Original Article

In Vitro and in Vivo Scolicidal Activities of *Holothuria leucospilota* Extract and CeO2 Nanoparticles against Hydatid Cyst

Safa ARYAMAND 1, *Shahram KHADEMVATAN 1, Khosrow HAZRATI TAPPEH 1, Behnam HESHMATIAN 2, Ali JELODAR 3

1. Cellular and Molecular Research Center, Department of Medical Parasitology and Mycology, Urmia University of Medical Sciences, Urmia, Iran
2. Department of Physiology, Urmia University of Medical Sciences, Urmia, Iran
3. Department of Medical Parasitology, Jondishapur University of Medical Sciences, Ahvaz, Iran

**Abstract**

**Background:** We aimed to investigate the scolicidal effects of *Holothuria leucospilota* extract and CeO2 nanoparticles against protoscoleces of hydatid cysts in-vitro and in-vivo.

**Methods:** Hydatid cysts were collected from, Urmia slaughterhouses between years 2016-2017 and the hydatid fluid aspirated from the fertile cysts. Various concentration of *H. leucospilota* extract, CeO2 NPs and combination of CeO2-NPs/*H. leucospilota* were used for 10-60 min to evaluate the viability of protoscoleces by 0.1% eosin method. CASPASE -3 activity measured for assessment of cell apoptosis in treated protoscoleces. BALB/c mice were infected intra-peritoneally with 2000 viable protoscoleces and treated daily for 4 wk by intra-gastrical inoculation with *H. leucospilota*, CeO2 NPs, combination of CeO2 NPs/*H. leucospilota* and Albendazole. Cyst development was macroscopically analyzed.

**Results:** *H. leucospilota* extract and combination of CeO2 NPs/*H. leucospilota* have potent scolicidal activity at concentration of 20 mg/ml and 15 mg/ml after 60 min treatment. Maximum caspase-3 activity was observed when protoscoleces expose with *H. leucospilota* and combination *H. leucospilota* & CeO2 NPs. After treatment of cyst infected mice with extract and CeO2 NPs, combination of CeO2 NPs/*H leucospilota* and albendazole, a significant decrease in number of cysts, size and volume of cyst (P<0.05) was observed.

**Conclusion:** This result shows an antihydatic and scolicidal effects of *H. leucospilota* extract and CeO2 NPs.
Introduction

Cystic echinococcosis (CE) or hydatidosis is a chronic zoonotic parasitic infection caused by *Echinococcus granulosus*. The life cycle of *E. granulosus* involves dogs and wild carnivores as a definitive host for the adult tapeworm and sheep, goats, cattle and camels as an intermediate host for the larval stage. Human can also be an accidental intermediate host for *E. granulosus* and infected through ingestion of parasite eggs in contaminated food, water or soil, or through direct contact with animal hosts (1).

Echinococcosis has a worldwide spread and considerable public health and economical concerns in many countries of world (1-3). Prevalence of disease was estimated at about 1-220/100000 in world. Hydatidosis in Iran is one of the most common zoonoses infection in endemic areas especially in rural and nomadic areas with sheep husbandry jobs (4-6). Human echinococcosis is reported from many parts of Iran. About 1% of patients referred to surgical wards, patients with hydatid cyst and prevalence of human hydatidosis is (1.2-0.6)/100000 in Iran (1, 4-6).

Hydatid cyst affects different organs. The most common organ is the liver (50%-70%) and lungs (20%-30%) and less frequently in the bones, kidneys, spleen, muscles, central nervous system and eyes (7,8). Thus, rapid diagnosis of disease and the use of effective therapies, before and after surgery, can be effective in controlling and treatment of disease (7,9).

Surgery is still main treatment of CE, however there is increasing risks of frequent recurrences, cyst rupture and spillage of protoscoleces fluid and secondary infections at the same organ or other organs (7, 8). Therefore, use of suitable drug therapy, before and after surgery and during aspirations of cysts, can be effective to reduce recurrence, secondary hydatidosis, further surgery and can completely cure the diseases (9). Now according to recommendations of WHO, albendazole and/or mebendazole used in treatment of CE but these synthetic drugs have various side effects, resistance to live, and active pathogens (10, 11).

To be more effective of drug therapies, the specified concentrations of the drugs must be able to pass through layers of cysts and remove the live protoscoleces of hydatid cyst (12). Thus, the use of new scolicidal drugs with high efficacy in a shorter time of exposure, fewer side effects, less toxicity and less drug resistance, is an essential need for surgeons (13). One of the helpful agents in treatment of hydatidosis is use of nanoparticles (14).

In recent years considerable attention to nanoparticles and used in treatment. Controlled delivery of the drug to the target organs reduce side effects, low toxicity, increase drugs absorptions and increase the effectiveness of treatment of diseases are the advantages of nanoparticles (15). Cerium oxide (CeO2) is one of the effective therapeutic nanoparticles with important rules in human health. So far, it has been used in different medical therapies and studies such as antioxidant effects, antiplasmodium reduction potential, wound healing, antibacterial and reducing nematode longevity (16, 17).

One of the other therapies use of bioactive compounds extracts from sponge, coral, crustacean, fish and echinoderm (18). Sea cucumbers (*Holothuria leucospilota*) are echinoderms from the class Holothuroidea located in the deep seas all over the world. Recently they have been the attention of many researchers. The various extracts derived from sea cucumbers have many activities such as antiviral, antibacterial, antiparasitic (19), anticancer, antioxidant, antifungal and anti-inflammatory (20-22). The presence of compounds such as saponin, chondroitin sulfate, Glucosamine, glycosphingolipid and essential fatty acid is the
origin of many biological properties in these animals. Based on existing research, sea cucumber extract can inhibit tumor activity through induction of apoptosis (23, 24).

Apoptosis is a process of programmed cell death, that in normal conditions would remove old, excess and harmful cells and it is essential for homeostasis. Apoptosis can be initiated through one of two pathways, intrinsic pathway (mitochondrial) and extrinsic pathway (death ligand) (25). Both pathways induce cell death by activating caspases, which are proteases, or enzymes that have basic rules in the initiation and execution of apoptosis. As an executioner caspase, the caspase-3 plays a central role in the execution-phase of cell apoptosis (26).

We investigated the scolicidal effects of *H. leucospilota* extract and CeO2 nanoparticles against protoscolices of hydatid cysts on in-vitro and in-vivo model.

**Methods**

**Chemicals**

CeO2-NPs (Cerium Oxide nanoparticles), Eosin powder and PBS (phosphate buffered saline) were purchased from Sigma-Aldrich, St Louis, MO, USA and were stored at room temperature until testing. CASPASE 3 activity ELISA kit was purchased from Zellbio, Germany. Albendazole was obtained from Kimiafaam, Tehran, Iran.

**Collection and Extraction of Sea Cucumber**

Sea cucumbers (Genus: *H. leucospilota*) were collected in 2016 from the Persian Gulf, around the coastal cities of Kish Island and Bandar Lengeh, Iran. Samples were transferred to Parasitology Laboratory of Urmia University of Medical Science. All of samples were washed and cleaned several times with distilled water. The different parts of *H. leucospilota* (cuvierian organs and body wall) were divided into small pieces. Samples were kept at room temperature a way from light and heat for 2-3 d until completely dry. After that, the different dried parts of *H. leucospilota* were powders by a homogenizer. Then all powders, separately, were put in methanol-water (50:50) for 48 h. The blends were filtered by Buchner funnel and then concentrated by rotary evaporation. Finally, all extracts were dried by a freeze dryer and kept them at refrigerator temperature until testing.

**Collection of cysts**

Hydatid cysts of *E. granulosus* were collected from infected livers and lungs of sheep and cattle slaughtered at Urmia abattoir between years 2016-2017 and transferred to Parasitology Laboratory of Urmia University of Medical Science. Cysts were washed and cleaned with distilled water and external part of cysts was sterilized 70% ethanol. The hydatid fluid aspirated from the fertile cysts with a sterile syringe, transferred into tubes, and waited 30 min to settle down protoscoleces. The supernatant phase was removed and the protoscoleces were washed 3 times with sterile phosphate buffered saline (PBS), pH 7.2 (27, 28).

**In-vitro scolicidal effects of CeO2 NPs and *H. leucospilota***

In present study to evaluate the viability of protoscoleces, 0.1% eosin staining was used. 1ml of 0.1% eosin was added to 1 mL of settled protoscoleces and mixed gently after a few minutes examined microscopically. Alive protoscoleces remained colorless and showed flame cells movements and dead protoscoleces colored red and become shrunken (Figs. 1, 2). In this investigation, four concentration of *H. leucospilota* extract, (1,5,10,20)mg/mL and four concentration of CeO2-NPs (1,5,10,15)mg/mL and four concentration of combination of CeO2 NPs/ *H. leucospilota* (1,5,10,15)mg/mL were used for 10,20,30 and 60 min. 0.2 mL of various concentrations of extract and NPs were exposed with protoscoleces in four times (All samples had viability >90% at the time of experiments), and in every experiment, the mortality of protoscoleces was determined (29).
Fig. 1: Dead protoscoleces of *E. granulosus* after exposure with 0.1% eosin

Fig. 2: Live protoscoleces of *E. granulosus* after exposure with 0.1% eosin

We also used normal saline as a negative control group and albendazole as a positive control group. All experiments were carried out in triplicate.

**CASPASE 3 activity assay detections**

Each concentration of *H. leucospilota* extract and CeO2 NPs, were more effective against protoscoleces, were used in CASPASE 3 activity ELISA test. Caspase-3 activity assay detections were performed according to the caspase-3 activity assay kit.

**In-vivo experiments**

Male BALB/c mice with weight from 25-30 gr and 8 week old, purchased from Razi institute Tehran, Iran. These mice were kept under normal conditions with a 12:12 h dark/light cycle with access to food and water. Mice were infected intraperitoneally with 2000 viable protoscoleces in 0.5 mL sterile PBS (29, 30). After two months of infection, 5 mice were selected randomly and anesthetized with ether and cysts were seen in peritoneal cavity.

Histopathological analysis was used to prove the layers of hydatid cyst. For this examination, small part of peritoneal cavity cyst fixed in 10% formalin and then stained with hematoxilin-eosin.

The study was conducted in accordance with the ethical standards of “Principles of Laboratory Animal Care with approval cod: Ir.UMSU.REC.1395.153.

**Experimental treatment of *E. granulosus*-infected mice**

Infected mice have divided into 5 groups of 10 mice was in each treatment group. Drug concentration were prepared (CeO2 NPs:50 mg/kg, *H. leucospilota*: 200mg/kg, Combination of CeO2 NPs /*H. leucospilota*: 300 mg/kg). Infected mice treated daily for 4 week by intragastrical inoculation (100 Microliter/day). Albendazole with concentration of 50mg/kg as control group. After 4 week, the mice were dissected and evaluated based on the presence or absence of cysts and morphological changes between groups.

**Statistics**

All the In-vitro and in-vivo experiments were performed in triplicate. Data analysis were carried out by using GraphPad Prism 7 and SPSS 16.0 (Chicago, IL, USA). The differences between groups were determined using...
t-test and ANOVA testing and P-values less than 0.05 were considered significant.

**Results**

**Determination of in vitro scolicidal effects of CeO2 NPs and H. leucospilota extract and combination of both by 0.1% eosin stain**

According to Table 1, The *H. leucospilota* extract and CeO2 NPs and combination of *H. leucospilota* & CeO2 NPs were shown potent scolicidal activity against protoscoleces of hydatid cysts under in-vitro conditions with reference to the known standard drug Albenazole in a dose-dependent and time-dependent. Figure 3 shows scolicidal effects of different concentration of the *H. leucospilota* extract (1,5,10,20) mg/mL and CeO2 NPs (1,5,10,15)mg/mL and combination of both (1,5,10,15) mg/mL after 10,20,30 and 60 min against protoscoleces of *hydatid cyst*.

| Time (mg/mL) Concentration | 10 min | 20 min | 30 min | 60 min |
|----------------------------|--------|--------|--------|--------|
| 10 min                     | 0      | 12     | 18     | 28     |
| 15                         | 20     | 25     | 55     | 70     |
| 20                         | 30     | 35     | 44     | 79     |

Table 1: Mortality rate of protoscoleces (%)

The maximum scolicidal effects of CeO2 NPs and *H. leucospilota* extract and combination of both were observed at the concentration of 10 mg/mL and 20 mg/mL and 15 mg/mL.

The mortality rate at the negative control group (hypertonic saline) after 60 min of exposure was 25% and the mortality rate at the positive control group (albendazole) at the concentration of 10 mg/mL after 60 min was 85%. All sea cucumber extracts body wall and cuvierian organs have potential antihydatid activity.
Fig. 3: Scolicidal effects of CeO2 NPs and H. leucospilota extract in different concentrations and different times
A: CeO2 ; B: H. leucospilota extract; C: H. leucospilota & CeO2 NPs; D: Albendazole, (positive control); E: PBS (negative control)
**Determination of in vitro effects by CASPASE -3 activity assay**

CASPASE -3 activity assay was done in the three induced groups and control groups. The concentrations of 10 mg/mL CeO2 NPs, 20 mg/mL H. leucospilota extract and 15 mg/mL combination of H. leucospilota extract & CeO2-NPs were exposure with protoscoleces for 60 min. After exposure time, Caspase-3 activity assay detections were performed according to the caspase-3 activity assay kit and the apoptotic cells were observed at 450nm with ELISA reader. High enzymatic Caspase-3 Activity correlates with the frequency of apoptotic cells. The number of apoptotic protoscoleces significantly correlated with caspase-3 activity. High caspase-3 activity, as measured using the enzymatic method, was observed when protoscoleces expose with H. leucospilota extract and combination H. leucospilota extract & CeO2 NPs. The minimum level of caspase-3 activity is when protoscoleces expose with CeO2 NPs and Albendazole (Fig. 4).

![CASPASE 3 activity assay](chart.png)

**Fig. 4:** CASPASE -3 activity assay in the three induced groups (CeO2 NPs: 10mg/ml, H. leucospilota extract: 20 mg/ml, combination CeO2 and H. leucospilota extract: 15 mg/ml) and control groups (albendazole as positive control group and PBS as negative control group)

**In-vivo effects of CeO2 NPs and H. leucospilota extract and combination of both on clinical parameters of secondary hydatidosis**

After 4 week of treatment period, the mice were euthanized and the peritoneal cavity was opened (Fig. 5), and the hydatid cysts were carefully removed. Size, number and weight of hydatid cysts in different treatment groups were calculated and compared with positive control group (albendazole) and negative control group. According to Table 2, the maximum size, number and weight of hydatid cysts were observed in the group of mice-infected with no treatment period, and the minimum size and number of hydatid cysts and volume range was observed in the group of mice-infected with albendazole and H. leucospilota extract and Combination of H. leucospilota extract & CeO2 NPs treatment.

Available at: [http://ijpa.tums.ac.ir](http://ijpa.tums.ac.ir)
Aryamand et al.: In Vitro and in Vivo Scolicidal Activities of Holothuria leucospilota …

Fig. 5: After two months, mice infected with cysts in peritoneal cavity and liver (by injection of 2000 viable protoscoleces in /0.5mL sterile PBS)

Table 2: Number, size and weight of hydatid cysts in the different groups

| Experimental groups | Number of cysts | Size range (mm) | Mean size | Weight of cysts (mg) | Mean weight | Percentage of infected mice (%) | Localization of cysts |
|---------------------|----------------|----------------|-----------|---------------------|-------------|-------------------------------|----------------------|
| Control - Control + | 0              | 0              | 0         | 0                   | 0           | 0                             | 0                    |
| Albendazole         | 10             | 2.7 – 6.8      | 0.90±3.27 | 2.8-4.2             | 0.51±3.41  | 100(10/10 mice)               | Liver and peritoneum cysts |
| CeO2-NPs            | 8              | 2.3 – 5.5      | 1.14±4.87 | 3.5-5               | 0.53±4.25  | 100(10/10 mice)               | Liver and peritoneum cysts |
| H. leucospilota extract | 6          | 2.3 – 5.2      | 0.98±3.63 | 2.7-4.4             | 0.54±3.54  | 100(10/10 mice)               | Liver and peritoneum cysts |
| Combination of H. leucospilota extract & CeO2-NPs | 8             | 2.8- 5.2       | 0.98±3.96 | 3-4.6               | 0.56±3.65  | 100(10/10 mice)               | Liver and peritoneum cysts |

Discussion

Cystic echinococcosis caused by the larval stage of the cestode *E. granulosus*. Now, surgery is the first choice and main treatment for treatment of hydatid cyst however there is increasing risks of frequent recurrences, therefore, use of suitable scolicidal agents with low side effects can be more effective for surgeons (2, 30,31). Antiparasitic drugs treatments such as albendazole and mebendazole are toxic in many cases, study based on the use of some scolicidal agents with no toxic, side effects and its protective abilities. This study for the first time has investigated the scolicidal effects of *H. leucospilota* extract and CeO2 nanoparticles against protoscoleces of hydatid cysts on in vitro and in vivo model.
Sea cucumber *H. leucospilota* isolated from Persian Gulf showed pharmacological activities such as antifungal, antivirus, anticancer, antitumor, antioxidant, anti-inflammatory and antibacterial. Thus, sea cucumber with anti-cancer and anti-tumor activities might be also suitable as anthelmintics agents that can induce apoptotic cell death suggested that can anti-helminthic and anti-lysidat activities (22, 31, 32). In addition, anti-parasitic effects of *H. leucospilota* have already been studied. Methanolic extract of *H. leucospilota* possesses lethal effects on *L. infantum* promastigotes and leads to the inducing of the apoptosis in parasites, *L. infantum* promastigotes were sensitive to *H. leucospilota* extract at different concentrations in time and in dose-dependent manner. Apoptosis occurs in Leishmania promastigotes after exposure with *H. leucospilota* extract (22) Our finding showed that *H. leucospilota* extract has a potent scolicidal in vitro and in vivo model (200 mg/kg in vivo and 20 mg/ml in vitro).

In vitro examination showed that effective scolicidal activity of *H. leucospilota* extract and combination of *H. leucospilota* extract at concentration 20mg/ml and 15 mg/ml. Finding demonstrated the in vivo treatment of infected mice with combination of *H. leucospilota* extract /CeO2 NPs and *H. leucospilota* extract had a reduction in the parasite number, size and weight in compared to the control groups with respective P-value<0.05 showed that the reduction was statistically significant (Table 2).

Similar to these results, many other new compounds and plants and nanoparticles are known to have activity against hydatid cyst, for example, Garlic (*Allium sativum*) (33), Artemisinin (34), Mentha spp. essential oil (35), Ajowan (*Trachyspermum*) (36), Panca granatum peel aqueous (30), Berberis vulgaris root (37), Mallotus philippinensis (38), Foeniculum vulgare Mill (27), selenium NPs (39), solid lipid NPs (40) and gold- NPs (41). Echinacea purpurea, Sambucaebulus and zinc oxide nanoparticles resulted in a significant decrease in the number of the developed cysts (42). *F. vulgare* essential oil had remarkable scolicidal effects in comparison with positive control; so that, at concentrations of 1 and 0.5 mg/ml killed 100% protoscoleces after 5 and 10 min, respectively (27).

**Conclusion**

We observed in vitro and in vivo scolicidal activity of *H. leucospilota* extract and CeO2 NPs for the first time in compared with the positive control albendazole, our extract has potent scolicidal effects against protoscoleces of *E. granulosus* in vitro and in vivo model, but these results should be examined by extent molecular and experimental studies before introducing them as a suitable drug in treating human and animal hydatidosis.

**Acknowledgements**

This study was supported financially by Urmia University of Medical Sciences, (1956). The authors would like to thank all staff of Department of Medical Parasitology of Urmia Universities of Medical Sciences, Iran.

This study was supported by the Research center for Department of Parasitology and Mycology, Urmia University of Medical Sciences as well.

**Conflict of interest**

The authors declare that there is no conflict of interests.

**References**

1. Khalkhali H, Foroutan M, Khademvatan S et al. Prevalence of cystic echinococcosis in Iran: a systematic review and meta-analysis. J Helminthol. 2018;92(3):260-8.
2. Mozezi SS, Fashi Harandi M et al. Sonographic and serological survey of hydatid disease in Rural regions of Shahdad and chatroud, Kerman province, 2006-2007. J Kerman Univ Med Sci . 2009;25:25-34.
3. Khademvatan S, Majidiani H, Foroutan M et al. *Echinococcus granulosus* genotypes in Iran: a systematic review. J Helminthol. 2019; 93(2):131-8.

4. Aliabady ZA, Berenji F, Jamshidi MR. A case report of muscle hydatidosis from Iran. Iran J Parasitol. 2015;10(1):132-135.

5. Sabzevari S, Badirzadeh A, Shahkaram R, Seyyedin M. Traumatic rupture of liver hydatid cysts into the peritoneal cavity of an 11-year-old boy: a case report from Iran. Rev Soc Bras Med Trop. 2017;50(6):864-867.

6. Spotin A, Mahami-Oskouei M, Harandi MF et al. Genetic variability of *Echinococcus granulosus* complex in various geographical populations of Iran inferred by mitochondrial DNA sequences. Acta Trop. 2017;165:10-6.

7. Pakala T, Molina M, Wu GY. Hepatic echinococcal cysts: a review. J Clin Transl Hepatol. 2016;4(1):39-46.

8. Khademvatan S, Yousefi E, Rafiei A, Rahdar M, Saki J. Molecular characterization of livestock and human isolates of *Echinococcus granulosus* from south-west Iran. J Helminthol. 2013;87(2):240-4.

9. Venukumar R. Clinical presentation of hydatid cyst of liver: descriptive study. Int J Surg. 2016;4(1):214-6.

10. Lötch F, Naderer J, Skuhala T et al. Intracystic concentrations of albendazole-sulphoxide in human cystic echinococcosis: a systematic review and analysis of individual patient data. Parasitol Res. 2016;115(8):2995-3001.

11. Organization WH. The World Health Report 1996. Fighting disease. Fostering development. Geneva: WHO; 1996. Centers for Disease Control and Prevention, Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States (Washington, DC: Department of Health and Human Services, 1994). 2015.

12. Lv H, Jiang Y, Liu G, Zhang S, Peng X. Surgical treatment of multiple hydatid cysts in the liver of a pediatric patient. Am J Trop Med Hyg. 2015;92(3):595-8.

13. Pensel PE, Gamboa GU, Fabbri J et al. Cystic echinococcosis therapy: albendazole-loaded lipid nanocapsules enhance the oral bioavailability and efficacy in experimentally infected mice. Acta Trop. 2015;152:185-94.

14. Cai K, He X, Song Z et al. Dimeric drug polymeric nanoparticles with exceptionally high drug loading and quantitative loading efficiency. J AM Chem Soc. 2015;137(10):3458-61.

15. Ling D, Lee N, Hyeon T. Chemical synthesis and assembly of uniformly sized iron oxide nanoparticles for medical applications. Acc Chem Res. 2015;48(5):1276-85.

16. Dahle JT, Arai Y. Environmental geochemistry of cerium: applications and toxicology of cerium oxide nanoparticles. Int J Environ Res Public Health. 2015;12(2):1253-78.

17. Rubio L, Annangui B, Vila L, Hernández A, Marcos R. Antioxidant and anti-genotoxic properties of cerium oxide nanoparticles in a pulmonary-like cell system. Arch Toxicol. 2016;90(2):269-78.

18. Yousefi E, Eskandari A, Javad Gharavi M, Khademvatan S. In vitro activity and cytotoxicity of *Crocus sativus* extract against *Leishmania major* (MRHO/IR/75/ER). Infect Disord Drug Targets. 2014;14(1):56-60.

19. Khademvatan S, Eskandari A, Saki J, Foroutan-Rad M. Cytotoxic Activity of *Holothuria leucospilota* extract against *Leishmania infantum* in vitro. Adv Pharmacol Sci. 2016;2016:8195381.

20. Lawrence A, Afifi R, Ahmed M, Khalifa S, Page T. Bioactivity as an options value of sea cucumbers in the Egyptian Red Sea. Conserv Biol. 2010;24 (1):217-25.

21. Bordbar S, Anwar F, Saari N. High-value components and bioactives from sea cucumbers for functional foods—a review. Mar Drugs. 2011;9(10):1761-805.

22. Foroutan-Rad M, Khademvatan S, Saki J, Hashemitabar M. *Holothuria leucospilota* extract induces apoptosis in *Leishmania major* promastigotes. Iran J Parasitol. 2016;11(3):339-349.

23. Adibpour N, Nasr F, Nematpour F, Shakouri A, Ameri A. Antibacterial and antifungal activity of *Holothuria leucospilota* isolated from Persian Gulf and Oman Sea. Jundishapur J Microbiol. 2014;7(1):e8708.

24. Li X, Roginsky A, Ding XZ et al. Review of the Apoptosis Pathways in Pancreatic Cancer Available at: http://ijpa.tums.ac.ir
and the Anti-apoptotic Effects of the Novel Sea Cucumber Compound, Frondoside A. Ann N Y Acad Sci. 2008;1138(1):181-98.

25. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495-516.

26. Hu H, Kang J, Chen R, Mamuti W, Wu G, Yuan W. Drug-induced apoptosis of Echinococcus granulosus protoscoleces. Parasitol Res. 2011;109(2):453-9.

27. Lashkarizadeh MR, Asgaripour K, Dezaki ES, Harandi MF. Comparison of scolicidal effects of nanoparticles and Foeniculum vulgare Mill on hydatid cysts protoscoleces. Iran J Parasitol. 2015;10(2):206-212.

28. Spicher M, Naguleswaran A, Ortega-Mora LM et al. In vitro and in vivo effects of 2-methoxyestradiol, either alone or combined with albendazole, against Echinococcus metacestodes. Exp Parasitol. 2008;119(4):475-82.

29. Urrea-Paris FM, Moreno MJ, Casado N, Rodriguez-Caabeiro F. Relationship between the efficacy of praziquantel treatment and the cystic differentiation in vivo of Echinococcus granulosus metacestode. Parasitol Res. 2002;88(1):26-31.

30. Labsi M, Kholifi L, Mezioug D, Soufli I, Touil-Boukoffa C. Antihydat and immunomodulatory effects of Punica granatum peel aqueous extract in a murine model of echinococcosis. Asian Pac J Trop Med. 2016;9(3):211-20.

31. Dhinakaran DJ, Lipton A. Pharmacological potentials of sea cucumber Holothuria atra extracts from the Indian Ocean. Asian J Biomed Pharm Sci. 2014;4(35):36.

32. Mokhlesi A, Saeidnia S, Gohari AR et al. Biological activities of the sea cucumber Holothuria leucospilota. Asian J Anim Vet Adv. 2012;7(3):243-9.

33. Sadjjadi SM, Zoharizadeh MR, Panjeshahin MR. In vitro screening of different Allium sativum extracts on hydatid cysts protoscoleces. J Invest Surg. 2008;21(6):318-22.

34. Spicher M, Roethlisberger C, Lany C et al. In vitro and in vivo treatments of Echinococcus protoscoleces and metacestodes with artemisinin and artemisinin derivatives. Antimicrob Agents Chemother. 2008;52(9):3447-50.

35. Maggiore MA, Albanese AA, Gende LB et al. Anthelmintic effect of Mentha spp. essential oils on Echinococcus granulosus protoscoleces and metacestodes. Parasitol Res. 2012;110(3):1103-12.

36. Moazeni M, Saharkhiz MJ, Hosseini AA. In vitro lethal effect of ajowan (Trachyspermum ammi L.) essential oil on hydatid cyst protoscoleces. Vet Parasitol. 2012;187(1):203-8.

37. Mahmouvdvand H, Dezaki ES, Sharififar F et al. Protoscolecidal effect of Berberis vulgaris root extract and its main compound, berberine in cystic echinococcosis. Iran J Parasitol. 2014;9(4):503-510.

38. Gangwar M, Verma VC, Singh TD, Singh SK, Goel R, Nath G. In vitro scolicidal activity of Mallotus philippinensis (Lam.) Muell Arg. fruit glandular hair extract against hydatid cyst Echinococcus granulosus. Asian Pac J Trop Med. 2013;6(8):595-601.

39. Mahmouvdvand H, Harandi MF, Shakibaie M et al. Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. Iran J Surg. 2014;12(5):399-403.

40. Ahmadnia S, Moazeni M, Mohammadi-Samani S, Oryan A. In vivo evaluation of the efficacy of albendazole sulfoxide and albendazole sulfoxide loaded solid lipid nanoparticles against hydatid cyst. Exp Parasitol. 2013;135(2):314-9.

41. Jahani Z, Meshgi B, Rajabi-Bzl M, Jalousian F, Hasheminasab S. Improved Serodiagnosis of Hydatid Cyst Disease Using Gold Nanoparticle Labeled Antigen B in Naturally Infected Sheep. Iran J Parasitol. 2014;9(2):218-225.

42. Razi Jm, Alborzi A, Najafzade Vh et al. Survey on effects of albendazole, Echinacea purpurea, Sambucus ebulus and zinc oxide nanoparticles on unilocular hydatid cyst in mice. SCIENTIFIC RESEARCH IRANIAN VETERINARY JOURNAL. 2015;11(2):68-125.