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RESEARCH ARTICLE

Efficacy and Safety *Curcuma zadoaria* L. to Inactivate the Hydatid Cyst Protoscoleces

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Abstract: ***Background***: The present work aimed to evaluate the chemical composition of *Curcuma zadoaria* essential oil and to investigate its efficacy and safety against hydatid cyst protoscoleces.

Methods: Collected protoscoleces from liver fertile hydatid cysts of infected sheep were exposed to different concentrations of the essential oil (75, 150, 300 µl/mL) for 5-30 min *in vitro* and *ex vivo*. Then, by using the eosin exclusion assay, the viability of protoscoleces was studied. In the next step, 24 male NMRI mice were examined to assess the toxicity of *C. zadoaria* essential oil by measuring the biochemical and hematological parameters.

Results: Based on the obtained results, the LD_{50} value of intraperitoneal injection of the *C. zadoaria* essential oil was 1.76 mL/kg of body weight and the maximum non-fatal dose was 0.96 mL/kg of body weight. *C. zadoaria* essential oil had a strong proto scolicidal activity *in vitro* so that at the 300 and 150 µl/ml entirely eliminates the parasite after 5 and 10 minutes; whereas, weak proto scolicidal activity was observed at lower doses. *Ex vivo* assay, no similar effect with *in vitro* was observed, therefore, more time is required to show a potent proto scolicidal activity. *C. zadoaria* essential oil at the concentrations of 300 and 150 µl/mL after an exposure time of 7 and 12 min, killed 100% of protoscoleces within the hydatid cyst, respectively. After intraperitoneal injection of the *C. zadoaria* essential oil for 2 weeks, no significant difference ($p > 0.05$) was observed in the clinical chemistry and hematologic parameters at the doses of 0.15, 0.3, 0.6 mL/kg.

Conclusion: The obtained results *in vitro* and *ex vivo* exhibited that *C. zadoaria* essential oil had a favorable proto scolicidal activity on hydatid cyst protoscoleces. However, more supplementary works are required to verify these findings by assessing clinical subjects.

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1. INTRODUCTION

Hydatidosis is also known as Cystic Echinococcosis (CE). It is a neglected zoonotic helminthic disease formed by the larval stage of the dog tapeworm *Echinococcus granulosus* (*sensulato*) [1]. The transmission cycle normally occurs between definitive hosts, such as dogs, and other canine animals and livestock. Humans as intermediate hosts can be infected by eating eggs, excreted from the feces of definitive hosts, where the parasite grows in its larval form as hydatid cysts in various organs especially in the liver and the lungs. In humans, CE can create a number of clinical manifestations, ranging from asymptomatic to life-threatening [2].

Presently, the percutaneous techniques and chemotherapy treatment with benzimidazoles are considered as the main methods for the treatment of hydatid cysts [3]. However, because of unpredictable results, and diverse side effects including hepatotoxicity, severe leucopenia, and alopecia, their efficacy is not satisfactory [4]. Currently, surgery is considered as the most reliable method for CE treatment that has the potential to remove cysts and results in perfect therapy; but it includes dangers, such as those linked with any surgical intervention, anaphylactic responses, and secondary CE appears in approximately 10% of the postoperative patients due to the discharge of the cyst fillings and protoscole-

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ces [5]. Protoscolicidal substances used in operating and percutaneous methods have restrictions because of the severe adverse effects, for example, biliary tract fibrosis, and liver necrosis [6, 7]. Regarding these problems, a suitable protoscolicidal agent with no local or systemic toxicity is needed for use during the hydatid cyst surgery.

To date, attention towards medicinal plants has increased due to low side effects and high efficacy, developing a more scientific interest in the therapeutic usage of herbs for the treatment of illnesses and improving health [8].

Curcuma zedoaria Rosc, also known as famous white turmeric, belonging to the family Zingiberaceaeis, which is broadly cultivated in India, China, Japan, Brazil, Nepal and Thailand [9]. The plant is traditionally applied to treat a wide range of diseases and conditions including leucorrhoea discharge, menstrual disorders, dropsy, leprosy, dyspepsia, flatulence, vomiting, cold, cough and fever [10]. Previous studies demonstrated that various extracts and isolated components of *C. zedoaria* showed some pharmacological properties such as hepatoprotective, anti-cancer, anti-analgesic, anti-allergen, and antimicrobial effects in modern medicine [10].

The reviews have also demonstrated that in phytochemical analysis of *C. zedoaria,* essential oil, the major compounds are oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes; whereas, in Gas Chromatography-Mass Spectrometry (GC-MS), the main components of the *C. zedoaria* essential oil were b-tumerone, 1, 8-cineole, zingiberene, cymene, and a-phellandrene [10]. However, it has been reported that different factors including the environmental condition, location harvest, harvest time, and the method of extraction might change the chemical composition and the functional activity of essential oils [11].

Based on the reported biological activity, especially antimicrobial activities mentioned above, there is no documented study according to our knowledge on the proto scolicidal effects of *C. zedoaria*. The present work aimed to evaluate the chemical composition of *C. zedoaria* essential oil and investigate its efficacy and safety against hydatid cyst protoscoleces.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

The rhizomes of plants were prepared from a market in Kerman province, South East of Iran. They were identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy of School of Pharmacy, Kerman University of Medical Science, Iran.

2.2. Isolation of Essential Oil

Two hundred and fifty grams of powdered rhizomes were used in the hydro-distillation method (750 ml water as solvent) for 3h using an all-glass Clevenger-type apparatus. The acquired essential oil was then dried over anhydrous sodium sulfate, and stored in darkness at 4°C in airtight glass vials and closed until testing [12].

2.3. GC/MS Analysis of Essential Oil

GC analysis was carried out by a Hewlett-Packard 6890 with an HP-5MS column (30m × 0.25 mm, film thickness 0.25 mm). The column temperature was maintained at 55°C for 3 min and programmed at 180°C at a rate of 5°C per min, and kept constant at 220°C for 5 min. The injector and interface temperatures were 220°C and 290°C, respectively. The flow rate of Helium as carrier gas was (1 mL/min C.F). The percentages were calculated by the electronic integration of FID peak areas without the use of response factors correction. The linear retention indices for all components were determined by the coinjection of the samples with a solution containing a homologous series of C8-C22 *n*-alkanes.

GC/MS analysis was performed using a Thermoquest-Finnigan gas chromatograph equipped with a fused silica capillary DB-5 column (30m × 0.25 mm, film thickness 0.25 mm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with an ionization voltage of 70 eV. Ion source and interface temperatures were 220°C and 290°C, respectively. The mass range was from 40 to 400 u. The oven temperature program was similar to GC. The components of the essential oil were identified by comparison of their relative retention time and mass spectra with standards Wiley 2001 library data of the GC/MS system or with those reported in the literature data [13].

2.4. Collection of Protoscoleces and Viability

Hydatid cysts from the liver of naturally infected sheep slaughtered at Khorramabad abattoir, Iran were used to collect the protoscoleces. Under sterile conditions, the hydatid cyst fluid was drained by the syringe and was poured into a flask; and left for half-hour to precipitate the contents. In the next step, the protoscoleces were collected after disposing the supernatant; they were then washed twice with the PBS (pH 7.2) solution. Finally, the number of protoscoleces/mL was adjusted to 5×10^3 protoscoleces in a 0.9% NaCl solution with an at least 90% viability rate by eosin exclusion test.

2.5. *In Vitro* Protoscolicidal Activity

C. zedoaria essential oil with different concentrations (75, 150, and 300 µL/mL) (0.2 ml) were added to each test tube containing 0.2 ml of protoscoleces and then kept for 5, 10, 20 and 30 min at 37°C. After the incubation time, the supernatant was removed and 50 µL of 0.1% eosin stain (Sigma-Aldrich, St. Louis, MO, USA) was added to the protoscoleces. After 10 min of incubation, the protoscoleces were smeared on a glass slide, covered with a coverslip, and tested under a light microscope. The results were reported in the percentage of dead and live protoscoleces with the counting of 300 protoscoleces [14].

2.6. Eosin Exclusion Test

Dye exclusion assay is usually carried out by counting wet preparations of cells in a hemocytometer in the attendance of eosin. This experiment was used to determine the viability of protoscoleces. In this test, flame cell motility and impermeability at 0.1% eosin solution (1 g of eosin powder in 1000 mL of distilled water) were used to evaluate the viability rate protoscoleces. After staining the liver, pro-

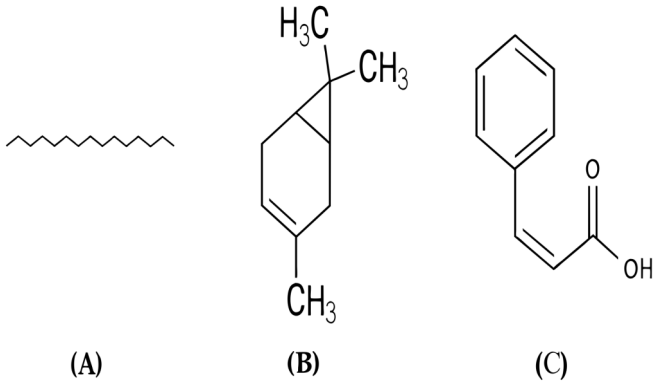

Fig. (1). The structure of main components of *C. zadoaria* essential oil; pentadecane (**A**), delta-3-carene (**B**), and cis-cinnamic acid (**C**).

toscoleces do not absorb color and display characteristic muscular movements and flame cell activity; while in dead protoscoleces, eosin enters the cell and protoscoleces become red (Fig. **1**).

2.7. *Ex vivo* Proto Scolicidal Activity

Ex vivo scolicidal effects of *C. zedoaria* essential oil and more than 50% of the liquid of fertile hydatid cysts (four cysts) were aspirated. And *C. zedoaria* essential oil at the concentrations of 75, and 15, and 300 µL/mL was injected into the cysts. In the next step, an aliquot from the liquid containing the protoscoleces was collected at 5, 10, 20, and 30 min after exposure. Subsequently and similar to the *in vitro* proto scolicidal assay, the viability of protoscoleces was determined by the eosin exclusion test [15].

2.8. Safety Evaluation

2.8.1. Animals

In total, 48 male NMRI mice (6-8 weeks old, weighting 35-40 g) were purchased from the Animal Breeding Stock Facility of Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, (Khorramabad, Iran). The animals were housed in a colony room with a 12:12-h light/dark cycle at 21 ± 2°C and were handled according to Guides for the Care and Use of Laboratory Animals; whereas, the study was administered by the Committee on the Ethics of Animal Experiments of the Lorestan University of Medical Science, Iran (98/756).

2.8.2. Acute Toxicity

Different doses of *C. zadoaria* essential oil (1-4 mL/kg) were injected intraperitoneally into four groups of six mice each to assess acute toxicity. The number of deaths were counted 24h after the treatment. LD_{50} values were determined by the Probit test in SPSS [5].

2.8.3. Sub-acute Toxicity

2.8.3.1. Study Design

Animals were randomly divided into four groups (6 mice per each group) as follows:

First group (control group, i): which received normal saline intraperitoneally for 14 consecutive days.

Second group (ii): mice that received the *C. zedoaria* essential oil at the dose of 0.15 mL/kg intraperitoneally for 14 consecutive days.

Third group (iii): mice that received the *C. zedoaria* essential oil at the dose of 0.3 mL/kg intraperitoneally for 14 consecutive days.

Forth group (iv): mice that received the *C. zedoaria* essential oil at the dose of 0.6 mL/kg intraperitoneally for 14 consecutive days.

2.8.4. Blood Sampling

On the fifteenth day, to collect the blood samples from each mouse, after anesthetizing the animals with ketamine (100 mg/kg)-xylazine (10 mg/kg) and opening their abdomen cavities, blood samples were collected from the animal's heart.

2.8.5. Measuring Hematological Parameters

To determine the hematological parameters, some blood was collected and poured into an anticoagulant tube with Ethylenediaminetetraacetic Acid (EDTA). Then, a number of hematological parameters including hemoglobin (Hb), hematocrit (HCT), White Blood Cell Counts (WBC), Red Blood Cell (RBC), and Platelet (PLT) counts were evaluated.

2.8.6. Measuring Biochemical Parameters

After separating the serum from the collected blood, some biochemical factors, such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Creatinine (Cr), Blood Urea Nitrogen (BUN), and bilirubin (direct and total) to evaluate the liver and kidney function were measured using the commercial diagnostic kits [16].

2.9. Statistical Analysis

All the tests were performed in triplicate in the present study. Data analysis was carried out using SPSS 17.0 statistical package (SPSS Inc., Chicago, IL, USA). The one-way ANOVA and descriptive statistics such as frequency calculation were used for data analysis and independent-samples *t* test was used for further analysis. $P<0.05$ was considered statistically significant.

3. RESULTS

3.1. GC/MS Analysis of *C. zadoaria* Essential Oil

Table **1** shows the results of GC/MS analysis of *C. zadoaria* essential oil. A total of 34 components were found; whereas the main components were pentadecane (29.6%), Delta-3-Carene (14.7%), and Cis-Cinnamic Acid (8.4%), respectively.

3.2. *In Vitro* Protoscolicidal Effects of *C. zadoaria* Essential Oil

Table **2** exhibits the proto scolicidal effects of *C. zadoaria* essential oil against the protoscoleces of hydatid cyst on *in vitro*. Based on the results, *C. zadoaria* essential oil had a powerful proto scolicidal activity therefore, at 300 and 150 µg/ml, it entirely eliminates the parasite after 5 and

Table 1. GC/MS analysis of *C. zadoaria* essential oil.

S. No.	Compound	RT[a]	Area (%)
1	Tricyclene	7.282	0.3
2	Alpha.-Thujene	7.430	0.2
3	Alpha-Pinene	7.671	2.7
4	Camphene	8.094	3.9
5	O-Cymene	8.694	1.9
6	Beta.-Pinene	8.982	1.1
7	Beta.-Myrcene	9.472	0.45
8	Alpha.-Phellandrene	9.895	0.7
9	**Delta-3-Carene**	**10.271**	**14.7**
10	P-Cymene	10.453	2.8
11	1,8-Cineole	10.707	1.3
12	Limonene	10.775	1.7
13	Gamma.-Terpinene	11.688	0.8
14	Styrene	12.233	0.7
15	Alpha.-Terpinolene	12.669	0.6
16	Bicyclo[4.1.0]Heptane	13.895	0.8
17	Beta.-Phellandren	14.572	0.7
18	Borneol	14.862	1.7
19	Eucarvone	15.802	0.8
20	Anisaldehyde	16.758	0.8
21	2,4-Cycloheptadien-1	17.024	0.2
22	Thymol	18.465	0.8
23	Carvacrol	18.764	0.2
24	Tridecane	19.418	0.3
25	Cyperene	21.985	2.3
26	Tetradecane	22.090	0.6
27	Alpha-Gurjunene	22.234	0.4
28	**Cis-Cinnamic Acid**	**22.911**	**8.4**
29	1-Tridecene	24.057	1.0
30	**Pentadecane**	**24.822**	**29.6**
31	Germacrene B	25.735	0.8
32	Heptadecadiene	28.395	1.8
33	8-Heptadecene	28.628	1.6
34	Acrylic Acid	29.436	7.5
	Total		94.35

10 minutes. Besides, *C. zadoaria* essential oil, at the dose of 75 µl/ml eliminates 41.3, 67.6, 82.3, and 98.6% parasites after 5, 10, 20, and 30 minutes of incubation. Subsequently, the 3.6 and 100% of protoscoleces were eliminated after 30 and 10 mins of exposure with normal saline and Ag-nitrate, respectively.

3.3. *Ex Vivo* Protoscolicidal Activity

Based on the *ex vivo* results, although *C. zadoaria* essential oil at the concentration of 300 and 150 µl/mL after 5 and 10 min completely killed 100% of protoscoleces *in vitro*, but did not reveal a similar effect in the *ex vivo* analysis, there-

Table 2. ***In vitro*** **protoscolicidal effects of** ***C. zadoaria*** **essential oil against protoscoleces of hydatid cyst at various concentrations following various exposure times.**

Mortality (%)	Time (min)	Concentration (µL/mL)
100 ± 0.0	5	**300**
100 ± 0.0	10	
100 ± 0.0	20	
100 ± 0.0	30	
84.3 ± 4.1	5	**150**
100 ± 0.0	10	
100 ± 0.0	20	
100 ± 0.0	30	
41.3 ± 2.51	5	**75**
67.6 ± 3.15	10	
82.3 ± 2.51	20	
98.6 ± 1.15	30	
0.0 ± 0.0	5	**Normal saline + Tween 20**
00 ± 0.0	10	
1.5 ± 0.5	20	
3.3 ± 1.15	30	
71.6 ± 2.88	5	**Ag-nitrate**
100 ± 0.0	10	
100 ± 0.0	20	
100 ± 0.0	30	

fore, it required more time to show potent proto scolicidal activity. *C. zadoaria* essential oil at the concentrations of 300, 150, and 75 µl/mL after an exposure time of 7, 12, and 40 min killed 100% of protoscoleces within the hydatid cyst, respectively (Table **3**).

3.4. Acute Toxicity

According to the results, the LD_{50} value of intraperitoneal injection of the *C. longa* essential oil was 1.76 mL/kg of body weight and the maximum nonfatal dose was 0.96 mL/kg of body weight.

3.5. Effect on Biochemical and Hematological Parameters

Determination of the toxicity of *C. zadoaria* essential oil was measured by the hematologic and chemical clinical parameters in the mice. The results showed that after 14 days of intraperitoneal injection of these 3 doses of *C. zadoaria* essential oil (150, 300, and 600 µL/kg), no mortality was observed. The hematologic and chemical parameters of the essential oil in doses of 0.15, 0.3 and 0.6 ml/kg, and the control group are shown in Tables **4** and **5**. The results showed that there was no significant difference between these parameters in comparison with the group receiving the essential oil of the plant and the control group (P> 0.05).

Table 3. ***Ex vivo*** **protoscolicidal effects of** ***C. zadoaria*** **essential oil against protoscoleces of hydatid cyst at various concentrations following various exposure times.**

Mortality (%)	Time (min)	Concentration (µL/mL)
57.6 ± 2.15	3	**300**
100 ± 0.0	7	
100 ± 0.0	12	
100 ± 0.0	20	
100 ± 0.0	40	
100 ± 0.0	60	
21.3 ± 1.15	3	
66.3 ± 4.3	7	
100 ± 0.0	12	**150**
100 ± 0.0	20	
100 ± 0.0	40	
100 ± 0.0	60	
8.6 ± 0.5	3	
24.6 ± 1.15	7	
51.3 ± 2.88	12	**75**
92.6 ± 4.51	20	
100 ± 0.0	40	
100 ± 0.0	60	
0.0 ± 0.0	3	
1.3 ± 0.57	7	**Normal saline + Tween 20**
2.3 ± 0.57	12	
3.6 ± 1.15	20	
4.6 ± 0.57	40	
6.0 ± 1.0	60	
42.3 ± 2.88	3	**Ag-nitrate**
100 ± 0.0	7	
100 ± 0.0	12	
100 ± 0.0	20	
100 ± 0.0	40	
100 ± 0.0	60	

4. DISCUSSION

From the beginning of mankind to now, natural products have been used to treat many diseases [17]. Of the natural products, medicinal herbs due to high availability, low cost,

Table 4. Clinical chemistry parameters in mice sera following the oral administration of *C. zadoaria* essential oil for two weeks.

Parameters	*C. zadoaria* Essential Oil (mL/kg)			Control	P value
	0.15	0.3	0.6		
AST (U/L)	145.3 ± 11.5	148.3 ± 9.3	154.6± 12.5	141 ± 13.5	0.442
ALT (U/L)	86.6 ± 3.3	94.3 ± 4.6	111.5 ± 7.6	93.4 ± 8.3	0.091
ALP (U/L)	250.3 ± 23.3	246.6 ± 19.5	261.5 ± 20.4	235.6 ± 16.5	0.325
Cr (mg/dL)	0.51 ± 0.08	0.47 ± 0.08	0.55 ± 0.1	0.38 ± 0.05	0.073
BUN (mg/dL)	28.3 ± 6.2	37.6 ± 6.3	42.3 ± 7.1	34.3 ± 3.4	0.21
TB (mg/dL)	0.56 ± 0.11	0.59 ± 0.15	0.83 ± 0.2	0.76 ± 0.2	0.165
DB (mg/dL)	0.28 ± 0.06	0.31 ± 0.03	0.41± 0.05	0.33 ± 0.01	0.563

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Cr, creatinine; BUN, Blood urea nitrogen; TB, Total bilirubin; DB, Direct bilirubin.

Table 5. Hematology parameters in whole blood of mice following the oral administration of *C. zadoaria* essential oil for two weeks.

Parameters	*C. zadoaria* Essential Oil (mL/kg)			Control	P value
	0.15	0.3	0.6		
RBC ($\times 10^6/\mu L$)	2.9 ± 0.15	3.1 ± 0.25	3.7 ± 0.41	3.4 ± 0.3	0.621
HGB (g/dL)	9.6 ± 0.7	10.1 ± 1.17	9.3 ± 0.6	11.3 ± 0.45	0.213
Hct (%)	27.6± 3.1	33.1 ± 2.51	29.2 ± 2.6	32.6 ± 2.18	0.092
WBC ($\times 10^3/\mu L$)	3.0 ± 0.35	2.6 ± 0.15	3.3 ± 0.25	2.9 ± 0.2	0.715
PLT ($\times 10^3/\mu L$)	159.3 ± 15.6	179.3 ± 18	166.6 ± 12.3	185 ± 17.3	0.125

RBC, red blood cell; HGB, hemoglobin; Hct, hematocrit; WBC, white blood cell; PLT, platelet.

high efficiency, and low toxicity have always been considered for therapeutic goals [17]. Presently, it has been reported that spices not only improve the taste, aroma, and color of food and drinks, but they can be applied to treat a wide range of diseases such as infectious diseases [18].

Hydatid cyst is a neglected parasitic infection that is considered a major health and economic problem in many countries around the world. It poses a threat to public health in many low and middle-income countries [1]. Surgery is one of the most effective and useful ways to treat hydatid cyst. But this method may associate with some complications such as the linkage of cysts that result in anaphylactic shock, and secondary hydatid cysts [3]. Nowadays, surgeons use protoscolicidal agents, such as Ag-nitrate and hypertonic saline agents to prevent these complications; however, recent studies showed some severe adverse side effects, for example, biliary tract fibrosis, and liver necrosis [5-7]. Therefore, there is an urgent need to discover a new alternative protoscolicdal agent with high efficiency and low toxicity. The present work aimed to evaluate the chemical composition of *C. zedoaria* essential oil and investigate its efficacy and safety against hydatid cyst protoscoleces.

Based on the obtained results, *C. zadoaria* essential oil had a powerful protoscolicidal activity *in vitro* so that at 300 and 150 µg/ml, it entirely eliminates the parasite after 5 and 10 minutes; whereas, at lower doses, it demonstrated weak protoscolicidal activity. As shown in *ex vivo* assay, although *C. zadoaria* essential oil at the concentration of 300 and 150 µl/mL after 5 and 10 min completely killed 100% of protoscoleces *in vitro*, but did not reveal a similar effect in the *ex vivo* analysis, therefore, it required more time to show a potent proto scolicidal activity. *C. zadoaria* essential oil at the concentrations of 300 and 150 µl/mL after an exposure time of 7 and 12 min, killed 100% of protoscoleces within the hydatid cyst, respectively.

Present procole demonstrated that an applicable protoscolicidal agent is described by some properties such as better performance at low doses, effectiveness at shorter exposure time, high stability in the presence of fluid cyst, easy access to agent, as well as no significant toxicity [8]. In recent years, a number of studies have been conducted on the proto scolicidal effects of natural products and herbal medicines such as *Zataria multiflora, Berberis vulgaris, Allium* spp. *Mentha* spp. and *etc*. [8]. The results of this work demonstrated that *C. zadoaria* can be a natural agent to produce a new proto scolicidal agent for use in hydatid cyst surgery.

The findings of the GC/MS analysis exhibited 34 components in *C. zadoaria* essential oil; therefore, pentadecane (29.6%- an alkane hydrocarbon), delta-3-carene (14.7%- a bicyclic monoterpene), and cis-cinnamic acid (8.4%- a white crystalline acid) had high percentage, respectively.

Today, it has been reported that antimicrobial effects a wide range of essential oils isolated from plants are related to

isoprene including monoterpenes, sesquiterpenes and other hydrocarbons and phenols [19, 20]. In terms of the antimicrobial mechanisms of these constituents, previous investigations have reported that these compounds exhibit their antimicrobial effects by penetrating into the parasite cytoplasm and through the destruction of its cell wall [21]; whereas, in some other studies, the antimicrobial effects of these constituents is attributable for entry into the pathogens and the disruption of their vital metabolism and interactions [22, 23]. Therefore, it is concluded that the proto scolicidal effects of *C. zadoaria* can be attributed to the presence of these constituents in its essential oil.

At present, it has been reported that on animal models; liver and kidney can be considered as one of the main organs which can be used to determine the toxicity of novel drugs; therefore, evaluating the function of this organ can be considered as a key approach to study the toxicity of some new drug agents [16]. The most important way to evaluate the liver and kidney damage is the the measurement of the serum levels of their enzymes such as ALT, AST, ALP, Bilirubin (total, direct), Cr, and BUN. The obtained results in this work revealed that the LD_{50} value of intraperitoneal injection of the *C. longa* essential oil was 1.76 mL/kg of body weight and the maximum nonfatal dose was 0.96 mL/kg of body weight. The results also showed that after 14 days of intraperitoneal injection of *C. zadoaria* essential oil (150, 300, and 600 µL/kg), no mortality was observed and also no significant difference was observed between the hematologic and chemical parameters in comparison with the group receiving the essential oil of the plant and the control group ($P > 0.05$); however, higher doses must be evaluated to reach the actual conclusion. Considering the toxicity of *C. zadoaria* essential oil, in a study conducted by Lai *et al*. (2004), it has been reported that *C. zadoaria* essential oil could inhibit the proliferation of human promyelocytic leukemia HL-60 cells and lead to the apoptosis of this human cancer cell line [24]. Based on the toxicity classification, these findings demonstrated that *C. zadoaria* essential oil had no significant toxicity against male mice [25].

CONCLUSION

The obtained results *in vitro* and *ex vivo* exhibited that *C. zadoaria* essential oil had favorable proto scolicidal activity on hydatid cyst protoscoleces. However, more supplementary works are required to verify these findings by assessing clinical subjects.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol was approved by the Committee on the Ethics of Animal Experiments of the Lorestan University of Medical Science, Iran with approval number: 2017-832.

HUMAN AND ANIMAL RIGHTS

No humans were involved in the study. All animal related experiments were followed in accordance with the standards set forth in the 8th Edition of Guide for the Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/Guidefor-the-care-and-use-of-laboratory-animals.pdf) published by the National Academy of Sciences, The National Academies Press, Washington DC, United States of America.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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