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

Kouidri Mokhtaria1,2*, Selles Sidi Mohammed Ammar1,3
1Laboratory of Parasitology of The Veterinary Institute, University of Tiaret, Algeria;
2Laboratory of Farm Animal Reproduction, University of Tiaret, Algeria;
3Laboratory of Research on Local Animal Products, University of Tiaret, Algeria.
*Email: mokhtariakouidri@yahoo.fr.

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 relapse, 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...
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.

**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 5 min, 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%, H₂O₂, 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).
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 10 min 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.

**ACKNOWLEDGEMENT**

The authors would like to thank the DGRSDT (Direction Générale de la Recherche Scientifique et du Développement Technologique) for its funding and Dr. Benhamed M and Dr. Khelil C for helping in the laboratory work and the veterinary staff of the slaughterhouse of Tiaret for their participation on the collect of the hydatid cysts.

**REFERENCES**

Adas G, Arikan S, Kemik O, Oner A, Sahip N, Karatepe O. 2009. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as
scolicidal agents on hydatid cysts (in vitro study). World J. Gastroenterol., 15: 112-116.

Anonymous. US Food and Drug Administration. Acetic Acid - Use in Foods. Available at: http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg562-100.html. Accessed March 9, 2006.

Brunetti E, Kern P, Vuitton DA. 2010. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Writing panel for the WHO IWGE. Acta Trop., 114(1): 1–16.

Budke CM, Deplazes P, Torgerson PR. 2006. Global socioeconomic impact of cystic echinococcosis. Emerg. Infect. Dis., 12: 296–303.

Chen H, Chen T, Giudici P, Chen F. 2016. Vinegar functions on health: constituents, sources, and formation mechanisms. Compr. Rev. Food Sci. Food Saf., 15: 1124e38.

Craig PS, McManus DP, Lightowlers MW, Chalbagoity JA, García HH, Gavidia CM, Gilman RH, Gonzalez AE, Lorca M, Naquira C, Nieto A, Schantz PM. 2007. Prevention and control of cystic echinococcosis. Lancet Infect. Dis., 7(6): 385-394.

Eckert J, Deplazes P. 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev., 17: 107–115.

Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y, Goda T. 2006. Dietary acetic reduced serum cholesterol and triacylglycerols in rats feed a cholesterol rich diet. Brit. J. Nutr., 95(5): 916-924.

Hajhossein R, Eslamirad Z, Mosayebi M, Ghasemikhah R, Didehdar M. 2015. In vitro effects of vinegar on protoscolices of hydatid cyst. Asian Pac. J. Trop. Dis., 5(3): 210-213.

Heitor OS, Wilson MAMd-M, Guilherme ARd-S, Jonato P, Brad J S. 2019. Vinegar (acetic acid) intake on glucose metabolism: A narrative review. Clin. Nutr. ESPEN., 32: 1-7.

Houshmand E, Kamalifar H, Elmi H. 2019. In vitro scolicidal effect of ginger (Zingiber officinale Roscoe) ethanolic extract against protoscolices of hydatid cyst. Iran J. Vet. Med., 13(1): 87-99.

Johnston CS, Gaas CA. 2006. Vinegar: medicinal uses and antiglycemic effect. Med. Gen. Med., 8: 61.

Johnston CS, Kim CM, Buller AJ. 2004. Vinegar improves insulin sensitivity to a high carbohydrate meal in subjects with insulin resistance or type 2 diabetes mellitus. Diabetes Care, 27: 281-282.

Junghanss T, da Silva AM, Horton J, Chiodini PL, Brunetti E. 2008. Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. Am. J. Trop. Med. Hyg., 79(3): 301–311.

Kavoosi G, Purfard A M. 2013. Scolicidal effectiveness of essential oil from Zataria multiflora and Ferula assafoetida: disparity between phenolic monoterpenes and disulphide compounds. Comp. Clin. Pathol., 22: 999–1005.

Kishi M, Fukaya M, Tsukamoto Y, Nagasaw T, Takenha K, Nishizawa N. 1999. Enhancing effect of dietary vinegar on the intestinal absorption of calcium in ovariectomized rats. Biosci. Biotechnol. Biochem., 63: 905-910.

Kohansal MH, Nouriana A, Rahimb MT, Daryanic A, Spotind A, Ahmadpour E. 2017. Natural products applied against hydatid cyst protoscolices: A review of past to present. Acta Trop., 176: 385–394.

Kondo S, Tayama K, Tsukamoto Y, Ikeda K Yamori Y. 2001. Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. Biosc. Biotech. Bioch., 65: 2690-2694.

Larki S, Jalali MHR, Goodarzi S. 2017. Scolicidal effects of gallic acid, one of the major compounds of plants, on
proteoscolices of hydatid cyst. Zaheden J. Res. Med. Sci., 19(5): e9791.

Laurimäe T, Kinkar L, Varcasia A, Dessi G, Sgroi G, D’Alessio N, Veneziano V, Saarma U. 2019. First detection of zoonotic tapeworm Echinococcus granulosus sensu lato genotype G7 in continental Italy. Parasitol. Res., 118(7): 2193-2201.

Mahmoudvand H, Harandi M F, Shakibaie M, Aflatoonian MR, ZiaAli N, Makki MS, Jahanbakhsh S. 2014. Scolicidal effects of biogenic selenium nanoparticles against proteoscolices of hydatid cysts. Int. J. Surg., 12: 399-403.

Mahmoudvand H, Kheirandish F, Dezaki ES, Shamsaddini S, Harandi MF. 2016. Chemical composition, efficacy and safety of Pistacia vera (var. Fandoghi) to inactivate proteoscolices during hydatid cyst surgery. Biomed. Pharmacother., 82: 393–398.

Mahmoudvand H, Mirbadie SR, Sadooghian S, Harandi MF, Jahanbakhsh S, Dezaki ES. 2017. Chemical composition and scolicidal activity of Zataria multiflora Boiss essential oil. J. Essent. Oil Res., 29(1): 42-47.

Moazeni M, Nazer A. 2010. In vitro effectiveness of Garlic (Allium sativum) extract on scolices of Hydatid cyst. World J. Surg., 34: 2677-2681.

Moazeni M, Larki S. 2010. In vitro effectiveness of acidic and alkline solutions on scolices of hydatid cyst. Parasitol. Res., 106: 853-856.

Moazeni M, Saharkhiz MJ, Hoseini AA, Alavi AM. 2012. In vitro scolicidal effect of Satureja khouzistanica (Jamzad) essential oil. Asian Pac. J. Trop. Biomed., 2(8): 616-620.

Murooka Y, Yamshita M. 2008. Traditional healthful Industrial fermented products of Japan. J. Ind. Microbiol. Biotechnol., 35: 791-798.

Naseri M, Akbarzadeh A, Spotin A, Akbari NA R, Mahami-Oskouei M, Ahmadpour E. 2016. Scolicidal and apoptotic activities of albendazole sulfoxide and albendazole sulfoxide-loaded PLGA-PEG as a novel nanopolymeric particle against Echinococcus granulosus protoscolices. Parasitol. Res., 115(12): 4595-4603.

Niazi M, Saki M, Sepahvand M, Jahanbakhsh S, Khatami M, Beyranvand M. 2019. In vitro and ex vivo scolicidal effects of Olea europaea L. to inactivate the protoscolices during hydatid cyst surgery. Ann. Med. Surg., 42: 7–10.

Rajabi MA. 2009. Fatal reactions and methaemoglobinaemia after silver nitrate irrigation of hydatid cyst. Surg. Pract., 13: 2–7.

Rostami A, Taheri M, Gholizadeh M, Seyyed Tabaei SJ, Raeghi S, Fallahi S. 2016. Scolicidal effect of some herbs on Echinococcus granulosus Protoscolices: a Systematic Literature Review. Herb. Med. J., 1(1): 1-7.

Sadjjadi SM, Sedaghat F, Hosseini SV, Sarkari B. 2009. Serum antigen and antibody detection in echinococcosis: application in serodiagnosis of human hydatidosis. Korean J. Parasitol., 47(2): 153-7.

Sahim M, Eryilmaz R, Bulbuloglu E. 2004. The effect of scolicidal agents on liver and biliary tree (experimental study). J. Investig. Surg., 17(6): 323.

Shahnazi M, Azadmehr A, Latifi R, Hajiaghaee R, Saeaei M, Alipour M. 2016. In vitro protoscolicidal effects of various concentrations of Ziziphora tenuior L. extract at different exposure times. Avicenna J. Phytochem., 6(4): 376–382.

Selles SMA, Koudri M. 2019. In vitro Scolicidal Activity of Citrus limon eureka Juice. Agricultura, 1–2 (109-110): 116-120.

Touhami M, Laroufi A, Elhabazi K, Loubna F, Zrara I, Eljahiri Y, Oussama A, Grases F, Chait A. 2007. Lemon juice has protective activity in rat's urolithiasis model. BMC Urology, 7: 1-10.
Vijayakumar C, Wolf Hall CE. 2002. Evaluation of household sanitizers for reducing levels of Escherichia coli on iceberg lettuce. *J. Food Prot.*, 65: 1646-1650.

Walker M, Rossignol JF, Torgerson P, Hemphill A. 2004. In vitro effects of nitazoxanide on Echinococcus granulosus protoscoleces and metacestodes. *J. Antimicrob. Chemother.*, 54: 609–616.

WHO. 2018. Echinococcosis. Fact Sheet (Updated February 2018). World Health Organization. Available at http://www.who.int/en/news-room/fact-sheets/detail/echinococcosis (last accessed 02.10.2018)

Zhang W, Li J, McManus DP. 2003. Concepts in immunology and diagnosis of hydatid disease. *Clin. Microbiol. Rev.*, 16: 18–36.