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### Short Communication

## In Vitro Efficacy of the Ankaferd Galenic Hemostatic Extract as a Germicidal Agent

\*Bayram METİN<sup>1</sup>, Nezih YILMAZ<sup>2</sup>, Yunus Emre BEYHAN<sup>3</sup>, Cahit BABÜR<sup>3</sup>, Mesut SİPAHİ<sup>6</sup>, Hüseyin EDE<sup>4</sup>, Yavuz Selim İNTEPE<sup>5</sup>, Ergin ARSLAN<sup>6</sup>, Tugay ATALAY<sup>7</sup>, Mustafa Yaşar ÖZDAMAR<sup>8</sup>

1. Department of Thoracic Surgery, Faculty of Medicine, Bozok University, Yozgat, Turkey
2. Department of Infectious Diseases, Faculty of Medicine, Bozok University, Yozgat, Turkey
3. Division of Public Health Affairs, Ministry of Health, Ankara, Turkey
4. Department of General Surgery, Faculty of Medicine, Bozok University, Yozgat, Turkey
5. Department of Cardiology, Faculty of Medicine, Bozok University, Yozgat, Turkey
6. Department of Chest Diseases, Faculty of Medicine, Bozok University, Yozgat, Turkey
7. Department of Neurosurgery, Faculty of Medicine, Bozok University, Yozgat, Turkey
8. Department of Pediatric Surgery, Faculty of Medicine, Bozok University, Yozgat, Turkey

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\*Correspondence  
Email:  
[drbaymet@hotmail.com](mailto:drbaymet@hotmail.com)

### **Abstract**

**Background:** Hydatid cysts are encountered frequently in regions endemic with livestock. The basic treatment for a hydatid cyst is total surgical removal of the cyst and its inner contents. Hypertonic NaCl or diluted betadine solution are used as germicidal agents for most hydatid surgeries. However, the germicidal efficacy of the Ankaferd Blood Stopper® (ABS) has not been investigated. Thus, we compared the efficacy of ABS for hydatid cysts with that of other germicidal agents.

**Methods:** Lung and liver tissues containing hydatid cyst liquid were collected from slaughterhouses. Six samples of each cyst were randomly allocated into different groups as follows: 20% hypertonic NaCl, betadine solution, ABS, 20% liquefied Andazole solution, 0.1% eosin, and distilled water. All groups were examined microscopically at 5, 10, and 15 min after treatment began to determine protoscolece viability rates.

**Results:** The most efficacious germicidal agent at 5 min was ABS, and betadine and hypertonic NaCl had similar efficacies. Betadine, ABS, and hypertonic NaCl showed similar efficacies at 15 min.

**Conclusion:** ABS was an effective germicidal agent to treat hydatid cysts.

## Introduction

Hydatid cysts are an infectious disease caused by *Echinococcus granulosus* larvae, whose adult form inhabits the small intestines of canids (1). Hydatid cysts are commonly encountered in Chile, Argentina, Cyprus, Hungary, Bulgaria, Turkey, and African and Asian countries where sheep and goat husbandry is widespread for meat production. Dogs, sheep, goats, camels, cattle, and other graminivorous animals play a major role transmitting the infection to humans. Animals whose meat is consumed are intermediate hosts for the parasite. Dogs are one of the most important transmission sources for infecting humans (2, 3). After consuming the eggs, bile salts in the duodenum dissolve the outer layer of the egg, which releases the embryo. The embryo attaches to the intestinal wall with hook-like structures, then passes through the mucosa, and enters the blood and lymphatic circulation. Embryos most likely migrate to the liver followed by the lungs. If the embryo escapes into systemic circulation, it can affect any organ (1, 4).

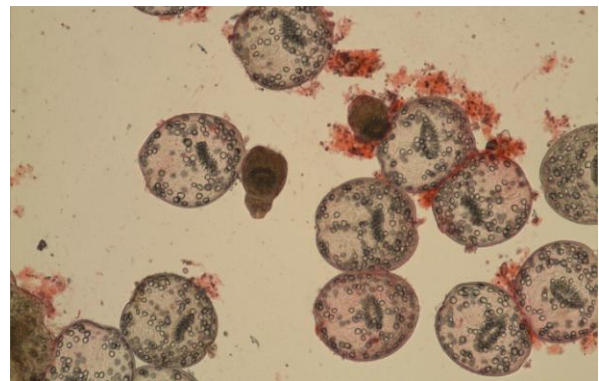
The Ankaferd Blood Stopper® (ABS) is a hemostatic agent obtained from the galenic extract of *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, and *Alpinia* sp. plants and is used traditionally in Turkish medical practice. The safety and efficacy of ABS have been demonstrated for traumatic dermal, postoperative, and dental bleeding.

Previous studies have reported the antimicrobial efficacy and hemostatic properties but no study has investigated the germicidal effect of ABS. Thus, we compared the efficacies of ABS and other germicidal agents to treat hydatid cysts (5).

## Materials and Methods

Lung and liver tissues containing hydatid cyst liquid were collected from slaughterhouses. Ten cysts were randomly obtained from

the relevant tissues. One-milliliter fluid samples from each of the 10 cysts were dyed separately with 0.1% eosin, examined microscopically, and all protoscoleces in the samples were counted to determine protoscolecocyte viability. The ratio of living to dead protoscoleces was expressed as percent cyst viability (6-7). Six samples of 5 ml each of cystic fluid without dye were drawn from each of the four cysts with the highest percent viability among the 10 cysts. All six samples from each cyst were randomly allocated into different groups as follows: Group 1 was mixed with 20% hypertonic NaCl, group 2 was mixed with betadine solution, group 3 was mixed with ABS, group 4 was mixed with liquefied 20% Andazole solution (2-g Andazole [albendazole] in 100-ml distilled water), group 5 was mixed with 0.1% eosin, and group 6 was mixed with distilled water (as a control group). Four 5-ml samples from each of the six groups were examined microscopically at 5, 10, and 15 min after treatment began to determine the protoscolecocyte viability rate after 0.1% eosin staining (7).



**Fig. 1:** Microscopic view of scoleces in the Ankaferd Blood Stopper-applied group 5 min after applying 0.1% eosin dye

Protoscoleces were assumed nonviable when they lost their ellipsoid shape by rounding or shrinking, and the vacuoles degenerated during uptake of eosin (6-7). The number of viable protoscoleces was counted under a micro-

scope and expressed as a percentage for the statistical analysis (Fig. 1).

### Statistical Analysis

The statistical analysis was performed using the SPSS software package (SPSS, Inc., Chicago, IL, USA). Percent viability is presented as mean  $\pm$  standard deviation. The Kruskal–Wallis test was used to detect differences between groups. The Mann–Whitney *U*-test was performed to test the significance of pairwise differences. *P*-values  $<$  0.05 were considered to indicate significance.

### Results

The mean viability rates of the groups after 5, 10, and 15 min are shown in Table 1. Signif-

icant differences in viability rates were detected between all six groups at 5, 10, and 15 min (*P*-values = 0.001, 0.001, and 0.002, respectively). Viability rates were significantly different between the betadine, ABS, and hypertonic NaCl groups at 5 and 10 min (*P*-values = 0.012 and 0.007 respectively), but they did not differ after 15 min (*p* = 0.119). The most efficacious germicidal agent at 5 min was ABS, and betadine and hypertonic NaCl had similar efficacies. Betadine, ABS, and hypertonic NaCl showed similar efficacies after 15 min. The protoscolece viability rates after the eosin, distilled water, and Andazole treatments were not different at 5, 10, and 15 min (*p*-values = 0.975, 0.496, and 0.131, respectively).

**Table 1:** Protoscolece viability rates 5, 10, and 15 min after treatment (mean  $\pm$  standard deviation)

	5 <sup>th</sup> minute	10 <sup>th</sup> minute	15 <sup>th</sup> minute
Andazole group	99.5 $\pm$ 1.0	98.0 $\pm$ 2.2	95.8.0 $\pm$ 3.3
Betadine solution group	62.5 $\pm$ 18.5	11.0 $\pm$ 1.2	3.0 $\pm$ 2.5
Hypertonic NaCl solution group	82.8 $\pm$ 6.6	21.8 $\pm$ 2.4	1.8 $\pm$ 2.4
Ankaferd group	25.0 $\pm$ 4.1	3.5 $\pm$ 1.3	0.0 $\pm$ 0.0
Eosin group	99.5 $\pm$ 1.0	98.5 $\pm$ 1.9	96.8 $\pm$ 1.0
Distilled water group	99.8 $\pm$ 0.5	99.5 $\pm$ 0.6	98.8 $\pm$ 1.0

### Discussion

The main hydatid cyst treatment is surgical removal of relevant tissue. The aim of the surgery is to eradicate the parasite, remove the related cavitory region, and save as much lung tissue as possible, considering all necessary precautions to prevent intraoperative rupture and dissemination (8). Cystic fluid contains thousands of protoscoleces and each can potentially form a new hydatid cyst during dissemination (9).

The area surrounding a cyst is generally covered with tissue compresses during surgery to avoid intraoperative contamination of cystic fluid to neighboring tissues. Then, the pericystic layer is incised to enucleate the cystic structure. After removing the cyst, the inner part of

the cavity is irrigated with one of several scolicidal solutions. Several scolicidal agents have been reported in the literature, such as hypertonic saline solution, 95% alcohol solution, Betadine solution, cetrimide bromide, hydrogen peroxide, and silver nitrate. Of them, alcohol and hypertonic saline solutions are the most often used agents (10-12). A 95% relapse rate of a 95% alcohol solution was lower than that of a hypertonic saline solution in a study comparing long-term relapse rates after testing different scolicidal agents (13). Another study reported that usnic acid has an insufficient germicidal effect after 15 min, whereas betadine, savlosol, and 96% alcohol solutions have a sufficient germicidal effect after 15 min; the savlosol solution was the most powerful agent among them (7). In our study, we did not observe any clear germicidal effect of eosin, dis-

tilled water, or Andazole after 5, 10, or 15 min. Although no significant difference was observed between the betadine solution, ABS, and hypertonic NaCl solution groups after 15 min, the groups differed significantly from each other after 5 and 10 min. As ABS was the most efficacious agent after 5 min, it could be considered the fastest acting germicidal agent among all of the solutions tested.

Eosin is a low toxicity dye that is inexpensive and easy to prepare and obtain. Thus, eosin has been the most frequently used agent to test protoscolecce viability in previous studies. The highest viability rates are obtained with 0.1% and 1% eosin solutions (6). Thus, we used eosin to minimize loss of scoloces due to the dyeing process.

Albendazole is another hydatid cyst treatment that causes death of cysts by preventing intracellular glucose uptake. Although one study reported good results using 10 mg/kg/day albendazole systemically for 1–3 months (14), no study has investigated applying albendazole locally. In our study, we observed that local application of liquefied 20% Andazole solution resulted in similar viability rates after 5, 10, and 15 min compared to those of distilled water and eosin but it had low germicidal efficacy.

Formalin and formaldehyde solutions have been commonly used as germicidal agents in the past. However, these solutions leak into the pericystic region and impair wound healing, resulting in the formation of bronchial fistulas and irritated tissue (15). Saline solutions < 10% do not have significant germicidal activity but solutions > 20% have sufficient germicidal activity (16). However, such high concentrations increase the risk for hypernatremia and sclerosing cholangitis in subjects with hepatic hydatid cysts (16). Previous studies related to liver and pulmonary hemorrhaging and leakage demonstrated that ABS is not toxic to liver or pulmonary tissues (17).

ABS is a galenic extract used to control bleeding in Turkish medical practice and has demonstrated antifungal, antibacterial, anti-

neoplastic, and pleurodesis activities in addition to its hemostatic effect (17-18).

## Conclusion

ABS could be a powerful germicidal agent in addition to its hemostatic property. Thus, we infer that ABS is an efficacious germicidal agent for percutaneous interventions applied to treat hydatid cysts; it reduces possible periprocedural hemorrhagic complications and inactivates protoscolecce concurrently. Further studies are needed to verify the effect of ABS on hydatid cysts in humans.

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## References

1. Gottstein B. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev.* 1992; 5(3): 248–261.
2. Matsaniotis N, Karpathios T, Koutoyzis J, Nicolaidou P, Fretzayas A, Papadellis F, Thomaidis T. Hydatid Disease in Greek Children. *Am J Trop Met Hys.* 1983; 32: 1075-78.
3. Kalaycı G, Genel Cerrahi, Nobel Tıp Kitabevi, II. Cilt. 2002; p. 1103-1109.
4. Şerefettin M Canda, Canda MŞ, Güray M, Canda T, Astarcoğlu H. The Pathology of Echinococcosis and the Current Echinococcosis Problem in Western Turkey (A Report of Pathologic Features in 80 Cases). *Turk J Med Sci.* 2003; 33: 369-374.
5. Akkoç N, Akçelik M, Haznedaroglu İC, Göker H, Turgut M, Aksu S, Kirazlı Ş, Fırat HC. In vitro anti-bacterial activities of ankaferd medicinal plant extract. *Türkiye Klinikleri J Med Sci.* 2009; 29: 410-15.
6. Mıman Ö, Aycan ÖM, Aydın C, Atambay M. What Should Be The Concentration of Eosin to Qualification of Ideal Staining for Viability Determination on Hydatid Cyst? *Turk Hij Den Biyol Derg.* 2010; 67(1): 21-26.

7. Esmel H, Çiftçi İH, Solak O, Dilek ON. Akciğer Hidatik Kistlerinde Usnik Asit, Betadine, Savlosol ve Desderman'ın Protoskoleksler Üzerine Germisid Etkinliğinin Araştırılması. Türkiye Parazitoloj Derg. 2007; 31: 101-104.
8. Yüksel M, Kalaycı G. Akciğer Kist Hidatiğinin Cerrahi Tedavisi. Yüksel M, Kalaycı G, editörler. Göğüs Cerrahisi, 1. Basım. İstanbul: Bilmedya Grup. 2001. p. 647-657.
9. Besim H, Karayalçın K, Hamamcı O, Güngör C, Korkmaz A, Scolicidal agents in hydatid cyst surgery. HPB Surg. 1998; 10: 347-351.
10. Arikoglu H. Histologic evaluation of the efficacy of different scoliceidal agents on the viability of scolices: an in vitro study. Postgraduate thesis. Selçuk University, Konya, Turkey 1996; 53.
11. Karaoğlanoğlu M, Akıncı ÖF, Ulukanlıgil M, metin MR, Çetin H, Çay N. Hydatid cyst viability: The effect of scoliceidal agents on the scolex in the daughter cyst. Turk J Med Sci. 2011; 41: 1001-1006.
12. Le Veen HH, Le Veen FR, Leveen GE. The mythology of povidone-iodine and the development of self-sterilizing plastics. Surg Gynecol Obstet. 1993; 176: 183-189.
13. Filice C, Brunetti E. Use of PAIR in echinococcosis. Acta Trop. 1997; 64: 95-107.
14. Çubuk S, Yücel O. Akciğer kist hidatiği Göğüs cerrahi Ders notları, Editör Orhan Yücel JCAM. 2012. p. 13-19.
15. Symbas PN, Aletras H. Hydatid disease of the lung. Shields TW. ed. General Thoracic Surgery. Philadelphia: Williams & Wilkins. 1994; p. 1021-1031.
16. Kayaalp C, Balkan M, Aydın C, Özgurtas T, Tanyuksel M, Kirimlioğlu V, Akoglu M, Oner K, Pekcan M. Hypertonic saline in hydatid disease. World J Surg. 2001; 25: 975-979.
17. Metin B, Altınok T, Menevşe E, Esen H. Evaluation of the effects of ankaferd blood stopper on rabbits with parenchymal damage: an experimental study. Turk Gogus Kalp Dama. 2013; 21: 428-433.
18. Bilgili H. Ankaferd Blood Stopper Araştırma Etkinlikleri Raporu: 2008; Bölüm 1; Versiyon 1: p. 18-20.