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Research Article

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Protoscolidical effects of *Zingiber officinale* and *Allium cepa* ethanolic extracts using hydatid cysts protoscolices and comparison of their activities with conventional anthelmintic

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Abstract

Hydatidosis, zoonotic disorder, is responsible for chronic sufferings of both human and animals. There is no other better treatment option without surgery and there is also a chance of recurrence of infection after surgery. As the existing drugs are not so effective, searching for new protoscolicidal agents become an urgent need. This study investigates the scolicidal activities of the *Zingiber officinale* and *Allium cepa* ethanolic extract on hydatid cysts protoscolices. The samples were collected from the infected livers and lungs of goats and sheep. Eosin stain (0.1%) was used to determine the viability of protoscolices and the result was observed in an optical microscope. Results revealed that *Z. officinale* extract (50 mg/ml) killed all the observed protoscolices after 120 min of exposure. However, if the concentration increases (100 mg/ml), the killing time becomes shorten gradually. Similar kinds of effects were observed in *A. cepa*, where more than 90% protoscolices were death at 25 mg/ml concentration at 120 min. The results showed that the protoscolicidal effects were dependent on concentration and time. When the scolicidal effects of these two plant extracts were compared to that of two anthelmintic albendazole and ivermectin, it was found that the ethanolic extracts of *Z. officinale* and *A. cepa* showed more scolicidal effects. Hence, these plant extracts can be considered as potent scolicidal agents for the treatment of hydatid cysts infection.

Keywords: *Allium cepa*, Hydatid cysts, Protoscolices, Viability, *Zingiber Z. officinale*

INTRODUCTION

Hydatid cyst, a parasitic infection, caused by *Echinicoccus granulosus* and considered as one of the leading zoonotic disorders with pernicious effects of human and animal. Although hydatid diseases may occur in different parts of the body, cystic echinococcosis mainly affects liver and lung (Zhou *et al.*, 2007). This disease may incur serious medical complications as well as financial concern to people; sometimes life-threatening if remains untreated for many years (Lahmar *et al.*, 2007). In most cases, there is no symptom or expression due to their tardy growth. Major clinical symptoms may appear eventually depending on the range and locations of infection and size of the cyst (Venukumar, 2017). Till now, surgery is the most common practice of treatment but rupture the cyst and intra-operative spillage of scolices after surgery are the common complications (Topcu *et al.*, 2006). Now-a-days, several scolicidal agents including silver nitrate, cetrimide, hypertonic saline, ethanol revealed many undesirable effects such as cholangitis, liver infarction, scleros-

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ing, methaemoglobinaemia etc. (Hosseini *et al.*, 2006; Rajabi, 2009). Furthermore, medications such as albendazole, mebendazole, amphotericin B and praziquantel are not so much effective and failure of treatment is also reported. The treatment has been interrupted due to adverse side reactions up to 10% cases while failure has been reported approximately 16% of patients (Reuter *et al.*, 2003). Vaccinations do not work in most instances and sometimes the parasites have become resistant to the available synthetic therapeutics (Hosseini *et al.*, 2006). As a result, explore new scolicidal agents from natural sources (with fewer side effects, lower cost, and higher efficacy) is a crying need to reduce the risk of intraoperative spillage of the scolices and posterior recurrence of *Cystic Echinococcus* (CE). Due to fewer side effects, low-cost, and high availability, plant extracts are a successful approach to treat a range of diseases.

A large number of plant extracts have antiparasitic activities of which some of them were found more effective than currently used therapeutics. These plants extract act interfering the parasites DNA (intercalation, alkylation), membrane integrity, microtubules, neuronal signal transduction etc. (Wink, 2012). Phytochemical screenings have shown that ginger is affluent in alkaloids, flavonoids, terpenes, saponins, steroids etc., which are mostly counted as bioactive ingredients (Rajabi, 2009; Sadhana und Gupta, 2013). The efficiency of antiparasitic action of *Z. officinale* against microfilariae of *Dirofilaria immitis, Schistosoma adults, Trichinella spiralis* muscular and intestinal phases in dogs and protoscolices of hydatid cyst were observed (Forouzan *et al.*, 2012). *Allium cepa* belonging to Alliaceae family is known as onion, recognized as a traditional medicine to cure intestinal infections and well-known for its antiparasitic, antibacterial, antithrombotic, antiviral, antifungal, antioxidant, antihypertensive, hypoglycemic, anti-inflammatory activities. It has flavonoids, spropylcycteine, cycloallicin, phenolic acids, sterols, saponins, sugars, volatile oils etc (Rahimi-Esboei *et al.*, 2016). The goals of our present study were to check scolicidal activity of the ethanolic extracts of *Z. officinale* and *A. cepa*, and compare effects of these extracts with conventional anthelmintic through the viability studies of protoscolices of *E. granulosus*.

MATERIALS AND METHODS

Collection of protoscolices

Hydatid cysts of *E. granulosus* were obtained from infected lungs and livers of assuredly infected goats and sheep slaughtered in Chittagong metropolitan area, Bangladesh. Hydatid fluid with protoscolices was collected and moved into Petri dish, and allowed to stand for 1 hour to precipitate at room temperature (Shahnazi *et al.*, 2014). By this time, protoscolices were settled down at Petri dishes. After collection, protoscolices were allowed to clean three times using saline water (0.9% NaCl). Viability of protoscolices was confirmed by observing their movements using light microscopy (Model: B-150, OPTIKA, Italy).

Viability test of protoscolices

To investigate the viability of protoscolices 0.1% eosin (a red fluorescence dye) dye was used. At first, 20 µl eosin solution was added to 20 µl of protoscolices solution. After 5 minutes of exposure, the protoscolices which didn't absorb stain considered as viable, while those absorbed color regarded as nonviable or dead protoscolices (Figure 1) (Smyth und Barrett, 1980; Shahnazi *et al.*, 2014). Then, live protoscolices were stored in a dark container containing the normal saline solution.

Collection of the plant parts

Fresh roots of ginger (*Z. officinale*) and bulb of onion (*A. cepa*) were purchased from the vegetable market of Chittagong, Bangladesh. These plant samples were identified, authenticated from Bangladesh National Herbarium, Dhaka, Bangladesh; and later deposited in the herbarium for future correspondence. The voucher specimen numbers were DACB 43721 for ginger, and DACB 43720 for onion respectively. The collected samples were cleaned by distilled water, cut into small pieces and dried in the sun for 10 days. After drying, the dried materials were pulverized. Powdered materials macerated individually in 99.5% ethanol for 15 days; and later filtration and drying (at room temperature) were performed. We have followed the cold extraction method according to Akter *et al.* (2018). Finally, the obtained sticky brown residues (crude extracts) were stored in a refrigerator at 4°C.

Studies on protoscolicidal effects of the extracts

Different concentrations (15, 25, 50 and 100 mg/ml) of the samples of *Z. officinale*, and *A. cepa* extracts were prepared by dissolving the extract in distilled water. Standard drugs like albendazole and ivermectin solutions of above-mentioned concentrations were also prepared. We had observed the protoscolicidal effects at

5, 30, 60, 90, and 120 minutes to determine the viability of protoscolices. Normal saline was used as a control group. 1ml of each concentration was taken in Petri dishes to which 1ml of protoscolices containing approximately 2000 protoscolices were affixed. The mixtures of the tubes were slightly consolidated and then incubated at 28°C for 5, 30, 60, 90 and 120 min respectively. After finishing each incubation period, the upper phase of the tubes was carefully removed without hampering the protoscolices. Then 0.1% eosin stain was added to the remaining settled protoscolices (Shahnazi *et al.*, 2014) and after 5 minutes, pellets of the protoscolices were smeared on a glass slip followed by covering by a coverslip and evaluating under an optical microscope (Model: B-150, OPTIKA, Italy). The percentages of viable protoscolices were computed by counting the protoscolices (usually >150). The efficacy of the ethanolic extract of both plants and conventional anthelmintic (standard drugs) on the viability of the protoscolices were determined through comparing with the control group. Non-treated protoscolices were considered as the control group.

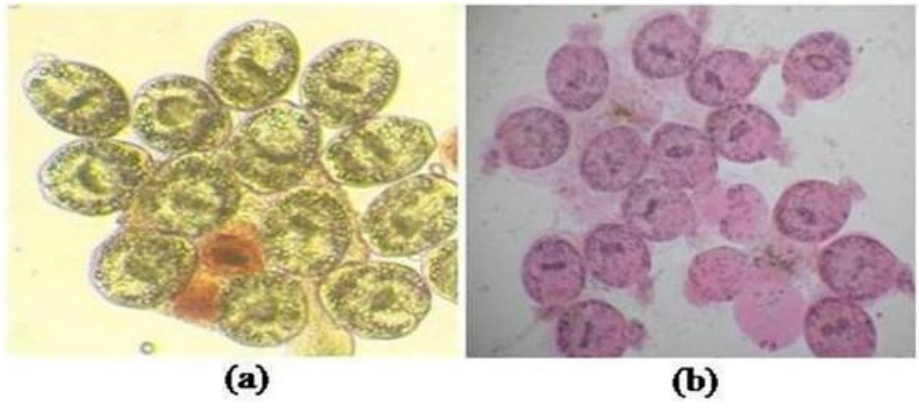

Figure 1: (a) Live protoscolices which didn't absorb color (remained its original color); (b) Dead protoscolices absorbed eosin dye (0.1%) and turned into purple color.

Statistical analysis

Data were presented as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Dunnet's t-test using SPSS 19.0 software. *$p<0.05$ were considered as significant values, whereas **$p<0.01$, ***$p<0.001$ were considered as highly significant values. Origin Pro-2018 (Origin Lab. Corp., USA) was used for preparing graphical representations.

RESULTS

Evaluation of the effect of *Z. officinale* on *E. granulosus* protoscolices

The scolicidal effects of the different doses of *Z. officinale* ethanolic extract on the viability of protoscolices are shown in Table 1. It was found that the viability of protoscolices was affected significantly when treated with different concentrations of *Z. officinale* extract. The scolicidal response follows dose-dependent and time-dependent manner. Figure 2 shows the live and dead protoscolices due to exposure of *Z. officinale* extract (100 mg/ml).

Evaluation of the effect of *A. cepa* on *E. granulosus* protoscolices

The effect of the ethanolic extract of *A. cepa* on the viability of protoscolices is shown in Table 2. Our experimental result showed that *A. cepa* possesses significant protoscolicidal activity when compared to control. In this case, the scolicidal response also follows dose-dependent and time-dependent manner. Figure 3 shows the live and dead protoscolices due to exposure of *A. cepa* extract (100 mg/ml).

Comparison of the protoscolice studies of the extracts with conventional anthelmintic

The study also observed the protoscolicidal effect of albendazole and ivermectin. The comparative studies were shown in Figure 4. Moderate protoscolicidal effects were found at lower concentrations of albenda-

zole. The viabilities of the protoscoleces were approximately 26.60%, 19.60% and 18.80% at the concentration of 400, 600 and 800 µg/ml respectively after 120 minutes of exposure. In contrast, about 87.20% protoscolices remained viable in the control group after the same exposure time (Figure 4c). Ivermectin possesses higher protoscolicidal activity when treated at a concentration of 15 µg/ml (viability- 17%) and 25 µg/ml (viability- 0%) at the same duration (Figure 4d).

Table 1: Scolicidal effect of *Z. officinale* extract on the viability of protoscolices (*E. granulosus*).

Concentration	**% of viability rate after exposure**				
	5 min	**30 min**	**60 min**	**90 min**	**120 min**
15 mg/ml	86.00 ± 4.94	75.20 ± 1.74	56.60 ± 2.71	6.60 ± 0.68**	1.80 ± 0.49**
25 mg/ml	85.00 ± 4.22	34.20 ± 2.29	16.80 ± 0.86***	4.60 ± 0.60**	1.20 ± 0.37**
50 mg/ml	84.20 ± 2.94	38.80 ± 2.08	4.60 ± 0.68***	0.60 ± 0.24*	0 ± 0.00**
100 mg/ml	83.80 ± 2.46	28.50 ± 1.02	2.60 ± 0.40**	0 ± 0.00***	0 ± 0.00**
Control	93.00 ± 5.17	89.20 ± 4.01	89.40 ± 6.10	88.40 ± 3.67	87.10 ± 2.78

Values are presented as mean ± SEM (n=5). Data was analyzed using one-way ANOVA followed by Dunnet's t-test. ***p <0.001, **p <0.01, *p <0.05 as compared with control.

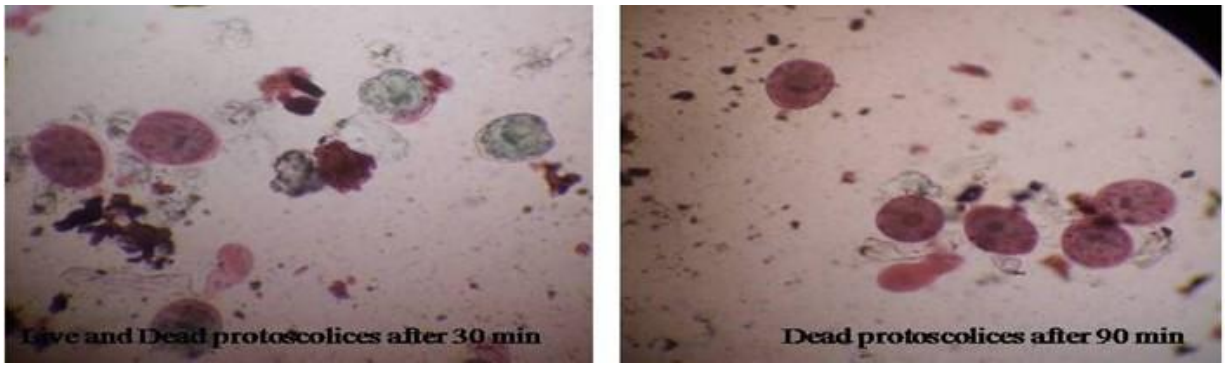

Figure 2: Live protoscolices (not stained) and Dead protoscolices (stained) after 30 min and 90 min of the exposure of *Z. officinale* extract (100 mg/ml).

DISCUSSION

Parasitic diseases are usually treated with synthetic anthelmintic in order to control hydatidosis. However, many of the scolicidal agents used nowadays have been reported not to be fully active against protoscolices. Moreover, most of them (e.g.: ethanol, albendazole sulfoxide, hypertonic saline, $AgNO_3$, cetrimide, formalin etc.) may provoke untoward complications and adverse side effects which may limit their applications (Rajabi, 2009). Recent years, plant extracts have considered with much attention for developing new scolicidal agents active against cystic Echinococcus. Many studies found that plant extracts of individual plant species may affect the viability of protoscoleces and survival of secondary hydatid cysts (Barzinji *et al.*, 2009). Gholami et al. (2013) found that methanolic extract of *Sambucus ebulus* fruits has high scolicidal efficacy. *Dendrosicyos socotrana* and *Jatropha unicostata* can kill Echinococcus species significantly (Barzinji *et al.*, 2009). The scocicidal efficacies of these extracts may differ due to the presence of different phytoconstituents in those plants.

Table 2: Scolicidal effect of *A. cepa* extract on the viability of protoscolices (*E. granulosus*).

Concentration	**% of viability rate after exposure**				
	5 min	**30 min**	**60 min**	**90 min**	**120 min**
15 mg/ml	91.20 ± 4.00	80.80 ± 3.97	70.20 ± 3.38	47.60 ± 2.50	10.20 ± 1.02*
25 mg/ml	88.40 ± 2.86	75.00 ± 4.40	64.80 ± 3.15	33.00 ± 2.55**	8.20 ± 0.66*
50 mg/ml	86.80 ± 4.45	78.60 ± 3.25	49.40 ± 2.42	10.40 ± 1.21*	6.60 ± 0.81**
100 mg/ml	82.40 ± 2.42	65.40 ± 3.19	26.80 ± 1.59	3.20 ± 0.37*	0 ± 0.00***
Control	94.00 ± 4.73	91.40 ± 6.34	90.60 ± 4.60	90.20 ± 3.64	89.60 ± 3.97

Values are presented as mean ± SEM (n=5). Data was analyzed using one-way ANOVA followed by Dunnet's t-test. ***p <0.001, **p <0.01, *p <0.05 as compared with control.

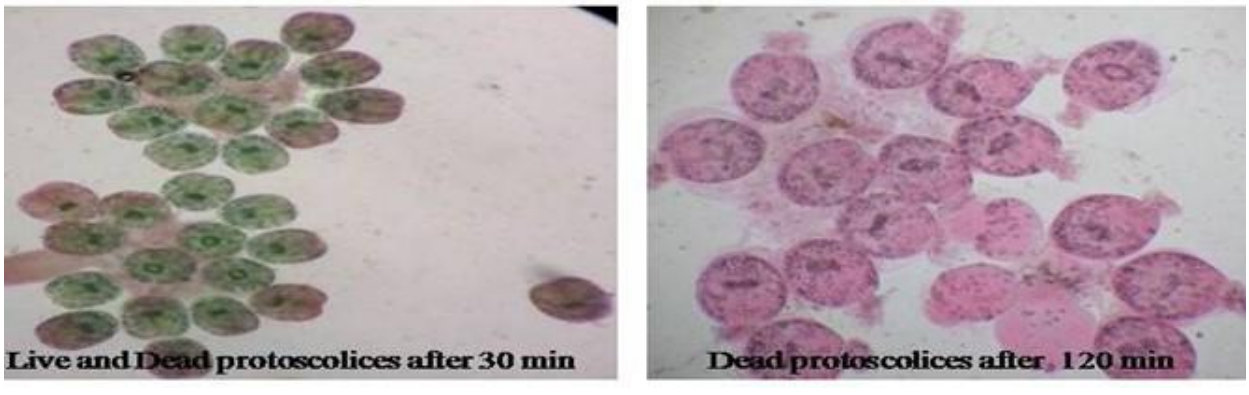

Figure 3: Live protoscolices (not stained) and Dead protoscolices (stained) after 30 min and 120 min of the exposure of *A. cepa* (100mg/ml)

In this study, we try to assess the protoscolicidal efficacy of *Z. officinale* and *A. cepa* for searching safe, cost-effective, less toxic, and potential sources of scolicidal agents. Our result revealed that ethanolic extract of *Z. officinale* and *A. cepa* possesses significant protoscolicidal efficacies.

We found that the viability of protoscolices decreases as the dose of the *Z. officinale* extract increases. At higher concentration (100 mg/ml), approximately 28.50% protoscolices remain viable after 30 minutes; however, after 90 minutes of the exposure, all protoscolices were expired. In contrast, approximately 87.10% protoscolices stay subsistent for the control group (untreated protoscolices) after 120 minutes of the exposure. Many phytoconstituents including alkaloids, flavonoids, terpenes, saponins, steroids etc. were found in *Z. officinale* (Sadhana und Gupta, 2013; Ahmad *et al.*, 2015; El-Sayed und El-Saka, 2015). Gingerols are the main phytocontituents in *Z. officinale* responsible for the antibacterial efficacy, and also for scolicidal efficacy probably (Rostami *et al.*, 2016; Sivasothy *et al.*, 2017). Other suspicious compounds in *Z. officinale* are shogaol, diarylheptanoids, phenylbutenoids, flavanoids, diterpenoids and sesquiterpenoids (Jitoe *et al.*, 1993; Park *et al.*, 2008; Zheng *et al.*, 2013). The results of scolicidal activity of *Z. officinale* extract are almost consistent with the report of Moazeni und Nazer (2011).

On the other hand, the viability percentage of protoscolices after exposing with *A. cepa* extract at a concentration of 15 mg/ml were 91.2%, 80.8%, 70.2%, 47.6%, 10.2% approximately after 5, 30, 60, 90 and 120 minutes respectively. A similar descending trend of the viability of protoscolices is also seen for the other doses of *A. cepa*. At higher concentration (100 mg/ml) of this extract, approximately 65.40% protoscolices remain viable after 30 minutes of the exposure; however,

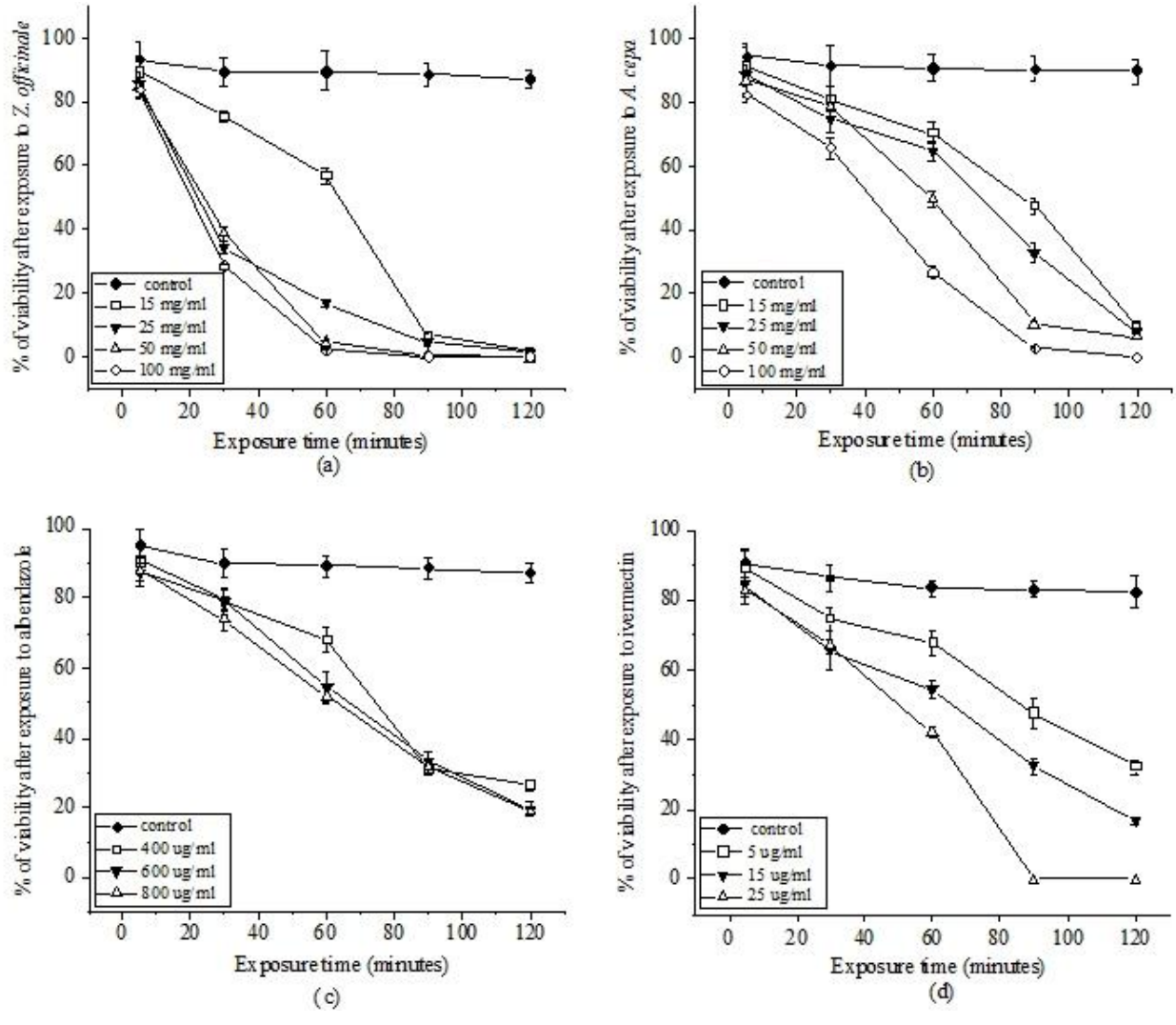

Figure 4: Scolidical effect of the different concentrations of (a) *Z. officinale* ethanolic extract (b) *A. cepa* ethanolic extract (c) Albendazole (d) Ivermectin

all the protoscolices died after 120 minutes. In control groups (untreated protoscolices), about 89.60% protoscolices still remain viable after 120 minutes. *A. cepa* possesses flavonoids, sterols, spropylcycteine, cycloallicin, phenolic acids, saponins, sugars, volatile oils etc. (Rahimi-Esboei *et al.*, 2016; Upadhyay, 2016). Barzinji *et al.* (2009) state that sterols, ketosteroids, terpenoids etc. may have an effect on the viability of certain organisms. The observed scolicidal effects of the ethanolic extracts of *A. cepa* may be due to the presence of above phytoconstituents. Our results endorsed the finding of Haghani *et al.* (2014).

We found that the scolicidal effects of the various concentrations of the ethanolic extract of *Z. officinale* and *A. cepa* were significant ($p<0.05$), when compared to the control group. The protoscolicidal effects are comparable to the standard drugs- albendazole and ivermectin. If we compare the 90 minutes data (exposure time- 90 minutes), it was found that protoscolices expose to *Z. officinale* (100 mg/ml) were killed comparatively faster than *A. cepa* (100 mg/ml). A similar trend of the effect was also seen for other concentrations. Albendazole at a concentration of 800 μg/ml, the viability percentage is 31.60% approximately after 90 minutes, which is lower than 100 mg/ml of *Z. officinale* and *A. cepa*. However, ivermectin (25 μg/ml) can expire all the protoscolices at 90 minutes. Considering 120 minutes data, the mortality rate of the protoscolices were 100% for *Z. officinale* (100 mg/ml), *A. cepa* (100 mg/ml), and ivermectin (25 μg/ml) respectively. However, about 18.80% of protoscolice remain viable for albendazole (800 μg/ml) after 120 minutes. We found that in all

cases, the protoscolicidal effects follow concentration-dependent and time-dependent manners.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Abstract in Bengali

আদা এবং পেঁয়াজের ইথানলিক নির্যাসের প্রটস্কলিসাইডাল প্রভাব এবং এর কার্যকারিতার সাথে প্রচলিত ক্রিমিনাশকের তুলনাঃ হাইডাটিডোসিস একটি জুনুটিক রোগ যা মানুষ এবং প্রাণী উভয়ের মারাত্মক দুর্ভোগের জন্য দায়ী। সার্জারি ছাড়া এর ভালো কোন চিকিৎসা নেই এবং সার্জারির পরেও পুনরায় সংক্রমন হওয়ার সম্ভাবনা থাকে। যেহেতু বিদ্যমান ওষুধগুলি এত কার্যকর নয়, তাই নতুন প্রটস্কলিসাইডাল ডাল এজেন্টদের সন্ধান করা জরুরি প্রয়োজন। এই গবেষণাটি হাইডাটিডসিস্ট প্রটস্কলিসেসর উপর আদা এবং পেঁয়াজের ইথানলিক নির্যাসের কার্যকারিতা অনুসন্ধান করে। আক্রান্ত ছাগল ও ভেড়ার যকৃত ও ফুসফুস থেকে নমুনা সংগ্রহ করা হয়। প্রটস্কলিসেস এর কার্যকরতা নির্ধারণের জন্য ০.১% ইওসিন স্টেইন ব্যবহার করা হয় এবং আলোক অনুবীক্ষণ যন্ত্রের নিচে তা পর্যবেক্ষণ করা হয়। ইহা প্রতিয়মান হয় যে, আদার রসে (৫০ মিগ্রা / মিলি) ১২০ মিনিট রাখার পর সব প্রটস্কলিসেস মারা যায়। ঘনত্ব যত বাড়ানো হয় (১০০ মিগ্রা/মিলি), মারা যাওয়ার সময় তত কমে যায়। একই ধরনের প্রভাব পেয়াঁজের ক্ষেত্রেও লক্ষ্য করা যায়, যেখানে ৯০% প্রটস্কলিসেস ১২০ মিনিট ধরে ২৫মিগ্রা/মিলি ঘনত্বের নির্যাসে রাখলে মারা যায়। ফলাফলগুলো দেখায় যে, প্রটস্কলিসাইডাল এর প্রভাব পাশাপাশি মৃত্যুর হার সময় এবং ঘনত্বের উপর নির্ভর করে। এই দুটো গাছের নির্যাসের স্কলিসাইডাল কার্যকারিতা বাজারে প্রচলিত ওষুধ এলভেনডেজল ও আইভারমেকটিন এর সাথেও তুলনা করে দেখা যায় যে, আদা এবং পেয়াঁজের ইথানলিক নির্যাসের অধিক স্কলিসাইডাল কার্যকারিতা আছে। সুতরাং হাইডাটিডসিস্ট সংক্রমণে ইহাদেরকে সম্ভাব্য স্কলিসাইডাল এজেন্ট হিসাবে বিবেচনা করা যেতে পারে।