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# Time-Related Comparison of Scolicidal Activity of the Different Substances Used In Hydatid Cyst

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**Abstract**

Inactivation of protoscoleces is one of the main principles of treatment in hydatid cysts. The aim of the present study is to evaluate the scolicidal efficacies of some frequently used scolicidal substances in hydatid cyst fluid.

Hydatid cyst fluids were aspirated from ten patients during surgical treatment sand the interventional radiology procedures. The suitable cyst fluids were examined for interactions with 0.9% NaCl, 15% NaCl, 10% povidone-iodine, savlon, crystalline, and 0.4% chlorhexidine gluconate (Chx-Glu), respectively. Scoleces in the mixtures were counted at minutes 2, 5, 10 and 20. The ratio of the number of living scoleces/total scoleces was calculated. Normal distribution of numerical data was analyzed using the Shapiro -Wilk test. ANOVA and LSD tests were used in the comparison of variables.

There were significant differences between each scolicidal substances and time in terms of the ratio of living scoleces ($p<0.05$). But all scolicidal substances failed to kill all of the scoleces in the second and fifth minutes. There were significant differences between Chx-Glu and other scolicidal substances in the second minute apart from povidone-iodine, and 99% of the scolexes died at 5 minutes with Chx-Glu. Chx-Glu, savlon, and 10% povidone-iodine showed full efficacy in minute 10. Crystalline and 15% NaCl were able to reach full efficacy in minute 20.

The efficacy of all substances was increasing with the waiting period. As soon as possible, the fastest active substance Chx-Glu seems to be used in conservative surgical interventions.

**Key Words:** chlorhexidine gluconate, hydatid cyst, protoscoleces, scolocidal substance, time

## Introduction

Hydatid cyst is a rare zoonotic disease can be seen throughout the world nowadays. Although it is seen in every region of Turkey, it is a significant health problem particularly in the regions of eastern and southeastern Anatolia.

The disease is relatively easy to diagnose with imaging modalities, but there is still no effective medical treatment. The curative treatment of the disease is generally provided by surgical or interventional radiology procedures (1). The inactivation of living scoleces is one of the main principles of curative treatment. In the literature, there are many drugs used as scolicidal agents. However, there is still no consensus on an agent that shows the most suitable and fastest efficacy without having any side effects.

The main reasons causing recurrence of hydatid cyst disease include primarily the spilling of cyst contents into the intraperitoneal area while draining the cyst, the improper use of scolicidal agents and not being able to reach the pockets in the pericyste during conservative surgery procedures (2-4). Conservative surgical procedures are more widely used in our country. In these procedures, it is important to know which scolicidal substance to be administered into hydatid cysts. In many hospitals, different chemicals are used, and in multiple cysts, there is a lot of waiting time for inactivity in surgery. We think that scolicidal substance is not kept into the cyst for enough time. This condition can lead to recurrent hydatid cyst.

The aim of this study is to evaluate the scolicidal efficacies of some frequently used different chemical substances in hydatid cyst fluid in vitro relative to time.

## Materials and Methods

The approval of The Ethics Committee No. 2016/93 dated 21.03.2016 was obtained for this prospective clinical in vitro study.

The study was conducted between January 2017 and July 2018 in department of general surgery and microbiology laboratory. Samples were collected from ten patients.

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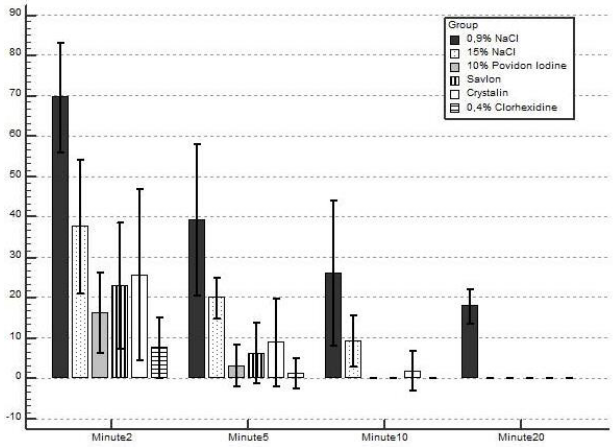


**Fig. 1.** Graph showing the ratio of living scoleces in hydatid cyst fluid

Patients who were planned to receive hepatic hydatid cyst were informed about our study and their consent was obtained. Then an appropriate treatment plan was created.

A cyst fluid was aspirated in a sterile manner with an 18 G needle - 50ml injector during surgical treatment. Similarly, in patients who underwent PAIR, a cyst fluid was aspirated in a sterile manner using an 18 G puncture needle guided by ultrasound.

The hydatid cyst fluids were immediately examined in the microbiology laboratory by the same physician at the different times. Scoleces that maintained their cestoid movement, oval shape and that were not stained with eosin dye under a microscope were accepted as living scoleces. Scoleces whose rostellum were sucked in, invaginated and rounded and that were stained with eosin dye were accepted as dead. It was found that scoleces in hydatid cyst fluids that were contaminated with bile were dead or mostly dead. Hydatid cyst fluids that had the typical rock water appearance contained a substantially high amount of living scoleces.

Then the stage of evaluating the interaction between scolicidal agents and hydatid cyst fluids that contained a sufficient number of living scoleces (>1000 scoleces/ml) was initialized. A known volume of the mix suspension (0.01 ml) was spread uniformly over a glass slide covering a specific area (1 sq. cm). The smear was then fixed, stained and examined under oil immersion lens, and the total scoleces are counted. Customarily, cells in a few microscopic fields are counted because it is not possible to scan the entire area of the smear. The counting of the total number of cells is determined by calculating the total number of microscopic fields per one square cm. area of the smear.

Centrifugation was not performed, so as to prevent the scoleces from mechanical disintegration.

The cyst fluids taken from three patients were eligible for quality and vitality. So, the study was repeated three times.

0.2ml of hydatid cyst fluid was placed in each of the six microtubes. Then, 0.2 ml scolicidal agents each containing 0.9% NaCl, 15% NaCl, 10% povidone-iodine, 0.15% chlorhexidine gluconate - 1.5% cetrimide (cetyl trimethyl ammonium bromide [Savlon solution]), 0.000024% Hydrogen peroxide - 0.24% sodium hypochlorite (crystalline solution), and 0.4% chlorhexidine gluconate (Chx-Glu), respectively, were added to the microtubes.

At minutes 2, 5, 10 and 20, samples were collected from the hydatid cyst fluid - scolicidal agent mixture and the scoleces were counted under a microscope at 100 (x) zoom. The ratio of the number of living scoleces/total scoleces was calculated.

For statistical analysis, normal distribution of numerical data was analyzed using the Shapiro-Wilk test. ANOVA and LSD tests were used in the comparison of variables that fitted the normal distribution. Tests were conducted using SPSS 22.0 package software and $p<0.05$ was considered significant.

## Results

The hydatid cyst fluids from ten patients; two of the cysts were localized in the left lobe and eight in the right lobe of the liver. According to Gharbi classification, three cysts were type 1 (active scoleces), three cysts were type 2 (active scoleces), three cysts were type 3 (one active scolex) and one cyst was type 4. Four the patients had one cyst, and six the patients had more than one cyst. Cyst diameter was smaller than 5cm in three patients and larger than 5cm in seven patients.

The ratio of living scoleces relative to time was calculated for five agents used as scolicidal and for the control group 0.9% NaCl (Table 1).

All the different scolicidal agents measured were significantly more effective than 0.9% NaCl at all time intervals (2, 5, 10, 20 min) ($p <0.001$).

When all the scolicidal study agents were compared, 15% NaCl was the most inactive agent (Table 2).

All scolicidal agents added to hydatid cyst fluids failed to kill all of the scoleces in the second and the fifth minute. Chx-Glu, savlon, and 10%

**Table 1.** Ratio of living scoleces by time in hydatid cyst fluid-scolicidal agent interaction

| Agents | The ratio of living scoleces (%) | | | |
|---|---|---|---|---|
| | Minute 2 | Minute 5 | Minute 10 | Minute 20 |
| 0.9% NaCl (control) | 69.67±5.51 | 39.33±7.50 | 26.00±7.21 | 18.00±1.73 |
| 15% NaCl | 37.67±6.66 | 20.00±2.00 | 9.33±2.52 | 0.00±0.00 |
| 10% Povidone iodine | 16.33±4.04 | 3.33±2.08 | 0.00±0.00 | 0.00±0.00 |
| Savlon | 23.00±6.24 | 6.33±3.05 | 0.00±0.00 | 0.00±0.00 |
| Crystalline | 25.67±8.50 | 9.00±4.36 | 2.00±2.00 | 0.00±0.00 |
| Chlorhexidine | 7.67±3.05 | 1.33±1.53 | 0.00±0.00 | 0.00±0.00 |

**Table 2.** Multiple comparisons of 15% NaCl and other agents relative to time

| | Time | Substances | p- value |
|---|---|---|---|
| 15%NaCl | Minute 2 | 0.9%NaCl | 0.000 |
| | | Povidone iodine | 0.001 |
| | | Savlon | 0.011 |
| | | Crystalline | 0.029 |
| | | Chlorhexidine | 0.000 |
| | Minute 5 | 0.9%NaCl | 0.000 |
| | | Povidone iodine | 0.000 |
| | | Savlon | 0.001 |
| | | Crystalline | 0.005 |
| | | Chlorhexidine | 0.000 |
| | Minute 10 | 0.9%NaCl | 0.000 |
| | | Povidone iodine | 0.004 |
| | | Savlon | 0.004 |
| | | Crystalline | 0.016 |
| | | Chlorhexidine | 0.004 |
| | Minute 20 | 0.9%NaCl | 0.001 |
| | | Povidone iodine | 1.000 |
| | | Savlon | 1.000 |
| | | Crystalline | 1.000 |
| | | Chlorhexidine | 1.000 |

povidone-iodine showed full efficacy in minute 10. Crystalline and 15% NaCl were able to reach full efficacy in minute 20.

In relation to time, the highest efficacy within the shortest time was exhibited by Chx-Glu (Figure 1).

When using Chx-Glu, nearly 93% of the scoleces died in the second minute. There were significant differences between Chx-Glu and other scolicidal agents apart from povidone-iodine in the second minute ($p<0.05$). Chx-Glu was still superior to crystalline and 15% NaCl in the fifth minute. In minute 10, Chx-Glu was only significantly different to 15% NaCl (Table 3).

There were significant differences between each scolicidal agent and time in terms of the ratio of living scoleces ($p<0.05$).

## Discussion

The most effective methods for preventing hydatid cyst disease consist of improving hygiene conditions, taking protective measures in endemic areas, and informing the individuals who are at risk about the disease.

The ideal treatment method for hydatid cyst should be selected according to the patient's clinical condition and cyst type. However, there is still a debate as to the treatment algorithms used in this period. Today, the preferred methods include surgical or interventional radiology methods, depending on the radiologic findings for the type of hydatid cyst. However, in all treatment methods, the recurrence rate of the disease is

**Table 3.** Multiple comparisons of chlorhexidine and other agents relative to time

| | Time | Substances | p- value |
|---|---|---|---|
| Chlorhexidine | Minute 2 | 0.9%NaCl | 0.000 |
| | | 15%NaCl | 0.000 |
| | | Povidone iodine | 0.098 |
| | | Savlon | 0.009 |
| | | Crystalline | 0.003 |
| | Minute 5 | 0.9%NaCl | 0.000 |
| | | 15%NaCl | 0.000 |
| | | Povidone iodine | 0.550 |
| | | Savlon | 0.150 |
| | | Crystalline | 0.036 |
| | Minute 10 | 0.9%NaCl | 0.000 |
| | | 15%NaCl | 0.004 |
| | | Povidone iodine | 1.000 |
| | | Savlon | 1.000 |
| | | Crystalline | 0.462 |
| | Minute 20 | 0.9%NaCl | 0.000 |
| | | 15%NaCl | 1.000 |
| | | Povidone iodine | 1.000 |
| | | Savlon | 1.000 |
| | | Crystalline | 1.000 |

higher than the desired healing.

In order to prevent recurrence of hydatid cysts, the scolicidal agent should be administered properly and within the appropriate time. Generally, physicians or surgeons select scolicidal agents according to their own experience.

In hydatid cyst patients who have undergone surgical treatment, it is reported that the cyst type according to radiologic classification is not associated with recurrence (4). In the present study, it was observed that the number of living protoscoleces was remarkably low in fluids obtained from Gharbi type 4 hepatic hydatid cyst cases. This was thought to stem from bile contamination in the hydatid cyst fluid. Although this might imply that recurrence rates might be lower in hydatid cysts that have a biliary communication, the literature data does not support this notion (5).

Another debatable issue is to provide scolicidal efficacy in hydatid cysts using only medical treatment. It has been asserted that 30% of the patients who were administered the only albendazole were cured, and 50% of the patients clearly responded to the treatment (6). The disease recurrence ratio is higher seen in a patient who did not receive any albendazole therapy (7). We think that medical treatment should be used for perioperative prophylaxis and to prevent spreading during surgery.

Hypertonic saline solutions are the most commonly used scolicidal agents throughout the world. NaCl is used in various concentrations. Use of different concentrations of the agent leads to change different efficiency times (8). Unfortunately, it is known that efficacy decreases in low concentrations. In a study comparing in vitro scolicidal agents, hypertonic saline was found to be significantly more effectivethan 0.9% NaCl (9). The full activity of 20% NaCl was in the 45th minute. Risk of hypernatremia and risk of prolongs the duration of the procedure increase at the use of 20% NaCl. A review published last year evaluating several different agents shows the effective scolicidal activities of hypertonic saline and Chx-Glu (10). Kayaalp et al. have shown that 0.9%, 3%, and 6.5% concentrations of hypertonic saline were not fully effective for 60 minutes, whereas 10%, 15%, 20%, and 30% concentrations showed full efficacy at minutes 75, 10, 6 and 3, respectively. On the other hand, they also emphasized that hypertonic saline was not safe to use due to its side effects (11). In the present study, it was found that 15% NaCl can show full efficacy at minute 20. In Turkey, for the treatment of cyst fluid, povidone-iodine and Ag nitrate were

previously used as scolicidal agents, though hypertonic solutions are presently preferred (12,13). In accordance with the literature, povidone-iodine was used in more than half of the patients until 1995, while hypertonic saline solutions were generally preferred later (14).

Chx-Glu is an antiseptic agent used in the local treatment of intraabdominal infection. This agent has a broad effect spectrum on gram-negative, gram-positive and fungal infections (15). It has been shown that lavage using 0.05% Chx-Glu is effective and nontoxic in patients with intraabdominal infection (16). A study indicated that 0.04% Chx-Glu solution shows high efficacy within a short period in vitro and achieves 100% efficacy in vivo in case of intraperitoneal hydatid cyst (17). Later Topcu et al. demonstrated that 0.04% Chx-Glu showed full efficacy within 5 minutes both in the cyst fluid and germinative membranes in 45 liver hydatid cysts in a study performed on 30 patients (18).

It was stated that the use of a sponge impregnated with 0.04% Chx-Glu showed both a mechanical and chemical barrier effect, killed all protoscoleces within 15 minutes and that it was the strongest agent (18).

10% povidone-iodine is used as a scolicidal agent by many surgeons. Bosanac et al. (19) used 10% povidone-iodine as a scolicidal agent in the percutaneous treatment of 52 patients with liver hydatid cysts. Drainage was performed after 30 minutes of interaction with the cyst fluid. No recurrences were seen during follow-ups for 6-9 years. There no significant major complications either. It was reported that three liver abscesses developed and were treated successfully. According to an earlier experimental study, it is supported that 10% povidone-iodine is more effective than saline as a scolicidal agent in intraperitoneal hydatidosis (20). Another in vitro and in vivo experimental study compared polyvinylpyrrolidone-iodine (Pvp-1) with normal saline and 2% taurolidine. It has been shown that Pvp-1 has almost full scolicidal effect in minute 5 (21). The present study also indicated that the faster-acting substance was 10% povidone-iodine after Chx-Glu.

Comparing 20% NaCl, 3% hydrogen peroxide, 10% savlon, 95% ethyl alcohol and 10% povidone-iodine, savlon was found to be the least concentration-dependent scolicidal agent among those studied (22). In the present study, savlon was significantly more effective than 15% NaCl solution.

In surgical treatment, the recurrence risk is higher in conservative approaches compared to radical surgery because the cyst cavity is opened and this may lead to spreading to nearby tissues/cavities of daughter vesicles, endocysts localized on the cyst wall. Therefore, the choice of the scolicidal substance becomes much more important, especially in conservative procedures. Conservative surgical procedures are preferred more often in Turkey because of the fact that radical surgery is a more major procedure requiring experience and its complications are severe. Hence, waiting time for complete inactivation is of very importance in order to let the scolicidal substance interact with the cyst.

An ideal agent should have full scolicidal efficacy at minimum concentration and within the minimum time period. It is preferred that the agent does not have local and systemic side effects, that it is easily accessible and cost-efficient. A completely ideal agent is yet to be found due to existing low scolicidal activities and side effects. There is no Cochrane review for scolicidal substances yet in hydatid cyst. The side effect profile, which is an important reason for selecting a scolicidal agent to use, should be investigated in animal models with new studies and an ideal scolicidal agent should be sought by performing effectivity and reliability analyses. In this way, it would be possible to lower the recurrence rates after surgical or percutaneous drainage.

In the present study, Chx-Glu showed the highest efficacy within the shortest time. Povidone-iodine and Savlon nearly reached the same efficacy. In terms of time, Chx-Glu, savlon, and 10% povidone-iodine showed more efficacy than 15% NaCl. Chx-Glu seems to be used in surgical and the interventional radiology procedure.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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