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In vitro effects of vinegar on protoscolices of hydatid cyst

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PEER REVIEW

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Comments

In this study the authors used commercial apple and balsamic vinegar. The results of the study show that vinegar has scolicidal activity and its activity is related to the concentration and exposure time. Details on Page 212

ABSTRACT

Objective: To determine the effect of apple vinegar and balsamic vinegar on protoscolices of hydatid cyst. In this study, different concentrations of vinegar were tested at different exposure times.

Methods: Liver hydatid cysts of naturally infected sheep were obtained from Arak abattoir. Protoscolices were aspirated from cyst and were transferred into a dark container and stored at 4 °C for further use. Eight concentrations of vinegars (1%, 5%, 10%, 15%, 20%, 25%, 50% and 100% v/v) were used for 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50 and 60 min. The viability of the protoscolices was confirmed by 0.1% eosin staining test under a light microscope.

Results: The scolicidal activity of apple and balsamic vinegar in concentration of $\geq 50\%$ was 100%. The scolicidal activity of vinegar was dose dependent and also time dependent. Therefore, by increasing the exposure time as well as increasing the concentration of vinegar, the mortality rate increased.

Conclusions: The results of our study show that vinegar has scolicidal activity and its activity is related to the concentration and exposure time.

KEYWORDS

Hydatid cyst, Scolicidal, Vinegar

1. Introduction

Hydatid disease is a parasitic infection caused by the larval stage of a tapeworm, *Echinococcus*[1]. This disease is still a major health problem in endemic areas over the world[2]. This parasite can cause zoonotic disease. The adult worms develop in the small intestine of dogs and other canine as definitive host. The larva stage develops

in the various parts of human body or other intermediate host[3]. There is currently no agreement about the ideal therapy for hydatidosis. Drug therapy is not very effective for hydatid cyst[4]. The best treatment is surgery[3]. The cyst may rupture during surgery and protoscolices that have been released from the secondary cysts in other organs[5]. So we need protoscolicidal solutions for inactivation of protoscolices during the hydatid cyst

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surgery^[6–8]. Such solutions should have the lowest risk and the greatest effect^[9]. Since no ideal material has been found to be safe and effective, this relation researchers are still looking for safe materials^[9]. The use of natural materials to inactivate protoscolices reduces their risk, because they are compatible with the human body. One of these substances is vinegar. From 2000 years ago, vinegar has been used to treat wounds, disinfection of surfaces and fight infections. Recent studies have clearly shown that vinegar has antimicrobial properties^[10–12].

Our study was designed to determine the effect of apple vinegar and balsamic vinegar on protoscolices of hydatid cyst. In this study, different concentrations of vinegar were tested at different exposure times.

2. Materials and methods

2.1. Collection of protoscolices

Liver hydatid cysts of naturally infected sheep were obtained from Arak abattoir in central of Iran. Protoscolices were aspirated from cyst under sterile condition. The hydatid fluid was transferred into glass cylinders and left to set for 30 min. The supernatant was removed and the sediment protoscolices were washed three times with normal saline. The viability of the protoscolices was confirmed by flame cell activity and 0.1% eosin staining test under a light microscope. The live protoscolices were finally transferred into a dark container containing normal saline solution and stored at 4 °C for further use^[2].

2.2. Preparation of vinegar

In this study, we used a commercial apple and balsamic vinegar. Vinegar was diluted with normal saline. Eight concentrations (1%, 5%, 10%, 15%, 20%, 25%, 50% and 100% v/v) of vinegar were used for 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50 and 60 min. Then, 2 mL of each concentration was placed in test tubes and 100 µL of protoscolices sediment was added. The contents of the tubes were gently mixed. The tubes were then incubated at 37 °C. At the end of each incubation time, the upper phase was carefully removed so as not to interrupt 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 glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead protoscolices were determined by counting a minimum of 300 protoscolices. Non-treated protoscolices were considered as a control group in each experiment. The experiments were performed in triplicate.

2.3. 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^[13]. After exposure to the stain for 15 min, the protoscolices with no absorbed dye were considered potentially viable (Figure 1); otherwise, they were recorded as dead (Figure 2).

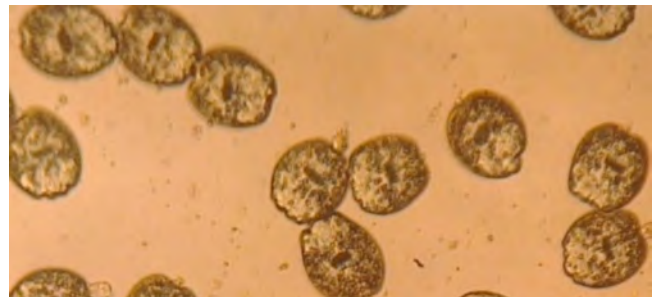


Figure 1. Protoscolices with no absorbed dye (viable protoscolices).

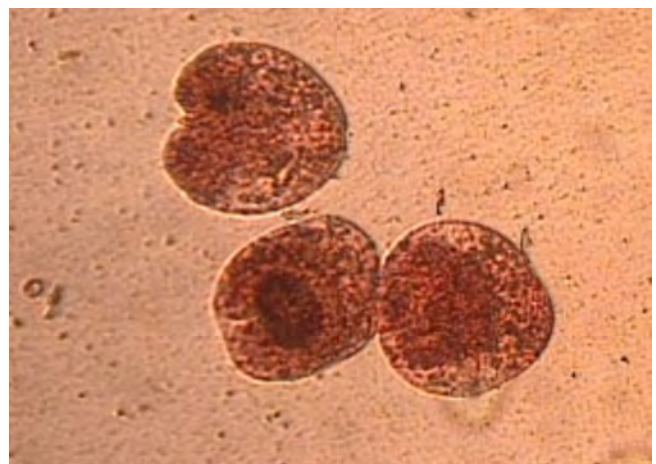


Figure 2. Protoscolices with absorbed dye (dead protoscolices).

2.4. Statistical analysis

The mortality rates of protoscolices were expressed as descriptive statistics, including frequencies table and

descriptive indices.

3. Results

The mortality rate of protoscolices after exposure to apple vinegar is shown in Table 1.

Table 1

The mortality rate of protoscolices in different concentrations of apple vinegar at different exposure times (mean of mortality rate %, in 3 trials).

Dilution (%)	Exposure time (min)											
	1	2	3	4	5	10	15	20	30	40	50	60
1	8.0	8.0	8.0	8.0	8.0	8.0	9.2	9.2	9.6	9.6	9.7	10.0
5	8.0	8.0	8.0	8.0	8.0	10.0	10.4	11.3	11.4	12.0	12.2	12.5
10	8.0	8.0	8.0	9.1	9.2	10.0	10.5	11.5	13.8	18.0	20.5	24.0
15	8.0	8.0	9.0	10.0	10.5	11.0	12.7	14.0	19.0	26.0	29.0	34.1
20	8.0	8.0	10.0	11.5	14.6	16.8	19.9	22.5	27.0	34.0	38.9	43.0
25	10.0	12.9	17.4	22.6	27.2	30.5	32.0	34.6	40.5	47.5	52.4	58.5
50	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
100	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Control	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0

The mortality rate of protoscolices after exposure to balsamic vinegar is shown in Table 2.

Table 2

The mortality rate of protoscolices in different concentrations of balsamic vinegar at different exposure times (mean of mortality rate %, in 3 trials).

Dilution (%)	Exposure time (min)											
	1	2	3	4	5	10	15	20	30	40	50	60
1	6.5	6.5	6.5	6.5	6.5	7.5	8.4	8.5	8.7	9.6	10.1	10.7
5	6.5	6.5	6.5	6.5	6.5	7.7	8.9	9.5	10.0	11.7	14.5	17.6
10	6.5	6.5	8.9	9.5	9.7	12.7	15.5	16.3	18.6	20.6	22.5	25.5
15	6.5	6.5	10.5	11.9	13.6	17.8	20.6	25.9	28.6	34.5	38.1	43.5
20	7.9	8.9	10.6	14.4	16.5	19.5	25.5	31.5	40.3	46.5	52.2	56.5
25	13.5	18.5	21.5	27.0	29.6	37.5	42.5	48.5	57.2	68.5	80.4	93.6
50	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
100	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Control	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5

According to our results, the apple and balsamic vinegar scolicidal activity were 100% in the concentration of $\geq 50\%$. The scolicidal activity of apple and balsamic vinegar in our experiments were dose dependent and also time dependent. Therefore, by increasing the exposure time as well as increasing the concentration of vinegar, the mortality rate increased.

4. Discussion

The first choice of treatment for hydatid disease is surgery. But it is associated with considerable recurrence rate[14]. Inactivation of the parasite with protoscolicidal

agents is an important component of the surgical treatment[15]. In current study, we used two types of vinegar as scolicidal agents. According to our results, the commercial apple and balsamic vinegar have scolicidal activity. Also scolicidal activity of vinegar related to concentration and exposure time.

Many scolicidal agents have been used during surgery, such as herbal extracts[2,13,16,17], hypertonic saline[9,18], hydrogen peroxide[18], 95% alcohol, povidone iodine, 5% formalin, silver nitrate, cetrimide, and albendazole[7,19]. The use of protoscolicidal substances for killing of protoscolices is questionable, as there is no ideal agent with both effective and safe[8]. For example, hypertonic saline has been used as a scolicidal agent in various concentrations. It is known that high concentration saline can cause sclerosing cholangitis and bile duct strictures[19]. Side effects of some of these agents like silver nitrate, formalin, and cetrimide-C have been proven[9]. A good scolicidal agent might do its lytic effect in lowest consternations, shortest time, and with the least side effects[9]. So far, no studies have been using vinegar as scolicidal agent and this is the first report on the scolicidal activity of vinegar. Vinegar is a natural material that is compatible with the human body, so perhaps it can be used during surgery to prevent recurrence of hydatid disease. However, it is necessary to do more studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The background is enough and explained the protoscolicidal solutions for inactivation of protoscolices during the hydatid cyst surgery.

Research frontiers

The field in this paper is related to using protoscolicidal solutions for inactivation of protoscolices by using natural vinegar.

Related reports

In the previous studies, the researchers used ethanol as protoscolicidal solutions. But in this study, the authors used vinegar, so I think there isn't related reports.

Innovations & breakthroughs

Using vinegar as the protoscolicidal solutions for inactivation of protoscolices is the innovation in this study.

Applications

The application for this study is replacement of ethanol with vinegar as the protoscolicidal solutions for inactivation of protoscolices.

Peer review

In this study the authors used commercial apple and balsamic vinegar. The results of the study show that vinegar has scolicedal activity and its activity is related to the concentration and exposure time.

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