

Evaluation of the efficacy of *Zataria multiflora* essential oil versus albendazole in patients infected with liver cystic echinococcosis: A nonrandomized clinical trial

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Background: Cystic echinococcosis (CE) is a life-threatening disease in many countries. Albendazole, as the drug of choice for medical treatment of CE, is accompanied by adverse effects and may be ineffective in 20%–40% of cases; hence, new and more effective compounds are urgently needed to optimize the management of the disease. This study was performed to evaluate the efficacy of *Zataria multiflora* essential oil (ZMEO) versus albendazole against human liver CE. **Materials and Methods:** In this nonrandomized and single-blinded clinical trial, thirty patients who were infected with liver CE were divided into two groups (15 in each) and treated with albendazole (800 mg daily) and ZMEO (60 mg daily), respectively. Albendazole and ZMEO were administered orally for 180 consecutive days. The volume of hydatid cysts was measured by ultrasonography before and 2, 4 and 6 months after the start of treatment. Simultaneously, biochemical analysis was performed on the blood samples of patients to assess the possible side effects of the two treatment regimens. **Results:** Two, 4 and 6 months after the start of treatment, ZMEO indicated a significantly higher ability in reduction of the volume of the hydatid cysts, compared to albendazole ($P < 0.05$). The mean values of aspartate aminotransferase, alanine transaminase and alkaline phosphatase were also significantly lower in the patients treated with ZMEO in comparison to those treated with albendazole ($P < 0.05$). No clinical adverse effects were observed in the patients treated by ZMEO. **Conclusion:** From the point of view of efficacy and safety, ZMEO indicated a significant superiority to albendazole. Hence, ZMEO may be considered as an alternative for albendazole in the medical treatment of liver CE.

Key words: Albendazole, cystic echinococcosis, essential oil, treatment, *zataria multiflora*

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INTRODUCTION

Cystic echinococcosis (CE) or hydatid disease is a serious public health concern with worldwide distribution.^[1] The causative agent for animal and human CE is the larval stage of *Echinococcus granulosus sensu lato* the parasite of the canine small intestine.^[2] CE is a chronic and life-threatening disease with poor prognosis. While the mortality rate of the disease is almost 2%–4%, this may increase significantly in countries with poor health

system.^[3] Hydatid cysts can occur in almost any part of the body, but infections are most commonly seen in the liver and lungs.^[4]

Treatment options for hydatid disease include surgery, medical therapy, and PAIR (Puncture, Aspiration, Injection of scolicedal agent, Reaspiration), whereas the clinically silent and inactive cysts are commonly monitored by “Watch-and-Wait” approach.^[3,5] Medical therapy may be the only treatment option for multiple, very small or inaccessible cysts and in old people

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as well as in those who do not accept surgery. Medical therapy may also be applied before surgery to reduce the cyst size and to decrease the recurrence rate after surgical operation.^[6] Albendazole is the drug of choice for medical therapy of hydatid disease, but it may completely cure only a third of infected patients. In 30%–50% of patients the size of the cyst decreases and the clinical signs are diminished. Conversely, in 20%–40% of cases, treatment with albendazole is ineffective.^[7] Regarding the concerns about the efficacy of albendazole, new and more effective compounds are urgently needed to optimize the treatment of CE in human beings.^[3]

A large number of chemical and natural compounds have been tested and found to be effective against the larval stage of *E. granulosus* in the *in vitro* and *in vivo* studies in recent years. However, these compounds have rarely been studied at the clinical trial level in human beings.^[2]

Zataria multiflora belongs to the Lamiaceae family and is a thyme-like plant that grows wild in Iran, Afghanistan and Pakistan. Aerial parts of *Z. multiflora* are used in traditional medicine for their analgesic, antiseptic, carminative, antidiarrheal, and anthelmintic activities. In addition, previous studies have shown that *Zataria multiflora* essential oil (ZMEO) has anti-nociceptive, antidiabetic, anti-aphthous, anti-inflammatory, antimicrobial, antifungal and antiprotozoal properties.^[8]

The methanolic extract of *Z. multiflora* has shown a high scolicidal activity *in vitro*.^[9] Accordingly, *in vivo* studies have confirmed the preventive and therapeutic effect of the methanolic extract, aromatic water, and the essential oil of *Z. multiflora* on the hydatid cysts in laboratory mice.^[10-12] This clinical trial therefore, was designed to evaluate the efficacy of ZMEO, in comparison with albendazole, in the treatment of liver CE in human cases.

MATERIALS AND METHODS

Study design and setting

This nonrandomized clinical study was performed during 2015–2017. All thirty patients who were infected with liver hydatid disease during this time and referred to Shiraz clinics, were included in this study. The patients were divided into groups 1 and 2 (15 in each) and treated with albendazole (800 mg daily) and ZMEO (60 mg daily), respectively. All patients were treated for 6 months. The diagnosis of CE was based on radiological imaging (ultrasonography) following the clinical history. To evaluate the therapeutic effect of albendazole and ZMEO on CE, ultrasonography was performed, and the length and width of the hydatid cysts were measured and recorded before and 2, 4 and

6 months after the start of treatment. The volume of the cysts was calculated through the following formula:

$$V = \frac{4}{3}\pi r^3 \quad (V: \text{volume}, \pi: 3.14, r: \frac{\text{cyst length} + \text{cyst width}}{4}).$$

Biochemical analysis was also performed on blood samples of all patients before and 2, 4 and 6 months after the start of treatment to assess the possible side effects of two treatment regimen on the liver function. The patients were regularly evaluated for probable side effects such as allergic reactions (e.g., pruritus, skin rash), bone marrow depression, anemia, infection, and liver problems (e.g., jaundice).

Selection of participants

In this clinical trial, all the patients who fulfilled the eligibility criteria were invited to participate in this study. In the first step, they were given detailed information about the aims, trials, and probable risks and benefits of two treatment methods, then their inquiries were answered. Inclusion criteria were as follows: patients who accepted the treatment protocol by signing the appropriate informed consent, those with liver hydatid cysts smaller than 5 cm in diameter, patients with noncalcified cysts and patients with noninfected cysts with bacterial infections. Therefore, thirty patients remained to be included in this study.

Intervention

Albendazole tablets were administered in two doses of 400 mg (800 mg daily) and ZMEO (20 mg soft capsules, each containing 5.5–6.5 mg thymol) was administered in three doses of 20 mg (60 mg daily). Albendazole and ZMEO were administered orally for 180 consecutive days.

Biochemical tests were performed before and 2, 4 and 6 months after the start of treatment. The activities of serum aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were assessed as the liver function tests. Biochemical measurements were done using a BS-200 Chemistry Analyzer (Mindray, China).

Outcomes

The primary outcome was reduction in the volume of hydatid cysts. Reduction in the cysts' volume was defined through measuring and comparing the cysts' volume before and 2, 4 and 6 months of the start of treatment. Lack of side effects on liver function was the secondary outcome. This was specified by comparing the results of biochemical liver function tests before and after treatment periods. To assess adverse events, participants remained under the supervision of the physician team during the course of treatment. All findings including the volume of hydatid cysts and the results of biochemical tests were recorded in the relevant record forms.

Sample size, randomization, and blinding

Because of the scarcity of human cases of hydatid disease as well as the inclusion criteria, the study was limited to only 30 patients (15 in each treatment group), however, *post hoc* calculation by power analysis on the obtained results showed that this sample size has an average power value about 82% with 5% error to detect the real differences between groups [Table 1].

In this study, we used the ZMEO produced by Barij Essence Pharmaceutical Company (Iran). This company supplies this herbal product to the market under the name of Gastrolit in the form of 20 mg soft capsules, each containing 5.5–6.5 mg thymol. These capsules are commonly administered for the treatment of irritable bowel syndrome in Iran.^[8,13] However, since it was the first time that these capsules were used for treatment of hydatid disease, the patients were allowed to choose their favorite medicine (Albendazole or ZMEO); therefore, random assignment

of the patients in two treatment groups was not feasible. Hence, the study was performed as a nonrandomized clinical trial. The researchers that analyzed the data were blinded to the groups. All the patients referred to ultrasound centers personally and the ultrasound staffs were unaware that the patients were involved in a research project. Therefore, the study is a single-blinded clinical trial.

Statistical analysis

The results are presented as mean \pm standard deviation. Normality of data distribution in each group was checked by one sample Kolmogorov–Smirnov test and accordingly parametric or nonparametric test were used. In order to compare the trend of changes in all laboratory values during the time between the groups, we applied repeated measures analysis of variance (RMANOVA) or repeated measures analysis of co-variance (RMANCOVA) to control the confounding factor (baseline values).

In addition, since the changes in the cyst's volume were not distributed normally over time, Friedman test was used instead of RMANOVA for each group (baseline values were controlled here, by subtracting from the after-treatment values to calculate the changes over time). To confirm the analysis, independent two samples *t*-test and Mann–Whitney U test were also used for between groups comparisons of the cyst volume and laboratory values in each time. Furthermore, effect size values were computed for all between groups comparisons based on Cohen's *d*.^[14] Subsequently, *post hoc* power analysis was done for just independent two samples *t*-tests as the sample to detect the power of sample size used in this study (Using G * Power calculator and $\alpha = 0.05$). All statistical analyses were done using IBM SPSS Statistics V22.0 (SPSS Amos; Chicago, Illinois, USA). $P < 0.05$ were considered statistically significant.

Ethical considerations

This study was approved by the Human Ethic Committee of Shiraz University of Medical Sciences (permit number: IR. Sums. Rec. 1396.335). Written informed consent was obtained from the patients before the commencement of the study. The study was registered retrospectively on the August 6, 2018 by Iranian Registry of Clinical Trials: IRCT20180219038798N1 (<https://en.irct.ir/user/trial/29767/view>).

RESULTS

Participant flow

The CONSORT diagram of this study is presented in Figure 1. As shown in this figure, of 50 eligible patients, 20 were excluded before the start of treatment.

Table 1: The results of liver function tests (means \pm standard deviation) (IU/L) in patients infected with hydatid disease before and after treatment by albendazole and *Zataria multiflora* essential oil

Treatment group	Albendazole (mean \pm SD)	ZMEO (mean \pm SD)	Effect size (power %)	<i>P</i> ^a
Liver enzyme				
AST				
BT	56.93 \pm 11.46	61.13 \pm 9.8	0.39	0.345 ^c
2MAT	61.67 \pm 12.16	57.40 \pm 9.19	0.40 (56)	0.001
4MAT	66.67 \pm 8.88	54.27 \pm 9.69	1.33 (99.9)	<0.001
6MAT	76.73 \pm 10.63	49.53 \pm 8.71	2.80 (100)	<0.001
<i>P</i> ^d	<0.001 (interaction effect)	0.007 (group effect)		
ALT				
BT	52.47 \pm 11.89	60.93 \pm 7.08	0.86 (99.5)	0.027
2MAT	58.60 \pm 11.29	56.00 \pm 6.63	0.28 (31.7)	<0.001
4MAT	67.20 \pm 10.08	51.27 \pm 5.92	1.92 (100)	<0.001
6MAT	73.27 \pm 9.73	47.73 \pm 6.44	3.09 (100)	<0.001
<i>P</i> ^d	<0.001 (interaction effect)	<0.001 (group effect)		
ALP				
BT	424.53 \pm 28.61	463.13 \pm 39.42	1.12 (99.9)	0.004 ^c
2MAT	437.87 \pm 29.0	446.67 \pm 32.7	0.28 (31.7)	0.015
4MAT	461.40 \pm 33.28	429.00 \pm 35.46	0.94 (99.9)	<0.001
6MAT	492.33 \pm 33.54	408.53 \pm 35.42	0.60 (88.8)	<0.001
<i>P</i> ^d	<0.001 (interaction effect)	<0.001 (group effect)		

^aIndependent two samples *t*-test, ^bRepeated measures ANOVA, ^cMann–Whitney U-test, ^dRepeated measure ANCOVA adjusted for baseline values where BT is significant, ^e*Post hoc* power analysis done with G*power calculator for independent *t*-tests based on effect size values and $\alpha=0.05$. BT: Before treatment, MAT: Months after treatment, ZMEO: *Zataria multiflora* essential oil, SD: Standard deviation, ALT: Alanine transaminase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

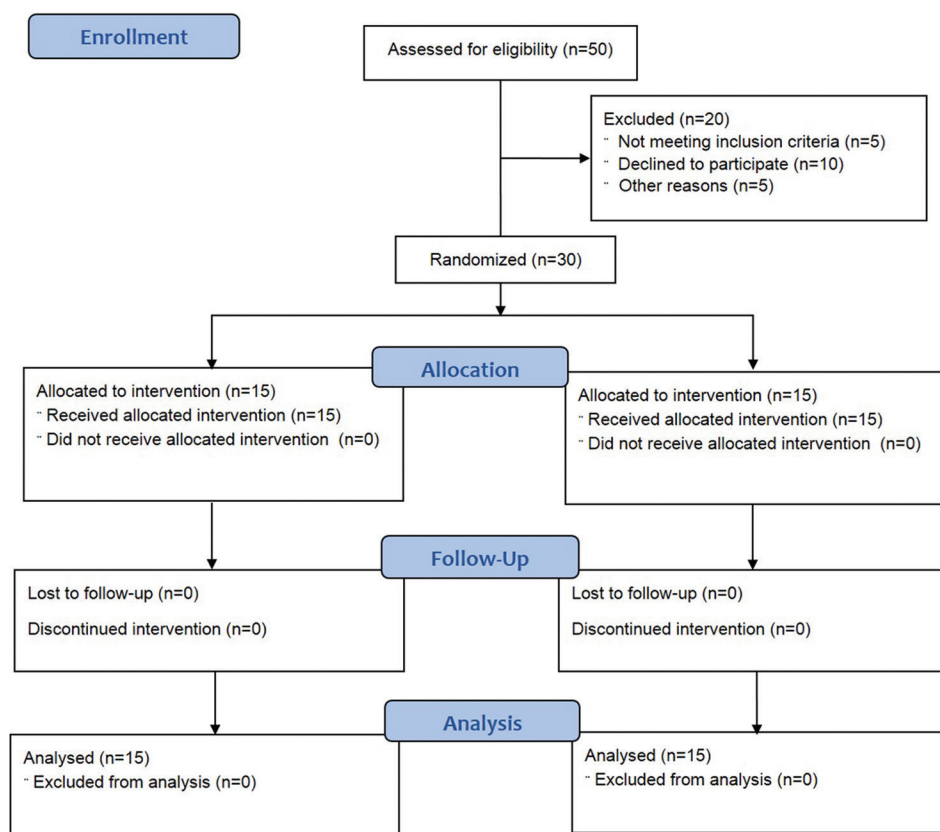


Figure 1: Consort flow diagram of patients infected with liver hydatid disease from allocation to analysis

Baseline data

The patients were from both gender (17 men and 13 women) and were 31–59 years old (mean: 49.2). The mean age in the albendazole (48.8 ± 4.85) and ZEMO (49.66 ± 8.15) groups was statistically the same (P = 0.72). In addition, the gender ratio did not significantly differ (P = 0.269).

Outcome and estimation

The volume of hydatid cysts (cm³) before and 2, 4 and 6 months after the start of treatment with albendazole and ZMEO is shown in Table 2. As the data presented in this Table show, even though the mean volume of the cysts was greater before the start of treatment in the ZMEO group, 6 months after the start of treatment, the mean volume of the cysts was greater in the albendazole group.

In order to evaluate the exact therapeutic effect of ZMEO in comparison with albendazole in the treatment of liver CE, the changes of the cyst’s volume were tested by Friedman test over time in each group separately. As a result, these changes were statistically significant in both albendazole (P = 0.03) and ZEMO (P = 0.000) groups. As between group comparisons, the ability of both agents in the reduction of the volume of the hydatid cysts from baseline values was compared 2, 4 and 6 months after the start of treatment [Table 3].

According to the data presented in this Table, ZMEO indicated a considerable statistically significant ability in reduction

Table 2: The volume of the hydatid cysts (cm³) before and after treatment of patients with albendazole and Zataria multiflora essential oil

Treatment period	Group	Cyst volume (mean±SD)	P ^a
Before treatment	Albendazole	8.43±8.93-2.30	0.436
	ZMEO	10.37±14.05-3.63	
2 mounts after treatment	Albendazole	8.12±8.61-2.22	0.325
	ZMEO	9.19±13.11-3.38	
4 mounts after treatment	Albendazole	7.72±7.82-2.02	0.174
	ZMEO	7.82±11.68-3.01	
6 mounts after treatment	Albendazole	7.61±7.98-2.06	0.161
	ZMEO	7.07±10.72-2.76	

^aMann–Withney U-test between ABZ and Zataria multiflora essential oil in each time. ZMEO: Zataria multiflora essential oil

of the hydatid cysts’ volume compared to Albendazole 2 months after the start of treatment (P = 0.012). Similarly, 4 months after the start of treatment, ZMEO represented a significantly higher power in the reduction of the hydatid cysts’ volume in comparison with albendazole (P = 0.005). In the same way, 6 months after the start of treatment, ZMEO decreased significantly the volume of the cysts compared with albendazole (P = 0.022) [Table 3].

The results of liver function tests are summarized in Table 1. As shown in this Table, the mean values of AST, ALT and ALP increased gradually from the beginning to the end of

the treatment in patients treated with albendazole, while these values decreased gradually in the patients treated with ZMEO. According to RMANOVA and RMANCOVA results, the pattern of changes during the time was significantly different between the groups (interaction effect). Due to the significant effect of group in this analysis and for more detailed research, univariate analysis was applied to compare the groups in each time. While before the start of treatment, the mean values of ALT and ALP were significantly lower in the albendazole group (hence, the baseline values considered as confounder and controlled by RMANCOVA analysis for these two measurements), conversely 2, 4 and 6 months after treatment, the values of AST, ALT and ALP were significantly lower in the patients treated with ZMEO [Table 1]. Table 4 shows the confounder-adjusted estimates and their 95% confidence interval for ALT and ALP.

Harms

No adverse clinical event was observed in the patients treated by ZMEO during the course of study. However, mild abdominal pain, gastrointestinal disorders, and nausea were observed in some patients treated with albendazole.

DISCUSSION

This study was performed to evaluate the therapeutic effect of ZMEO on the human liver CE. Fortunately, the results were encouraging. The complexity and dynamic nature of CE make its treatment difficult.^[15] A lot of attempts have been made to introduce innovative therapeutic agents for treatment of the disease all over the world. According to

the results of previous studies, the therapeutic effect of different drugs on hydatid disease decreases when the cysts are larger.^[5,16,17]

In the present study, before the start of treatment, the mean volume of the hydatid cysts in the patients in the ZMEO group was almost 1.2 times greater than those of albendazole group [Table 2]. Nevertheless, 6 months after the start of treatment, the mean volume of the hydatid cysts was smaller in the ZMEO group, demonstrating that this herbal product has better therapeutic effect on the liver CE than albendazole. As the data presented in Table 3 indicate, ZMEO showed a significantly higher efficacy than albendazole against liver CE, 2, 4 and 6 months after the start of treatment ($P < 0.05$).

The existing medical therapeutic choices for hydatid disease are limited to benzimidazoles (albendazole and mebendazole), having only parasitostatic properties. Therefore, after the administration of these drugs, complete cure does not occur;^[18] hence, following medical therapy with albendazole, the rate of recurrence may reach 80%. On the other hand, duration of treatment with albendazole has an important role in the therapeutic outcomes. Longer course treatment with albendazole may result in a greater therapeutic potential, but continuation of treatment with this drug may result in adverse side effects including nausea, vomiting, abdominal pain, and enhanced liver transaminases. These complications, when albendazole is administered systemically, are dose dependent. This is in a situation where benzimidazoles should be administered for long periods of time and with high doses to be effective against CE.^[6,17-19]

According to the above issues, the therapeutic effect of ZMEO on liver CE could be considered promising compared to albendazole that is presently known as the drug of choice for treatment of human CE.

Previous chemical analysis by gas chromatography (GC) and GC-mass spectrometry (GC-MS) indicated that thymol and carvacrol were the main components of ZMEO.^[18,20]

Table 3: Reduction in the volume of the hydatid cysts (means±standard deviation/cm³) after treatment of patients with albendazole and *Zataria multiflora* essential oil

Treatment group	Albendazole (means±SD)	ZMEO (means±SD)	Effect size ^a	P ^b
2MAT	0.42±0.61	1.19±1.32	0.75	0.012
4MAT	0.79±1.32	2.58±2.95	0.78	0.005
6MAT	0.96±1.32	3.31±3.89	0.81	0.022

^aCohen's $d = (mean_1 - mean_2) / S_{pooled}$, ^bMann-Whitney U-test. MAT: Months after treatment, ZMEO: *Zataria multiflora* essential oil, SD: Standard deviation

Table 4: Adjusted estimates (adjusted mean difference) and 95% confidence intervals (for alanine aminotransferase and alkaline phosphatase) for the treatment effect of outcomes (aspartate aminotransferase did not need adjustment because it did not have any confounding factor)

	2 months: Estimate (95% CI)	4 months: Estimate (95% CI)	6 months: Estimate (95% CI)
ALP			
Albendazole	454.61 ^a (450.01-459.21)	478.17 ^a (468.29-488.05)	506.48 ^a (492.76-520.19)
ZMEO	429.92 ^a (425.32-434.52)	412.23 ^a (402.35-422.10)	394.39 ^a (380.67-408.10)
ALT			
Albendazole	64.93 ^b (60.58-69.28)	68.99 ^b (64.69-73.28)	78.52 ^b (73.55-83.50)
ZMEO	54.13 ^b (49.78-58.49)	51.94 ^b (47.65-56.24)	47.74 ^b (42.77-52.72)

^aCovariates appearing in the model are evaluated at the following values: ALK_before=443.8333, ^bCovariates appearing in the model are evaluated at the following values: ALT_before=56.7000. ALT: Alanine transaminase, AST: Aspartate aminotransferase, CI: Confidence interval, ZMEO: *Zataria multiflora* essential oil, ALP: Alkaline phosphatase

Additionally, the scolicidal property and antihydatid effect of thymol^[21,22] and carvacrol^[23] have been previously documented. Thymol and carvacrol, as the main compounds of ZMEO, are lipophilic in nature and could easily enter the cell membrane, changing its permeability and causing the release of cellular content.^[8] This property may elucidate the anti-hydatid property of ZMEO.

In this study, the mean values of AST, ALT and ALP were higher than the normal ranges before the start of treatment [Table 1]. Presence of hydatid cysts in the liver may be toxic to this organ and CE has been known as a causative agent for oxidative stress in different animal species and humans.^[24] While the mean values of AST, ALT and ALP increased 2, 4 and 6 months after the start of treatment in patients treated with albendazole, these values decreased in patients treated with ZMEO. Accordingly, the level of the mentioned transaminases was significantly lower in the patients treated with ZMEO, 4 and 6 months after the start of treatment compared to those treated with albendazole [Table 1].

Fatal drug-induced liver injury (DILI) has become a serious health problem in the USA,^[25] and albendazole has been reported as a causative agent for DILI.^[26] Liver damages and elevation of liver transaminases is one of the most important adverse side effects of long-term albendazole administration in patients infected with CE.^[24,27] This event may result in interruption of treatment.^[24] Liver damages produced by albendazole are categorized as hepatocellular injury type.^[28] *Z. multiflora* contains compound such as thymol, carvacrol, zatrinal, oleanolic acid, betulic acid, rosmarinic acid, and monoterpenoids, which act as scavengers of free radicals.^[29] Previous studies have shown that *Z. multiflora* has antioxidant,^[8] immunostimulatory^[30] and hepatoprotective^[8] activities. Atayi *et al.*, reported that in mice infected with CE, the aromatic water of *Z. multiflora*, when administered along with albendazole, could diminish the oxidative stress and hepatic injury induced by hydatid cysts and also by long-term administration of albendazole.^[24] However, our results in this human trial were in accordance with those of all referenced studies reporting the safety of *Z. multiflora* in laboratory animals. Previous studies also confirmed that thymol and carvacrol, as the main component of ZMEO, are safe compounds.^[8]

Our study had some limitations. It was a nonrandomized trial; furthermore, the patients and investigators could not be blinded because ZMEO (soft capsules) and albendazole (tablets) have different shapes. In addition, because of the scarcity of human cases of hydatid disease, our study was limited to 30 patients (15 in each treatment group). Hereof, the results of this study may encourage the researchers to do further randomized clinical trials in larger size in this topic.

CONCLUSION

This is the first report of a clinical trial in the treatment of liver CE in human beings using a herbal product. ZMEO indicated a significantly higher therapeutic effect than albendazole on the liver CE. In addition, this herbal product induced no elevation in the liver enzymes. Hence, from two points of view; i.e., (a) therapeutic effect and (b) the results of liver function tests, ZMEO indicated a statistically significant superiority to albendazole. These findings could be considered as an encouraging step forward in the medical treatment of liver CE using a herbal medicine. However, additional studies for ZMEO with a significantly larger sample size and a longer follow-up period are suggested to be conducted. These studies could be helpful to find the optimum dosage of ZMEO and to clarify the exact efficacy or toxicity of this herbal product after its long-term consumption.

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Conflicts of interest

There are no conflicts of interest.

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