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# Hypertonic Saline in Hydatid Disease

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**Abstract. The objective of this study was to determine the scolicidal effects of saline in different concentrations using different exposure times and to examine whether hypertonic saline can be used to irrigate the abdomen when there is a free intraperitoneal perforation of hydatid disease. Various concentrations of saline solutions (0.09%, 3.0%, 6.5%, 10%, 15%, 20%, 25%, 30%) were added to concentrated *echinococcus granulosus* sediments for the following times: 1, 2, 3, 4, 5, 10, 15, 30, 45, and 60 minutes. Normal (0.09%), 3.0%, and 6.5% saline resulted in high viability ratios after 60 minutes' exposure. Complete lethality for 10%, 15%, 20%, 25%, and 30% saline occurred at the end of 75, 10, 6, 3, and 3 minutes, respectively. During the second part of the study, 20 Sprague-Dawley rats were used for abdominal saline irrigation in four groups: 30% NaCl for 3 minutes; 20% NaCl for 6 minutes; intravenous isotonic dextrose water and furosemide plus 30% NaCl irrigation for 3 minutes; the same prophylactic therapy plus 20% NaCl irrigation for 6 minutes. Sodium and chloride values rose significantly (20–30%) shortly after hypertonic saline irrigation in each group ($p < 0.01$). Support with isotonic dextrose and furosemide before irrigation did not have any beneficial effect on biochemical values or mortality. The 24- and 48-hour mortality rates were 70% and 90%, respectively. These studies illustrate that the scolicidal effect of hypertonic saline is limited in low concentrations, but an increase in the concentration can augment its adverse effects. Peritoneal irrigation with hypertonic saline should be avoided for intraabdominal perforated hydatid disease. Therefore, we concluded that hypertonic saline is not a good scolicidal agent to prevent recurrence of hydatid disease.**

Hydatid disease is endemic to the Middle East, the areas bordering the Mediterranean Sea, South Africa, Northern Canada, Australia, and New Zealand. Although most hydatid disease are found in liver and lung, the disease can arise anywhere in the body. Despite low recurrence rates after complete removal, in most cases total cystectomy involves a major organ resection, with its attendant increase in operative risk for a benign disease [1]. For this reason, radical methods are not popular, especially in the endemic areas [2]. The conservative approach includes evacuation of the cyst cavity and irrigation with a scolicidal agent. This type of therapy is generally simpler and can be done using radiologic intervention [3]. Recurrence during long-term follow-up is the most common problem with this therapeutic approach. Avoiding spillage of the cyst contents and the use of effective scolicidal agents are essential to lessen the recurrence rate [2]. Inadequate scolicidal concentration or exposure time, inadequate evacuation of the hydatid membranes, and dilution of the scolicidal agent by the cyst fluid are the main causes of diminished scolicidal effect [4].

Hypertonic saline is one of the most common scolicidal agents in the world. The rationale for its use is simply that it effects a sufficiently strong osmotic gradient across the outer cuticular membrane of the scolex to cause lysis. No doubt lethality can be achieved if the concentration of the salt solution is high enough and the exposure time long enough [5, 6]. Although hypertonic saline has been used as a scolicidal 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 time. The objective of this study is to determine the scolicidal effect of saline in different concentrations using different exposure times. In the second part of the study we examined whether hypertonic saline can be used to irrigate the abdomen when there is free intraperitoneal perforation of the hydatid disease.

## Materials and Methods

Hydatid cysts from the livers of sheep were obtained from the slaughterhouse the same day the animals were slaughtered. Hydatid fluid was aspirated from the cysts, collected in a bottle, and the supernatant removed. Centrifugation was never applied as it might destroy the protoscolices; cyst fluid, rather than saline, was used for suspension so the surgical working conditions were reproduced. The viability of this suspension was confirmed prior to the experiments. Scolicidal activity was determined using a previously described method [7]. Protoscolices stained with 0.01% eosin were examined under ×100 magnification at room temper-

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ature. Because flame cell activity disappears before taking in the dye [8], we did not use this early criterion for lethality. We 0preferred the strict criterion of staining the protoplasm to confirm lethality. Each protoscolex that did not take the dye in was accepted as potentially viable. Concentrated (10,000 protoscoleces/$mm^3$) cyst fluid was used, and eight samples of 0.5 cc sediment were obtained. Various concentrations of 9.5 cc sodium chloride solutions were added to each sediment (0.09%, 3.0%, 6.5%, 10%, 15%, 20%, 25%, 30%, respectively). We waited 1, 2, 3, 4, 5, 10, 15, 30, 45, and 60 minutes for each concentration, following which the upper portions of the solutions were removed. The remaining settled protoscolices were then washed in normal saline and, after staining, examined for viability. The first 100 protoscolices were counted in the microscopic field and the live/dead ratio was calculated. At first the experiment was repeated five times and then for some periods during which total lethality was critically important. Test times were prolonged to 6 minutes for 20% saline and to 75 to 90 minutes for 10% saline to attain total lethality. The tests were then repeated five times.

For the invivo part of the study, we examined whether hypertonic saline can be used to irrigate the abdomen when there is a free intraperitoneal perforation of the hydatid disease. First we showed that 30% sodium chloride was 100% lethal to protoscolices in 3 minutes, whereas 20% sodium chloride took 6 minutes to be totally effective, and saline concentrations less than 20% were not totally effective in 6 minutes. These concentrations and constant times were examined in the experiment.

Female Sprague-Dawley rats ($n = 20$) weighing 140 to 270 g were used for the experiment. The rats were obtained from the Gulhane Military Medical Academy, Research and Development Center, Department of Experimental Animals Breeding in Ankara, Turkey. The animals were housed in a climate-controlled animal-care facility with free access to standard rat chow (Rat feed, Korkutelim Yem Gida Sanayii A.S. Korkutelim Feed Factory, Afyon, Turkey) and tap water. All research was conducted in compliance with Gulhane Military Medical Academy Surgical Research Laboratory Review Board standards. The subcommittee on research animal care at our institution approved the study.

The rats received enflurane inhalation anesthesia. Phlebotomy was performed by cardiac puncture. A 2 ml blood sample was obtained and centrifuged immediately. Sodium and chloride ion concentrations were established from the plasma with an autoanalyzer (EST Light plus, Belford, Ma, USA). After shaving the abdomen, a 5 cm long subxiphoid midline incision was made through the skin and peritoneum. The rats were then randomized into four groups as follows.

1. Hypertonic saline (30%) was used to irrigate the abdomen for 3 minutes.
2. Hypertonic saline (20%) was used to irrigate the abdomen for 6 minutes.
3. 5% Dextrose water (2 ml) and 0.25 mg furosemide were injected into the inferior vena cava for prophylactic therapy of acute hypernatremia. The abdomen was then irrigated with 30% hypertonic saline for 3 minutes.
4. After the same prophylactic therapy protocol described above (dextrose and furosemide), the abdomen was irrigated with 20% hypertonic saline for 6 minutes.

Scolicidal agents were aspirated immediately at the end of the exposure time and the abdomen was washed out with 20 ml normal saline. After cleansing the abdomen, a 2 ml blood sample was obtained from the inferior vena cava for sodium chloride determination, and the abdomen was closed in the usual way. All the groups was followed closely and separately, and they were permitted free rat chow and tap water.

Preoperative and postoperative sodium and chloride ion concentrations and mortality rates were followed for 7 days. Brain tissue samples were obtained immediately after death, and living animals were sacrificed at the end of the experiment for brain histopathology. Tissues were preserved in formalin solution and examined at the Gulhane Military Medical Academy Department of Pathology.

To compare preoperative and postoperative sodium chloride values, we used the dependent-two-samples *t*-test. We used the Mann-Whitney U-test to compare the numeric values among groups. For mortality rates, we used Fisher's exact chi-square test.

## Results

The viability ratio for all concentrations of the protoscolices is summarized in Figure 1. Normal saline (0.09%) and 3.0% and 6.5% hypertonic saline had a high viability ratio after 60 minutes of exposure. Saline 10% had a progressive scolicidal effect within 60 minutes. During the early period (1–10 minutes) more than half of the protoscolices were viable; at the end of 60 minutes, viability was reduced to 10%. When the exposure time was extended, complete lethality for 10% saline was observed at the end of 75 minutes. The 15% saline killed all of the protoscolices at the end of 10 minutes. The 20% saline was highly effective during the first 5 minutes. At the end of 3 and 5 minutes, 90% and 98% to 99% of the protoscolices died, respectively; but it took at least 6 minutes for complete lethality by 20% saline. Both 25% and 30% saline are totally lethal in 3 minutes. The only differences between the 25% and 30% saline were the live/dead ratios during the first and second minutes.

Tables 1 and 2 summarize the biochemical values of the in vivo study. There were no significant differences in any of the weights of the groups. Nor was there any difference between the preoperative sodium and chloride ion concentrations of the groups.

Sodium and chloride values rose significantly after hypertonic saline irrigation in each group ($p < 0.01$). We found the degrees of the rise to be approximately 20% for sodium and 30% for chloride. The chloride increase was greater than the sodium increase in every group. Support with isotonic dextrose and furosemide before irrigation did not have any beneficial effect on biochemical values or mortality.

Six minutes of 20% sodium chloride irrigation increased the sodium and chloride levels more than did 3 minutes of 30% sodium chloride irrigation (sodium 172.6 vs. 178.3 mEq/L; chloride 137.7 vs. 141.1 mEq/L; sodium increase 19.7% vs. 22.7%; chloride increase 28.7% vs. 31.9%). This increase did not reach statistical significance. The 24- and 48-hour mortality rates were 70% and 90%, respectively. Some rats had convulsions during follow-up, usually 15 to 20 minutes postoperatively.

## Discussion

Hypertonic saline has been used as a scolidical in various concentrations (3–30%) for 50 years [6]. The scolidical effect decreases substantially in low concentration (< 20%) [4], and the use of high

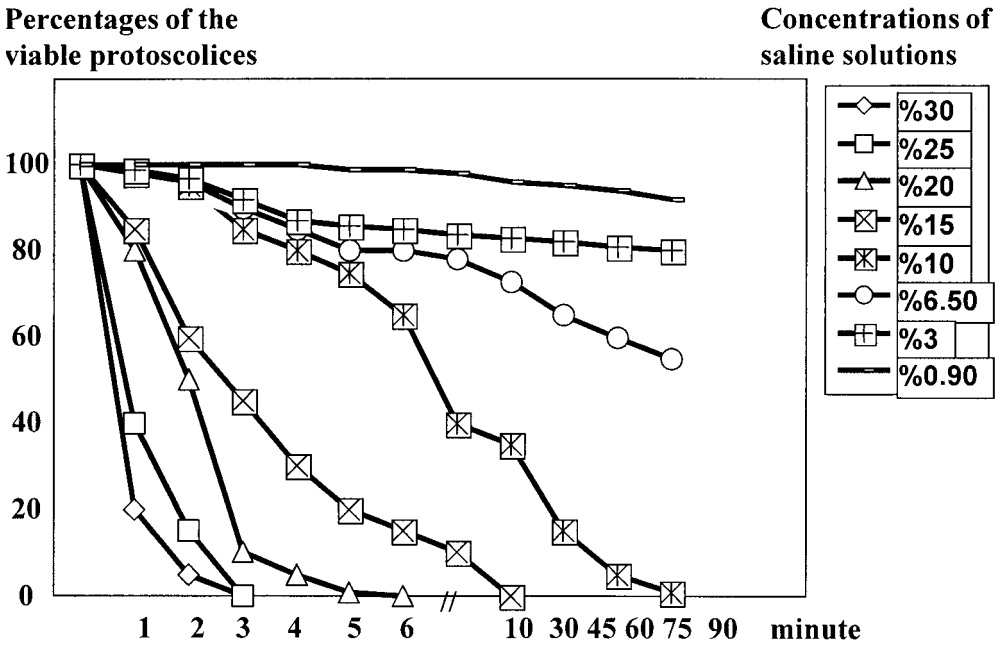


**Fig. 1.** Viability ratios of the protoscolices in different saline concentrations and for different exposure times.

**Table 1.** Serum sodium ion concentrations before and after peritoneal hypertonic saline irrigation.

| | Sodium ion levels (mEq/L) | | |
|---|---|---|---|
| Group | Before irrigation | After irrigation | Increase ratio (%) |
| I | 144 ± 2 | 175 ± 4* | 21 ± 3 |
| II | 144 ± 1 | 171 ± 8* | 26 ± 7 |
| III | 145 ± 1 | 178 ± 7* | 23 ± 5 |
| IV | 146 ± 2 | 178 ± 8* | 22 ± 5 |
| Total | 145 ± 2 | 175 ± 7* | 21 ± 5 |

* $p < 0.01$.

**Table 2.** Serum chloride ion concentrations before and after peritoneal hypertonic saline irrigation.

| | Chloride ion levels (mEq/L) | | |
|---|---|---|---|
| Groups | Before irrigation | After irrigation | Increase ratio (%) |
| I | 107 ± 1 | 140 ± 3* | 31 ± 3 |
| II | 107 ± 1 | 135 ± 6* | 26 ± 7 |
| III | 107 ± 1 | 141 ± 7* | 32 ± 7 |
| IV | 107 ± 2 | 142 ± 6* | 32 ± 8 |
| Total | 107 ± 1 | 140 ± 6* | 30 ± 6 |

* $p < 0.01$.

concentrations risks of inducing sclerosing cholangitis in the bile ducts and acute hypernatremia [9]. Perez Fontana in Uruguay first described hypertonic saline as a scolidical. He described hypertonic saline as a specific killer of the protoscolices and as not harmful to tissues. According to Martinez Peralta, use of hypertonic saline in the clinic has been initiated without prior examination. According to him, the disadvantages of formalin comprised the mean reason for the rapid acceptance of hypertonic saline as a scolicidal [6].

Although there is no experimental evidence on the effectiveness of 3% saline in the surgical literature, many surgeons use this concentration for scolicidal purposes [10–12]. We found 3% saline to be ineffective even at the end of 60 minutes. Little et al. observed a 22% recurrence rate during a 30-month follow-up when 3% saline was used [11].

Five percent saline was described as an effective scolicidal by Heslop [13], but we found even 6.5% saline to be ineffective in 60 minutes. Saidi also demonstrated that 5% saline was ineffective and recommended more concentrated solutions [5].

We found 10% saline to be 100% lethal only at the end of 75 minutes. The study by Landa Garcia et al. supported our findings that after intraperitoneal inoculation of inactivated protoscolices with 10% saline for 5 minutes there was no decrease in the number of cysts in comparison with the control group [14].

Although no experimental study on the effect of 15% saline has been reported in the literature, some recommend this concentration as an effective scolicidal [3, 15]. According to our study, 15% saline requires at least 10 minutes' exposure for total lethality of the protoscolices.

In vitro studies had shown that 20% saline was effective in 5 minutes [4]. We also found that 20% saline was highly effective in 5 minutes, but we recommend that the minimal waiting period for 20% saline should be at least 6 minutes. In repeated tests we observed 1% to 2% undyed protoscolices at 5 minutes. When we increased the exposure time to 6 minutes, all the protoscolices accepted the dye. A 30% solution is used in clinical practice [16]. This concentration is totally effective in 3 minutes.

Adding scolicidals to the cyst cavity without evacuation can cause dilution problems. For example, a 10 cm diameter cyst contains almost 500 cc of cyst fluid (from the volume of a sphere: $4/3\ \pi\ r^3$). If only 250 cc of cyst fluid is aspirated and is replaced by 20% saline, the end concentration decreases to half (10%), and the time required for total lethality is prolonged 12-fold (from 6 minutes to 75 minutes). We concluded that the scolicidal effect of hypertonic saline depends on the concentration rather than the exposure time. So, if the surgeon wishes sufficient effectiveness

from saline, he or she must increase the concentration rather than the exposure time.

Twenty percent saline should be used only if the cyst cavity is emptied completely. If more than half of the cyst can be emptied, 30% saline is preferred and the exposure time must be prolonged to 10 minutes (efficacy time for 15% saline). If less than the half of the cyst cavity can be emptied, using hypertonic saline does not seem rational. Hypertonic saline can be used only in the completely or almost completely emptied cysts and in high concentrations (20–30%).

There are no data to indicate which scolicidal agent is best when there is free perforation of hydatid disease in open wounds, muscles, abdomen, pleura, or cranium. Although Kune recommended 0.5% cetrimide and hydrogen peroxide for peritoneal irrigation [17]. Besim's group showed that peritoneal irrigation with a cetrimide 0.5% chlorhexidine 0.05% combination in rats results in a high mortality rate [18].

Hypertonic saline might have given a false sense of security to surgeons as if it were saline. Ersahin et al. used hypertonic saline for irrigation of perforated brain hydatid disease in a child. The child died of convulsions 2 days after the operation. The death was attributed to the formalin irrigation of the cyst cavity, not to the hypertonic irrigation of the brain tissue; and they recommended irrigating contaminated areas with hypertonic saline (20%) when free perforation occurred [19].

Hypertonic saline is a dangerous agent for washing out normal tissues. Sodium ion concentrations reach values of 170 to 180 mEq/L in a short time. Acute hypernatremia causes convulsions due to intracranial bleeding, necrosis, and myelinolysis. About 90% of the rats in this experiment died within 2 days because of acute hypernatremia. Prophylactic intravenous isotonic dextrose and furosemide supplement has no beneficial effect on either decreasing the degree of acute hypernatremia or the mortality.

Peritoneal irrigation with hypertonic saline should be avoided for intraabdominal perforated hydatid disease. Nor should it be used to wash out other tissues (pleura, intracranial or spinal organs, muscles) until further studies demonstrate conclusively that the practice is not harmful.

It is known that high-concentration saline can cause sclerosing cholangitis and bile duct strictures. The scolicidal effect of hypertonic saline is limited in low concentrations, and increasing the concentration can augment its adverse effects. Additionally, hypertonic saline is not a safe agent for peritoneal irrigation. We concluded, therefore, that hypertonic saline is not a good scolicidal agent for preventing recurrence of hydatid disease.

**Résumé**

Les buts de cette étude ont été de déterminer l'effet scolicidal du sérum salé à des concentrations et des durées d'exposition différentes et de déterminer si on peut I'utiliser pour laver la cavité péritonéale en cas de perforation libre d'un kyste hydatique. On a employé des concentrations différentes de sérum salé (0,09%, 3%, 6,5%, 10%, 15%, 20%, 25% et 30%) au contact de sédimentations d'echinococcus granulosus pendant 1, 2, 3, 4, 5, 10, 15, 30, 45 et 60 minutes. La viabilité du parasite après 60 minutes d'exposition à une solution de sérum salé physiologique (0,09%), à 3%, ou à 6,5% était élevée. La mort parasitaire a été de constatée, respectivement, 75, 10, 6, 3, et 3 minutes après contact pour les concentrations de 10%, 15%, 20%, 25%, et 30% de sérum salé. Dans la deuxième partie de l'étude, on a étudié l'irrigation péritonéale chez 20 rats de Sprague Dawley, divisés en quatre groupes selon qu'ils ont eu une irrigation péritonéale de NaCl à 30% pendant 3 minutes, Na Cl à 20% pendant 6 minutes, une solution aqueuse isotonique de dextrose en intraveineuse et une irrigation de furosémide plus du NaCl à 30% pendant 3 minutes, et enfin la même prophylaxie plus une irrigation du NaCl à 20% pendant 6 minutes. La natrémie et la chlorémie se sont élevées de façon significative (20–30%) peu après l'irrigation par du sérum hypertonique dans chaque groupe ($p < 0{,}01$). Ajouter du dextrose et de la furosémide avant l'irrigation n'a rien changé en ce qui concerne les taux sanguins ou la mortalité. La mortalité à 24 h et à 48 h a été, respectivement, de 70% et de 90%. Ces études indiquent que I'effet scolicidal du sérum hypertonique est limité à des concentrations basses mais qu'on peut éviter des effets secondaires non désirables en augmentant sa concentration. De même, une irrigation péritonéale avec du sérum hypertonique doit être évitée en cas de perforation de maladie hydatique. Ainsi, nous concluons que le sérum hypertonique n'est pas un bon agent scolicidal pour prévenir la récidive de kyste hydatique.

**Resumen**

El propósito del presente estudio fue determinar los efectos escolicidas de la solución salina en diferentes concentraciones y utilizando diferentes tiempos de exposición e investigar si la solución salina hipertónica puede ser utilizada para irrigar el abdomen en los casos en que se produzca perforación libre intraperitoneal de la enfermedad hidatidica. Se anadió solución salina en diferentes concentraciones (0.09%, 3%, 6.5%, 10%, 15%, 20% 25% y 30%) a sedimentos concentrados de equinococus granulosos por los siguientes periodos de tiempo: 1, 2, 3, 4, 5, 10, 15, 30, 45 y 60 minutos. La solución salina en concentraciones normal (0.09%), al 3% y al 6.5% resultó en tasas variables de viabilidad luego de 60 minutos de exposición. Se registró letalidad total con concentraciones al 10%, 15%, 20%, 25% y 30% luego de exposiciones por 75, 10, 6, 3, y 3 minutos, respectivamente. En la segunda fase del estudio se emplearon 20 ratas Sprague Dawley para estudiar el efecto de la irrigación peritoneal en cuatro grupos: NaCl 30% por 3 minutos, NaCl 20% por 6 minutos; solución intravenosa de dextrosa en agua y furosemida, más irrigación con NaCl 30% por 3 minutos; y la misma terapia profiláctica más irrigación con NaCl 20% por 6 minutos. Los valores de sodio y de cloruro ascendieron significativamente (20–30%) al poco tiempo de la irrigación con solución salina, en cada grupo ($p < 0.01$). El soporte con dextrosa isotónica y furosemida administradas antes de la irrigación no mostró efecto benéfico en cuanto a los valores bioquimicos o a la mortalidad. Las tasas de mortalidad a 24 y 48 horas fueron 70% y 90%, respectivamente. Tales resultados indican que el efecto escolicilico de la solución salina hipertónica es limitado con las concentraciones bajas pero que aumentar la concentración puede incrementar los efectos adversos. También indican que la irrigación con solución salina hipertónica debe ser evitada en los casos de enfermedad hidatidica.

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