thymol in humans was determined [36]. The metabolites thymol sulfate and thymol glucuronide were found in urine, however only thymol sulfate was detectable in plasma with a maximum plasma concentration of 93.1 ng/ml reached after 1.97 h. It should be stated that the single dose in that study was very low: 1.08 mg per human volunteer. Furthermore, a maximum plasma concentration of 3 μg/ml was detected in piglets after an oral administration of 13.2 mg/kg of thymol [37].

No data related to the pharmacokinetics after oral administration of thymol in mice are published; therefore it is impossible for us to discuss how plasma values in mice correspond to our previously published in vitro efficacy studies. We demonstrated a marked effect of thymol against *E. granulosus* even as using small concentrations such as 1 and 5 μg/ml [20]. Coincidentally, these values are similar to the maximum plasma concentration of 3 μg/ml detected in piglets [37].

The in vitro and in vivo cytotoxicity activities of thymol were evaluated by Robledo et al. (2005) [18]. The cytotoxic activity (50% lethal concentration) in U-937 human promonocytic cells was 400 ± 0 μg/ml. In addition, at an oral dosage of 40 mg/kg of body weight/day, thymol was not toxic to Golden hamsters based on corporal weight, behavior and serum levels of bilirubin, uric acid and glucose. In our experiments on murine echinococcosis, this lack of toxicity was also evident since none of the mice exhibited adverse effects during the entire treatment period. Moreover, mice behaviors remained unaltered, with no changes in mobility, feeding habits, etc.

In conclusion, we demonstrated the efficacy of thymol against murine experimental cystic echinococcosis. Short periods of treatment were sufficient to achieve a pharmacological effect. Due to the lack of toxicity observed in mice at the tested doses, we consider that thymol is a potential alternative to be applied for the treatment of human hydatid disease. In the future, it would be interesting to adjust the dose and duration of treatment in order to further enhance drug efficacy.

Acknowledgments

This work was supported by the PICT 2012 No. 1164 (ANPCyT, Argentina), PIP 0029 (CONICET, Argentina) and Universidad Nacional de Mar del Plata (Grants EXA 581/12 and EXA 672/14), Argentina.

References

[1] C.M. Budke, H. Carabin, P.C. Ndimubanzi, H. Nguyen, E. Rainwater, M. Dickey, et al., A systematic review of the literature on cystic echinococcosis frequency worldwide and its associated clinical manifestations, Am. J. Trop. Med. Hyg. 88 (2013) 1011–1027.
[2] E. Brunetti, H.H. Garcia, T. Junghanss, Cystic echinococcosis: chronic, complex, and still neglected, PLoS Negl. Trop. Dis. 5 (7) (2011) e1146.
[3] M. Stojkovic, M. Zwahlen, A. Teggi, K. Vutova, C.M. Cretu, R. Virdone, et al., Treatment response of cystic echinococcosis to benzimidazoles: a systematic review, PLoS Negl. Trop. Dis. 3 (2009) e524.
[4] D.P. McManus, D.J. Gray, W. Zhan, Y. Yang, Diagnosis, treatment, and management of echinococcosis, BMJ 344 (2012) 39–44.
[5] P. Moro, P.M. Schantz, Echinococcosis: a review, Int. J. Infect. Dis. 13 (2009) 125–133.
[6] J. Hammond, D. Fielding, S. Bishop, Prospects for plant anthelmintics in tropical veterinary medicine, Vet. Res. Commun. 21 (1997) 213–228.
[7] A.E. Edris, Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review, Phytother. Res. 21 (2007) 308–323.
[8] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, Biological effects of essential oils—a review, Food Chem. Toxicol. 46 (2008) 446–475.
[9] J.B. Githiori, S. Athanasiadou, S.M. Thamsborg, Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants, Vet. Parasitol. 139 (2006) 308–320.
[10] A.L. Camurca-Vasconcelos, C.M. Bevilaqua, S.M. Morais, M.V. Marciel, C.T.C. Costa, I.T.F. Macedo, et al., Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils, Vet. Parasitol. 148 (2007) 288–294.
[11] N. Jeyathilakan, K. Murali, A. Anandraj, B. Latha, B. Abdul, Anthelmintic activity of essential oils of *Cymbopogon nardus* and *Azadirachta indica* on *Fasciola gigantica*, J. Vet. Anim. Sci. 6 (2010) 204–209.
[12] I.T. Macedo, C.M. Bevilaqua, L.M. de Oliveira, A.L. Camurca-Vasconcelos, L.S. Vieira, F.R. Oliveira, et al., Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes, Vet. Parasitol. 173 (2010) 93–98.
[13] A. Albanese, M. Elissondo, L. Gende, M. Eguaras, M. Denegri, *Echinococcus granulosus*: in vitro efficacy of *Rosmarinus officinalis* essential oil on protoscoleces, Int. J. Essent. Oil Ther. 3 (2009) 69–75.
[14] M. Taran, E. Azizi, A. Shikhvaisi, N. Asadi, The anthelmintic effect of *Pistacia khinjuk* against protoscoleces of *Echinococcus granulosus*, World J. Zool. 4 (2009) 291–295.
[15] M. Moazeni, M.J. Saharkhiz, A.A. Hosseini, In vitro lethal effect of ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces, Vet. Parasitol. 187 (2012) 203–208.
[16] M.A. Maggiore, A.A. Albanese, L.B. Gende, M.J. Eguaras, G.M. Denegri, M.C. Elissondo, Anthelmintic effect of *Mentha* spp. essential oils on *Echinococcus granulosus* protoscoleces and metacestodes, Parasitol. Res. 110 (2012) 1103–1112.
[17] C.C. Liolios, O. Gortzi, S. Lalas, J. Tsaknis, I. Chinou, Liposomal incorporation of carvacrol and thymol isolated from the essential oil of *Origanum dictamnus* L. and in vitro antimicrobial activity, Food Chem. 112 (2009) 77–83.
[18] S. Robledo, E. Osorio, D. Muñoz, L.M. Jaramillo, A. Restrepo, G. Arango, In vitro and in vivo cytotoxicities and antileishmanial activities of thymol and hemisynthetic derivatives, Antimicrob. Agents Chemother. 49 (4) (2005) 1652–1655.
[19] I. Hierro, A. Valero, P. Pérez, P. González, M.M. Cabo, M.P. Montilla, Action of different monoterpenic compounds against *Anisakis simplex* s.I.L3 larvae, Phytomedicine 11 (2004) 77–82.
[20] M. Elissondo, C. Albani, L. Gende, M. Eguaras, G. Denegri, Efficacy of thymol against *Echinococcus granulosus* protoscoleces, Parasitol. Int. 57 (2) (2008) 185–190.
[21] M.C. Elissondo, P.E. Pensel, G.M. Denegri, Could thymol have effectiveness on scolices and germinal layer of hydatid cysts? Acta Trop. 125 (2013) 251–257.
[22] M.V. Andresiuk, F. Ponce Gordo, M. Saarma, M.C. Elissondo, A. Taraborelli, C. Casalongue, *Echinococcus granulosus* genotype G1 dominated in cattle and sheep during 2003–2006 in Buenos Aires province, an endemic area for cystic echinococcosis in Argentina, Acta Trop. 127 (2013) 136–142.
[23] M. Elissondo, M. Dopchiz, M. Brasesco, G. Denegri, *Echinococcus granulosus*: first report of microcysts formation from protoscoleces of cattle origin using the in vitro vesicular culture technique, Parasite 11 (4) (2006) 415–418.

[24] M.T. Rogan, K.S. Richards, Development of the tegument of *Echinococcus granulosus* (Cestoda) protoscoleces during cystic differentiation in vivo, Parasitol. Res. 75 (4) (1989) 299306.
[25] M.C. Elissondo, L. Ceballos, M. Dopchiz, M.V. Andresiuk, L. Alvarez, S. Sánchez Bruni, et al., In vitro and in vivo effects of flubendazole on *Echinococcus granulosus* metacestodes, Parasitol. Res. 100 (2007) 1003–1009.
[26] A. Hemphill, B. Stadelmann, R. Rufener, M. Spiliotis, G. Boubaker, J. Müller, N. Müller, D. Gorgas, B. Gottstein, Treatment of echinococcosis: albendazole and mebendazole — what else? Parasite 21 (2014) 70.
[27] Q.A. McKellar, P. Harrison, E.A. Galbraith, H. Inglis, Pharmacokinetics of fenbendazole in dogs, J. Vet. Pharmacol. Ther. 13 (4) (1990) 38692.
[28] L. Ceballos, C. Elissondo, L. Moreno, M. Dopchiz, S. Sánchez Bruni, G. Denegri, Albendazole treatment in cystic echinococcosis: pharmacokinetics and clinical efficacy of two different aqueous formulations, Parasitol. Res. 103 (2008) 355–362.
[29] M. Urrea-Paris, N. Casado, M. Moreno, F. Rodriguez-Caabeiro, Chemoprophylactic praziquantel treatment in experimental hydatidosis, Parasitol. Res. 87 (2001) 510–512.
[30] L. Ceballos, M.C. Elissondo, S. Sánchez Bruni, A. Confalonieri, G. Denegri, L. Alvarez, et al., Chemoprophylactic activity of flubendazole in cystic echinococcosis, Chemotherapy 56 (2010) 386–392.
[31] M. Moazeni, S. Larki, A. Oryan, M.J. Saharkhiz, Preventive and therapeutic effects of *Zataria* multiflora methanolic extract on hydatid cyst: an in vivo study, Vet. Parasitol. 205 (2014) 107–112.
[32] P.E. Pensel, S. Castro, D. Allemandi, S. Bruni, S.D. Palma, M.C. Elissondo, Enhanced chemoprophylactic and clinical efficacy of albendazole formulated as solid dispersions in experimental cystic echinococcosis, Vet. Parasitol. 203 (2014) 80–86.
[33] W. Kammerer, M. Perez-Esandi, Chemotherapy of experimental *Echinococcus granulosus* infection. Trials in CF-1 mice and jirds (*Meriones unguiculatus*), Am. J. Trop. Med. Hyg. 24 (1975) 90–95.
[34] E. Ernst, R. März, C. Sieder, A controlled multi-centre study of herbal versus synthetic secretolytic drugs for acute bronchitis, Phytomedicine 4 (1997) 287–293.
[35] H. Matthys, C. de Mey, C. Carls, A. Rys, A. Geib, T. Wittig, Efficacy and tolerability of myrtol standardized in acute bronchitis: a multi-centre, randomised, double-blind, placebo-controlled parallel group clinical trial vs. cefuroxime andambroxol, Arzneim. Forsch. 50 (2000) 700–711.
[36] C. Kohlert, G. Schindler, R.W. März, G. Abel, B. Brinkhaus, H. Derendorf, Systemic availability and pharmacokinetics of thymol in humans, J. Clin. Pharmacol. 42 (2002) 731–737.
[37] J. Michiels, J. Missotten, N. Dierick, D. Fremaut, P. Maene, S. Smet, In vitro degradation and in vivo passage kinetics of carvacrol, thymol, eugenol and trans-cinnamaldehyde along the gastrointestinal tract of piglets, J. Sci. Food Agric. 13 (2008) 2371–2381.