

Evaluation the Effects of *Eucalyptus* Essential Oil and Hypertonic Saline as Scolicidal Agents in Induction of Sclerosing Cholangitis in Rabbits

Abstract

Background: There are several scolicidal agents for inactivation of hydatid cyst protoscolices during surgery, but most of them are associated with adverse side effects such as sclerosing cholangitis (SC) and liver necrosis. The present study aimed to evaluate the adverse effects of *Eucalyptus* essential oil to induce SC in animal models. **Materials and Methods:** Eighteen rabbits weighting 2000–3500 g were included in three groups for this study. About 0.3 ml of *Eucalyptus* essential oil (1%), or 5% hypertonic saline, or normal saline was injected for 5 min in the gallbladder. After 4 months, the liver, common bile duct, and duodenum were resected and immediately sent for cholangiography and pathologic studies. **Results:** According to pathological studies, inflammation is more common in *Eucalyptus* and hypertonic saline groups. Due to cholangiographic studies, stricture was slightly higher in the hypertonic saline group than the *Eucalyptus* group. **Conclusion:** Thus, 1% *Eucalyptus* essential oil is at least as safe as 5% hypertonic saline, but for clinical uses, it must be confirmed with further studies.

Keywords: *Eucalyptus* essential oil, sclerosing cholangitis, scolicidal agents

Introduction

The hydatid cyst is caused by the larval stage of *Echinococcus granulosus*. The normal habitat of the mature stage of *E. granulosus* is the small intestine of the dog. Sheep is the usual intermediate host harboring the cystic larval form of this tapeworm. Human is the accidental intermediate host.^[1] The most important sheep breeding countries, for example, Greece, Australia, New Zealand, South America, Turkey, Iran, and Iraq, are in extreme risk for the hydatid cyst infection. About 75% of all hydatid cysts in humans are found in the liver.^[1]

Hydatid disease is a major health disorder, which is symptomatic in many patients. Hydatid disease can be seen in any organ of the human body, but the liver and lung are the main sites of parasitic infection.^[2,3] There is a high incidence of accidental diagnosis of liver hydatid disease.^[4] Several studies have been performed on liver hydatid disease diagnosis and treatment. Surgery remains the mainstay of treatment of hepatic echinococcosis either by traditional opened surgery or recently by laparoscopic

management of the liver hydatid cyst.^[5] It is important to control the load of parasites before the surgery, so, in some studies, albendazole had been administered before and after the surgery.^[6,7] The most common surgical procedure is evacuation of the cyst and instillation of scolicidal agent into the cyst cavity to sterilize it and to prevent its spread. Thus, it is important to control parasitic insemination during surgery. Many surgeons use scolicidal agents to prevent parasitic insemination during surgery.

Several scolicidal agents, such as Savlon, hypertonic saline, silver nitrate, chlorhexidine, formaldehyde, ethyl alcohol, hydrogen peroxide, mebendazole, albendazole, *Eucalyptus globulus*, *Mesobuthus eupeus* venom, and Betadine have been practiced throughout the world, and many of them have been abandoned due to early and late complications.^[8-11]

Our routine surgical approach for the treatment of hydatid cyst infection is evacuation of the cyst and instillation of 5% hypertonic saline which is the most common surgical procedure in the treatment of hepatic hydatid cyst. One of

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the most frequent late complications after evacuation of the cyst and instillation of various scolical agents is sclerosing cholangitis (SC), which is a lethal outcome. There are some investigations on the incidence of this complication after administration of various agents.^[12,13] Other studies showed that 4 months is good enough for the development of SC.^[14,15] We compared the incidence of SC following injection of 5% hypertonic saline, *Eucalyptus* essential oil (it is experimentally approved as a scolical agent in our previous study),^[9] and normal saline 0.9% transhepatically into the gallbladder of the rabbits.

Materials and Methods

This study approved by the ethical and operative principles of laboratory animal care of Shiraz University of Medical Sciences. The Ethics Committee of Shiraz University of Medical Sciences approved the study (IR. SUMS. REC. 1397.448).

Chemicals

Formalin, Tween 80 (Merck, Darmstadt, Germany), ketamine, and xylazine 2% (Alfasan, Woerden, The Netherlands) were used. *Eucalyptus* essential oil (Barij Essence Co., Kashan, Iran) and hypertonic saline 5% (Shahid Ghazi Co., Tabriz, Iran) were also used in our study.

Eucalyptus essential oil preparation

Essential oil by 100% purity was purchased from Barij Essence Company and diluted by distilled water and Tween 80 to make a 1% suspension of *Eucalyptus* essential oil. The amount of Tween 80 for this dilution was <0.1% that was safe for biological systems.

Experimental design

Eighteen male/female Dutch rabbits weighting 2000–3500 g were randomly divided into three equal groups. All rabbits were anesthetized with intramuscular injection of 44 mg/kg of ketamine and 8 mg/kg xylazine. In supine position, the right upper quadrant of the abdomen was incised and the common bile duct was clamped. Then, transhepatic injection of scolical agent to the gallbladder was performed with a 27G needle, and clamp was opened after 5 min. Fascia and skin were closed in two layers. About 0.3 ml of *Eucalyptus* essential oil (1%) was injected into common bile duct as scolical agent in the first group, 0.3 ml of 20% hypertonic saline in the second group, and 0.3 ml of normal saline as a control group. After 4 months, the rabbits were euthanized by a high dosage of chloroform inhalation. Following immediate autopsy, the liver, common bile duct, and duodenum were resected and immediately sent for cholangiographic study. After clamping of the ampulla of the common bile duct, 1 ml of 50% diluted

contrast (76% of meglumine compound, Darupakhsh Co., Tehran, Iran) was injected into the gallbladder and X-ray was taken. Cholangiograms were scored none, mild, moderate, or severe SC.^[16] Then, the specimens were placed in 10% buffered formalin and sent for histopathology evaluation. Hepatocyte injury was morphologically assessed. Confluent necrosis, interface hepatitis, portal inflammation, bile duct damage, and fibrosis were independently scored. We used a semi-qualitative scoring system according to the importance of each pathological finding: absent (0), mild (+), moderate (++), and severe (+++). Both pathologists and radiologists were blind to the drugs administered.

Results

Three rabbits died during the 1st week excluded from the study postoperation that one of them was from hypertonic saline and two of them were from the *Eucalyptus* oil group.

Radiological results

The scoring system was according to the intrahepatic and extrahepatic cholangiographic findings.^[16] Depending on cholangiographic findings, no visible abnormalities were shown with Score 0, normal or minimal caliber of bile ducts with multiple strictures was exhibited with Score 1, secular dilation and segmental stricture were demonstrated with Score 2 which is shown in Figure 1, focal segmental irregularity and dilation of bile ducts were compatible with Score 3 as shown in Figure 2, and severe irregularity and narrowing of the bile ducts were compatible with Score 4 as shown in Figure 3.

Forty percent of the rabbits in the hypertonic saline group and 33% in the *Eucalyptus* group developed radiological findings in favor of SC [Table 1].

Pathologic results

According to histological study, all cases in the *Eucalyptus* and hypertonic saline groups had some findings in favor of early stages of SC [Table 2]. There was no fibrosis in both the groups. Portal inflammation was observed in nearly all of the rabbits. Histological pictures are shown in Figures 4-7.

Pathological scoring

For statistical analysis, we used a semi-qualitative scoring system according to the importance of each pathological finding: absent (0), mild (+), moderate (++), and severe (+++).

In the *Eucalyptus* essential oil (1%) group, mild bile duct damage was seen in three rabbits, and in the hypertonic saline group, mild bile duct damage was seen in four rabbits. Two rabbits had moderate portal inflammation, and two had mild portal inflammation in the *Eucalyptus*

essential oil (1%) group, and in the 5% hypertonic saline group, three rabbits had mild portal inflammation and one

had moderate portal inflammation. One rabbit had a mild interface in the *Eucalyptus* essential oil (1%) group.

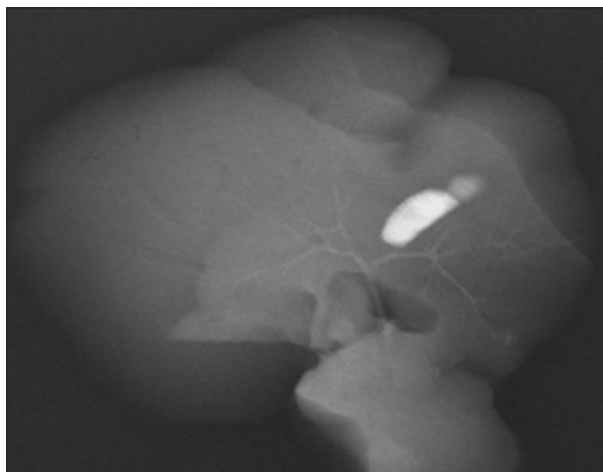


Figure 1: Mild narrowing and irregularity of the central intrahepatic bile ducts, compatible with Score 2



Figure 2: Focal segmental irregularity and dilation of the bile ducts, compatible with Score 3

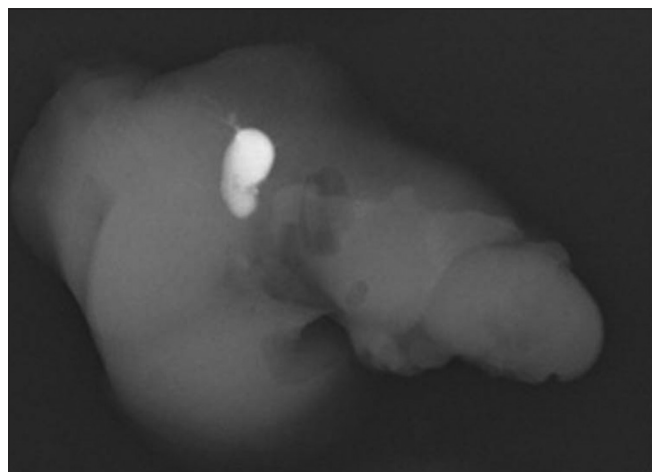


Figure 3: Severe irregularity and narrowing of the bile ducts, compatible with Score 4

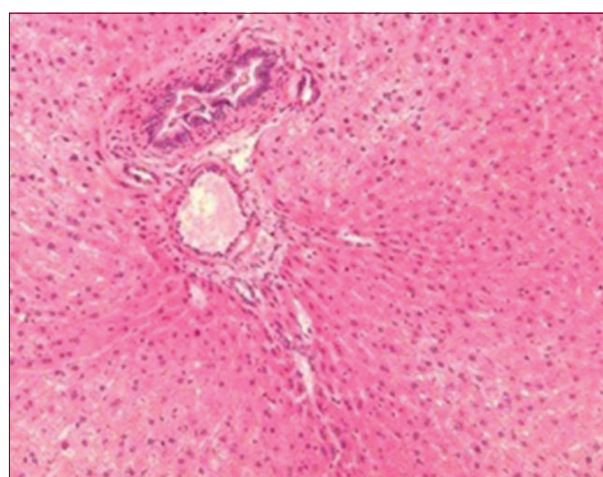


Figure 4: Normal bile duct with no portal inflammation H and E, ×100

Table 1: Cholangiographic results of the groups

Scores	<i>Eucalyptus</i> essential oil (1%) group (n=6), n (%)	5% hypertonic saline group (n=5), n (%)	0.9% normal saline group (n=4), n (%)
No abnormality (1 score)	0	2 (40)	3 (75)
Mild defect, narrowing irregularity (score 2)	4 (67)	1 (20)	0
Segmental stricture and dilation (score 3)	2 (33)	0	1 (25)
Severe stricture of the entire length (score 4)	0	2 (40)	0

Table 2: Pathological findings in biopsies done after 4 months

Findings	<i>Eucalyptus</i> essential oil (1%) group (n=6)	5% hypertonic saline group (n=5)	0.9% normal saline group (n=4)
Fibrosis	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0
Bile duct damage	+, *, +, +, 0, 0, 0	+, +, +, +, 0	0, 0, 0, 0
Portal inflammation	+, +, ++, ++, 0, 0	+, +, +, ++, 0	0, 0, 0, 0
Confluent necrosis	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0
Interface	+, 0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0

*+, ++, +++ are degree of intensity in each liver and 0 showed absent sign

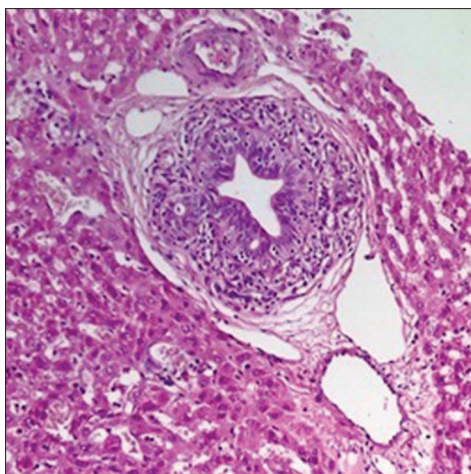


Figure 5: Moderate portal inflammation and bile duct damage, hypertonic solution group H and E, $\times 200$

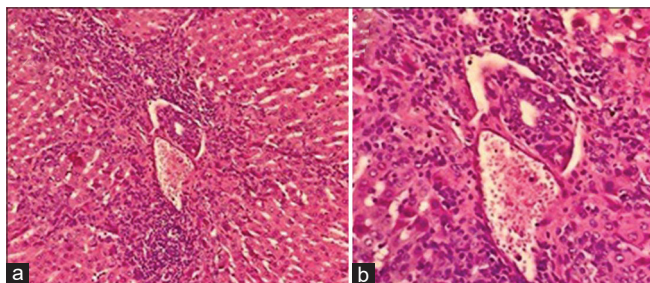


Figure 6: (a and b) Moderate portal inflammation, interface hepatitis, and bile duct damage, H and E, $\times 200$ and $\times 400$, respectively

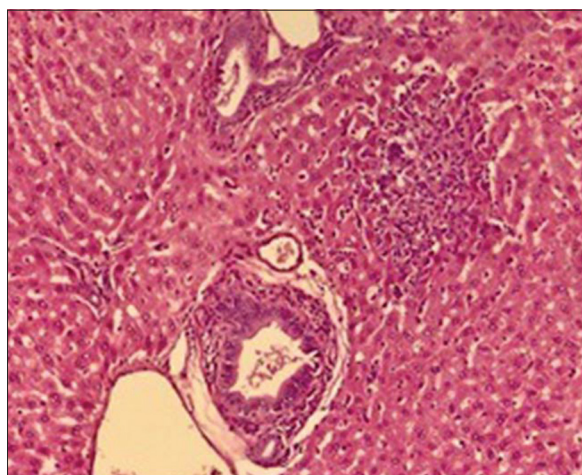


Figure 7: Mild portal inflammation and focal spotty necrosis and bile duct damage, *Eucalyptus* 1% group H and E, $\times 200$

Discussion

Hydatid disease is a common worldwide problem, which is endemic in developing countries, especially in the Middle East.^[17,18] Surgery is the best choice of treatment of hepatic hydatid disease, either by traditional open surgery or laparoscopic approach.^[5] Partial hydatid cystectomy, evacuation of the contents, instillation of scolicidal agents, and filling the cavity with greater omentum are the most

routine procedures for the treatment of hepatic hydatid disease.^[14] There is an important role for scolicidal agents to prevent echinococcus scolices contamination during the surgery and reduce the chance of recurrence of Hydatid cysts.

There are many studies on scolicidal agents. Due to the side effects of most of scolicidal agents and efficacy, hypertonic saline solution (20%) is the most accepted scolicidal agent that is used nowadays.^[19] Based on some studies, hypertonic saline solution can cause SC. In our previous study, *Eucalyptus* essential oil (0.5%, 1%, and 0.1%) had scolicidal effect, and *Eucalyptus* essential oil 1% had the best efficacy.^[9]

Secondary SC is one of the most feared postoperation complications. Some surgeons hypothesized that periductal abscess and surgical biliary tree trauma were the main causes of secondary SC, but studies mention that scolicidal agent which is used during surgical intervention is the most effective cause of secondary SC.^[1,14] Therefore, several studies were performed on the best effective scolicidal agent that has the lowest risk for postoperation SC.^[1-10,18]

Many of hydatid cysts have connection to biliary trees, also called cystobiliary fistula, so it is important that scolicidal agents do not cause SC.^[14]

In cystobiliary fistula and unusual color of cyst fluid, hypertonic saline and silver nitrate should not be used.^[2-4] In our experiment, the scolicidal solutions were directly injected into the gallbladder and the common bile duct was clamped for 5 min. Histological examination showed that both 5% hypertonic saline and 1% *Eucalyptus* essential oil cause intra- and extrahepatic bile duct damage and portal inflammation. However, in the control group that was treated by normal saline solution, no bile duct injury or portal inflammation was seen. Thus, both 5% hypertonic saline and 1% *Eucalyptus* essential oil may induce SC in long term.

Our cholangiographic result showed that both 5% hypertonic saline and 1% *Eucalyptus* essential oil can cause some degrees of bile duct stenosis; however, two cases in the 5% hypertonic saline group developed with severe bile duct stenosis. As shown in pathological study, most cases of the normal saline group remained normal in the cholangiographic study, so cholangiographic study data of bile duct damage are the same as pathological data.

Although the pathogenesis of SC and relation between scolicidal agents with SC is not completely known, immunologic reaction seems to have a major role in this process. Nowadays, it has been shown that direct exposure of many scolicidal agents to bile ducts is one of the main causes of SC.^[7] Some animal studies, such as the one performed by Houry *et al.*, showed that 5% hypertonic saline solution had no or minimal effect on inducing SC,^[20] but our study showed that 5% hypertonic solution caused some bile duct damage and could cause SC in long term.

Furthermore, our previous animal study supported our new results and showed that 5% hypertonic saline could cause SC in long term.^[14]

Therefore, both 1% *Eucalyptus* essential oil and 10% hypertonic saline can potentially produce SC and must not be used as scolical agent in cases with ductobiliary fistula. We showed that the effect of 1% *Eucalyptus* essential oil was almost like 5% hypertonic saline. Since hypertonic saline is routinely used as scolical agent, 1% *Eucalyptus* essential oil could be an acceptable scolical agent which can be used during hydatid cyst evacuation operation. For the use of 1% *Eucalyptus* essential oil solution in human studies, further quantitative and qualitative studies are recommended.

Conclusion

According to histological study, all cases in the *Eucalyptus* and hypertonic saline groups had some findings in favor of early stages of SC. Furthermore, when the cyst has a connection with the biliary tree, the usage of 1% *Eucalyptus* essential oil and 5% hypertonic saline should be avoided, due to the risk of SC.

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Conflicts of interest

There are no conflicts of interest.

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