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The scolocidal effect of propolis on protoscoleces and daugther cysts

Propolisin protoskoleksler ve kız kistler üzerine skolosidal etkisi

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SUMMARY

Objective: Scolocidal elements are used in the aim of not scattering the protoscoleces' outside the cyst during the treatment of cyst hydatid in our present-day. It is hoped from the scolocidal materials; besides its effect on protoscoleces, not being toxic, being easy practicable, not causing sclerozan cholanjit and if possible, penetrating in the daughter vesicules. In this research, the effect of propolis which is a bee product on protoscoleces were investigated.
Methods: In our work, daughter vesicules and protoscoleces obtain from the liver of a patient during surgery and propolis from the Black Sea Region were used. Viability tests were done by using 0.1% eosine solution. After propolis disolved in dimethylsulfoxide, within the range of 50 mg/mL and 0.010 mg/mL using by 0.9% serum physiological solution 100 µl into each of the tube and in 1.,3.,5.,10.,20.,and 30. minutes the rates of viability of protoscolex (4000 protoscolex/mL) are fixed. The research were subjected to daughter vesicules that is 4-7 mm in diameters. The concentration of propolis at 0.80 and 3.12 mg/mL were investigated viability rates of protoscolex at different incubation time and whether the possibilty of penetration to the daughter vesicules.
Results: It was seen that protoscolexs were effected by the Propolis at 0.100 mg/mL concentration in the first minute, at 0.050 mg/mL concentration in the 3. minutes, at 0.025 mg/mL concentration at 10. minutes and at 0.010 mg/mL concentration in 20. minutes. For protoscolex in the vesicules at 0.80 mg/mL concentration in 20. minute, in 3.12 mg/mL concentration in 10. minutes were found to be lethal.
Conclusions: As the protoscolex could be killed by Propolis which penetrate to daughter cysts in a very short time as 10 minutes, we thing that it will be beneficial completing advanced studies.
Keywords: Propolis, protoscolex, daughter cyst, cyst hydatid

ÖZET

Amaç: Günümüzde skolosidal ajanlar hidatik kist sıvısının tedavi sırasında kistin dışına dağılmasını önlemek amacıyla kullanılmaktadır. Etkili bir skolosidin protoskoleksler üzerindeki etkisinin yanı sıra, toksik olmayan, kolay uygulanabilen sklerozan kolanjit oluşturmayan ve kız veziküllerin de içine nüfuz edebilen özelliklere sahip olması beklenmektedir. Bu çalışmada doğal bir arı ürünü olan propolisin skolosidal etkisinin araştırılması amaçlanmıştır.
Yöntem: Çalışmada, karaciğer hidatik kisti bulunan bir hastadan operasyon sırasında elde edilen, kız veziküller ve protoskoleksler ve Karadeniz bölgesinde elde edilen propolis kullanılmıştır. Canlılık tesbiti %0,1'lik Eosin solüsyonu kullanılarak yapılmıştır. Propolis DMSO içinde çözülerek, %0,9'luk serum fizyolojik içinde 50 mg/mL ile 0,010 mg/mL arasında sulandırılmıştır (4000 protoskoleks/mL) ve tüplere 100'er µl dağıtılmıştır. Farklı konsantrasyonlardaki propolisin 1.,3.,5.,10.,20.,ve 30. dakikalarda protoskoleksler üzerine etkisi hemositometre lamında sayım yapılarak belirlenmiştir. Ayrıca, 4 ila7 mm boyutlarında olan çok sayıdaki kız veziküllere 0,80 ve 3,12 mg/mL'de hazırlanan propolis uygulanmış,5.,10.,20.,30., dakikalarda kistler açılarak içlerindeki protoskolekslerin canlılık durumları araştırılmıştır.

Bulgular: Propolisin 0,100 mg/mL'de ilk dakikada, 0,050 mg/mL'de 3. dakikada, 0,025 mg/mL'de 10. dakikada, 0,010 mg/mL'de 20. dakikada protoskoleksler üzerine öldürücü etkisi gözlenmiştir. Kız veziküller içindeki protokoleksler 0,80 mg/mL'lik konsantrasyonda 20. dakikada protoskolekslerin %50'si, 3,12 mg/mL'lik konsantrasyonda 10. dakikada ise tamamı etkilenmiştir.
Sonuç: Özellikle, 10 dakika gibi kısa bir sürede kız veziküllere de etki edebilen doğal madde propolisin skolosidal ajan olarak kullanılabileceği ve bu konuda daha ileri çalışmalara gereksinim olduğu kanısına varılmıştır.

Anahtar sözcükler: Propolis, protoskoleks, kız vezikül, kist hidatik

INTRODUCTION

Hydatid cyst disease or hydatidosis is one of the most serious parasitic diseases of man. It is more prevalent in man raising areas such as Australia, New Zeland, America, Europe, Asia, Africa and Middle East countries[1]. Humans are infected with hydatid cysts when they accidentally ingest Echinococcus granulosus eggs either as a results of fondling dogs or by eating food contaminated with infected dog dung. In general more than 15 years may elapse between infection and overt pathogenesis[1].

Although most hydatid disase are found in liver and lung, the disease can arise anywhere in the body. Hydatidosis constitutes an important public health problem in Turkey. Some of the operated people undergo second or more operation due to complications of the first operation[2]. Despite low recurrence rates after complete removal in most cases total cystectomy involes a major organ resection, with in attendant increase in operative risk for a benign disease. For this reason, avoiding spillage of the cyst contents and the use of effective scolocidal agents are essential to lessen the recurrance rate. So, the scolocidal effects of some chemicals have been studied by researhers in Turkey[2-5].

Propolis is a resinous substance that honey bees collect from different plant exudates and use to fill gaps and to seal parts of the hive. Propolis has attracted much attention in recent years as a useful natural substance applied in medicine, even if it is known in folk medicine since ancient times[6,7]. Propolis is a sticky dark colored material that honey bees collect from plants. The chemical composition of propolis is very complex and is dependent upon the source plant. The main sources of propolis in the temperate zone, including Europe, Asia and North America. Samples originating from these regions are characterized by similar chemical composition; the most important constituents appeared to be phenolics: Flavonoids, aromatic acids and their esters[8-13].

With respect to propolis scolocidal activity, relatively little is known and data in literature are scarce. Considering the propolis therapheutic potential and the need for new alternatives for scolosidal treatment, this work was carried out with the aim to evaluate the in vitro effects of a DMSO exract of propolis on the viability of protoscoleces.

MATERIAL AND METHODS

The origin of protoscolex: In our work, daughter vesicules and protoscolex obtain from the liver of a patient during surgery. Hydatid fluid was aspirated from the cysts, are collected. Concentrated (4000 protoscolex/mL) cyst fluid was used. Protoscolices stained with 0.1% eosine were examined x40 magnification at room temperature. Because flame cell activity disappears before taking in the dye.

Propolis and polen origins: Polen and propolis samples were produces by honeybees (Apis mellifera L.) in the region of Trabzon, Turkey rich in Rubus caucasicus, Castenea sativa, Fagus orientalis, Rhododendron luteum, Picea orientalis. They were provided by Trabzon Agricultural Development Cooperative.

Preparation of extracts of propolis: We used an extraction procedure by using dimethylsulfoxide (DMSO) as solvent. Propolis samples were grinded (Retsch, 2M 200) and bottled in 5 g portions. Portions were dissolved in 5 mL of DMSO (100% w/v) by continuous mixing for 5 h, then kept at 370C in water bath overnight. Extracts of 4 mL (1000 mg/mL) obtained by centrifuging at 3000 rpm for 15 minutes were filtered and final volumes were completed to 10 mL using deionized water.

Filter procedure was repated and final extract adjusted to 10 mL by deionized water to give a stock concentration of propolis extracts of 240 mg/mL. Working solutions were then prepared in 0.9% serum physiologycal. After propolis solved in DMSO, beginning from 50 mg/mL until 0.010 mg/mL ½ dilution is done in 0.9% serum physiologycal. 4000 protoscolex/mL suspention is out 100 µl into each of the tube and in 1., 3., 5., 10., 20., and 30., minutes the rates of viability of protoscolices are fixed. Each experiments was repeated three times.

To test the efficacy of propolis on the viability of the integrity of daughter cysts and the viability of the protoscolices contained in these cysts. 20 daughter cysts obtained from a patient with Gharbi type III hydatid cysts of the liver were used. The cysts were divided into two groups, in the first of which cysts were placed into 3.12 mg/mL propolis and the second into 0.80 mg/mL propolis; they were kept there for 5, 10, 15, 20 and 30 minutes. Integrity of the cyst wall and viability of the contents were evaluated using a vital staining technique with 0.1% eosin.

20 µl sample was removed from tubes and placed under a coverslip on a hemocytometer, and both viable and the non viable protoscolices were counted under the microscope.

The number of protoscolices from each tubes and daughter cysts were obtained by mathematical equation: UPx Dx 4.103/SQ where, UP: Unstained Protoscolices (viable) D: Dilution of the protoscolices suspention SQ: Number of squares of the hemocytometer counted. The viability percentage of protoscolices population of each tube was obtained by applying the following mathematical equation: UPx TPx100 (TP: Total Protoscolex).

Statistical analysis: Statistical Analysis of the data was accomplished by using one-way analysis of variance complemented by the Tukey test.

RESULTS

The viability ratio for all concentration of protoscolices is summarized in Table 1. During the early period, in low concentration of propolis (0.010 mg/mL) more than half of protoscolices were viable; at the end of 20 minutes, viability in all concentration was not seen.

When the exposure time was extended, complete lethality for 0.010 mg/mL propolis was observed at the end of 15 minutes. The 0.025 mg/mL propolis killed all of protoscolices at the end of 5 minutes. The 0.050 mg/mL propolis was highly effective during the first 3 minutes. 3.12 mg/mL propolis killed all of the protoscolices in daughter cysts at the end of 10 minutes. On the other hands, 0.80 mg/mL propolis killed all of the protoscolices (in daughter cysts) at the end of 30 minutes (Table 2).

Table 1: Viability ratios of the protoscolices (%) in different propolis concentration and for different exposure times.

Propolis/time	1 min	3 min	5 min	10 min	15 min	20 min
0.100 mg/mL	0	0	0	0	0	0
0.050 mg/mL	50	0	0	0	0	0
0.025 mg/mL	80	20	0	0	0	0
0.010 mg/mL	80	70	20	10	0	0

Table 2: Viability ratios of the protoscolices in daughter cysts (%) in different propolis concentration and for different exposure times.

Propolis	5 min	10 min	15 min	20 min	30 min
3.12 mg/mL	80	0	0	0	0
0.80 mg/mL	80	60	50	30	0

DISCUSSION

Cyst hydatatid is a zoonotic infection caused by Echinococcus spp. and is one of the most important helminthic diseases worldwide. According to Turkish Ministry of Heath records, 21 303 patients had operations to treat or confirm cyst hidatid in the period 1987-1994 which corresponds to approximately 2663 patients per year. The estimated surgical case rate of cyst hydatid is 0.87-6.6 per 100 000 in Turkey[1]. As these are surgically confirmed cases, the real number of patients was never known. All the above stated data clearly show that cyst hydatid is not limited to certain region but a widespread problem in Turkey. Surgery is still the most widely used for cyst hydatid therapy, although there are some problems related to rec-

curence. 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. For this reason, radical methods are not popular. The conservative approach includes evacuation of the cyst cavity and irrigation with a scolocidal agent. 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. Inadequate scolocidal concentration or exposure time, inadequate evacuation of the hydatid membranes, and dilutions of scolocidal agent by the cyst fluid are the main causes of diminished scolocidal effect[2-5]. Thus, the search for new alternative scolocidal agents is necessary, such as natural products. Propolis, a bee product, has various biological and therapeutic activities, which have been associated with the presence of flavonoids and aromatic acids and esters[6,7]. Content of propolis taken from several studies are presented in the following table[11].

Table 3: Groups and number of identified compounds of propolis.

Compounds	No
Alcohols, ketones	8
Aliphatic acids and esters	6
Amino acids	24
Aromatic acids and esters	12
Chalcones and dihydrochalcones	2
Flavanones	38
Flavones	27
Flavonols	11
Hydrocarbons esters	11
Minerals	22
Steroids	6
Sugars	7
Terpenoids	7

In the present study we used Turkish propolis and researched their in vitro activity on protoscolices and daugter cysts.

The wide use of propolis is due to its biological properties, such as antimicrobial, immunomodulatory, antitumoral activity[14-21]. Moreover, some clinical and experimental investigations have pointed to the antiparasite properties of propolis extracts[22,23]. Previous studies have shown propolis in vitro activity against Trypanosoma spp.[24-27], Giardia intestinalis[28], Leishmania spp.[29-33], Trichomonas vaginalis[32]. In vitro effects of this product on protoscoleces have been reported in two study[34,35]. We determined that 0.50 mg/mL concentration of propolis killed all of the protoscoleces at the end of the 3rd minute. We concluded that propolis can be used as a scolicidal agent after studies which will determine in vivo studies. Our results correborate these findings. But in vitro effects of daughter cysts have not been reported. Thus the present work was carried out to evaluate the in vitro activity of propolis extract (prepared in DMSO) both on protoscoleces and protoscoleces in daughter cysts.

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REFERENCES

1. Saygı G. Hydatidosis in Turkey within the Last Fourteen Years (1979-1993). Cumhuriyet University press, Sivas, Turkey 1996.
2. Besim H, Karayalçın K, Hamamcı O, Güngör Ç, Korkmaz A. Scolicidal agents in hydatid cyst surgery. HPB.Surgery 1998; 10: 347-51.
3. Karayalçın K, Besim H, Sonisik M, Erverdi N, Korkmaz A, Aras N. Effect of Hypertonic Saline and Alcohol on Viability of Daughter cysts in Hepatic Hydatid Disease. European J Surg 1999; 165: 1043-4.
4. Kayaalp C, Balkan M, Aydın C, Özgürtaş T, Tanyüksel M, Kirimlioğlu V, Akoğlu M, Öner K, Pekcan M. Hypertonic Saline in Hydatid Disease. World S.Surg 2001; 25: 975-9.
5. Puryan K, Karadayı K, Topçu S, Canbay E, Sümer Z, Turan M, Karayalçın K, Şen M. Chlorhexidine Glucanote. An ideal Scolicidal agent in the treatment of intraperitoneal Hydatidosis W.J. Surgery 2005; 29: 227-30.
6. Aliyazıcıoğlu Y, Değer O, Ovali E,

Barlak Y, Hosver I, Tekelioğlu Y, Karahan SC. Effects of Turkish polen and propolis extracts on respratory burst for K-562 cell lines. International Immonopharmacology 2005; 5: 1652-7.
7. Bankova V. Recent trends and important developments in propolis research. Evid Based Complement Alternat Med 2005; 2: 29-32.
8. Uzel A, Sorkun K, Önçağ Ö, Çoğulu D, Gençay Ö, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res 2005; 160: 189-95.
9. Salomão K, Dantas AP, Borba CM, Campos LC, Machado DG, Aquino Neto FR, de Castro SL. Chemical composition and microbicidal activity of extracts from Brazilian and Bulgarian propolis. Lett Appl Microbiol 2004; 38: 87-92.
10. Marcucci MC, Ferreres F, Garcia-Viguera C, Bankova VS. Phenolic compounds from Brazilian propolis with pharmacological activities J. Ethnopharmacol 2001; 74: 105-12.
11. Marcucci MC. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 1995; 26: 83-99.
12. Mercan N, Kıvrak I, Duru ME, Katırcıoğlu H, Gülcan S, Mala S, Acar G, Salih B. Chemical composition effects onto microbial and antioxidant activities of propolis collected from different regions of Turkey. Annals of Microbiology 2006; 56: 373-8.
13. Miyataka H, Nishiki M, Matsumoto H, Fujimoto T, Matsuka M, Satoh T. Evaluation of propolis. I. Evaluation of Brazilian and Chinese propolis by enzymatic and physico-chemical methods. Biol Pharm Bull 1997; 20: 496-501.
14. Amaros M, Lurton E, Boustie J, Gire L, Sauvager F, Cormier M. Comparison of the anti-Herpes simplex virus activities of propolis and. J Nat Prod 1994; 57: 644-7.
15. Bosio K, Avanzini C, D'avolio A, Ozino O, Savoia D. In vitro activity of propolis against Streptococcus pyogenes. Lett Appl Microbiol 2000; 31: 174-7.
16. Kartal M, Yıldız S, Kaya S, Kurucu S, Topçu G. Antimicrobial activity of propolis samples from two different regions of Anatolia. J Ethnopharmacol 2003; 86: 69-73.
17. Murad JM, Calvi SA, Soares AMVC, Bankova V, Sforcin JM. Effect of propolis from Brasil and Bulgaria on fungucidal activity of macrophages against Paracoccidiodies brasiliensis. J Ethnopharmacol 2002; 79: 331-4.
18. Orsi RO, Sforcin JM, Funari SRC, Bankova V. Effects of Brazilian and Bulgarian propolis on bactericidal activity of macrophages against Salmonella Typhimurium. Int Immunopharmacol 2005; 5: 359-68.
19. Popova M, Silici S, Kaftanoğlu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine 2005; 12: 221-8.
20. Sforcin JM, Fernandes J.R.A, Lopes C.A.M, Bankova V. Seasonal effect on Brazilian propolis antibacterial activity, J. Ethnopharmacol 2000; 73: 243-9.
21. Yıldırım Z, Hacıevliyagil S, Kutlu NO, Aydın NE, Kurkcuoglu M, Iraz M, Durmaz R. Effect of water extract of Turkish propolis on tuberculosis infection in guinea-pigs. Pharmacol Res 2004; 49: 287-92.
22. Özan F, Polat ZA, Er K, Özan Ü, Değer O. Effect of propolis on survival of periodontal ligament cells: New storage Media for avulsed Teeth. Journal of Endodontics 2007; 33: 570-3
23. Öztürk F, Kurt E, Cerci M. The effect of propolis extract in experimental chemical corneal injury. Ophthalmic Res 2000; 32: 13-8.
24. Cunha IBS, Salamo K, Shımızu M, Bankava VS. Antitrypanosomal activity of Brazilian propolis from Apis mellifera. Chem Pharm Bull 2004; 52: 602-4.

25. De Castro L, K.O. Higashi, Effect of different formulations of propolis on mice infected with Trypanosoma cruzi, J. Ethnopharmacol 1995; 46: 55-8.
26. Higashi KO, S.L. de Castro. Propolis extracts are effective against Trypanosoma cruzi and have an impact on its interaction with host cells, J. Ethnopharmacol 1994; 43: 149-55.
27. Prytzyk E, Dantas AP, Salomao K. Flavonoids and trypanocidal activity of Bulgarian propolis. J. Ethnopharmacol 2003; 88: 189-93
28. Freitas SF, Shinohara L, Sforcin JM, Guimares S. In vitro effects of propolis on Giardia duodenalis trophozoites. Phytomedicine 2006; 13: 170-5.
29. Ferreira FM, CastroRA, Batista MA, Rossi FM, Silveira-Lemos D, Frézard F, Moura SA, Rezende SA. Association of water extract of green propolis and liposomal meglumine antimoniate in the treatment of experimental visceral leishmaniasis. Parasitol Res 2014; 113: 533-43.
30. Duran N, Muz M, Culha G, Duran G, Ozer B. GC-MS analysis and antileishmanial activities of two Turkish propolis types. Parasitol Res 2011; 108: 95-105.
31. da Silva SS, Thomé Gda S, Cataneo AH, Miranda MM, Felipe I, Andrade CG, Watanabe MA, Piana GM, Sforcin JM, Pavanelli WR, Conchon-Costa I. Brazilian propolis antileishmanial and immunomodulatory effects. Evid Based Complement Alternat Med 2013; 673058.
32. Monzote Fidalgo L, Sariego Ramos I, García Parra M, Cuesta-Rubio O, Márquez Hernández I, Campo Fernández M, Piccinelli AL, Rastrelli L. Activity of Cuban propolis extracts on Leishmania amazonensis and Trichomonas vaginalis. Nat Prod Commun 2011; 6: 973-6.
33. Duran G, Duran N, Culha G, Ozcan B, Oztas H, Ozer B. In vitro antileishmanial activity of Adana propolis samples on Leishmania tropica: a preliminary study. Parasitol Res 2008; 102: 1217-25.
34. Kismet K, Kilicoglu SS, Kilicoglu B, Erel S, Gencay O, Sorkun K, Erdemli E, Akhan O, Akkus MA, Sayek I. The effects of scolicidal agent propolis on liver and biliary tree.J Gastrointest Surg 2008; 12: 1406-11.
35. Kismet K, Kilicoglu B, Koru O, Tanyuksel M, Oruc MT, Sorkun K, Salih B, Akkus MA. Evaluation on scolicidal efficacy of propolis. Eur Surg Res 2006.