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Inhibitory Effectiveness of Musk on Viability of Protoscolices of Hydatid Cysts

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ABSTRACT

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Hydatid disease is considered as one of the important zoonotic diseases, due to the complications and the difficulties of the diagnosis and treatment. The present work evaluates the effect of musk concentrations on the viability of *Echinococcus granulosus* protoscolices *in vitro*. Three different concentrations of musk 25, 50 and 100% were used, in addition to control for 10, 20, 30, 40, 50 and 60 min. While the rate of dead protoscolices was 6.98% in the control group, when protoscolices were exposed to musk concentration of 25% , the rate of dead protoscolices increased to 21.44, 34.20, 43.00, 51.40, 79.34 and 100% after 10, 20, 30, 40, 50 and 60 minutes, respectively . The mortality rate of protoscolices increased to 53.93, 81.36, 97.04 and 100% when they were exposed to the concentration of 50% after 10, 20, 30, and 40 min, respectively. One hundred percent mortality rate was observed at concentration of 100% after 30 min of exposure. This *in vitro* study showed that musk can be a promising source of potent antiprotoscolices.

Introduction

Parasitic infections of plants, humans, and other animals pose a worldwide problem. For example, more than 650 million people are at risk for gastrointestinal parasitic infection, and about 200 million are actually infected (Pakshir *et al.*, 2009). 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; Fasihi Harandi *et al.*, 2012). Clinical manifestation of hydatidosis is

characterized by tumor-like growths that occur mostly in the liver and lungs, with varying degrees of infestation of other organs (Abdel-Hafez and Al-Yaman 1989). These growths are usually filled with a watery fluid known as 'hydatid cyst fluid.' The released embryos penetrate the intestinal wall and via the portal system enter mainly into the liver (50- 70%), lungs (20-30%), or any other organs where the hydatid cysts grow up (Eckert and Deplazes, 2004). Unfortunately, numerous reports

indicate that hydatidosis incidence has increased in various regions of the world (Eckert *et al.*, 2000). It has been identified as a global public health and economic problem particularly in developing regions including Iraq, which causes serious morbidity and death if left untreated. Measurement of the socio-economic impact of echinococcosis/ hydatidosis represents an important challenge in the involved societies. In humans, the quantifiable items of disease are those connected with preoperative diagnosis, surgical treatment, hospitalization, post surgical examination and drugs (Cook and Zumla, 2003). In many countries, hydatid disease is more prevalent in rural areas where there is close contact between people, dogs, and various domestic animals which act as intermediate hosts (Frider and Larrieu, 2010). Since the mid-1970s, significant advances have been made in the chemotherapy of the metacestode stage of *Echinococcus*. *In vitro* and *in vivo* techniques for drug testing are now available, and considerable clinical, pharmacological, and parasitological knowledge has accumulated (Moazeni and Roozitalab, 2010).

Musk was extensively used not only as a fragrance but also for medicinal purposes since ancient times (Takaoka, 2007). Musk is formed from several compounds, the main compound which causes the odour is muscone (3- methylcyclopentadecan-1-one) the active ingredient of musk (Lai,1976) has medicinal properties. Other compounds present in musk include steroids, paraffins, triglycerides, waxes, muco pyridine, other nitrogenous substances and fatty acids (Oh *et al.*, 2002; Thevis *et al.*, 2013). Musk is an effective detoxification agent and can be used to reduce pain, inflammation and swelling. It can also be used to treat seizures (Zhu 1989; Cheng *et al.*, 2011). The activity of Musk was studied on the parasitic

Trichomonas vaginalis by Badawy *et al.* (2014), fungi and bacteria by Saddiq (2014) and AL-Jobori *et al.*(2014,2016). There are currently three treatment options for hydatid disease, surgery, ultrasound-guided aspiration, and chemotherapy (WHO,1996). There is clearly a need for drugs that are more effective and easier to administer. Many efforts have been made to discover new antiparasitic compounds from various kinds of sources such as plants, animals and microorganisms. The present study was conducted to evaluate the *in vitro* effect of Musk on the protoscolices of hydatid cysts.

Materials and Methods

Parasite Material

Fresh hydatid cysts were obtained from livers of naturally-infected sheep, which had been slaughtered at local abattoirs in Baghdad province . They were wrapped carefully in clean plastic bags, placed in an ice box, and in the same day of slaughter transported to the Department of Parasitology, University of Baghdad, where protoscoleces were extracted.

Preparation of Protoscolices

Protoscoleces were extracted according to Smyth (1967). The outer surfaces of the cysts were sterilized with 70% ethanol before being dissected and its withdrawn fluid sac by syringe (10 ml) then wash the cysts from the inside with a solution of phosphate (pH of 7.2) and collected in Phosphate buffer solution (PBS) in test tubes ,then underwent washing process by centrifuge three times per 3000 c/m and take the precipitation contain the protoscoleces.

Musk

Synthetic musk (a Pakistani product) was

purchased from local Iraqi markets. To determine the scolical activity of Musk upon the protoscoleces of hydatid cysts, various concentrations of Musk 25, 50, 100% and alcohol as control were used for 10, 20, 30, 40, 50 and 60 min.

***In vitro* Scolicidal Effects of Musk**

In this study four concentrations of Musk (0, 25, 50 and 100 %) were used. A total of 2.5 mL of each Musk concentration was placed in test tubes, to which a drop of protoscolex-rich sediment was added. The contents of the tubes were gently mixed. The tubes were then incubated at 37°C for 10, 20, 30, 40, 50 and 60 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 500 protoscolices. Non treated protoscolices were considered as a control group in each experiment. The experiments were performed in five replications.

Viability Test

The viability of protoscolices was determined prior to the experiments. A 0.01 ml solution of pooled protoscolices was transferred over a slide and mixed with 0.01 ml of 0.1% aqueous eosin stain, as a vital staining, and was evaluated by low power microscopy after 5 min. Unstained protoscolices were considered as viable while stained protoscolices were considered

as non-viable (Smyth and Barrett, 1980). When the percentage of viable protoscolices in the sediment was 95% or more, they were considered to be appropriate for experiments. The percentage of viable protoscolices (viability rate) was determined by counting a minimum of 100 protoscolices (as a ratio of number of viable protoscolices to total protoscolices).

Statistical Analysis

The recorded data were statistically analyzed using Statistical Analysis System-SAS -computer package program (SAS, 2012). The mean values were compared with the least significance difference (LSD) test following (Steel and Torrie, 1980) at 5% level.

Results and Discussion

Viability of Protoscolices

The viability of protoscolices was tested prior to the experiments using 0.1% aqueous eosin stain. Dead protoscolices absorbed eosin and colored red, but alive protoscolices remained colorless and showed characteristic muscular movements and flame cell activity (evaluated under light microscope). Viability of protoscoleces Untreated protoscoleces remained viable for 60 min prior to treatment, with no significant differences in viability between 0 and 60 min ($P < 0.05$).

***In vitro* Treatment of Protoscoleces**

The viability of protoscoleces was significantly affected when treated with musk. As shown in Figures 1 that different concentrations of musk following different exposure times had potent scolical effects against protoscoleces of hydatid cysts. Musk showed high scolical activity

against protoscolices at all three concentrations tested. While the mortality rate of protoscolices was 6.98% in the control group (untreated), when protoscolices were exposed to the musk at concentration of 25%, the mortality rate increased to 21.44, 34.20, 43.00, 51.40, 79.34 and 100% after 10, 20, 30, 40, 50 and 60 minutes, respectively (Table 1). The mortality rates of hydatid cyst protoscolices after exposure to concentration of 50 % of musk were 53.93, 81.36, 97.04 and 100% after 10, 20, 30, and 40 minutes, respectively (Table 2). The protoscolices treated with concentrations of 100% started to show a decrease in viability at 10 min post-treatment, one hundred percent mortality rate was observed after 30 min of exposure (Table 3). The difference between the scolicidal effect of musk was statistically significant ($P < 0.05$) for all three concentrations and at various exposure times, comparing to the control group.

Clinical management procedures had 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). To date, many scolicidal agents have been used for inactivation of the hydatid cyst content, but there has been no ideal agent that is both effective and safe (McManus *et al.*, 2003).

A number of drugs are being used and various degrees of success have been claimed. However, the metabolites of certain drugs including benzimidazole, mebendazole, albendazole, and albendazole sulfoxide are potentially toxic in some subjects (Pawlowski 1997). Also adverse side effects has been reported for 20% hypertonic saline, 20% silver nitrate, 0.5% - 1% cetrimide, ethyl alcohol, and 20 mg/mL albendazole sulfoxide (Moazeni and

Nazer, 2010). Among the most promising advances in the field of drug development is discovering new molecules or novel uses of the already available compounds with known safety and without any side effects (Mathew *et al.*, 2008). Considerable attention to alternative therapies, particularly using natural sources derived compounds for the treatment of diseases has been paid as many studies reviewed the effect of different medical plants on *Echinococcus granulosus* (Barzinji *et al.*, 2009; Yones *et al.*, 2011; Moazeni and Mohseni, 2012 ; Mahmoudvand *et al.*, 2014).

In the present study we investigated the potency of musk concentrations on the protoscolices of hydatid cyst. The results of our study showed that musk has a significant scolicidal activity ($P < 0.05$) at the concentrations of 25, 50 and 100 % after various exposure times of application (figure 1 and tables 1, 2, 3). In previous studies, we investigated the inhibitory effect of musk at concentrations 25, 50, 75, and 100% on bacteria and fungi (AL-Jobori *et al.*, 2014, 2016).

The inhibitory effect of Musk is due to its content of compounds, the main compound which causes the odour is muscone (3-methylcyclopentadecan-1-one) the active ingredient of musk (Lai, 1976), has medicinal properties. Other compounds present in musk include steroids, paraffins, triglycerides, waxes, muco pyridine, other nitrogenous substances and fatty acids (Ekwenye and Elegalam, 2005; Thevis *et al.*, 2013). The antimicrobial properties of musk have been established against wide spectra of microbes, including bacteria and fungi, using a variety of experimental methods (Cheng *et al.*, 2011; Saddiq, 2011, 2014). Also revealed motility inhibition of *Trichomonas vaginalis* trophozoite (Badawy *et al.*, 2014).

Table.1 Scolicidal Effect of Musk at the Concentration of 25 % Following Various Exposure Times

Time (min.)	Live Protoscolices	Dead Protoscolices	Mortality rate (%)
0	2000	150	6.98
10	1334	364	21.44
20	1314	683	34.20
30	1458	1100	43.00
40	1352	1430	51.40
50	1180	4540	79.37
60	0.00	2600	100.00
L.S.D 0.05	115.76	113.25	7.33

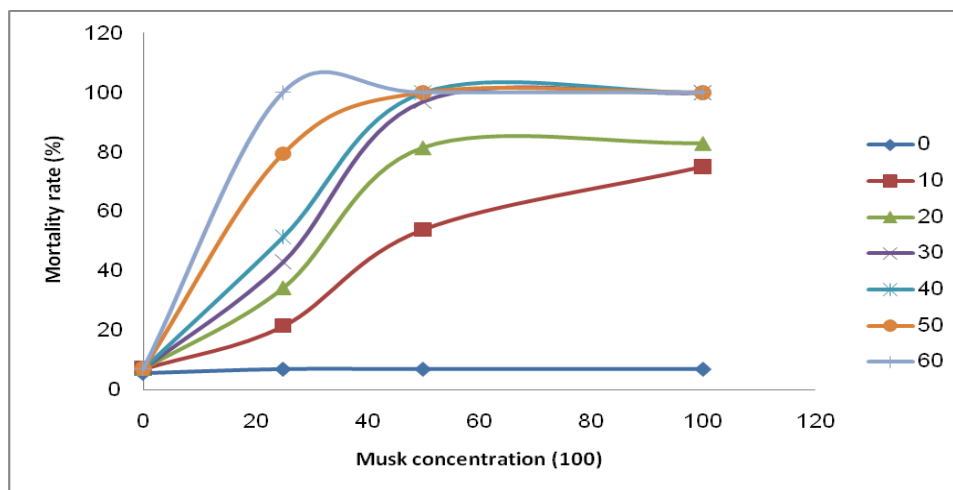
Table.2 Scolicidal Effect of Musk at the Concentration of 50 % Following Various Exposure Times

Time (min.)	Live Protoscolices	Dead Protoscolices	Mortality rate (%)
0	2000	150	6.98
10	1230	1440	53.93
20	710	3100	81.36
30	39	1280	97.04
40	0.00	2628	100.00
50	-	-	-
60	-	-	-
L.S.D. 0.05	103.22	100.99	6.56

Table.3 Scolicidal Effect of Musk at the Concentration of 100 % Following Various Exposure Times

Time (min.)	Live Protoscolices	Dead Protoscolices	Mortality rate (%)
0	2000	150	6.98
10	1420	4300	75.17
20	1140	5520	82.88
30	0.00	3340	100.00
40	-	-	-
50	-	-	-
60	-	-	-
L.S.D. 0.05	92.13	98.11	5.67

Figure.1 In vitro Lethal Effects of Musk against Protoscolices of Hydatid Cyst at the Various Concentrations Following Various Exposure Times



Efficiency of the medicinal plant is referred to its content of many efficient compounds similar to musk content such as flavonoids, peptids and peptide alkaloids and saponin and sugars (lactose, glucose galactose) as well as glycosides and vitamins A and C (Abdel-Zaher *et al.*, 2005). They may have target sites, such as inhibitors of protein or DNA synthesis, or within cytoplasmic components, like B-lactam antibiotics (Walker *et al.*, 2004). Suthar *et al.* (2010) demonstrated that *Nigella. sativa* oil could inhibit DNA synthesis by inhibiting histone deacetylase (HDAC) enzyme interacting with the chromosomes.

The ideal treatment for patients with hydatid disease should be complete elimination of the parasite and prevent recurrence of the disease with minimal morbidity and mortality. In this study the results showed that the concentration 25% of musk completely eliminated the parasite after 60 minutes, and the concentration of 50% completely eliminated the parasite after 40 minutes, whilst the concentration of 100% completely eliminated the parasite after 30 minutes (figure 1 and tables 1,2,3). This can be a promising source of potent

antiprotoscolices. The dose-dependent protoscolicidal effects of musk concentrations seem to be due to their role in breakdown of biological activities of protoscolices through interference with their metabolism.

In conclusion, this in vitro study showed that musk is an effective scolicidal agent. To the best of our knowledge, this is the first report that investigates the scolicidal efficacy of musk concentrations on the protoscolices of hydatid cysts. The results of this study allowed us to suggest that musk is likely source of new compounds that could be used as an effective scolicidal agent. Our data suggest that musk can be a promising source of potent antiprotoscolices.

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