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Anti-giardial activity of *Sambucus ebulus*

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Abstract. – OBJECTIVES: *Giardia (G.) lamblia* is a parasite that causes giardiasis in humans and other mammals. The common treatment produces unpleasant side effects. The ethnopharmacology for management of parasitic infections accelerates and guides the search for new chemical objects. This study assessed the *in vitro* cytotoxicity of *Sambucus (S.) ebulus* fruit against Cysts of *G. lamblia*.

MATERIALS AND METHODS: Giardia cysts were isolated from patients' fecal specimens; the cysts were isolated by sucrose 0.85 M solution. The plant extract was used at concentrations of 1, 10, 50 and 100 mg/mL throughout the experiments. The extracts were incubated with several isolates of *G. lamblia* for 5, 10, 30 and 60 minutes and then the viability were distinguished by eosin 0.01%.

RESULTS: *S. ebulus* extract at the concentration of 100 mg/ml for 60 minutes had the most anti-giardial activity (78 ± 4%) than other concentrations.

CONCLUSIONS: Considering excellent anti-giardial activity of *S. ebulus in vitro*, it seems to have potential for the treatment of the parasitic disease caused by the protozoan *G. lamblia*.

Key Words:
Sambucus ebulus, Giardia lamblia, Parasitic disease.

Introduction

Giardiasis is a major diarrheal disease found throughout the world. The flagellate protozoan *Giardia (G.) lamblia*, is the most commonly identified intestinal parasite in the US and the most common protozoan intestinal parasite isolated worldwide especially at resource-poor settings[1]. Giardiasis is more common in children than in adults[2]. The organism has been found in as many as 80% of raw water supplies from lakes, streams, and pools and in as many as 15% of filtered water samples[2]. Giardiasis usually represents a zoonosis with cross-infectivity between animals and humans[1]. Clinical manifestations of giardiasis include acute or chronic diarrhea, abdominal pain, and malabsorption, leading to malnutrition and weight loss, particularly in children[3]. The common treatment for this sickness includes different drugs such as metronidazole and furazolidone, that have unpleasant side-effects such as metallic taste, headache and pruritus[4]. In addition, some strains of *G. lamblia* have developed resistance to some of these remedies[5]. Thus, the search for new antiprotozoal compounds with high activity, low toxicity, cheaper and more effective is still a necessary aim. To improve this therapy, ethnopharmacological studies disclose a variety of plants used for the treatment of gastro-intestinal (GI) disorders such as diarrhea and dysentery, caused, in some cases, by *G. lamblia*[6]. Iranian traditional medicine uses the leaves, fruits and rhizomes of *S. ebulus* in treating some inflammatory cases such as, bee and nettle bites and arthritis[7]. In addition, it has been reported to be an insect repellent, anti-protozoa, anti-bacterial toward *H. pylory*, convenient in treatment of burns and infections wounds, edema, eczema[8-9]. Considering the severity of giardiasis, particularly in children, and the contribution of the ethnopharmacological knowledge for the treatment of parasitic infections, the aim of the present study is to investigate the efficacy of *S. ebulus* fruit against cysts of *G. lamblia*.

Materials and Methods

Purification of G. lamblia Cysts

G. lamblia cysts were isolated from faeces of giardiasis patients from the different hospitals of Mazandaran Medial University. All samples

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were processed immediately after arrival, ordinarily within 48 h after excretion. A highly purified cyst suspension was achieved by combining the sucrose flotation method with a simplified sucrose gradient method[10]. Stools were broken up in tap water and filtered through a 300 urn filter. 3 ml of faecal suspension were layered on 3 ml of 0.85M sucrose and centrifuged at 600 g for 10 min at 4°C. The cysts at the sucrose-water interface were aspirated with a Pasteur pipette and washed 3 times with water. Washed cysts were carefully added to the top of a discontinuous density gradient, consisting of two3-ml lavers of 0.85 and 0.4 M sucrose. After centrifugation at 600g for 10 min at 4°C cysts concentrated at the 0.85-0.4M sucrose interface were collected and washed again. Purified cysts were resuspended in normal saline and stored at 4°C for a maximum of 3 days prior to use.

Preparation of S. ebulus Extract

Fruits of *S. ebulus* were dried under shade, and powdered mechanically using a commercial electrical blender. 70 g of dry powder was added to 350 ml of pure methanol and left at room temperature for 24 h. Mixture was filtered and solvent was removed by evaporation in a rotating evaporator. The remaining semisolid material was then freeze-dried. The obtained filtrate (5.5 g) was placed into a sterile glass container and stored at 4°C for further use.

Effectiveness of S. ebulus Extract on Cysts of Giardia lamblia

Four concentrations (1, 10, 50 and 100 mg ml^{-1}) of the *S. ebulus* extract were used for 5, 10, 30 and 60 min. 2 ml of each solution was placed in test tubes, to which 10,000 washed cysts was added. The contents of the tubes were gently mixed. The tubes were then incubated at 37°C for 5, 10, 30 and 60 min. At the end of each incubation time the upper phase was carefully removed so as not to interrupt the cysts. 2 ml of 0.1% eosin stain was then added to the remaining settled cysts and mixed gently. The upper portion of the solution was discarded after 15 min of incubation. The remaining pellet of cysts was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead cysts were determined by counting 500 cysts. Nontreated cysts were considered a control group in each experiment. The experiments were performed in triplicate.

Viability Test

In the present study eosin stain with the concentration of 0.1% (1 g of eosin powder in 1000 ml distilled water) was used to check the viability of the cysts. Fifteen minutes after exposure to the stain the cysts with no absorbed dye were considered potentially viable otherwise, they were recorded as dead.

Statistical Analysis

Statistical analysis was conducted by a one-way ANOVA (analysis of variance) considering a level of significance of 95% ($p < 0.05$), with SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Antigiardial effects of *S. ebulus* extract are summarized in Table I. *S. ebulus* extract at the concentration of 1 mg ml^{-1} killed 25, 30, 35, and 39% of the cysts, at the concentration of 10 mg ml^{-1} killed 33, 36, 40 and 41% of the cysts, at the concentration of 50 mg ml^{-1} killed 48, 50, 50 and 64% of the cysts and at the concentration of 100 mg ml^{-1} killed 58, 63, 69 and 78% of the cysts after 5, 10, 30, and 60 min of application, respectively. Antigiardial effect of any concentrations of the methanolic extract of *S. ebulus* was extremely significant compared to the control groups at all exposure times ($p < 0.001$). In addition to the present study show the increased of *Giardia* cysts inactivation with times (Table I). The results of our study improved high antigiardial activity of methanolic extract of *S. ebulus* fruit showed *in vitro*.

Table I. Anti-giardial effect of *S. ebulus* fruit extract at different concentration and times.

Time/concentration	After 5 min	After 10 min	After 30 min	After 60 min
1 mg/ml	25 ± 2.5	30 ± 2.3	35 ± 1	39 ± 1.8
10 mg/ml	33 ± 1.3	36 ± 2.1	40 ± 1.6	41 ± 3.1
50 mg/ml	48 ± 2.7	50 ± 2.2	50 ± 3.2	64 ± 4.1
100 mg/ml	58 ± 3.7	63 ± 3.5	69 ± 3.9	78 ± 4

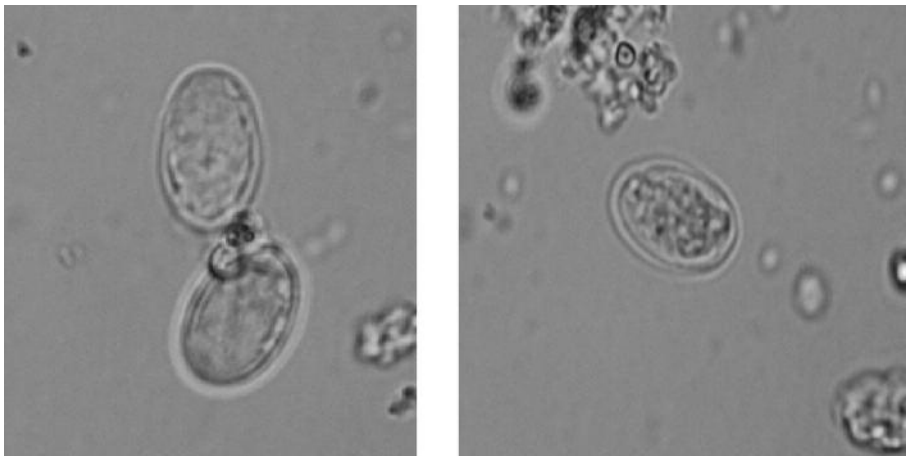

Figure 1. Viable cysts with no absorbed dye (*left*) and dead cysts with absorbed dye (*right*).

Discussion

Giardiasis is caused by the flagellate protozoan *G. lamblia*. Infection is transmitted through ingestion of infectious cysts. *G. lamblia* is one of the most common, universal pathogenic intestinal protozoan parasites of humans[11]. *Giardia* species are endemic in most areas of the world that have poor sanitation. In developing countries, the disease is an important cause of morbidity, and water-borne and food-borne outbreaks are common. Because ingestion of as few as 10 *Giardia* cysts may be sufficient to cause infection, giardiasis is common in daycare center attendees and institutionalized patients in developed countries. *G lamblia* is a particularly significant pathogen for people with malnutrition, immune deficiencies, or cystic fibrosis[1]. The necessity to search new, safe, and effective agents for the treatment of giardiasis is imperative due to the side effects and resistance of the reference drugs[12-14]. Plants used for the treatment of gastrointestinal disorders, such as diarrhea and dysentery, raise the possibility of new alternative therapies[14]. Several experimental studies have investigated the antigiardial effects of medical plants[15]. Anti-giardia activity of phenolic-rich essential oils gained from some aromatic plants have been reported[16]. The tested essential oils inhibited the growth of *G. lamblia*. *Thymbra (T.) capitata* essential oil was the most active. The tested essential oils inhibited parasite adherence since the first hour of incubation and were able to kill almost 50% of the parasites population[16]. Also, anti-giardial activity of *Citrullus (C.) lanatus* fruit has been reported[17]. All crude extracts and isolated compounds were active against *G. lamblia*[17]. Costa Brandelli et al[18], reported the antiprotozoal activity of 27 crude methanolic extracts derived from 26 plants used in Mexican traditional medicine for treatment of diarrhea and dysentery. Erenow many works had been down for antigiardial activity of some plants. The most effective natural product has been garlic[19,20]. In addition various study revealed the antiprotozoal, antibacterial, insect repellent, anti-hemorrhoid activities of *S. ebulus* extract[7-9].

Conclusions

In this study anti-giardial activity of methanolic extract of *S. ebulus* on cyst of *G. lamblia*, was evaluated. Our experiments improved a strong anti-giardial activity of *S. ebulus*. These findings should assist in initiating therapy for such patients to reduce the morbidity and mortality caused by this pathogen.

Competing Interest

None to declare.

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