

The effect of silver nitrate, chloroformic garlic extract, and normal saline in induction of sclerosing cholangitis in rabbits

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ABSTRACT

الأهداف: تقييم آثار نترات الفضة بنسبة 0.5%، ومستخلص الثوم المخمر بالكلوروفورم بنسبة 20%، والمحلل الملحي العادي بنسبة 0.9%، في تحفيز الالتهاب التصليبي لقناة الصفراء لدى الأرانب.

الطريقة: أجريت هذه الدراسة خلال ستة أشهر، في الفترة من أبريل 2006م وحتى سبتمبر 2006م، بمركز أبحاث الحيوانات في مختبر جامعة شيراز - شيراز - إيران. كان هناك ثلاث مجموعات متساوية من الأرانب وتم حقنها بنترات الفضة بنسبة 0.5%، ومستخلص الثوم المخمر بالكلوروفورم بنسبة 20%، والمحلل الملحي العادي بنسبة 0.9%، في قناة الصفراء لكل مجموعة على التوالي. تم تخدير الحيوانات وتشريحها بعد أربعة أشهر، حيث استؤصل كلا من الكبد وقناة الصفراء وتم دراستها نسيجياً. كما أُجري تخطيط قناة الصفراء لتقييم وجود ومقدار الالتهاب التصليبي في قناة الصفراء.

النتائج: في مجموعة نترات الفضة، ومستخلص الثوم المخمر بالكلوروفورم، والمحلل الملحي العادي، تم الكشف عن وجود التهاب تصليبي في قناة الصفراء لدى سبعة أرانب (58%)، وأرنب واحد (8%)، وأرنب واحد (7%) على التوالي. كان الفرق بين مجموعتي نترات الفضة ومستخلص الثوم المخمر بالكلوروفورم ذو دلالة إحصائية، ولوحظت نتائج مماثلة بين مجموعة مستخلص الثوم المخمر بالكلوروفورم ومجموعة المحلل الملحي العادي.

خاتمة: لقد كان لمستخلص الثوم المخمر بالكلوروفورم مضاعفات أقل بنسبة (20%) مثل الالتهاب التصليبي لقناة الصفراء مقارنة بالمواد الأخرى.

Objective: To evaluate the effects of 0.5% silver nitrate, 20% chloroformic garlic extract, and 0.9% normal saline in induction of sclerosing cholangitis in the bile ducts of rabbits.

Methods: During a 6-months period from April to September 2006 in Shiraz University Laboratory Animal Research Center, we selected 3 equal groups

of rabbits. We injected 0.5% silver nitrate, 20% chloroformic garlic extract, and 0.9% normal saline into the bile ducts of each group. The animals were euthanized, and autopsied after 4 months and the liver and bile ducts were removed and studied histopathologically. Cholangiography was undertaken to evaluate the presence and extent of any sclerosing cholangitis.

Results: Animals showed sclerosing cholangitis in silver nitrate group (7 [58%]), one (8%) in chloroformic garlic extract group and one (7%) in normal saline group. The difference between silver nitrate and chloroformic garlic extract groups were statistically significant and similar results were noticed between chloroformic garlic extract and normal saline groups.

Conclusion: Twenty percent of chloroformic garlic extract had fewer complications such as sclerosing cholangitis, compared to other materials.

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Hydatid disease of the liver is still endemic in many parts of Iran.¹ The treatment of hydatid cyst is largely surgical, with medical treatment being served as adjuvant treatment.² Sclerosing cholangitis encompasses a spectrum of chronic cholestatic diseases of the intrahepatic and extrahepatic bile ducts characterized by patchy inflammation, fibrosis, and

structuring.³ Injection of different scolical agents into the hydatid cysts has been used to sterilize the cyst and to prevent intra-abdominal dissemination of the parasite during surgery.^{2,4,5} These agents including hypertonic saline, silver nitrate, formalin, may result into occurrence of sclerosing cholangitis.^{5,6} Twenty percent chloroformic garlic extract was previously used as a protoscolical agent,^{7,8} and there is no data available in this relation in induction of sclerosing cholangitis. So, this study was undertaken to compare the effects of silver nitrate, chloroformic garlic extract, and normal saline in induction of sclerosing cholangitis in bile ducts of rabbit as an experimental animal model.

Methods. During a 6 months period from April to September 2006 in Shiraz University Laboratory Animal Research Center, 45 Dutch rabbits weighing 1.5-2 kg were randomly divided into 3 equal groups receiving 0.5% silver nitrate, 20% chloroformic garlic extract (prepared by percolation method), and 0.9% normal saline in their bile ducts. The study adhered to the principles of laboratory animal care established by Ethic Committee of Shiraz University of Medical Sciences. The animals were anesthetized using 44 mg/kg of ketamine hydrochloride and 8 mg/kg of xylazine intramuscularly. Under anesthesia, the abdomen was opened from the right side and 0.5 ml of 20% chloroformic garlic extract was injected transhepatically by a 25 G needle into the gall bladder and then the common bile duct was clamped for 5 minutes. Similar methods were applied for injection of 0.5% silver nitrate and 0.9% normal saline in the other 2 groups. The abdomen was closed after all injections. Tetracycline (2% OCT, Darupakhsh, Iran) was sprayed on the incision area with parenteral administration of penicillin (33 mg/kg, Darupakhsh, Iran) and streptomycin (30 mg/kg, Darupakhsh, Iran) for 5 days. The abdomen was opened again after 4 months and the animal was euthanized. Liver, biliary system, and duodenum were transferred into a normal saline solution mixed with ice and underwent a cholangiography immediately. Then, the tissue samples were transferred into a 10% buffered formalin solution for histopathological studies while both the radiologist and the pathologist were blind to all samples. In cholangiography, one ml of 50% contrast medium (76% meglumin compound, Darupakhsh, Iran) diluted in normal saline was injected into the gall bladder while the ampula was clamped. Two specimens were provided from the liver for histopathological studies. Five rabbits died during the first month of the study period (2 in chloroformic garlic extract, 2 in silver nitrate and one in normal saline groups) and were excluded from the study.

The collected data were analyzed by Chi-Square method using the SPSS Version 13. The *p* value less than 0.05 was considered statistically significant.

Results. Histopathological findings were demonstrated in Table 1 showing no sclerosing cholangitis in any of the groups. Ballooning and periportal degeneration was visible in the hepatocytes in silver nitrate group (69% of samples). Portal space inflammation was observed in all rabbits of silver nitrate group followed by 64% in normal saline and 38% in chloroformic garlic extract groups (Figure 1). Radiological findings based on cholangiography of the 3 groups were shown in Table 2. Sclerosing cholangitis was significantly more in silver nitrate group. Mild stenosis was noticed just in one case of chloroformic garlic extract and moderate stenosis in one case of

Table 1 - Pathological findings in 3 groups of rabbits 4 months post-operation.

Findings	Chloroformic garlic extract group	Silver nitrate group	Normal saline group
		n (%)	
Ballooning degeneration of hepatocytes	-	6 (46)	-
Periportal degeneration of hepatocytes	-	3 (23)	-
Kupffer cell change	-	-	-
Central vein change	-	-	-
Portal space infiltration (lymphocyte)	5 (38)	13 (100)	9 (64)
Sinusoidal change	-	-	-
Paranchymal change	-	-	-

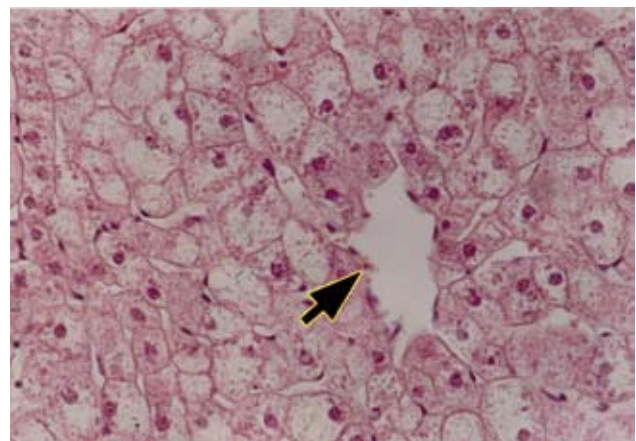


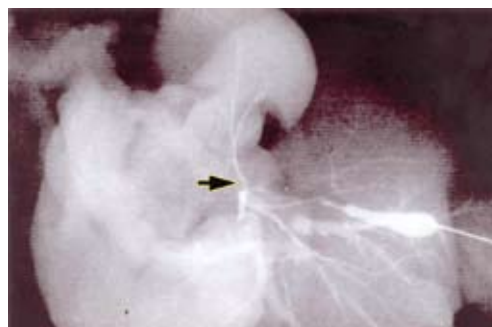
Figure 1 - Inflammation and ballooning degeneration around central vein.

Table 2 - Cholangiographic findings in 3 groups of rabbits 4 months post-operation.

Findings	Chloroformic garlic extract group	Silver nitrate group	Normal saline group
		n (%)	
Normal	12 (92)	5 (39)	12 (86)
Mild narrowing of hepatic ducts more in hilum	1 (8)	1 (8)	0
Moderate narrowing of hepatic ducts more in hilum	0	1 (8)	1 (7)
Mild irregularity and multiple beading of bilateral intra and extra hepatic bile ducts plus narrowing in hilum	0	2 (15)	0
Moderate narrowing of bilateral intra and extra hepatic bile ducts and h hilum	0	1 (8)	0
Mild narrowing and beading of right hepatic duct	0	1 (8)	0
Mild distal narrowing of right hepatic duct and common bile duct	0	1 (8)	0
Non-conclusive	0	1 (8)	1 (7)

normal saline, while the remained changes were visible just in silver nitrate group (58%). Sclerosing cholangitis was observed in 7 cases of silver nitrate group, which was statistically significant compared to chloroformic garlic extract group ($p=0.01$). The difference between the garlic extract and normal saline groups was not statistically significant ($p>0.05$) (Figures 2 & 3).

Discussion. Hydatid cyst is an endemic parasitic disease in middle east including Iran,^{1,9} while the treatment of choice is still surgery^{6,9} by evacuation of the cyst or evacuation of the cyst with omentoplasty, partial cystectomy, liver resection, cyst evacuation through the skin, and injection of protoscolicidal agents into it.^{3,8,10} The important point in hydatid surgery is preventing dissemination of protoscolices into the peritoneal cavity. Several methods were applied in this relation such as administration of oral albendazole before^{6,11} or after the surgery,¹² administration of H_2O_2 and pads soaked in protoscolicidal agents.¹¹ In majority of cases, protoscolicidal agents are injected into the cyst before it is opened.⁵ Sclerosing cholangitis is one of the complications occurring after injection of almost all protoscolicidal agents.¹³ The first reports of sclerosing cholangitis dates from 1981 after injection

**Figure 2** - Moderate narrowing of hepatic ducts, more in hilum.**Figure 3** - Mild multiple beading and narrowing of bilateral intra and extra hepatic bile ducts and narrowing of the hilum.

of formalin into the cyst.⁵ The reason for occurrence of sclerosing cholangitis is the presence of a connection between the cyst and the bile ducts^{14,17,18} resulting into penetration of protoscolicidal agents into the biliary system and development of sclerosing cholangitis due to caustic effects of these agents or due to immunological mechanisms.^{4,14} The connection between hydatid cysts and biliary ducts was reported to be 25% and 50%.⁶ Several efforts were undertaken to administer a potent protoscolicidal agent and to prevent any sclerosing cholangitis. The protoscolicidal activity of 20% chloroformic extract of chloroformic garlic was previously shown in our area.⁷ Our study was undertaken in animal model to evaluate any sclerosing cholangitis induced by the 20% chloroformic chloroformic garlic extract. Cholangiographic findings are similar in primary and secondary sclerosing cholangitis¹⁶ showing focal strictures, irregularities, beading and fraction of small bile ducts. Cholangiography is the standard method of diagnosis for sclerosing cholangitis.¹⁶⁻²¹ Pathological findings are unspecific and cannot separately be used for diagnosis of sclerosing cholangitis, and the sampling methods are also quite different,^{15,17,21} This method was reported to be used in some researches in diagnosis of sclerosing cholangitis to demonstrate any periductal or

concentric fibrosis, biliary hyperplasia in portal space, ductal obliteration, or inflammation in portal tracts.^{21,22} However, the morphological and functional features of sclerosing cholangitis have not been defined completely in any animal model.²² We used cholangiographic findings as major criteria of diagnosis of sclerosing cholangitis. Our results showed that sclerosing cholangitis was significantly less in chloroformic garlic extract group (8%) in comparison with the silver nitrate (58%) group and similar to the normal saline group (7%). The only case of sclerosing cholangitis in normal saline group may be due to administered agents,^{3,16} and can be similarly explained in the chloroformic garlic extract group too. The histopathological findings including Ballooning and periductal degeneration of hepatocytes were just visible in the silver nitrate group (69%) although these findings may have viral or therapeutic causes. According to the high incidence of sclerosing cholangitis in silver nitrate group, these pathological findings may be primary signs of pathological changes in the tissue. The unspecific pathological findings of portal space inflammation were observed in all cases of silver nitrate group (100%), which was significantly more than normal saline (64%) and chloroformic garlic extract (38%) groups which may be due to administered agents.

In conclusion that the chloroformic garlic extract can be safely administered as a protoscolicidal agent with least occurrence of sclerosing cholangitis.

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References

- Mehrabani D, Oryan A, Sadjjadi SM. Prevalence of Echinococcus granulosus in stray dogs and herbivores in Shiraz, Iran. *Vet Parasitol* 1999; 86: 217-220.
- Linda Garcia JI, Alonso E, Gonzalez-Uriate J, Rodriguez Romano D. Evaluation of scolicalidal agents in an experimental hydatid disease model. *Eur Surg Res* 1997; 29: 202-208.
- Gossard AA, Angulo P, Lindor KD. Secondary sclerosing cholangitis: A comparison to primary sclerosing cholangitis. *Am J Gastroenterol* 2005; 100: 1330-1333.
- Belghiti J, Perniceni T, Kabbej M, Fekete F. Complications of peroperative sterilization of hydatid cysts of the liver. Apropos of 6 cases. *Chirurgie* 1991; 117: 343-346.
- Prasad J, Bellamy PR, Stubbs RS. Instillation of sclicidal agents into hepatic hydatid cysts: can it any longer be justified? *NZ Med J* 1991; 104:336-337.
- Pappalardo G, Frattaroli FM, Ioppolo A, Conti E, Reggio D, DiMacco G, Castrini G. Secondary sclerosing cholangitis: an experimental study. *Eur Surg Res* 1988; 20: 358-363.
- Zoharizadeh MR. In vitro protoscolicidal effect of garlic extract. MD thesis. Shiraz, Iran: Shiraz University of Medical Sciences; 2000. p. 126-129.
- Barzin Z, Sadjjadi SM, Hosseini SV, Mehrabani D. In vitro and in vivo protoscolicidal effect of chloroformic garlic extract on hydatid cyst. MSc thesis, Shiraz, Iran: Shiraz University of Medical Sciences; 2006. p. 75-76.
- Sadjjadi SM. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int* 2006; 55 :197-202.
- Sahin M, Eryilamaz R, Bulbuloglu E. The effect of scolicalidal agents on liver and biliary tree (experimental study). *J Invest Surg* 2004; 17: 323-326.
- Castellano G, Moreno-Sanchez D, Gutierrez J, Moreno-Gonzalez E, Colina F, Solis-Herruzo JA. Caustic sclerosing cholangitis. Report of four cases and a cumulative review of the literature. *Hepatogastroenterology* 1994; 41: 458-470.
- Meshikhes AWN. Surgical treatment of hydatid cysts of the liver. *Br J Surg* 2003; 90: 1536-1541.
- Tozar E, Topcu O, Karayalcin K, Akbay SI, Hengirmen S. The effects of cetrimide-chlorhexidine combination on the hepato-pancreatico-biliary system. *World J Surg* 2005; 29: 754-758.
- Houry S, Languille O, Huguier M, Benhamou JP, Belghiti J, Msika S. Sclerosing cholangitis induced by formaldehyde solution injected into the biliary tree of rats. *Arch Surg* 1990; 125: 1059-1062.
- Taranto D, Beneduce F, Vitale LM, Loguerico C, DelVacchio Blanco C. Chemical Sclerosing cholangitis after injection of scolicalidal solutions. *Ital Gastroenterol* 1995; 27: 78-79.
- Vitellas KM, Keogan MT, Freed KS, Enns RA, Spritzer CE, Baillie JM, et al. Radiologic Manifestations of sclerosing cholangitis with emphases on MR Cholangiopancreatography. *Radiographics* 2000; 20: 959-975.
- Harrison PM. Diagnosis of primary sclerosing cholangitis. *J Hepatobiliary Pancreat Surg* 1999; 6: 356-360.
- Colle I, Van Vlierberghe H. Diagnosis and therapeutic problems of primary sclerosing cholangitis. *Acta Gastroenterol Belg* 2003; 66: 155-159.
- Engler S, Elsing C, Flechtenmacher C, Theilmann L, Stremmel W, Stiehl A. Progressive sclerosing cholangitis after septic shock: a new variant of vanishing bile duct disorders. *Gut* 2003; 52: 688-693.
- Rodriguez HJ, Bass NM. Primary sclerosing cholangitis. *Semin Gastrointest Dis* 2003; 14: 189-198.
- Nakazawa T, Ohara H, Sano H, Aoki S, Kobayashi S, Okamoto T, et al. Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis. *Gastrointest Endosc* 2004; 60: 937-944.
- Goetz M, Lehr HA, Neurath MF, Galle PR, Orth T. Long-term evaluation of a rat model of chronic cholangitis resembling human primary sclerosing cholangitis. *Scand J Immunol* 2003; 58: 533-540.