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In-Vitro Scolicidal Effects of Grape and Apple Vinegar on the Hydatid Cyst Protscoleces

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Abstract

At present, surgery remains the preferred treatment for hydatid cyst. Various chemical scolicidal agents have been used for inactivation of protoscoleces during surgery, but most of them are associated with adverse side effects. In this study we investigated the effect of two commercial grape and apple vinegar on the viability of hydatid cyst protoscoleces. The protoscoleces of *E. granulosus* were aspirated from the infected livers and lungs of sheep slaughtered at Tiaret abattoir. 0.5 mL of two commercial grape and apple vinegar was used for 5, 10, and 15 min in the experiments. 0.1% eosin staining assessed viability of protoscoleces. All tests were carried in triplicate. The mortality rates of protoscoleces were 100% after 5min, 10 min and 15 min of incubation with grape vinegar and apple vinegar. To conclude, the results of the present survey indicated high scolicidal activity of apple vinegar and grape vinegar against protoscoleces of hydatid cyst and can be used in hydatid cyst surgery. However, further studies will be needed to confirm these results by checking this scolicidal activity in an in-vivo model.

Keywords: Hydatid cyst; protoscolece; surgery; vinegar; apple; grape.

INTRODUCTION

The larval stage of the tapeworms belonging to the species complex *Echinococcus granulosus sensu lato* (s.l.) are the etiological agents of a globally widespread zoonotic disease known as cystic echinococcosis (CE) (Budke *et al.*, 2006; WHO 2018; Laurimäe *et al.*, 2019).

Dogs and carnivores are the main host of this parasite and humans are infected by ingesting parasite eggs released from these animals (Sadjjadi *et al.*, 2009). Hydatidosis is recognized by long-term growth of hydatid cysts in the different organs (liver, spleen, lung, muscle and brain) of human and other intermediate hosts (Zhang et *al.*, 2003; Rostami *et al.*, 2016). The infection is usually diagnosed by imaging and serologic tests (Sadjjadi *et al.*, 2009).

Hydatidosis still remains a major public health problem in many parts of the world with pastoral communities, especially in regions of South America, East Africa, Eastern Europe, Russia, the Mediterranean littoral and Middle East, Central Asia and China (Eckert and Deplazes 2004; Rostami *et al.*, 2016).

Annual costs associated with cystic echinococcosis (CE) are estimated to be 3 billion US$ for treating cases and losses to the livestock industry can include liver condemnation, reduction in carcass weight, decrease of milk production and reduced fertility (WHO, 2018; Houshmand *et al.*, 2019).

Currently there are three-treatment options for CE: surgery, ultrasound-guided aspiration, and chemotherapy. The recommended chemotherapy drugs for treatment of hydatidosis are benzimidazole derivatives, such as mebendazole and albendazole. However, due to increase of their resistance protoscoleces to and drug side effects, their uses are limited (Kohansal *et al.*, 2017; Naseri *et al.*, 2016; Walker *et al.*, 2004).

Surgery is one of the best choices for the treatment of hydatidosis and to prevent re-lapse, effective scolicidal agents must be used after surgery (Brunetti, 2010). There are several agents, which have been used

for inactivation of the cyst contents, for example, hypertonic saline, silver nitrate, cetrimide, and ethanol (Houshmand *et al.*, 2019). However, recent studies have confirmed severe complications such as necrosis, fibrosis and impaired performance of liver and gallbladder following the use of these agents (Rajabi, 2009; Sahim *et al.*, 2004). So, it is necessary for surgeons to try to achieve new scolicidal agents with increased efficacy and low side effects in order to maintain the human health (Niazi *et al.*, 2019).

Vinegar is a sour-tasting liquid obtained from the anaerobic conversion of sugars to ethanol by yeasts and aerobic oxidation of ethanol to acetic acid by bacteria. It may be classified in accordance with raw materials 'grain vinegar', such as those obtained from rice and wheat, or as 'fruit' vinegars, including juices from grape, apple and coconut (Chen *et al.*, 2016; Heitor *et al.*, 2019).

The chemical and organoleptic properties of vinegars are a function of the starting material and the fermentation method. Acetic acid, the volatile organic acid that identifies the product as vinegar, is responsible for the tart flavor and pungent, biting odor of vinegars (Anonymous, 2006). Other constituents of vinegar include vitamins, mineral salts, amino acids, polyphenolic compounds (eg, gallic acid, catechin, caffeic acid, ferulic acid), and non volatile organic acids (eg, tartaric, citric, malic, lactic) (Junghanss *et al.*, 2008; Mahmoudvand *et al.*, 2016).

For centuries, vinegar has been widely used as a dietary spice and natural remedy for various ailments in folk medicine. Moreover, it is considered a "super food" by laypersons, purported to improve weight loss, digestion and skin quality; so much so that there are even vinegar diets. The earliest report dates back 2300 years whereby Hippocrates (c. 420 BC) used vinegar for wound care (Johnston et Gaas, 2006).

The aim of this study was to evaluate the effect of two commercial grape and apple vinegar against protoscoleces of hydatid cyst, to explore their potential as natural scolicidal agent.

RESEARCH METHOD

Collection of protoscoleces

The protoscoleces of *E. granulosus* were aspirated from the infected livers and lungs of sheep slaughtered at Tiaret abattoir, western Algeria and carried to the parasitology laboratory of the veterinary institute, University of Tiaret (Algeria). The hydatid fluid was transferred into glass cylinders under sterile condition (Moazeni *et al.*, 2012) and left to set for 30 min (Kavoosi and Purfard, 2013). The protoscoleces settled down at the bottom of the cylinders. The supernatant was removed and the sedimented protoscoleces were washed three times with normal saline. The viability of the protoscoleces was confirmed by their flame cell motility and impermeability to eosin solution (0.1%) under a light microscope (Mahmoudvand *et al.*, 2014).

Scolicidal assay

In this study, we evaluate the scolicidal effects of two commercial vinegars: grape vinegar (bottle of 250 ml, degree of acidity not less than 4%, made in Syria) and apple vinegar (bottle of 250 ml, degree of acidity 5%, made in Algeria).

The method used was that of Mahmoudvand *et al.* (2017) slowly modified. Briefly, 0.5 ml of a rich protoscoleces solution was placed in test tubes. Then 0.5 ml of vinegar was added to each test tube. The contents of the tubes were gently mixed and then incubated at 37 °C for 5, 10 and 15 minutes. At the end of each incubation times, the upper phase was carefully removed so as not to interrupt the protoscoleces. Then 0.5 ml of 0.1% eosin stain was then added to their remaining settled protoscoleces and mixed gently. The upper portion of the solution was discarded after 15 min of incubation. The remaining pellet of protoscoleces was smeared on a glass slide, covered with a cover glass and

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examined under a light microscope. The percentages of dead protoscoleces were determined by counting an average of 1000 protoscoleces. In the control, protoscoleces were treated only with normal saline. All tests were carried out 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 protoscoleces. After exposure to the stain, the protoscoleces which excluded and did not take the dye eosin were considered as potentially viable (Figure 1); if they allow entry of eosin and are colored red, they have been recorded as dead (Moazeni and Nazer, 2010) (Figures 2 and 3). The protoscoleces death rate was determined as a percentage of dead protoscoleces compared to the total protoscoleces.

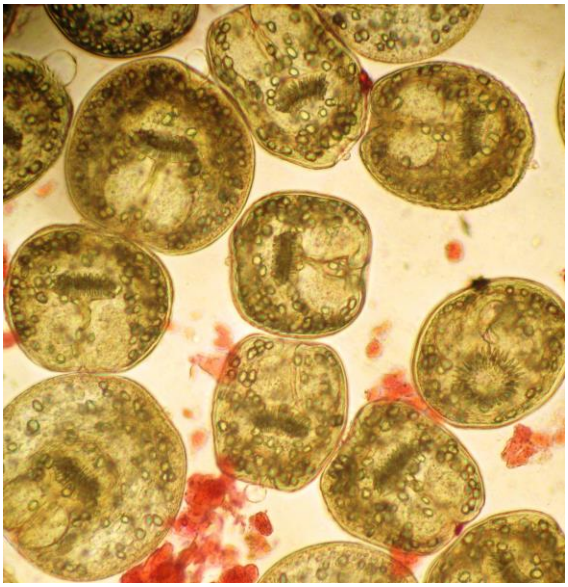

Figure 1. Live protoscoleces of hydatid cysts after exposure with 0.1% eosin

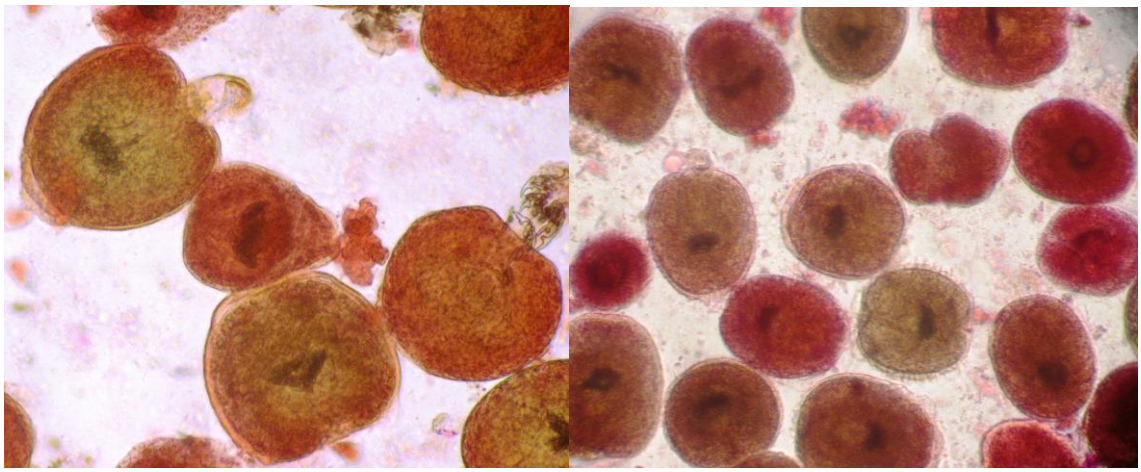

Figure 2: Died protoscoleces after 5 min (at right) and 15 min (at left) of exposure with grape vinegar

RESULTS AND DISCUSSION

The scolicidal effects of grape and apple vinegar with various exposure times were shown in Table 1 and Table 2. The mortality rates of protoscoleces were 100% after 5min, 10 min and 15 min of incubation with grape vinegar and apple vinegar.

Cystic echinococcosis (hydatid disease) continues to be a substantial cause of morbidity and mortality in many parts of the world (Craig *et al.*, 2007). The basic treatment of the hydatidosis in humans is the surgical procedure and removal of cysts from the body (Mahmoudvand *et al.*, 2016). Due to laceration of the cyst and spread of the content within it (protoscoleces) during the surgery that can put the patient at the risk of re-infection, immunological reactions such as anaphylaxis shock and even death (Junghanss *et al.*, 2008), surgeons use several chemical agents with scolicidal effect to solve this problem, as formalin, povidone-iodine, hypertonic saline 10% - 20%, H_2O_2, cetrimide and

alcohol (Larki *et al.,* 2017), but many of these substances may cause unwanted side effects that limit their usage (Moazeni and Nazer, 2010; Shahnazi *et al,* 2016). Therefore, finding new scolicidal agents with fewer side effects, low cost and higher efficacy are an urgent need for surgeons (Adas *et al.,* 2009).

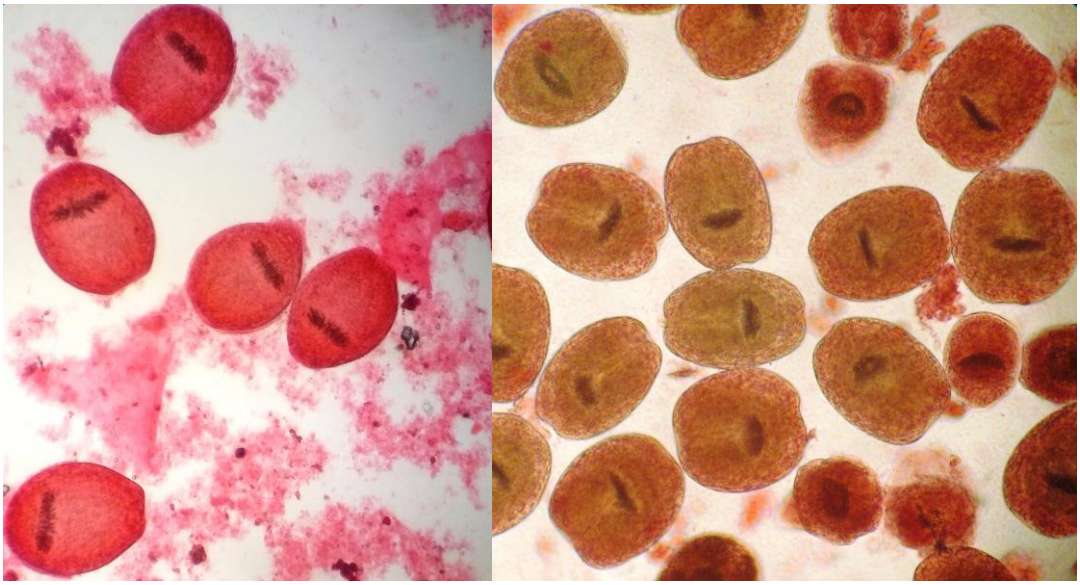

Figure 3. Died protoscoleces after 5 min (at right) and 15 min (at left) of exposure with apple vinegar.

Table 1. Scolicidal effect of apple vinegar at different exposures time

	Experiments	Control	1st Test	2nd Test	3rd Test
5 min	Protoscoleces	1697	1263	812	932
	Dead protoscoleces	149	1263	812	932
	Mortality rate	8.78%	100%	100%	100%
	Motility	+	-	-	-
10 min	Protoscoleces	1451	1390	999	1030
	Dead protoscoleces	216	1390	999	1030
	Mortality rate	14.88%	100%	100%	100%
	Motility	+	-	-	-
15 min	Protoscoleces	1120	1157	913	1066
	Dead protoscoleces	185	1157	913	1066
	Mortality rate	16.52%	100%	100%	100%
	Motility	+	-	-	-

Some scientific investigation clearly states the benefits of vinegar such as antimicrobial properties (Vijayakumar and Hall, 2002), prevent inflammation and hypertension (Murooka and Yamshita, 2008), lower serum cholesterol (Fushimi *et al.,* 2006), reduction in systolic blood pressure (Kondo *et al.,* 2001), enhanced calcium absorption and retention (Kishi *et al.,*1999), decrease the glycemic index of carbohydrate food for people with and without diabetes (Johnston *et al.*, 2004).

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Table 2. Scolicidal effect of grape vinegar at different exposures time

	Experiments	Control	1st Test	2nd Test	3rd Test
5 min	Protoscoleces	1433	1426	1170	1143
	Dead protoscoleces	302	1426	1170	1143
	Mortality rate	21.07%	100%	100%	100%
	Motility	+	-	-	-
10 min	Protoscoleces	1396	1522	1279	990
	Dead protoscoleces	313	1522	1279	990
	Mortality rate	22.42%	100%	100%	100%
	Motility	+	-	-	-
15 min	Protoscoleces	1800	1070	1120	1389
	Dead protoscoleces	326	1070	1120	1389
	Mortality rate	18.11%	100%	100%	100%
	Motility	+	-	-	-

In the current study, we used two types of vinegar as scolicidal agents. According to our results, the commercial grape and apple vinegar have high scolicidal activity from 5 min of exposure time.

So far, only one study has been using commercial vinegar of apple and balsamic (Hajihossein *et al.*, 2015), but no study has evaluated that of grape vinegar as scolicidal agent and this is the first report on his scolicidal activity.

Hajihossein *et al.* (2015) reported that the apple and balsamic vinegar scolicidal activity was 100% in the concentration of ≥50%. They also suggested that vinegar is a natural material and is compatible with the human body, so perhaps it can be used during surgery to prevent recurrence of hydatid disease.

Similar results were reported by Moazeni and Larki (2010), who showed that the use of acidic solutions was 100% effective after 5 minutes of exposure with a pH of 1 and after 10 minutes for those with pH of 2 and 3.

Selles and Kouidri (2019) reported that *Citrus limon eureka* juice presented high scolicidal activity (100%) at the dose of 0.5 ml with a pH of 1.38 after 10min of exposure. The therapeutic effects of Lemon juice can be related to its citric acid content that gives the lemon a bitter taste and a pH of 2 - 3 (Touhami *et al.*, 2007).

CONCLUSION

To conclude, the results of the present survey indicated high scolicidal activity of apple vinegar and grape vinegar against protoscoleces of hydatid cyst and can be used in hydatid cyst surgery. However, further studies will be needed to confirm these results by checking these scolicidal activities in an in vitro model.

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