

***In vitro* Effectiveness of 3% Hydrogen Peroxide, 0.5% Silver Nitrate and 20% Hypertonic Saline on Protoscoleces of Hydatid Cyst**

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Several scolocidal agents have been used to eradicate the infective protoscoleces, however recurrences occur. The objective of this study was determining of the scolocidal effects of 3% Hydrogen peroxide, 0.5% Silver nitrate and 20% hypertonic saline on protoscoleces of hydatid cyst.

Protoscoleces were collected aseptically from sheep and cattle livers containing hydatid cyst.

Hypertonic saline was used as positive control, while normal saline was used as negative control. These scolocidal solutions were used for 5,10,15 and 20 minutes in the experiments. Viability of protoscoleces was assessed by 0.1% eosin staining. Normal saline had no significant protoscolocidal effect. However, 20% Hypertonic saline and 0.5% Silver nitrate solution revealed higher protoscolocidal effect than 3% Hydrogen peroxide. The results showed that hypertonic saline 20% and 0.5% Silver nitrate solution is highly effective in killing protoscoleces of *Echinococcus granulosus in vitro*.

Key words: Hydatid cyst, Protoscoleces, Protoscolocidal agents, Hypertonic saline, Silver nitrate, Hydrogen peroxide.

Hydatidosis is one of the most important parasitic zoonoses caused by *Echinococcus granulosus*. The disease has a worldwide distribution and is endemic in several countries including Mediterranean countries, the middle and Far East, South America, Australia, New Zealand and East Africa¹. Humans are infected by ingesting

of parasite eggs leading to cyst formation mainly in the liver and lung^{2,3}. The most confident way for treatment of hydatid cyst is surgical operation, but dissemination of protoscoleces rich fluid during surgery is a major cause of recurrence⁴.

The best treatment is considered surgery. During the surgery and before evacuation of the cyst, protoscolocidal agents are used to be injected into the cyst in order to prevent secondary cyst formations⁵. The most common protoscolocidal agents used are hypertonic saline, Silver nitrate, cetrimide and formalin; each has a variety of dangerous complications. In this regard, World Health Organization (WHO) purposed an Urgent

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need to find new protoscolicidal agents which are more effective and with less side effects⁶.

Hypertonic saline is one of the most common scolocidal agents in the world. The rationale for its use is simply that it affects a sufficiently strong osmotic gradient across the outer cuticular membrane of the scoleces to cause lyses. No doubt lethality can be achieved if the concentration of the salt solution is high enough and the exposure time long enough^{5,7}. Although hypertonic saline has been used as a scolocidal agent in various concentrations (3–30%) using various exposure times (5–30 minutes), there are no data about which concentration is suitable for which exposure time⁸. The objective of this study is to determine the scolocidal effect of 20% saline, 0.5% Silver nitrate and 3% Hydrogen peroxide using different exposure times.

MATERIAL AND METHODS

Hydatid cysts from the livers and lungs of sheep and cattle were obtained from the slaughterhouse of Urmia city and transferred to parasitology laboratory of faculty of medicine. Hydatid cyst fluid was aspirated by 20cc syringe from the cysts, collected in a bottle, sterile cysts were deleted. The cyst fluid stayed in room temperature and the supernatant removed. Centrifugation was never applied as it might destroy the protoscoleces. Protoscoleces stained with 0.1% eosin were examined under $\times 100$ magnification at room temperature. Dead protoscoleces absorbed eosin and colored red but

alive protoscoleces remained colorless, also live protoscoleces had movement in their flame cells. When live protoscoleces with enough volume were obtained, 1 drop of concentrate protoscoleces was poured into a test tube and 1ml of mentioned agents added. Totally we had 5 tubes. First tube 3% Hydrogen peroxide, second tube Silver nitrate 0.5%, third tube 20% hypertonic saline, fourth tube normal saline as negative control, fifth tube saturated saline as positive control. We waited 5, 10, 15, and 20 minutes for each scolocidal agent, following which the upper portions of the solutions were removed. The remaining settled protoscoleces were stained and examined for viability via the movement of flame cells and dye absorption ability. The first 100 protoscoleces were counted in the microscopic field and the live/dead ratio was calculated.

RESULTS

After 5, 10, 15 and 20 min, the protoscolicidal effects were determined by 0.1% eosin and the movement of flame cells under $\times 100$ magnification. Normal saline as negative control had no significant protoscolicidal effect. However, 20% hypertonic saline and 0.5% Silver nitrate solution revealed higher protoscolicidal effect than 3% Hydrogen peroxide. Saturated hypertonic saline killed all of protoscoleces in 5 minutes as positive control. The results showed that hypertonic saline 20% and 0.5% Silver nitrate solution is highly effective in killing protoscoleces of *Echinococcus granulosus in vitro*.

Table 1. Effect of 20% hypertonic saline on protoscoleces in 5,10,15 and 20 minute

Scoliciadal aganet	5 th minute	10 th minute	15 th minute	20 th minute
20%heypertonic saline	Dead	Dead	Dead	Dead
Colored by 1% eosin	+	+	+	+
Movement of flame cells	-	-	-	-

Table 2. Effect of normal saline on protoscoleces in 5,10,15 and 20 minute as negative control

Scoliciadal aganet	5 th minute	10 th minute	15 th minute	20 th minute
Normal saline	Live	Live	Live	Live
Colored by 0.1% eosin	-	-	-	-
Movement of flame cells	+	+	+	+

Table 3. Effect of 0.5% silver nitrate on protoscoleces in 5,10,15 and 20 minute

Scoliciadal aganet	5 th minute	10 th minute	15 th minute	20 th minute
0.5%Silver nitrate	Dead	Dead	Dead	Dead
Colored by 0.1% eosin	+	+	+	+
Movement of flame cells	-	-	-	-

Table 4. Effect of Saturated hypertonic saline on protoscoleces in 5,10,15 and 20 minute as positive control

Scoliciadal aganet	5 th minute	10 th minute	15 th minute	20 th minute
Saturated hypertonic saline	Dead	Dead	Dead	Dead
Colored by 0.1% eosin	+	+	+	+
Movement of flame cells	-	-	-	-

DISCUSSION

The traditional treatment of liver hydatid cysts is surgery. However, the surgical results indicate a high rate of mortality, morbidity, and recurrence (9-11). Surgical procedures range from simple punctures and aspirations of the cyst, to liver resections or even transplantation, but the most common technique is total or partial cystectomy (12). The mortality rate ranges from 0% to 6.3% and the complication rate of surgery varies between 12.5% and 80% in accordance with the surgical technique performed and the nature of the cysts (9,13).

However, in the early 1980s several reports of accidental punctures of abdominal hydatid cysts with no severe complications contributed to the application of deliberate puncture of abdominal cysts followed by the introduction of a scolocidal agent. WHO consultants recommended this method as an alternative method to surgery (11) but it has some limitations as well. To begin with, it is not possible to remove all of the components of the cyst, to destroy the cavity completely, and to drain the cyst which contains solid material (14). One of the remarkable findings was to observe that alcohol, commonly used as a scolocidal agent, did not produce the desired effect on any of the 3 components of the hydatid cyst. They thought should be inactivated for effective treatment and for preventing recurrences by finding a better scolocidal agent.

So we tried to examine some different agents on protoscoleces in order to find the most effective scolocidal agent. Hydrogen peroxide 3% had a strong effect on scoleces and killed all of them after 15 minutes. 0.5% Silver nitrate started to effect on scoleces after 5 minutes and saturated saline as a positive control killed all of scoleces in 5 minutes, but 20% hypertonic saline killed scoleces in 5 minutes. Normal saline had no effect even after 20 minutes.

In a similar study done by Besim et al. in 1998, 20% hypertonic saline and 3% hydrogen peroxide introduced as effective scolocidal agents that kill in 15 minutes they reported that 3% saline and normal saline make scoleces inactive but do not kill them¹⁵. In other study by Paksoy and his group 20% hypertonic saline starts its affect after injection and kills scoleces in 5 minutes. The results of this study were similar to ours¹⁶. In 1963, protoscolocidal effects of silver nitrate were studied by Meymerian et al. It was shown that 0.05% silver nitrate had higher protoscolocidal effect than 1% concentration¹⁷. The protoscolocidal effect of 0.5% silver nitrate was confirmed in *in vivo* through injection of exposed protoscoleces into the mice peritoneum by Saidi in 1976(5). In our study, 0.5% silver nitrate could kill all of protoscoleces after 5 min of exposure *in vitro*.

Hypertonic sodium chloride with production of osmotic gradient was able to destroy protoscoleces¹⁸. Other studies by Abbasi Dezfuli and Ghazanfari showed that 3% and 10% concentrations didn't have any protoscolocidal

effect. However, 20% concentration could kill all protoscolecocytes after 5 min of exposure^{18,19}. In the present study, 20% sodium chloride could kill almost all protoscolecocytes after 5 min.

In conclusion, many substances used and examined as scolicidal in our study, However Most effective agents were hypertonic saline 20% and silver nitrate 0.5%.

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