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International Journal of Current Research
Vol. 9, Issue, 06, pp.53404-53409, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

SCOLICIDAL EFFECTS OF SOME HERBS USED IN IRAQ TRADITIONAL MEDICINE ON PROTOSCOLICES OF HYDATID CYSTS

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ARTICLE INFO

Article History:
Received 25th March, 2017
Received in revised form
08th April, 2017
Accepted 06th May, 2017
Published online 30th June, 2017

Key words:

Hydatid Cyst,
Scolicidal,
Ethanolic Extract,
Mentha longifola.

ABSTRACT

Background: Because there is no effective drug therapy for hydatid cyst yet, assessment and finding of some new agents especially from herbal origin with a desired scolicidal effect attracts great attention for treatment and pre-surgical use to prevent the hydatid cyst recurrence. In the present study, the scolicidal effect of ethanolic extract of *Allium sativum*, *Mentha longifola* and *Zingiber officinale* were investigated.

Methods: Suspension of protoscolices was obtained an aseptically from sheep livers containing hydatid cysts. Three concentrations of Allium sativum, Mentha longifola and Zingiber officinale extract (10, 25 and 50 mg/ml) were used for 5,10,15, 20, 25 and 30 min. Viability of protoscolices was confirmed by 0.1% Eosin staining.

Results: Present study showed that ethanolic extract of garlic (*Allium sativum*) had more potent protoscolicid effects among all the 3 plants and killed 100% of protoscolices in 25 mg/ml, 50 mg/ml on a minimum of 30, 15 min of application, respectively. While the rate of dead protoscolices was 13.7% in the control group, while protoscolices were exposed to *Mentha longifola* extract at the concentration of 25 mg/ml, the rate of dead protoscolices increased to 83.1%, 87.1%, 98.0%, 99.2%, 99.9%, and100% after 5,10,15, 20, 25 and 30 min respectively. One hundred percent mortality rate was observed at concentration of 50 mg/ml after 20 min of exposure. The scolicidal activity of *Zingiber officinale* extract at the concentration of 25 mg/ml killed (19.1, 22.5, 31.5, 41.0, 50.6 and 60.2) % of the protoscolices after 5, 10, 15, 20, 25 and 30 min of application respectively, but scolicidal activity of *Zingiber officinale* extract increased at the concentration of 50 mg/ml to 98.8% after 30 min of application then 100% after 35 minutes.

Conclusions: Ethanolic extract of garlic, *Mentha longifola* and ginger showed high protoscolicidal activity in vitro. It may be considered as an effective natural agent used in hydatid cyst treatment and pre-surgery to prevent secondary cyst recurrence. However, further biological and phytochemical investigations aiming to identify the active compounds of these extracts and other pure compounds from these plants to find other ant parasitic agents that are not affected when treated with common therapeutic agents.

Citation: May A. I. AL-Mosa, Kifah Fedhil Hassoon Al- Shabaa and Nadia K.J.AL-Dawah, 2017. "Scolicidal effects of some herbs used in Iraq traditional medicine on Protoscolices of Hydatid cysts", *International Journal of Current Research*, 9, (06), 53404-53409.

INTRODUCTION

Hydatidosis is a chronic infection of medical and veterinary importance caused by the larval stage of a cosmopolitan parasitic cestode *Echinococcus granulosus* (Eckert and Deplazes, 2004). Human cystic echinococcosis (hydatid disease) continues to be a substantial cause of morbidity and mortality in many parts of the world (Craig *et al*., 2007). Although scientists and clinicians have accumulated much experience in the diagnosis and treatment of human echinococcosis, there are still many questions and problems. In general, radical resection of the parasitic mass, if possible, represents the preferred treatment strategy. Surgery is usually complemented by pre-, and/or post-surgical chemotherapy, and in inoperable cases, chemotherapy is the only option. The poor response of this infection to most chemotherapeutic agents has made hydatidosis primarily a surgical disease, and thus the role of chemotherapy is for prophylaxis against spillage during surgery, for the treatment of inoperable cases, or for use in areas without adequate surgical facilities, benzimidazoles have to be applied in high doses for extended periods of time, and adverse side effects are frequently observed. Most studies indicate that the efficacy of albendazole as measured by the disappearance of a cyst is generally less than 30% under ideal circumstances. There is clearly a need for drugs that are more effective and easier to administer (Blanton, 1998; Wen *et al*., 1993; Walker *et al*., 2004). Scolicidal solutions remain indispensable in the treatment of hydatid cyst disease and surgeons need less harmful but more effective drugs in hydatid disease (Adas *et al*., 2009). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as plants, animals and microorganisms. Recently, herbal medicines have increasingly been used to treat many diseases including several infections (Khan *et al*., 2010). The use of natural materials to inactivate protoscolices reduces their risk, because they are compatible with the human body. One of

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these substances is garlic (*Allium sativum*) belongs to the family of Liliaceae. Garlic has been known to possess dietary and medicinal properties (Eja *et al*., 2007). For instance, its antiviral (Weber *et al*., 1992; Waldman, 1993), antifungal (Ghannoum, 1990; Cai, 1991), antibacterial (Eja *et al*., 2007; Ross *et al*., 2001; Martin and Ernst, 2003), antiprotozoal (Soffar and Mokhtar, 1991; Harris *et al*., 2000), and antihelminthic (Abdel-Salam *et al*., 2008). The second substances *Mentha longifola* know herbal has elongated oval or spear leaves arranged on the stem of the plant in smooth cross pairs or wrinkled to fluff regularly serrated, upper leaves sitting either lower with short stem (PDR for herbal medicines, 1998). It has many therapeutic uses it was repelling gas, anti-colic, sweating helps, anti-vomiting, treats indigestion accompanied by gas and dysmenorrhoea, as for the external uses sterile and anti –itch (AL- Zoubidy *et al*., 1996). In pharmacological studies *Mentha* species showed variety of effects like anti- flat worm (Sharathchandra *et al*., 1995) anti- microbial and anti – fungal (Mucciarelli *et al*., 2001). The last substances ginger (*Zingiber officinale*) family Zingiberaceae, which showed insecticidal, growth regulating, reducing fecundity, development edifying properties and repellent activity against many tested insects (Abdul Rahuman *et al*., 2008). Scientific reports show that *Zingiber officinale* has carminative, antipyretic, anticancer, cardio tonic, antispasmodic, anti-diabetic, antioxidant and anti-hepatotoxic activities (Lakshmi and Sudhakar, 2010). Our study was designed to determine the effect of garlic, Stachys and ginger on protoscolices of hydatid cyst. In this study, different concentrations of all substances were tested at different exposure times.

MATERIALS AND METHODS

Collection of Protoscolices

Hydatid cysts from livers of naturally infected sheep were obtained from Al-Najaf abattoir in Iraq. The hydatid fluid was aseptically transferred into glass cylinders and left to set for 30 min. The protoscolices settled down at the bottom of the cylinders. The supernatant was removed and the yielded protoscolices were washed three times with normal saline. Viability was assessed by muscular movements and 0.1% eosin staining test. The live protoscolices were finally transferred into a dark container containing normal saline solution and stored at 4°C for further use.

Preparation of Extracts

The garlic (*Allium sativum*) cloves were sliced, dried under shade, air-dried aerial parts of the Stachys (*Mentha longifola*) and fresh rhizomes of ginger (*Zingiber officinale*), were purchased from a local herbal market in Najaf. Then, the rhizomes were peeled, cut into pieces, dried under shade and all other materials were powdered mechanically using a commercial electrical blender. Ethanol extracts was depending on ethanol by using soxhlet apparatus and continuous hot extraction for 72 hours at (60-50)c° then, dried and collected as powder (Harborne, 1983).

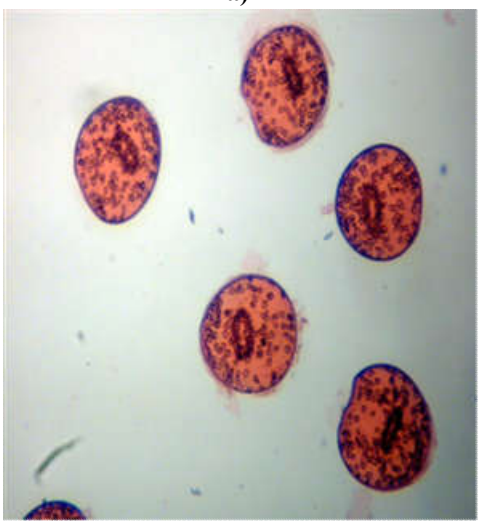
Scolicidal Assay

In this study, three concentrations of Stachys extract (10, 25 and 50 mg/ml) were used for 10, 15, 20, 25 and 30 min**.** To prepare the extract solution at 10, 25 and 50 mg/ml concentrations, 0.1, 0.3 and 0.5 g of dried extract was dissolved in 10 ml of distilled water, respectively. Then 2.5 ml of each Stachys solution was placed in test tubes, to which a drop of protoscolex rich sediment was added. After gently mixed, the tubes were incubated at 37°C for 10, 15, 20, 25 and 30 min. At the end of each incubation time the upper phase was carefully removed so as not to disturb the protoscolices. One milliliter of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. The upper portion of the solution was discarded after 15 min of incubation. The remaining pellet of protoscolices was then smeared on a manually scaled glass slide, covered with a cover glass (24 × 50 mm), and examined under a light microscope. The percentages of dead protoscolices were determined by counting a minimum of 400 protoscolices. Non treated protoscolices were considered as a control group in each experiment. The experiments were performed in triplicate.

Viability Test

In the present study, eosin stain with the concentration of 0.1% (1 g of eosin powder in 1000 ml distilled water) was used to check the viability of the protoscolices. After exposure to the stain, the protoscolices that excluded the eosin dye were considered potentially viable otherwise, they were recorded as dead (Figure 1).

a)



b)

Figure 1. A live protoscolices (A); Dead protoscolices (B) after exposure to ethanolic extracts and staining with 0.1% eosin

Statistical Analysis

Differences between the test and control groups were analyzed with Anova test. Statistical analysis was performed with LSD. P values less than 0.05 were considered to be significant.

RESULTS

Results of the effectiveness of different concentrations of *Allium sativum* extract as a scolicidal agent are shown in tables 1 and 2, scolicidal activity of *Allium sativum* extract at concentration of 25 mg/ml was 82.8 %, 86.9%, 92.9%, 98.0%, 99.2% and 100 % after 5,10,15, 20, 25 and 30 min of application respectively. *Allium sativum* extract at concentration of 50 mg/ml killed 95.9%, 98.3% and 100%of protoscolices after 5, 10 and 15 min respectively. While the death rate in the control group was 16.5%, thus the scolicidal effect of both concentrations of the ethanolic extract of *Allium sativum* was extremely significant compared to the control groups at all exposure times ($P \leq 0.0001$). The results of our study indicated that ethanolic extract of *Allium sativum* showed a high scolicidal activity in vitro. The mortality rate of protoscolices after exposure to different concentrations of the ethanolic extract of *Mentha longifola* following various exposure times are presented in tables 3and 4. *Mentha longifola* showed high scolicidal activity and its ethanolic extract was found to be effective against protoscolices at all concentrations tested. While the mortality rate of protoscolices was 13.7% in the control group, when protoscolices were exposed to the *Mentha longifola* extract at concentration of 25mg/ml, the mortality rate increased to 83.1%, 87.1%, 98.0%, 99.2%, 99.9%, and100% after 5, 10, 15, 20, 25 and 30 minutes respectively.One hundred percent mortality rate was observed with *Mentha longifola* extract at concentration of 50 mg/ml after 20 min of exposure. The difference between the scolicidal effect of *Mentha longifola* extract was statistically highly significant ($P \leq 0.0001$) for all concentrations and at various exposure times, comparing to the control group.

Table 1. Scolicidal effect of garlic (*Allium sativum*) extract at the Concentration of 25 mg/ml following various exposure times*

Exposure time (min)	Experiments	Protoscolices	Dead protoscolices	Mortality rate (%)
5	1	500	410	82.0
	2	560	450	80.4
	3	450	390	86.7
	Total	1510	1250	82.8
10	1	400	345	86.3
	2	450	400	88.9
	3	450	385	85.6
	Total	1300	1130	86.9
15	1	420	385	91.7
	2	450	420	93.3
	3	400	375	93.8
	Total	1270	1180	92.9
20	1	490	480	97.9
	2	509	500	98.2
	3	449	440	98.2
	Total	1448	1420	98.0
25	1	551	543	98.6
	2	423	420	99.3
	3	579	578	99.8
	Total	1553	1541	99.2
30	1	500	500	100
	2	504	504	100
	3	508	508	100
	Total	1512	1512	100
Control	NO.	1580	260	16.5

* $P \leq 0.0001$

Table 2. Scolicidal effect of garlic (Allium sativum) extract at the concentration of 50 mg/ml following various exposure times

Exposure time (min)	Experiments	Protoscolices	Dead protoscolices	Mortality rate (%)
5	1	460	440	95.7
	2	542	525	96.9
	3	540	513	95.0
	Total	1542	1478	95.9
10	1	463	452	97.6
	2	555	550	99.1
	3	490	480	98.0
	Total	1508	1482	98.3
15	1	417	417	100
	2	485	485	100
	3	419	419	100
	Total	1321	1321	100
20	1	473	473	100
	2	422	422	100
	3	536	536	100
	Total	1431	1431	100
25	1	512	512	100
	2	540	540	100
	3	463	463	100
	Total	1515	1515	100
30	1	523	523	100
	2	542	542	100
	3	520	520	100
	Total	1585	1585	100
Control	NO.	1580	260	16.5

Table 3. Scolicidal effect of *Mentha longifola* extract at the concentration of 25 mg/ml following various exposure times

Exposure time (min)	Experiments	Protoscolices	Dead protoscolices	Mortality rate (%)
5	1	466	450	96.6
	2	512	489	95.5
	3	525	310	97.1
	Total	1503	1249	83.1
10	1	532	470	88.3
	2	445	388	95.5
	3	450	385	85.6
	Total	1427	1243	87.1
15	1	490	480	97.9
	2	509	500	98.2
	3	449	440	98.2
	Total	1448	1420	98.0
20	1	551	543	98.6
	2	423	420	99.3
	3	579	578	99.8
	Total	1553	1541	99.2
25	1	512	510	99.6
	2	429	429	100
	3	445	445	100
	Total	1386	1384	99.9
30	1	500	500	100
	2	504	504	100
	3	508	508	100
	Total	1512	1512	100
Control	NO.	1460	200	13.7

* $P \leq 0.0001$

The scolicidal effects of *Zingiber officinale* extract are summarized in tables 5 and 6. *Zingiber officinale* extract at the concentration of 25 mg/ml killed (19.1, 22.5, 31.5, 41.0, 50.6 and 60.2) % of the protoscolices after 5, 10, 15, 20, 25 and 30 min of application, respectively. The scolicidal activity of *Zingiber officinale* extract at the concentration of 50 mg/ml was 96.0 % after 30 min of application. The scolicidal effect of both concentrations of the methanolic extract of *Zingiber officinale* was extremely significant compared to the control groups at all exposure times ($P \leq 0.001$). The result showed that 50 mg/ml of *Zingiber officinale* extract have high scolicidal activity in vitro.

Table 4. Scolicidal effect of *Mentha longifola* extract at the concentration of 50 mg/ml following various exposure times

Exposure time (min)	Experiments	Protoscolices	Dead protoscolices	Mortality rate (%)
5	1	514	470	91.4
	2	460	415	89.0
	3	460	414	90.0
	Total	1434	1299	90.5
10	1	560	532	95.0
	2	560	540	96.4
	3	472	450	95.3
	Total	1583	1524	96.2
15	1	465	460	98.9
	2	525	520	99.1
	3	480	474	98.0
	Total	1470	1454	98.9
20	1	473	473	100
	2	422	422	100
	3	536	536	100
	Total	1431	1431	100
25	1	512	512	100
	2	540	540	100
	3	463	463	100
	Total	1515	1515	100
30	1	523	523	100
	2	542	542	100
	3	520	520	100
	Total	1585	1585	100
	Control	1460	200	13.7

Table 5. Scolicidal effect of Zingiber officinale extract at the concentration of 25 mg/ml following various exposure times

Exposure time (min)	Experiments	Protoscolices	Dead protoscolices	Mortality rate (%)
5	1	420	75	17.9
	2	450	90	20.0
	3	410	80	19.5
	Total	1280	245	19.1
10	1	400	95	23.8
	2	420	100	23.8
	3	400	80	20.0
	Total	1220	275	22.5
15	1	530	175	33.0
	2	400	110	27.5
	3	450	150	33.3
	Total	1380	435	31.5
20	1	510	200	39.2
	2	400	150	37.5
	3	553	250	45.2
	Total	1463	600	41.0
25	1	440	220	50.0
	2	500	255	51.0
	3	450	228	50. 7
	Total	1390	703	50.6
30	1	440	270	61.4
	2	447	250	55.9
	3	450	285	63.3
	Total	1337	805	60.2
Control	NO.	1390	155	11.2

* $P \leq 0.001$

DISCUSSION

Cystic echinococcosis was among the most neglected parasitic diseases. Development of new drugs and other treatment modalities receives very little attention, if any, and is slow. Clinical management procedures have evolved over decades without adequate evaluation of important features such as efficacy, effectiveness, rate of adverse reactions, relapse rate, and cost (Junghanss *et al*., 2008). The control of helminthosis and generally of all parasitic diseases is usually made with synthetic anithelmintics. Up to date, many chemical scolicidal agents have been used for inactivation of the hydatid cyst protoscolices.

Table 6. Scolicidal effect of Zingiber officinale extract at the concentration of 50 mg/ml following various exposure times

Exposure time (min)	Experiments	Protoscolices	Dead protoscolices	Mortality rate (%)
5	1	450	215	47.8
	2	500	210	42.0
	3	430	175	40.7
	Total	1380	600	43.5
10	1	530	310	58.5
	2	420	225	53.6
	3	470	260	55.3
	Total	1420	795	55.9
15	1	466	350	75.1
	2	512	344	67.2
	3	525	330	62.9
	Total	1503	1024	68.1
20	1	445	350	78.7
	2	470	400	85.1
	3	500	410	82.0
	Total	1415	1160	81.9
	1	450	385	85.6
25	2	532	470	88.3
	3	440	400	90.9
	Total	1422	1255	88.3
30	1	483	480	99.4
	2	505	500	99.0
	3	449	440	98.0
	Total	1437	1420	98.8
35	1	445	445	100
	2	500	500	100
	3	450	450	100
	Total	1395	1395	100
Control	NO.	1390	155	11.2

Many of these scolicidal agents may cause undesirable complications that limit their use, because they need a higher concentration or longer time to have a notable effect. For example adverse side effects have been reported for 20% hypertonic saline, 20% silver nitrate, 0.5% - 1% cetrimide, ethyl alcohol (95%), and 20 mg/ml albendazole sulfoxide (Moazeni and Nazer, 2010). The appearance of resistance to synthetic anthelmintics stimulated the research of alternatives, such as medicinal plants (Pessoa, 2002). According to circumstances and depending on their efficacy, naturally produced plant anthelmintics offer an alternative that can overcome some of these problems and is both sustainable and environmentally acceptable (Elissondo, 2008). An ideal scolicidal agent was define as being potent in low concentrations, acting in a short period time, being stable in cyst fluid, not affected by dilution with the cyst fluid, being able to kill the scolex in the cyst, being non - toxic, having low viscosity, and readily available and easily prepared, as well as being inexpensive (WHO, 1996). The results of our study showed the potency of ethanolic garlic (*Allium sativum)* extract on the scolices of hydatid cyst (high scolicidal activity) at the concentrations of 25 and 50 mg/ ml after 30 and 15 min of application, respectively. Sadjjadi *et al*., investigated the protoscolicidal activity of aqueous, chloroformic and hydro-alcoholic extracts of garlic, they concluded that the chloroformic extract of garlic had a 97.9% scolicidal effect at a concentration of 200 mg/ ml after 30 minutes of exposure (Sadjjadi *et al*., 2008). While Moazeni observed a higher scolicidal effect (100%) with ethanolic extract of garlic at a lower concentration (50 mg/ ml) and in a shorter exposure time (10 minutes) (Moazeni and Nazer, 2010). Also the garlic had more potent scolicidal effects among all the 3 plants and the chloroformic extract of garlic was the most potent protoscolicid among all of the extracts and killed 98% of protoscolices in 50 mg/ml on a minimum of 20 minutes exposure (Eskandarian, 2012). The results of these in vitro studies are in agreement

with our findings and showed that extracts of garlic are effective scolicidal agents and therefore may be used in hydatid cyst surgeries. Garlic has been used as herbal medicine for thousands of years for different medical purposes (Sadjjadi *et al*., 2008) .However, the in vivo efficacy of this extract remains to be explored. Even though garlic is edible, it's possible side effects when used as a scolicidal agent need more investigation. Based on our searches, we couldn't find any special study that investigates the scolicidal efficacy of *Mentha longifola*, although they have some other medicinal effects. Significant advances have been made in the chemotherapy of the metacestode stage of *Echinococcus*. Experiences from animal experiments and human trials provide an encouraging basis for future studies, which should include research into new formulations of benzimidazoles, combinations of existing anti-hydatid drugs, and a continued search for new and effective agents (Wen *et al*., 1993).

According to the results of our study, the scolicidal activity of *Mentha* extract at a concentration of 25 mg/ml (30min) and 50 mg/ml (20 min) is comparable with scolicidal power of 95% ethyl alcohol (15 min) (Erzurumlu *et al*., 1998). hypertonic saline (45 min) (Caglar *et al*., 2008) and 0.5–1% cetrimide (10 min) (Frayha *et al*., 1981). To the best of our knowledge, this is the first report that investigates the scolicidal efficacy of *Mentha* extract on the protoscolices of hydatid cysts. The results of this study allowed us to suggest that *Mentha* is likely source of new compounds that could be used as an effective scolicidal agent, metabolites includes: flavonoids, polyphenols, tannins, antioxidant and free radical scavenging activity of various polyphenols. Polyphenols also posses many biological effects and these were generally attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Javidnia *et al*., 2004). In the present study, we investigated the effectiveness of ethanolic extract of ginger (*Zingiber officinale*) on the protoscoleces of hydatid cyst, Results during the present work pointed out that *Zingiber officinale* extract at a concentration of 50 mg/ml can kill all protoscoleces after 35 min of application, although *Zingiber officinale* showed lower scolicidal power in comparison with *Allium sativum* and *Mentha longifola*, it is comparable with results of Moazeni and Nazer who find that concentration 25 mg/mL, 50mg/mL and 100 mg/mL concentration killed 100% of protoccoleces after 60min, 40min and 30min of application respectively (Moazeni and Nazer, 2011). Our data suggest that medicinal plants can be a promising source of potent antiprotoscolices. We recommend further research on the in vivo efficacy of *Allium sativum*, *Mentha longifola* and *Zingiber officinale* extract and its potential side effects, at low concentration with few time.

Acknowledgement

A special debt gratitude to the staff of Al-Najaf abattoir in Iraq and technicians in the laboratory of microbiology department of faculty of Veterinary Medicine in the University of Kufa. Also my sincere thanks to all staff in the faculty for providing me this opportunity to complete our research program.

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