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Research Article

In vitro evaluation of protoscolicidal effects of cetrimide and povidone-iodine in hydatid cyst disease

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Abstract

Echinococcosis is an endemic zoonotic disease caused by the dog tape-worm Echinococcus granulosus. Surgical treatment and percutaneous drainage have been used for the treatment of hydatid cysts. However, inactivation of the parasite with protoscolicidal agents is a crucial part in the treatment in both methods. The aim of this study was to evaluate the protoscolicidal effect of cetrimide and povidone-iodine in hydatid cyst disease. Fifty intact cysts of pulmonary hydatid disease of patients not received preoperative antihelmenthic were included in the study. While those cysts of patients who received preoperative antihelmenthic were excluded from the study. The protoscolicidal effect of cetrimide (0.05%, 0.1%, 0.5%) and povidone-iodine (10%) were assessed in this in vitro study using 1, 2 and 5 minutes as exposure time. Cetrimide (0.1%, 0.5%) have a higher protoscolicidal effect than 10% povidone-iodine that is statistically significant after different exposure time. The data concluded that cetrimide (0.1%) is a very effective protoscolicidal agent even with short exposure time, so it is the least concentration dependent and the least time dependent to achieve its protoscolicidal effect.

Keywords: Hydatid cyst; Viability; Protoscolicidal agents

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Introduction

Echinococcosis is an endemic zoonotic disease caused by the dog tape-worm Echinococcus granulosus. It particularly affects people living in the rural sheepfarming area. The disease

commonly occurs in the Middle East, the Mediterranean countries, Eastern Europe, South America, Australia and New Zealand [1]. Hydatid cyst fluid is actively secreted by the parasite thus a tense cyst wall indicates a living hydatid cyst, while a flaccid but intact wall is typical of non-viable cyst. The fluid pressure within a living hydatid cyst can reach 70 cm of water. Typically, this fluid is crystal clear, slightly alkaline, with specific gravity 1.005-1.009. However opaque fluid may be obtained from dead cyst as well as those secondarily infected or bile-stained fluid [2]. Fertile hydatid cysts contain myriads of scolices, which can be visible as grains of hydatid sand in otherwise clear or slightly yellowish cyst fluid [3]. However, the recurrence rate of this disease is still high, in the range of 920% [4]. Surgical treatment and percutaneous drainage have been used for the treatment of hydatid cysts [5, 6] However inactivation of the parasite with protoscolicidal agents is a crucial part in the treatment in both methods.
Many agents have been used intraoperatively as well as in percutaneous drainage to prevent the risk of spillage, and reduce the recurrence rate. The most frequently used agents are hypertonic saline, alcohol, and povidone iodine. During surgical treatment and percutaneous drainage, it is important that the agents injected into the cyst be effective on the

protoscolices both in the crystal-clear fluid and in the daughter cysts. As the daughter cysts are small samples of the cyst, they are extremely resistant structures and responsible for the survival of the cyst. The effect of protoscolicidal substances on protoscolices in the daughter cysts are not sufficiently well known, though they are, in practice, used very frequently [7]. The aim of this study was to evaluate the protoscolicidal effect of cetrimide and povidone-iodine in hydatid cyst disease.

Method

This is a prospective study were conducted at the al-Sader medical city during the period from 1st of January 2012 to 1st of January 2015.A total of 50 intact cysts of pulmonary hydatid disease of patients not received preoperative anti-helmenthic were included in the study. While those cysts of patients who received preoperative anti-helmenthic were excluded from this study. The cysts transferred to the laboratory of hospital immediately after surgery. These cysts divided into 5 groups, each group contained 10 cysts, these groups instilated with0. 05% cetrimide, 0.1% cetrimide, 0.5% cetrimide, 10% povidone-iodine and 0.09% NaCl as control group respectively. The cyst fluid aspirated completely by syringe and put in falcon tube and centrifuging with 500 round per minute for one minute where the protoscolices precipitated and separated. The viability of the protoscolices was assessed by motile behavior (flame cell activity) were slides has been prepared on which a drop of hydatid cyst sediment (precipitated protoscolices) examined with light microscope under 40X,100X and oil emersion power to detect this flame cell activity which

is flickering movement exhibited by the scolices and it mean viable protoscolex while if no flickering exhibited so it is non-viable protoscolex. Also to test the viability of protoscolices, eosin exclusion test was used [8]. 0.01 ml of precipitated protoscolices put over a slide and mixed with 0.01ml of 0.1% eosin and covered with cover slide and examined under light microscope under 10x and 40x after one minute. Viable protoscolices remain unstained where as non-viable protoscolices have damaged membrane and take up the eosin so stained (red). At least 100 protoscolices were counted for each test. When the percentage of viable scolices were more than (95 %), they considered appropriate for this study. When viable protoscolices with enough volume were obtained, 0.1 ml of precipitated protoscolices was put in a test tube and one ml of the scolicidal agent was mixed. After one minute of exposure, 5 ml of distilled water was added and centrifuged for one minute with 500 round per minute and the sediment taken to test the viability of protoscolices by the two methods mentioned. These steps were repeated for each agent (0.05 % cetrimide, 0.1% cetrimide, 0.5% cetrimide, 10% povidone-iodine) with each specific concentration using different exposure time (1, 2, 5 minutes). At least 100 protoscolices were counted for each test. The test repeated for each agent with its specific concentration and exposure time 20 times and the average number of non-viable and total number of protoscolices was counted.

Statistical analysis was performed using SPSS (statistical package for social sciences) version 17. Chi square test was used for categorical data. Set $P < 0.05$ as significant, in addition to the descriptive statistics for non-parametric data for comparing the protoscolicidal effect of different

agents. The mean percentage of nonviable protoscolices and the agents used to the exposure time were recorded. These tests were performed under aseptic technique where the solutions, including the eosin were sterilized. The test repeated for each cyst several times.

Result

The mean percentage of dead protoscolices and the protoscolicidal agents used with the time exposure were shown in table (1) which revealed that after 1 minute of exposure time, the protoscolicidal effects of 0.05%, 0.1 % and 0.5% cetrimide were 69.44%, 92.45% and 96.55% respectively while the protoscolicidal effects of 10% povidone-iodine was 57.40% compared with 8% for 0.9% Nacl as shown in figure (1). After 2 minutes of exposure time the scolicidal action of 0.05 %, 0.1 % and 0.5% cetrimide were 79.09%, 92.85% and 98.46% respectively, while the effect of 10% povidone-iodine was only 57.62%. After 5 minutes the scolicidal action was 89.84%, 97.14% and 99.31% for 0.05 %, 0.1 % and 0.5% cetrimide respectively, but 10% povidone-iodine showed only 59.31% protoscolicidal effects. The study showed that the scolicidal effects of 0.05% cetrimide are higher than 10% povidone iodine after different exposure time. However, its protoscolicidal effects are statistically not significant after one minute of exposure time, but statistically significant difference was observed in the protoscolicidal effects after 2 and 5 minutes, so cetrimide at this concentration need longer exposure time to achieve the higher protoscolicidal effect as shown in

figure (1). The study revealed that 0.1% Cetrimide has a much higher protoscolicidal effect than 10% povidone-iodine that is statistically significant after 1, 2 and 5 minutes' exposure time as shown in figure (1) and figure (2). However, this study showed that the protoscolicidal effects of 0.5% cetrimide are slightly higher than 0.1 cetrimide that is statistically not significant after different exposure time as revealed in figure (3). Therefore, it is worthy to say that lower concentrations of cetrimide can achieve similar protoscolicidal effects. However, this study showed that the protoscolicidal effects of 10% povidone-iodine were 57.40%, 57.62% and 59.31% after exposure times of 1, 2 and 5 minutes respectively, which revealed that 10% povidone-iodine have low protoscolicidal effects that is statistically not significant after different exposure time as shown in figure (4). So 10% povidone-iodine have the same protoscolicidal effect even after longer exposure time. Also the study showed that the protoscolicidal effects of 0.1% cetrimide are slightly higher with increasing the time of exposure of scolicidal agent that is statistically not significant after 2 and 5 minutes, so it is worthy to say that lower concentrations of cetrimide can achieve the similar protoscolicidal effect.

Figure 2. show the protoscolicidal effect of different agents after 5 minutes' exposure time.

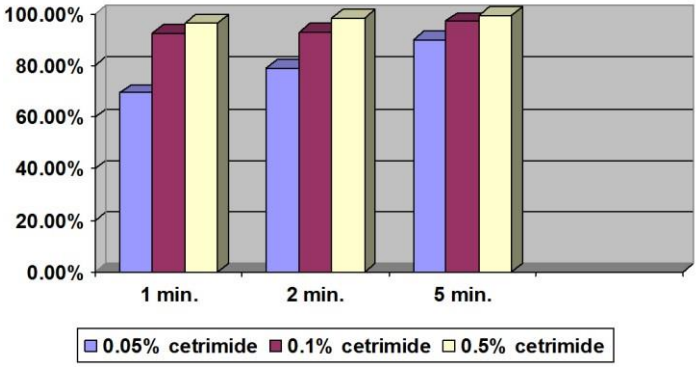

Figure 3. show the protoscolicidal effect of different concentration of cetrimide at different exposure time.

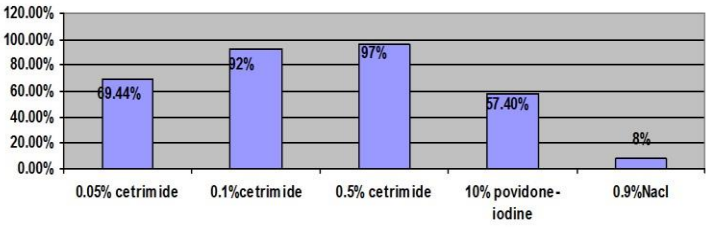

Figure 1.
Show the protoscolicidal effect of different agents after 1 minutes exposure time.

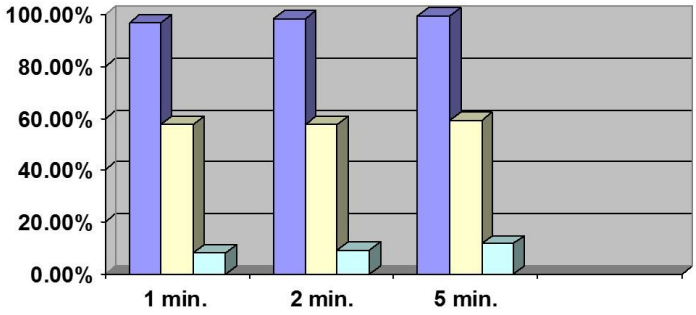

Figure 4.

Show the protoscolicidal effect of different agents at different exposure time.

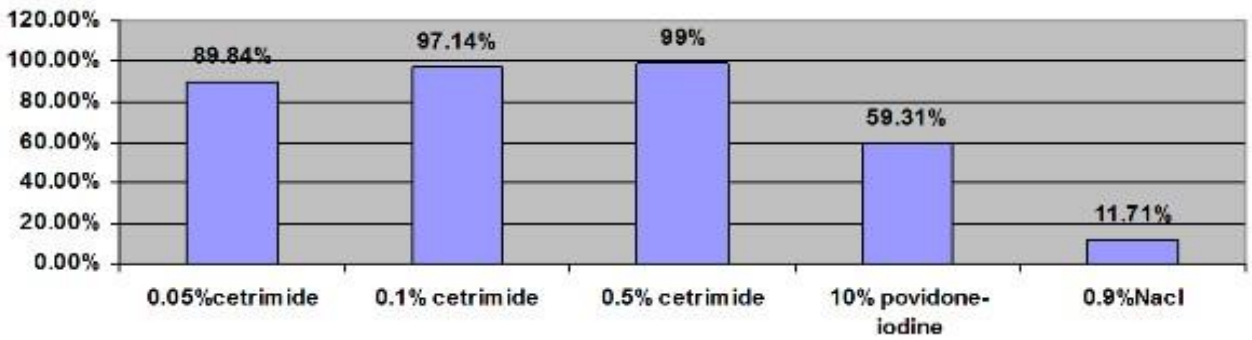

Table 1.

Shows protoscolicidal effects of different concentrations of cetrimide in comparison to 10% povidone iodine.

Protoscolicidal agents	Mean% of dead protoscolices (No. of dead /No. tested)			P value
	1 minute	2 minutes	5 minutes	
0.05% cetrimide	69.4%(75/108)ϕ¥β	79.09%(87/110)*ϕ¥β	89.84%(115/128)*ϕ¥β	0.0004
0.1% cetrimide	92.45%(98/106)*ϕ	92.45%(104/112)*ϕ	97.14%(136/140)*ϕ	0.195
0.5% cetrimide	96.55%(112/116)*ϕ	98.46(128/130)*ϕ	99.31%(145/146)*ϕ	0.473
10% povidone iodine	57.4%(62/108)ϕ	57.62%(68/118)ϕ	59.31%(86/145)ϕ	0.943
0.9% NaCl	8.33%(10/120)	9.32%(11/118)	11.71%(15/128)	0.653

*vs povidone iodine ϕvs NaCl ¥vs 0.5% cetrimide βvs 0.1% cetrimide

Discussion

Hydatid disease is still an endemic disease in particular regions of the world, its treatment using percutaneous aspiration injection and reaspiration (PAIR), laparoscopic or open method requires effective protoscolicidal agents as an integral part specially in a PAIR method so it is important to find the ideal protoscolicidal agent. To evaluate the effect of protoscolicidal agents used in this study the results were analyzed in

term of agent's factors, time factor and concentration factor. This study showed that the scolicidal effects of 0.05% cetrimide are higher than 10% povidone iodine after different exposure time. However, its protoscolicidal effects are statistically not significant after one minute of exposure time, but statistically significant difference was observed in the protoscolicidal effects after 2 and5 minutes, so cetrimide at this concentration need longer exposure time to achieve higher protoscolicidal effect as shown in figure [1]. This is consistent with the result of Powlowski et al, (2001) study, which reported that 0.05% cetrimide was a potent protoscolicidal agent [10]. Also the study reported that increasing the concentration of cetrimide from 0.05% to 0.1% will increase the effect of the agent that is statistically significant after any exposure time, but increasing the concentration of cetrimide from 0.1 % to 0.5% will increase the effect of the agent that is statistically significant after 1 and 2 minutes but statistically not significant after 5 minutes' exposure time. This is comparable to the result obtained by Frayha GJ, et al (1981) study, which found that the scolicidal effect of cetrimide at a low concentration [11]. Also, it is consistent with the result of Besim H et al (1998) study, which found that cetrimide has the least concentration dependent scolicidal agent among other agents studied [12]. Also, it is reported in previous studies that cetrimide has low toxicity because its low degree of absorption by host tissue make it the scolicidal agent of choice in hydatid cyst surgery [11]. Other previous study showed that cetrimide is a good protoscolicidal agent to prevent hydatid cyst recurrence after 2 years of follow up [13]. This study showed that increasing the time of exposure of protoscolices

to cetrimide (0.5%, 0.1%) will increase the effect of cetrimide that is statistically not significant after any exposure time. This is consistent with the results of Seyed Vahid et al (2001) study, which found that 0.5% cetrimide had the same effect after 1, 2 and 5 minutes [14]. Also, this is comparable to the result obtained by Frayha GJ. Et al (1981) study, which found that 0.1% cetrimide, has rapid action against protoscolices [11]. So 0.1% cetrimide has the highest protoscolicidal effect even after short exposure time. This is might be explained that cetrimide act rapidly and it is the least time–dependent protoscolicidal agent. However, increasing the time of exposure of protoscolices to 0.05 % cetrimide will increase the effect of the agent that is statistically not significant after 1 and 2 minutes, but statistically significant after

5-minutes so cetrimide at this concentration is time dependent. However, this study showed that the protoscolicidal effects of 10% povidoneiodine were 57.40%, 57.62% and

59.31% after exposure times of 1,2 and 5 minutes respectively which revealed that 10% povidone-iodine have low protoscolicidal effects. Also, this study reported that increasing the time of exposure of protoscolices to 10% povidone-iodine will increase the effect of the agent that is statistically not significant after any exposure time as shown in figure [4]. So 10% povidoneiodine have the same protoscolicidal effect even after long exposure time. The time factor is important in hydatid cyst surgery because previous studies showed that one of the factors that increase the risk of development of sclerosing cholangitis is the condition that prolongs the exposure of the biliary tree to the scolicidal solution [15]. The study

concluded that the cetrimide (0.1%) is a very effective protoscolicidal agent even with short exposure time, so it is the least concentration dependent and the least time dependent to achieve its protoscolicidal effect while 10% povidone-iodine has a lower protoscolicidal effect than 0.1 % cetrimide even with longer exposure time.

Competing interests

Author declare that I have no competing interests.

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