Histopathological examination of the impact of sodium hypochlorite on the hepatobiliary system.
An experimental study

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BACKGROUND: *The liver is the most common organ for settlement of hydatic cyst disease. All acknowledged protoscolicidals that are used for echinococcus degeneration have a risk of caustic secondary sclerosing cholangitis. The sodium hypochlorite is an effective protoscolicidal agent for treatment of hydatid liver cysts in vitro.*
OBJECTIVE: *This study aimed to investigate the safe usability of sodium hypochlorite for the treatment of hydatid cyst in the hepatobiliary system in an experimental rat model.*
METHODS: *This experimental study designed as one side blinded animal study. Study was carried out between October 2017 and August 2018. Rats were randomly allocated to the study (n=7), control (n=7), and sham (n=7) groups. A duodenotomy was performed, and a catheter was inserted through the ampulla. The tip of the catheter was placed to instill 0.15 ml sodium hypochlorite (0,25%) solution, and 0.15 ml isotonic saline solution were into the common bile duct in the study and control groups, respectively. After three months, all rats were sacrificed. Livers, biliary tracts, pancreas, and duodenum were investigated for histopathological changes by blinded two pathologists.*
RESULTS: *No significant difference was found between groups for periductal portal inflammation (p=0.077), parenchymal inflammation, and focal necrosis (p=0.119). There was not any histopathological change in 71.4 % of the subjects in control and experimental groups.*
CONCLUSION: *Sodium hypochlorite (0,25%) did not cause any unfavorable changes in the hepatobiliary system, and this reminds that sodium hypochlorite can be a safe alternative in percutaneous drainage, laparoscopic, and open surgery in the treatment of hydatid cyst.*

KEY WORDS: Hepatobiliary system, Hydatid disease, Sodium hypochlorite, Treatment

Introduction

Hydatid disease, also known as echinococcosis, is a parasitic disorder characterized by cysts in the liver, lungs and/or other organs and to be continues a major endemic problem in many parts of the world. Liver cyst hydatid disease can be treated surgically or percutaneously. The spread of the protocolex rich fluid during these processes is the most important reason for relapse [1]. Several studies showed that hydrated membranes of cysts were completely lysed by different concentrations of sodium hypochlorite solution (NaOCl) [2]. Sodium hypochlorite has been used in a patient with nephrolithiasis to sterilize pyogangrenous foci during kidney surgery. It was also used during tooth canal therapy. Also, catheter surgery was investigated for lens cleaning with 0.6% hypochlorite. Girardo et al. have shown the strong antiseptic effect of sodium hypochlorite on Gram-negative

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microorganisms. In another study, similar results were seen for *Legionella species* [3,4]. Studies in the literature have shown that sodium hypochlorite is effective on live larvae and can be used for food decontamination. Sodium hypochlorite has long been used for water sanitation. Despite the widespread clinical use of sodium hypochlorite, there is no reported case of sclerosing cholangitis expect in one experimental study. This study aims to investigate the effects of sodium hypochlorite injection into choledochus on the hepatopancreatobiliary system of the rats.

Materials and Methods

Study Design

This experimental study was carried out by the rules for the care and use of laboratory animals determined by the ethical committee of the experimental animals in Ege University (2017-002). The study was conducted on Wistar -Albino male rats weighing 250-300 g. The groups were randomly selected, and composed of 3 groups; experimental group (n = 7), control group (n = 7) and sham group (n = 7). A 3-mm duodenotomy was performed after passing through a midline abdominal incision. A catheter with a diameter of 0.7 mm was inserted through the ampulla, and the catheter tip was advanced 1.5 cm to prevent the escape of the solution into the common pancreatic duct. Then in the study group 0.15 ml sodium hypochlorite (0.25%) solution, and in the control group 0.15 ml, isotonic saline solution were injected into the common bile duct without pressure. Three months later, laparotomy was performed on the rats in the study, control and sham groups. The liver, bile ducts and pancreas of rats were histopathologically examined in three groups.

Surgical Procedure

Rats were anesthetized with an intramuscular injection of ketamine HCl (40 mg/kg body weight) (*Ketalar, Parke-Davis, Eczacibasi, Istanbul, Turkey*), and Xylazine (5 mg/kg body weight) (*Rompun Bayer, Leverkusen, Germany*). All animals were left to spontaneous breathing during the experiment. A midline laparotomy incision was performed after the abdominal region was shaved and cleansed with povidone-iodine. The intestines are coated with sterile gauze soaked with isotonic saline at 37°C to minimize tissue evaporation. The body temperature was maintained between 36°C and 38°C using a heating lamp. Also, 5 ml of Ringer's lactate solution was administered subcutaneously to prevent water loss in animals during the experiment. A 3-mm duodenotomy was performed after a midline abdominal incision. A catheter with a caliber of 0.7 mm was inserted through the ampulla, and the catheter tip was advanced 1.5 cm to prevent the escape of the solution into the main pancreatic duct. Then in the study group 0.15 ml sodium hypochlorite (0.25%), and in the control group 0.15 ml, isotonic saline solution was injected into the main bile duct without exerting pressure.

Immediately after the injection, the main bile duct occluded with the atraumatic vascular clamp (bulldog) to prevent backflow. The catheter was then withdrawn. The clamp was opened after 5 min, and the duodenotomy was closed with 8-0 polypropylene suture. The animals in the sham group will be laparotimized, and the liver bile ducts and pancreas of the rats were removed for histopathologic examination. Study and control group animals were kept in the laboratory for three months during that time the animals were fed with tap water and rat chow ad libitum and maintained in separate cages at room temperature (18°-20°C) [3,4,5]. Since the experiment to be performed may cause pain during and after the experiment, animals have undergone anesthesia to make them suffer the least pain immediately before the experiment. For premedication, xylazine was administered subcutaneously at a dose of 10 mg/kg, For anesthesia ketamine (90 mg/kg/ip) and xylazine 10 mg/kg/im were administered. As analgesic buprenorphine was given subcutaneously at a dose of 0.01-0.1 at 8-12 hour-intervals.

Assessment Methods

Three months later, the rats in the study, control, and sham groups were sacrificed by cervical dislocation under ether anesthesia and a second laparotomy was performed. Histopathological examination of the liver, bile ducts and pancreas of rats was performed in two groups in that order. Liver right and left lobe specimens as well as the main bile duct, pancreas, duodenum were resected en bloc, and all tissues were immediately fixed in 10% formalin solution, embedded in a paraffin block, sectioned, and stained with hematoxylin and eosin. Sections were evaluated under light microscopy by two experienced hepatobiliary system pathologists who were unaware of the group data and decision of each other.

Outcome Parameters

Sham, control and study groups, each consisting of 7 subjects, were compared regarding six histopathological parameters. These parameters were portal or periductal inflammation, parenchymal inflammation / focal necrosis, ductal proliferation, zonal confluent necrosis, fibrosis, and ductal stricture. These parameters were scored as 1-2-3 according to the severity of the change, 1: no change, 2: slight changes, 3: significant changes.

Statistical Analysis

Since the data are categorical, numerical and, percentage values were given as descriptive statistics. The difference between the groups was examined by the chi-square test. Level of significance was considered as $p < 0,05$. Analyzes were addressed using SPSS v21.

Results

The incidence and severity of portal or periductal inflammation, parenchymal inflammation / focal necrosis, ductal proliferation, zonal confluent necrosis, fibrosis, and ductal stricture according to the groups are presented in Table I, Figs. 1-4 demonstrate normal tissue structures, and various degrees of severe inflammation and necrosis in the sections of the common bile duct, liver, pancreas, and duodenum, respectively. Comparison of sham, control and study groups regarding the incidence and severity of the above- mentioned histopathologic changes are presented in Table II. There was no significant difference between groups regarding periductal portal inflammation ($p = 0.077$) and parenchymal inflammation and focal necrosis ($p = 0.119$). Since ductal proliferation, zonal confluent necrosis, fibrosis, and ductal stricture were not observed in any case; statistical analysis was not performed for these variables.

In Table III, an analysis was made of the group from which the histopathological changes originated, and it was determined that the differences were originated from the sham group. No change in histopathologic parameters was observed in any of the subjects in the sham group, but in 71.4% of the subjects in the control and experimental groups any change in histopathological parameters was not seen

In Fig. 1, portal periductal inflammation and in Fig. 6 the distribution of the frequency and severity of parenchymal inflammation and focal necrosis according to groups are seen.

Discussion

The diagnosis of hydatid disease is easy especially when it is confined to organs like the liver. However, it may be challenging when it simulates other lesions such as pseudocyst, a choledochal cyst, serous or mucinous cystadenoma, and cystadenocarcinoma. Also, there is no consensus on the surgical management of hydatid disease in the medical literature [6].

Surgery is the mainstay of the treatment of hydatid disease. The surgical procedures should be tailored according to the location of the cyst, presence or not of a pancreatic ductal fistula communicating with the lesion. In a systemic review by Dziri et al., the open approach had

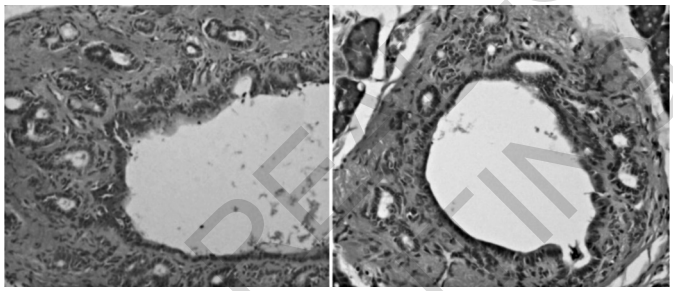

Fig. 1: Choledochus sections stained with hematoxylin and eosin (HE X 100). Study group at left and control group at right showing the regular architecture.

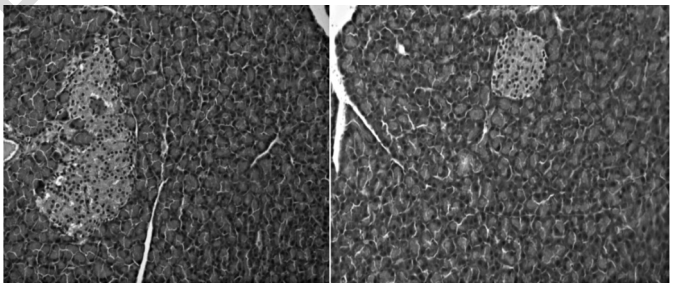

Fig. 3: Pancreas sections stained with hematoxylin and eosin (HE X 40). Study group at left and control group at right showing the regular architecture.

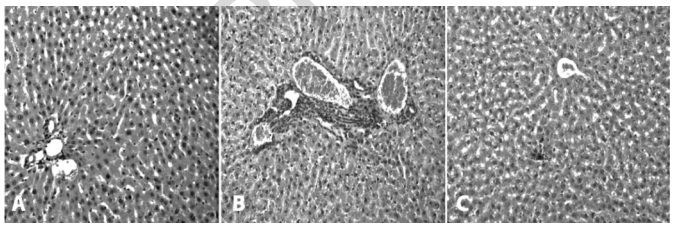

Fig. 2: Liver sections stained with hematoxylin and eosin (HE X 40). A) Control group at left showing the regular architecture. B) Portal mild/ moderate inflammation and C) Focal necrosis in the rats of study group (HE X 100).

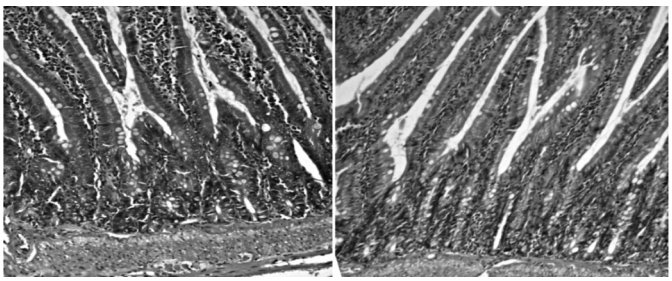

Fig. 4: Duodenum sections stained with hematoxylin and eosin (HE X 100). Study group at left and control group at right showing the regular architecture.

TABLE I - *An overview of histopathological variables under investigation in sham, control and study groups.*

Group	Subject no.	Portal or periductal inflammation	Parenchymal inflammation/Focal necrosis	Ductal proliferation	Zonal confluent necrosis	Fibrosis	Ductal stricture
Sham	1	1	1	1	1	1	1
	2	1	1	1	1	1	1
	3	1	1	1	1	1	1
	4	1	1	1	1	1	1
	5	1	1	1	1	1	1
	6	1	1	1	1	1	1
	7	1	1	1	1	1	1
Control	1	2	1	1	1	1	1
	2	1	2	1	1	1	1
	3	1	2	1	1	1	1
	4	1	1	1	1	1	1
	5	2	1	1	1	1	1
	6	1	1	1	1	1	1
	7	1	2	1	1	1	1
Study	1	1	3	1	1	1	1
	2	1	1	1	1	1	1
	3	2	1	1	1	1	1
	4	2	1	1	1	1	1
	5	1	2	1	1	1	1
	6	2	1	1	1	1	1
	7	1	1	1	1	1	1

Hint: 1: rare changes which may occur under normal circumstances; 2: mild inflammatory changes in some portal zones accompanied with few necrotic areas; 3: widespread necrotic areas and mild inflammation in many portal zones.

TABLE II - *Comparison of histopathological changes in sham, control and study groups.*

Variable	Degree of change	Group Sham	Control	Study	p-value
Portal periductal inflammation	None	7 (100%)	5 (71.4%)	4 (57.1%)	0.077
	Mild	0	2 (28.6%)	3 (42.9%)	
Parenchymal inflammation / focal necrosis	None	7 (100%)	4 (57.1%)	5 (71.4%)	0.119
	Mild	0	3 (42.9%)	1 (14.3%)	
	Significant	0	0	1 (14.3%)	
Ductal proliferation	None	7 (100%)	7 (100%)	7 (100%)	N/A
Zonal confluent necrosis	None	7 (100%)	7 (100%)	7 (100%)	N/A
Fibrosis	None	7 (100%)	7 (100%)	7 (100%)	N/A
Ductal stricture	None	7 (100%)	7 (100%)	7 (100%)	N/A

Hint: N/A: not applicable

TABLE III - *A general overview of presence of histopathological alterations in sham, control and study groups.*

Variable		Groups Sham	Control	Study	p-value
Histopathological change	Yes	7 (100%)	2 (28.6%)	2 (28.6%)	0.011*
	No	0	5 (71.4%)	5 (71.4%)	
	Total	7	7	7	

Hint: *: statistically significant. There is a statistically significant difference between 3 groups with respect to the frequency of histopathological changes under investigation. This difference is found to be due to the sham group.

been validated; however, the laparoscopic approach could not be supported by the available information. Moreover, the laparoscopic approach for the treatment of liver hydatid disease was associated with an increased rate of extrahepatic and peritoneal recurrence [7].

Percutaneous drainage has many advantages; the hydatic membranes of the cyst, consisting of a laminar layer and a germinative layer, cannot be removed by this method. Cyst hydatid membranes may limit the efficacy of percutaneous catheter drainage. In some patients,

the needle may become clogged during aspiration due to membrane fragments that obstruct the lumen. Catheter blockage with membrane fragments can be a major problem when drainage is needed. Studies show that substances such as alcohol and hypertonic saline cannot make hydatic membranes of the cysts that can be easily drained through the catheter. In a series, the inadequate percutaneous drainage of cyst hydatid content resulted in the referral of 6.3% of the patients to general surgery. A pharmacologic agent that can eradicate cyst hydatic membranes can resolve these problems. An agent that can lyse hydatid cyst membranes without damaging the host tissue may enhance the efficacy of percutaneous drainage [8,9].

Spread of protosccolex-rich fluid during operation is the most important reason for recurrence after surgery. The administration of scolisidal substance into the hydatid cyst of the liver before opening it is the most commonly used measure to prevent this serious complication. In up to 80%, of the cysts with a diameter of 5 cm, there is a communication with bile ducts in the form of a thin channel while all cysts over 7 cm in size communicate with bile ducts. Sclerosing cholangitis has been reported after surgical treatment of liver cyst hydatid, which is theoretically attributed to the caustic effect of the scolicidal solution. Passing through a biliary fistula. Several scolicidal agents have been used all over the world for years. In a study by Karaoglanoglu et al., sodium hypochlorite has proven to have a strong scolisidal activity.[5,10].

Topical use of sodium hypochlorite has been reported to be a safe and effective antiseptic measure, particularly in dentistry. No systemic toxicity or severe local reaction has been reported with its topical use. However, at higher concentrations such as 3-5.25%,) interstitial inoculation of sodium hypochlorite can exhibit toxic action. Sodium hypochlorite triggers immediate hemolysis of erythrocytes when given in isotonic solutions, and this action is supposed to be related to its potent oxidizing impact on cell membranes [11]. Many other studies have demonstrated dose-dependent toxicity associated with sodium hypochlorite when there is direct injection into the tissue [12]. Sodium hypochlorite can be fatal if a considerable quantity is absorbed systemically, and the dose causing death in 50% of animals tested was found to be 33.3 mg/kg when administered intravenously to rats [13].

In this study, possible use of sodium hypochlorite is investigated in percutaneous drainage, laparoscopic and open surgery in the management of hydatid cyst. According to our findings, sodium hypochlorite does not cause different and more negative pathological changes than the isotonic saline in the hepatobiliary system. We believe that these findings, despite being preliminary findings of an experimental study, are promising, which indicated that sodium hypochlorite could be used in the treatment of hydatid cyst disease of the hepatobiliary system.

Sodium hypochlorite is widely used in the irrigation of root canals of teeth in dentistry. The ability to dissolve necrotic tissue has favored strong bactericidal action and low toxicity when used at the appropriate dose. There is not much information about the irritating effect of sodium hypochlorite on tissue [14,15]. It has been reported that sodium hypochlorite, the most commonly used irrigation agent in endodontics, can cause an allergic reaction [15,16].

To overcome possible toxic and deleterious effects of sodium hypochlorite, determination of the ideal concentration of sodium hypochlorite is critical.

Karaoglanoglu et al. implied that a concentration of 0.25% of sodium hypochlorite was the lowest concentration capable of melting the membrane of the hydatid cyst completely [2]. Sodium hypochlorite has been shown to be safe for host tissue, and it may serve as an adjunct measure for percutaneous drainage of hydatid cysts attributed to its antiparasitic and membrane dissolution features. Sodium hypochlorite may provide expansion of the indications for percutaneous drainage of hydatid cysts [2].

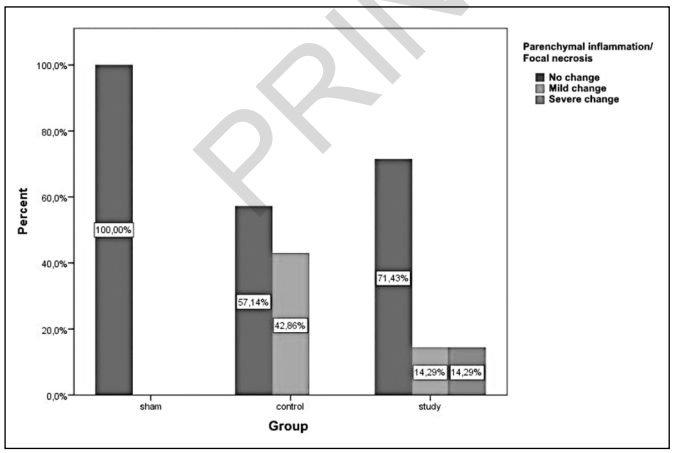

Fig. 5: Degree of portal periductal inflammation in sham, control and study groups.

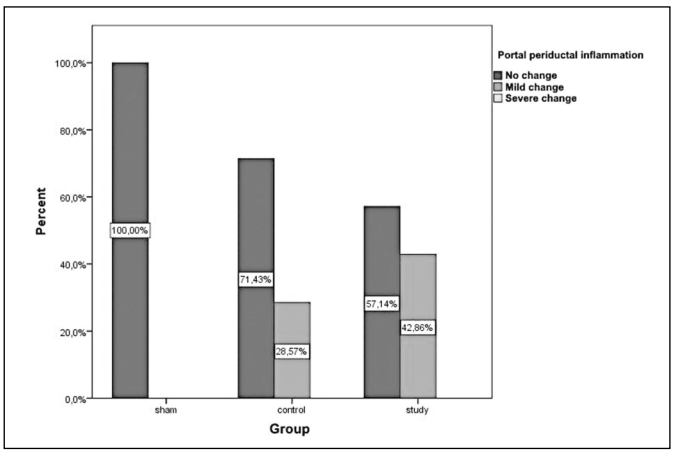

Fig. 6: Degree of inflammation and focal

In our study, histopathologic changes at the tissue level did not show any allergic or inflammatory effect different of excess than that of the isotonic saline. This suggests that sodium hypochlorite can be safely used in the treatment of hydatid cyst. Sodium hypochlorite is a strong alkaline, showing the bactericidal effect by stimulating the formation of hypochloric acid by removing chlorine from the reaction. Consideration of careful use and biocompatibility will reduce the complication and treatment failure to a minimum. Sodium hypochlorite, the active ingredient in bleach, is a useful chemical that has been used since the 18th century as a disinfectant. In medical practice, it has been utilized as an antiseptic agent in the treatment of gangrene, burns, and wounds. In the circulation, sodium hypochlorite may combine with water to yield hypochlorus acid, exhibiting substantial antibacterial and antifungal features. Hypochlorus acid produces superoxide radicals, leading to oxidative injury and bacterial death [10,11].

Our results may open new horizons that may improve the therapeutic success without causing substantial burden regarding mortality and morbidity. Selection of treatment must be made concerning factors such as the location of the cyst, patient compliance, and recurrence [17].

To the best of our knowledge, this is the first study in the medical literature to investigate the role of sodium hypochlorite in the treatment of hydatid disease, and thus has a unique value.

Limitations of the present study involve experimental design and necessity for further research to understand the applicability of our results to the human population. The dose-effect relationship must be studied in different models for the determination of the optimal dose and possible toxic effects. In this context, permanence of the inflammatory process following administration of sodium hypochlorite observed in the acute phase must be monitored closely. On the other hand, it should be kept in mind that there is a need for further studies to investigate the efficacy and reliability of the results in clinical practice.In conclusion, the findings of our study show that sodium hypochlorite does not cause any negative changes in the hepatocyte tissue. From this point of view, we think that sodium hypochlorite may be a safe alternative to percutaneous drainage, laparoscopic and open surgery in hydatid cyst treatment. Further research is needed to investigate the efficacy and verify the clinical relevance of the findings.

Riassunto

Il fegato è l'organo più comune per la localizzazione delle cisti idatidee. Tutti i protoscolicidi efficaci che sono utilizzati per la distruzione dell'echinococco presentano il rischio di provocare una colangite caustica sclerosante. L'ipoclorito di sodio è un agente protoscolicidico efficace in vitro per il trattamento delle cisti idatidee.

Questo studio si è proposto di studiare l'utilizzazione dell'ipoclorito di sodio per il trattamento della cisti idatidea nel sistema epatobiliare in un modello sperimentale di ratto, senza conseguenze negative.

Si è trattato di uno studio sperimentale progettato come uno studio cieco su animali da esperimento, ed è stato condotto tra ottobre 2017 e agosto 2018.

21 ratti sono stati assegnati in modo casuale ai tre gruppi di studio (n = 7), di controllo (n = 7) e sham (n = 7). È stata eseguita una duodenotomia e un catetere è stato inserito attraverso l'ampolla per instillare 0,15 ml di soluzione di ipoclorito di sodio (0,25%) e 0,15 ml di soluzione salina isotonica nel dotto biliare comune rispettivamente nei gruppi di studio e di controllo. Dopo tre mesi, tutti i ratti sono stati sacrificati. Fegato, vie biliari, pancreas e duodeno sono stati studiati per gli effetti istopatologici da due diversi patologi in cieco.

Come risultato nessuna differenza significativa è stata trovata tra i gruppi rispetto alla infiammazione portale periduttale (p = 0,077), infiammazione parenchimale e necrosi focale (p = 0,119). Non c'è stato alcun cambiamento istopatologico nel 71,4% dei soggetti nei gruppi di controllo e di sperimentazione.

Si convlude che l'ipoclorito di sodio (0,25%) non ha causato cambiamenti sfavorevoli nel sistema epatobiliare e ciò indica che esso può essere un'alternativa sicura nel drenaggio percutaneo, nella chirurgia laparoscopica e a cielo aperto nel trattamento della idatidosi.

References

1. Gomez I Gavara C, López-Andújar R, Belda Ibáñez T, et al.: *Review of the treatment of liver hydatid cysts*. World J Gastroenterol, 2015; 21:124-31.

2. Karaoglanoglu M, Akinci OF, Bozkurt S, et al.: *Effect of different pharmacologic and chemical agents on the integrity of hydatid cyst membranes*. AJR Am J Roentgenol, 2004; 183:465-69.

3. Tozar E, Topcu O, Karayalcin K, et al.: *The effects of cetrimide-chlorhexidine combination on the hepato-pancreatico-biliary system*. World J Surg, 2005; 29:754-58.

4. Kismet K, Kilicoglu SS, Kilicoglu B, et al.: *The effects of scolicidal agent propolis on liver and biliary tree*. J Gastrointest Surg, 2008; 12:1406-411.

5. Sahin M, Eryilmaz R, Bulbuloglu E: *The effect of scolicidal agents on liver and biliary tree (experimental study)*. J Invest Surg, 2004; 17:323-26.

6. Bruch MK: *Toxicity and safety of topical sodium hypochlorite*. Contrib Nephrol, 2007; 154:24-38.

7. Dziri C, Dougaz W, Bouasker I: *Surgery of the pancreatic cystic echinococcosis: Systematic review*. Transl Gastroenterol Hepatol, 2017; 2:105.

8. Aydın C, Kayaalp C, Nessar G, et al.: *Is cetrimide-chlorhexidine risky for secondary sclerosing cholangitis?* Adv Clin Exp Med, 2014; 23:395-98.

9. Clarkson RM, Moule AJ, Podlich H, et al.: *Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations*. Aust Dent J, 2006; 51:245-51.

10. Sezer A, Hatipoglu AR, Usta U, et al.: *Effects of intraperitoneal melatonin on caustic sclerosing cholangitis due to scolicidal solution in a rat model*. Curr Ther Res Clin Exp, 2010; 71:118-28.

11. Peck BW, Workeneh B, Kadikoy H, Abdellatif A: *Sodium hypochlorite-induced acute kidney injury*. Saudi J Kidney Dis Transpl, 2014; 25:381-84.

12. Mohammadi Z: *Sodium hypochlorite in endodontics: An update review*. Int Dent J, 2008; 58:329-41.

13. Nath KA, Norby SM: *Reactive oxygen species and acute renal failure.* Am J Med, 2000; 109:665-78.

14. Wong DT, Cheung GS: *Extension of bactericidal effect of sodium hypochlorite into dentinal tubules.* J Endod, 2014; 40:825-29.

15. Baumgartner JC, Cuenin PR: *Efficacy of several concentrations of sodium hypochlorite for root canal irrigation*. J Endod, 1992; 18:605-12.

16. Essner MD, Javed A, Eleazer PD: *Effect of sodium hypochlorite on human pulp cells: An in vitro study*. Oral Surg Oral Med OralPathol Oral Radiol Endod, 2011; 112(5):662-66.

17. Jerraya H, Khalfallah M, Osman SB, et al.: *Predictive factors of recurrence after surgical treatment for liver hydatid cyst*. Surg Endosc 2015; 29:86-93.