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Research Article

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EVALUATION OF SCOLICIDAL EFFECT OF *Teucrium polium, Zingiber officinale* AND *Nigella sativa In-Vitro* ON *Echinococcus granulosus*

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ABSTRACT

Hydatid disease is still a serious public health concern for developing countries. It is one of public health and economic problem. The basic approaches for treatment of hydatid disease are surgery and chemotherapy. Due to risk of surgery and cost of chemical treatments, it is recommended on alternative. The purpose of this study is to evaluate the effect of alcoholic extracts of *Teucrium polium, Zingiber officinale* and *Nigella sativa in-vitro*. Protoscolices of *Echinococcus granulosus* were collected from sheep liver containing hydatid cysts and were exposed to four different concentration extract of *T. polium, Z. officinale* and *N. sativa* for 10, 20, 30, 40, 50 and 60 minutes. The effect of these extracts had been studied on the viability of protoscolices of *E. granulosus in-vitro* in comparison with unexposed control. The extract of three treatments (*T. polium, Z. officinale* and *N. sativa)* had significant effect on hydatid cyst protoscolices in comparison with unexposed control. The effect of three extracts was statistically significant. It is revealed that the scolicidal activity of *T. polium* extract was greatest followed by *Z. officinale* and *N. sativa*. It is concluded that the alcoholic extract of *T. polium, Z. officinale* and *N. sativa* could be a natural scolicidal agent of hydatid cyst.

Keywords: Hydatid disease; *T. polium*; *Z. officinale*; *N. sativa*; *Echinococcus granulosus*; Protoscolices.

INTRODUCTION

Hydatid cyst is a major public health problem in developing countries[1]. The larval infection is characterized by long term growth of the metacestod in the intermediate host[2]. Cystic echinococcosis in widely distributed throughout the world and is still an important public health challenge in many countries of the world[3]. The disease in Iraq regarded as one of the most important public health and socio-economic problem[4].

The chemotherapeutic treatment echinococcosis remains unsolved since no currently available drugs are totally effective against hydatid cyst[5]. Until now surgical removal of the cyst is the only accepted way for the treatment[6]. However this line of treatment is relatively costly in addition to that spillage of hydatid fluid during surgery may lead to secondary cyst formation due to implantation of the protoscolices and/or development of allergic reaction which may lead to anaphylactic shock[7]. Precutaneous drainage is a method for hydatidosis and has provided alternative to surgery[8].

Several trials have been conducted on the use of chemotherapy most of them were of no proven efficacy[9].

The attention of many researchers was divided toward herbal medicine in treatment of parasitic disease, because of occurrences of drug resistance to chemical treatments. Yones, *et al*, reported that naturally produced plant anthelmintic offer an alternative that can overcome some of these problem[10].

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Nigella sativa L is commonly known as black seed, it is grown in different parts of the world. Traditionally used as a natural treatment for different pathological conditions[11]. In addition to its antibacterial, antifungal, antiviral and antiparasitic effects[12]. The active principle of *N. sativa* includes thymoquinone, carvacral, p- cymere and thymal[13].

Mahmoudvand et al. carried study on *in-vitro* scolicidal effect of *N. sativa* essential oil and its active principles thymoquinone, against protoscolices of hydatid cysts. They revealed that the essential oil of *N. sativa* at the concentration of 10 mg/ml and its main component, thymoquinone, at the concentration of 1 mg/ml had potent scolicidal activities against protoscolices of *E. granulosus* after 10 minutes exposure[12].

It has been reported that seeds of *N. sativa* a dicotyledone of the Ranunulaceae family, have pharmacological effect against Giardia, Blastocystis hominis, Trichomonas, malaria and some nematode and cestodes[14].

Ginger is a common name of *Zingiber officinale*; belong to family Zingiberaceae which contains a variety of compound which has insecticidal effect[15]. It is also shown that, it has carminatives, antipyretic, anticancer, cardiac tonic, antispasmodic, antidiabetic, antioxidant and antihepatotoxic activities[16].

Scolicidal activity of methanolic extract of *Zingiber officinale* against protoscolices of hydatid cyst from sheep liver has been reported. It was found that the scolicidal activity of *Z. officinale* extract at concentration of 100mg/ml was greatest than 50 mg/ ml and 25mg/ ml, killing the protoscolices after 10, 20, 30 and 40 minutes of

exposure[17].

Teucrium polium known as felty germander is a sub- shrub and herb native to the Western Mediterranean region. Its leaves are used in cooking and for medicine, particularly for the treatment of stomach ailments, and also used in visceral pain. In traditional medicine, it is used as an antihypertensive, antibacterial, carminative, anti-inflammatory, antidiarrhea, antidiabetes, anticonvalscent and antimalarial agent.[18]

The aim of the present study was to evaluate the scolicidal activity of *T. polium*, *Zingiber officinale* and *Nigella sativa* extracts *in-vitro*.

MATERIALS AND METHODS

Hydatid cysts

The cysts were obtained from infected liver of sheep naturally infected with hydatid disease, slaughtered at Kirkuk slaughterhouse and carried in cooled boxes to the Kirkuk hospital laboratory. All the tissues covered the hydatid cyst were removed and then the external surface was sterilized with 70% ethanol. The fluid was aseptically drawn from cysts. 50ml of fluid was transferred to glass cylinder and left for half an hour to settle the protoscolices at the bottom of cylinder. The supernatant was discarded and settled protoscolices were collected and washed several times in phosphate buffer solution (PBS).

The viability of protoscolices was determined by their motility and flame cell activity by staining method using 0.1% eosin solution and examined under light microscope (x10). The experiments were performed in triplicate[21,22].

Collection and Preparation of Alcoholic Plant Extract:

The plants were cleaned from impurities and kept at laboratory temperature in a dry place. 20g of plants (*Nigella sativa* seeds ground by blender to get on fine powder, powder of *Z. officinale* and flowers of *T. polium*) were put in Thimbles of Soxhlet Apparatus according of the method of (Al- Zohyri, 1982)[21], added 200 ml of 96% ethanol and for 24 hours. After that the extracted material was concentrated by the rotary evaporator at temperature 40-45°C. After evaporation of ethanol thick textured gelatinous material was observed. The stoke solution was prepared, by dissolving 1gm of the extract in 5ml of distill water to give a concentration of 200mg/ml.

Four different concentration were prepared (25, 50, 75 and 100 mg/ml for *T polium* and *Z officinale* while 125, 150, 175, 200) mg/ml for *Nigella sativa*) to study the effects on the vitality of protoscolices[24].

Statistical analysis

Statistical Analysis System (SAS)[24], was used to show the effect of different factors in the study. The data were presented as mean ± standard deviation, using statistical software. Chi- square test used to compare between control and experimental groups.

RESULTS AND DISCUSSION

Table**1** showed high scolicidal activity of alcoholic extract of *Teucrium polium* at four concentrations 25, 50, 75 and 100 mg/ml after different exposure for 10, 20, 30, 40, 50 and 60 minutes. The mortality rate of protoscolices exposed to 25mg/ml was 34 %, 48.6 %, 57.3 %, 67.5 % , 75.6%, and 87.3% at 10, 20, 30, 40, 50 and 60 minute. While at 50 mg/ml the mortality rate increased to 100% were seen after exposure for 50 & 60 minutes. At 75mg/ml concentration 100% mortality were seen at 40, 50 & 60 minutes of exposure time. The mortality rate at concentration 100mg/ml had 100% scolicidal effects at 30, 40 and 50 minutes of exposure time. The difference between scolicidal effects of *T. polium* extract was statistically highly significant in all treatmet groups.

Table 1. Mortality rate of protoscolices using different concentration of *Teucrium polium* at various exposure time.

Exposure time (min.)	Control	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
10	4	34	73.33	83.35	87.43
20	7.93	48.6	90.2	94.86	99.5
30	11	57.3	93.66	99.56	100
40	12.6	67.5	99.7	100	100
50	15.6	75.6	100	100	100
60	18.9	87.3	100	100	100
Anova D. F. F. Value	14.316 5 P<0.001	19.9 5 P<0.0001	123.71 5 P<0.0001	119.39 5 P<0.0001	155.58 5 P<0.0001

Zingiber officinale extract, at various exposure times was found to be effective against protoscolices at all four test concentrations. The mortality rate also increased in all four treatment group and reached 100% at 75 mg/ml after exposure of 60 minutes while at 100 mg/ml the mortality rate reached 100 % on exposure to 40, 50 and 60 minutes. Statistically there was significant difference between treatment groups, as shown in table **2**.

Table 2. Mortality rate of protoscolices using different concentration of *Zingiber officinale* at various exposure time.

Exposure time (min.)	Control	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
10	4.0	38.9	55	69	77
20	7.93	54	75	77	86
30	11.0	68	80	87	97
40	12.6	75	86	93	100
50	15.6	84	93	99.5	100
60	18.9	96	99.5	100	100
Anova D. F. F. Value	14.316 5 P<0.001	32.75 5 P<0.0001	3.385 5 P<0.05	6.097 5 P<0.01	9.678 5 P<0.01

Concerning alcoholic extract of *Nigella sativa*, the mortality rate of hydatid cyst protoscolices at various time of exposure is shown in table **3**. In all treatment groups, the mortality rate increased with increasing the concentration, and exposure time. Although the scolicidal effect increased but did not reach to 100% in all treatment groups and increasing the time of exposure. At 200mg/ml the mortality rate reached to 94% after exposure for 60 minutes. Statistically there was highly significant difference between different concentrations of treatment at various times of exposure.

Several chemical scolicidal agents have been used for

inactivation of hydatid cyst protoscolices, such as albendazole sulfoxide, 20% hypertonic saline, 20% silver nitrate, 0.5–1% cetrimide, chlorhexidine gluconate, ethyl alcohol, unfortunately many of these agents have dangerous adverse side effects and their efficacy is controversial[24].

Table 3. Mortality rate of protoscolices using different concentration of *Nigella sativa* at various exposure time.

Exposure time (min.)	Control	125 mg/ml	150 mg/ml	175 mg/ml	200 mg/ml
10	4.0	10	17	35	49
20	7.93	14	26	40	58
30	11.0	26	38	57	67
40	12.6	40	46	64	77
50	15.6	54	60	75	86
60	18.9	64	73	84	94
Anova D. F. F. Value	14.316 5 P<0.001	58.16 5 P<0.0001	41.5 5 P<0.0001	26.48 5 P<0.0001	9.39 5 P<0.001

The control of helminthes including hydatid cyst is usually made with antihlmintics. The appearance of resistance to synthetic antihelmintics stimulates the researchers to investigate herbal alternatives treatment[25].

In the present study, the potency of alcoholic extract of *Teucrium polium, Zingiber officinale* and *Nigella sativa* on protoscolices of hydatid was investigated *in-vitro*, using different concentration at various time of exposure.

It seems that the scolicidal effect of *T. polium* of different concentration increased with increasing the concentration and time of exposure. The death rate of protoscolices reached 100 % at concentration of 50 mg/ml after 50 minute; at concentration of 75 mg/ml after 40 minutes while at concentration 100 mg /ml after 30 minutes.

The plant liquid extract is used in the treatment of abscesses[26]. In Kirkuk (Iraq), a study done on the effect of medical herbs on protoscolices. She found that effect of *T. polium* on activity of protoscolices increased with the increasing of their concentration, and the effect of *herba-alba* was greater than *T. polium*[27].

Our study is in agreement with a study done in Mousl city, who found that the alcoholic extract of *T. polium* at the concentration 5, 10, 15, 20, 25 and 30 mg/ml had significant effect on viability of protoscolices of *E. granulosus* of sheep origin in vitro, Hence the concentration that caused death of all protoscolices was at 30, 45, 60 minutes respective[18].

The effectiveness of different concentration of alcoholic extract of *Z. officinale* showed the death rate increased with increasing the concentration and exposure time. The scolicidal effect at concentration of 25, 60, 75 & 100 mg/ml reached 96%, 99.5 %, and 100% after exposure for 60 minutes, while at concentration 100% mortality occur at 40 minutes of exposure. The exact mechanism of scolicidal effect of *Z. officinale* is not clear, further study is required to identify and isolate its active compounds.

It is shown that the alcoholic extract of *N. sativa* seed posses scolicidal activity. Although its effect of different concentration was increased with increasing the exposure in comparison with control, but it did not reach to 100% motility.

The scolicidal activity of 125, 150, 175, and 200 mg/ml reached to 64 %, 73 %, 84 % and 94 % on exposure for 60 minute. This is in contrast to study of Mahamoud *et al*[12], who found that the scolicidal effect was 100% with methanolic extract at concentration of 50 and 25 mg/ml. This might be due to difference in extraction used in both studies. The exact mechanism of antihelmintic effect of *N. sativa* is not clear. Never the less[28], showed that *N. sativa* could inhibit synthesis by inhibiting bistone deacetylase (HDAC) enzyme interacting with the chromosome. It seems from the results of this study that the scolicidal effect of *N. sativa* was less than *T. polium* and *Z. officinale*.

In Baghdad Iraq, Al- Basheer, *et al*[29] studied the effect of *N. sativa* extract on the viability of *Echinococcus granulosus in-vitro* using five concentrations of *Myrtus communis* leaves and *Nigella sativa*, they found that the effect of *N. sativa* was less than *Myrtus communis*.

CONCLUSION

The finding of the present study demonstrated a potent scolicidal activity of various alcoholic extracts (*Teucrium polium*, *Zingiber officinale* and *Nigella sativa*). The efficacy of *T. polium* and *Z. officinale* alcoholic extracts was greater than *N. sativa*. *T. polium* and *Z. officinale* can be considered as a natural scolicidal agents against hydatid cyst protoscolices. Further studies are required to evaluate the efficacy of more medical plants, mode of actions and *in-vivo* effects of these medical plants.

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