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Efficacy of *Capparis spinosa* Linn Leaf and Fruit Extracts on Giardia Lambia Cysts *In vitro*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AM, JA, RN, AM and NA designed the study. Author AK performed the statistical analysis. Author JF, NM and PK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Giardia lamblia (G. lamblia)* is one of the most common intestinal parasites worldwide. There are some side effects and the reports of parasite resistance to metronidazole as the first line treatment of giardiasis. Therefore, it is essential to discover an effect and safe drug to treat giardiasis.
Methodology: In this study, the anti-parasitic activity of hydroalcoholic extracts of *Capparis spinosa Linn (C. spinosa)* leaves and fruits with *different* concentrations (4 to 0.125 mg/ml) was assessed against human isolates of the *G. lamblia* cysts and incubated at 37℃. After staining Giardia cysts with 0.1% eosin, the lethal percentage and 50% lethal concentration (LC_{50}) of fruit

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and leave *C. spinosa L* extracts and metronidazole on *G. lamblia* cysts were calculated after 3, 6, 24 and 48 hours.

Results: Anti-giardia activity of fruit and leaf extracts of *C. spinosa* was different between concentrations and time points ($p<0.005$). The lethal effect of both *C. spinosa L* extracts and metronidazole increased significantly in a concentration - and time–dependent response ($P<0.0001$). The highest lethal percentage of *G. lamblia* cysts was observed a concentration of 4 mg / ml of fruit (100%) and leaf (44%) extracts of *C. spinosa L* after 48 hours, respectively. LC_{50} values were 0.38 ± 0.02mg/ml for fruit extract, 2.32 ± 0.1 mg/ml for leaf extract and 0.53 ± 0.03 µg/ml for metronidazole after 48hours.

Conclusion: *C. spinosa* can be effective in eliminating *Giardia* cyst of contaminated environments and water.

Keywords: Giardia lamblia; herbal extract; Capparis spinosa L; medicinal plants; anti-parasitic activity; pathogenic.

1. INTRODUCTION

Giardia lamblia is one of the most common pathogenic protozoa causing giardiasis. Its annual prevalence was estimated about 280 million people worldwide. Giardia cysts were transmitted through the consumption of contaminated water and food. In human, the parasite can cause asymptomatic infections to severe diarrhea or chronic diarrhea syndrome, weight loss, bloating, anorexia, steatorrhea, abdominal pain and malabsorption [1,2]. The drugs such as metronidazole, furazolidone, tinidazole and quinacrine are currently prescribed to treat giardiasis. Although these medications are often helpful, they may have side effects such as bad taste in the mouth, gastrointestinal distress, nausea, headache, leukopenia, neurotoxicity, restlessness, seizure and dizziness, and may interfere with other treatments. On the other hand, the carcinogenic and mutagenic effects of some of these drugs have been demonstrated in laboratory animals [3]. Also, the increased resistance of the parasite to current drugs led to the efforts to find more effective and safe drugs for the treatment of this disease. Plants were the main sources of bioactive products for centuries, providing in the meanwhile fruitful inspiration for the discovery of conventional drugs] [4]. Numerous studies have been conducted around the world on the administration of medicinal herbs to treat giardiasis. Some studies have reported lethal effects of *Zingiber officinal* and *Curcuma longa*, *Mentha x piperita* and *Cannabis sativa*plants against *G. lamblia* cyst or trophozoite forms [5-7]. The caper bush, *Capparis spinosa*, belongs to the dicotyledon and apopetalous plants of the family Capparaceae. In traditional medicine, this herb has been used as a medicine for the treatment of gout, rheumatism and liver disease [8]. *The most important chemical compounds of C. spinosa* that have pharmacological properties are quercetin (flavonoid compounds), rutin, kaempferol, pectin, glycoside, alkaloid, compestrol, beta-sitosterol and stigmasterol. Rutin and quercetin are found in large amounts in the plant fruits and buds [4,9,10]. Some studies have confirmed the antifungal and antibacterial effects of this plant; the growth of two fungal species of *Microsporum canis* and *Trichophyton violaceum* has been completely inhibited by the aqueous extract of *C. spinosa* [11]. Other studies have showed limited activity of gram-positive and gram-negative bacteria (*Helicobacter pylori*, *Escherichia coli*, *Mycobacterium tuberculosis* and *Bacillus cereus)* by *C. spinosa* [12,13]. In addition, total polyphenols of *C. spinosa* alone or in together with praziquantel exhibited schistosomicidal and hepatoprotective effects in mice infected with *Schistosoma mansoni* [14]. Therefore, the present study was designed to evaluate the effect of hydroalcoholic extracts of *C. spinosa* leaves and fruits on *G. lamblia* cysts in vitro.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of *G. lamblia* Cysts

Fresh stool specimens were collected from medical diagnostic laboratories in Ilam city. The presence of *G. lamblia* cysts was confirmed by direct smear preparation and microscopic examination of the specimens. To concentrate the number of the cysts in fecal specimen, the flotation was performed using 0.85 M sucrose solution. Briefly, 10 grams of stool samples were mixed with 10 times the volume of water and passed double-layered gauze. The coarse particles were discarded and the filtered

suspension was transferred into 15ml tubes and centrifuged at 1500 rpm for 5 min. The supernatant was discarded, and the resulting precipitate was washed again in the same way. Finally, the precipitate was dissolved in 1 ml of distilled water and added by 3 ml of 0.85 M sucrose solution. After centrifugation at 2200rpm for 10 min, it was formed three layers, the cysts were collected from the first and second layers by a Pasteur pipette. Then, the cysts were washed twice and were kept in 1 ml sterile normal saline solution containing 100IU/ml penicillin and 100µg/ml streptomycin and 1.25 µg/ml of Amphotericin B for 4 hours at 4°C. The cysts were counted using a Neubauer chamber. The viability rate of cysts was determined using staining of cysts helping 0.1% eosin (Image. 1) [15,16].

2.2 Preparation of *C. spinosa* Extraction

In this experimental study, the leaves and fruits of *C. spinosa* were collected from Kabir Kuh ranges of Darreh Shahr city located in Ilam province, Iran, and were cleaned and dried under shad at ambient temperature and away from direct sunlight. The dried leaves and fruits were powdered and passed through a sieve No. 10. To prepare the hydroalcoholic extract, 150 g of each powder were soaked in equal volumes of absolute ethanol and water and incubated at room temperature for 48 hours. Then, the extracts were concentrated using a rotary evaporator and dried in an oven at 40°C. The extracts were stored at -20°C until use.

2.3 Measuring the Lethal Effect of Extracts on *G. lamblia* Cysts

The viability rate and counting live *G. lamblia cysts* was determined using 0.1% eosin and a Neubauer chamber [17]. Microscopically, the dead cysts were red, while the live cysts did not absorb the stain and appeared completely colorless (Image 1).

2.4 Testing the Effect of Extracts on *G. lamblia* Cyst

In the present study, it was used the different concentrations (0.125 to 4mg/ml) of hydroalcoholic extracts of *C. spinosa* leaves and fruits. To evaluate lethal activity of *C. spinosa* extracts on *G. lamblia* cysts, 500 µl of each leaves and fruits extracts were poured into separate tubes and was added 500µl of Giardia cyst suspension containing 10,000 cysts and incubated at 37℃ for. 3, 6, 24 and 48 hours. All experiments were repeated three times. Saline buffer containing *G. lamblia* cysts was used as negative control and standard drug of metronidazole was used as a positive control. IC50 values were measured using GraphPad PRISM software version 5.04 (San Diego, USA). Mean lethal activity of the groups according various concentrations and times was compared using two-way repeated measures ANOVA and Tukey's test for Post-Hoc analysis. P values ≤ 0.05 were considered significant.

3. RESULTS AND DISCUSSION

The lethal activity of hydroalcoholic extracts of *C. spinosa* leaves and fruits were different in various times and concentrations ($p<0.005$) (Figs. 1, 2). The highest lethal percentage (100%) of *G. lamblia* was seen after treating with *C. spinosa* fruit extract (100%) and then followed by leaf extract (44.6%) at 4 mg concentration after 48 hours and then, maximum lethal effect (83.8%) exhibited at 2 mg/ml concentration of *C. spinosa* fruit extract after 48 hours and the lowest killing percentage (17.5%) was related to 1.25mg/ml concentration of *C. spinosa* leaf and fruit extracts. Also, standard drug of metronidazole induced a lethal efficacy on *G. lamblia* cysts in a dose – and time dependent response ($P < 0.00$) (Fig. 3). LC_{50} values were 0.38 ± 0.02mg/ml for fruit extract, 2.32± 0.1 mg/ml for leaf extract and 0.53±0.03 µg/ml for metronidazole after 48hours.

The mean lethal effect of hydroalcoholic extracts of *C. spinosa* leaves and fruits on *G. lamblia* cysts increased over time so that there was a statistically significant difference between lethal effect of each concentration of leaf or fruit extract of *C. spinosa* after 24 hours ($P = 0.012$) and 48 hours ($P<0.001$). The highest levels of lethality (100%) of 4 mg/ml concentration of *C. spinosa* fruit extract on Giardia cyst was observed after 48h and the lowest lethal activity (20.50%) of the same concentration was seen after three hours. Then, the highest killing percentage of *C. spinosa* fruit extract was related to 2 mg/ml concentration after 48 hours (83%) and 24 hours (50%) respectively. Mean lethal activity in equal concentrations of three treated groups was significantly different after 12, 24 and 48h ($P < 0.001$) while there was no significant difference between all groups after 3 and 6 hours ($P> 0.05$). Therefore, the mean lethal effect of hydroalcoholic extract of *C. spinosa* fruits and leaves as well as metronidazole increased over time.

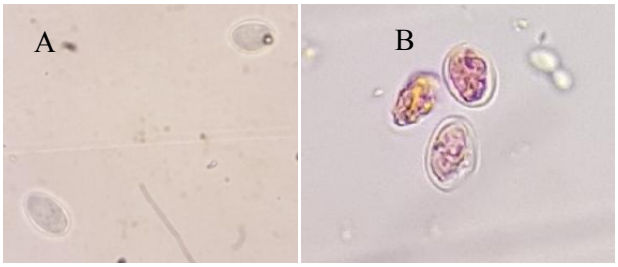

Image 1. It shows *G. lamblia* cysts after staining with 0.1% eosin. Panels A and B display live cysts (colorless) and dead cysts (pink) respectively

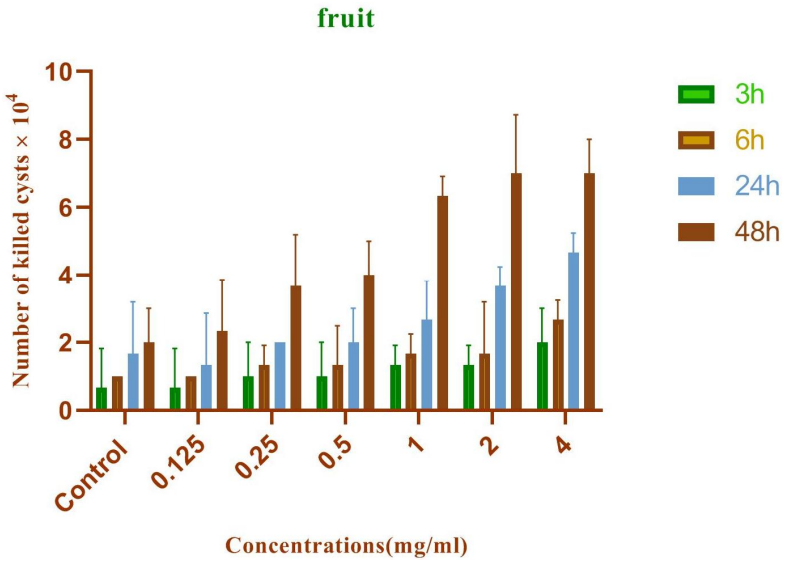

Fig. 1. Anti-giardia activity of different concentrations of *Capparis spinosa* fruit extract on Giardia cysts after 3, 6, 24 and 48 hours

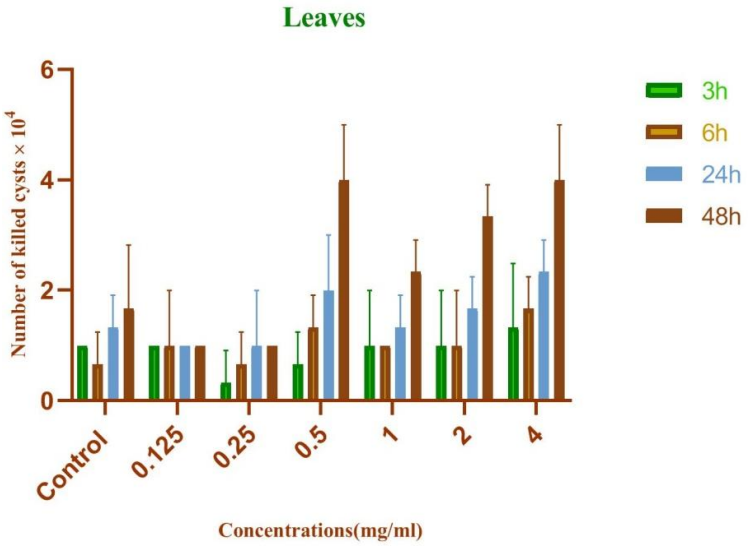

Fig. 2. Anti-giardia activity of different concentrations of Capparis spinosa leaf extract on Giardia cysts after 3, 6, 24 and 48 hours

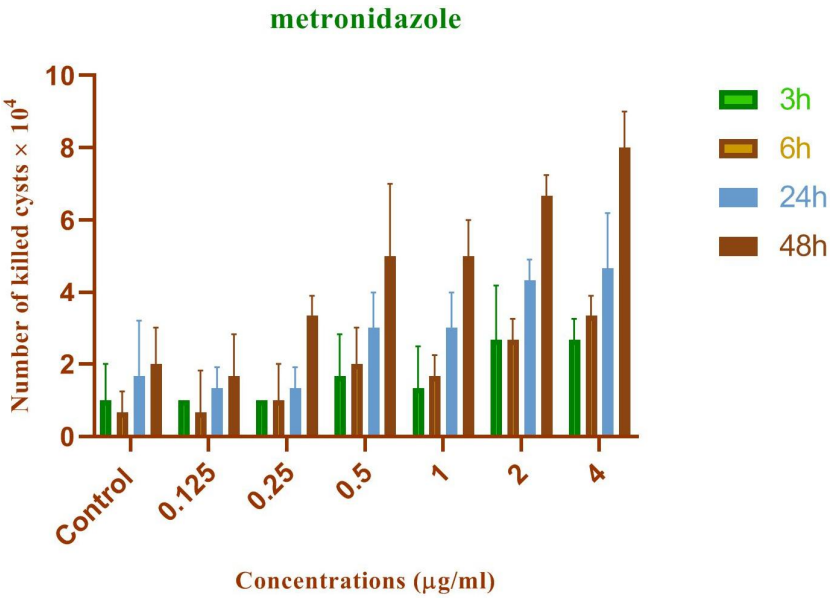

Fig. 3. Anti-giardia activity of different concentrations of metronidazole on Giardia cysts after 3, 6, 24 and 48 hours

Many studies have reported effect of some medicinal herbs such as garlic, rosemary, lavender, trachyspermum against *G. lamblia* cysts and trophozoites [18,19].

In traditional medicine, oral or poultice *C. spinosa* fruits and leaves are used in the treatment of some diseases (e.g. diabetes, fungal infection, gastrointestinal infection and skin diseases) (references). It is sometimes used to treat skin dryness and inflammation and to increase subcutaneous blood flow as a poultice [9]. The snailcide [20], antibacterial [12], anti-Leishmania [21], and anti-helminthic effects of *C. spinosa* extract were observed [22]. Shemshadi et al. (2015) investigated the effect of hydroalcoholic extract of *C. spinosa* root against Leishmania *major* promastigotes and amastigotes and 0.9 mg/ml concentration was able to kill 97.8% of promastigotes after 72hours. In addition, the mean wound size in the mice receiving hydroalcoholic extract of *C. spinosa* root had a good effect on preventing wound development [22].

In another study, the ethanolic extract of *C. spinosa* limited significantly growth of *Salmonella typhimurium* bacteria. A protein isolated from *C. spinosa* seeds has shown a potent anti-proliferative activity against tumour cells and some antifungal activities (29). The hydroalcoholic extract of *Lavender* on *G. lamblia* infection induced in mice led to 77.7%, 84.3% and 95.1% reduction in the cysts in animals received the concentrations of 100, 200 and 400 mg/ml for 10 days, respectively. Therefore, high concentrations of extract were more effective in eliminating the cysts and treating giardiasis [18].

In our study, higher concentrations of the extract increased the lethal percentage of *G. lamblia* cysts. In an *in vivo* study, Bahri Najafi et al [23] investigated the effects of some medicinal plants on *Giardia* cysts in comparison with metronidazole. According to the results, the mean lethality of the essential oils in the studied plants was higher at 60 minutes than at 30 minutes. So, the effect of time on the lethality of extract of plants was shown to be consistent with the effect of time in our study. It is noteworthy in this study and similar studies that the longer the exposure of the parasite to the extract, the lower the concentration of the extract inhibits the growth of the parasite, and the cysts are destroyed by proximity to the plant concentration. Hydroalcoholic extract of *C. spinosa* fruit exhibited agreater lethality on *G.lamblia* cysts than its leaves that may be due to their different anti-giardia compounds. Moreover, it was found that the lethal effect of these extracts had a direct relationship with increasing concentration and time. The greatest effect of both the extracts and especially the hydroalcoholic extract of *C. spinosa* fruit was reported in the 4mg/ml

concentration and 48hours. Some of the reasons for the differences between the results and effective doses of different plant extracts on *Giardia* parasite include differences in genera and family of different plants, different extraction methods, type of extractor (aqueous, chloroform, hydroalcoholic) as well as study conditions (in vitro or in vivo). For example, Rahimi Esboei et al. (2012) investigated the in vitro effect of different concentrations (1, 10, 50 and 100 mg/ml) of *Artemisia annua* hydroalcoholic extract on *Giardia* cyst. the concentrations of 50 and 100 mg/ml had the highest lethal effect after 3 and 24 hours and 100 mg/ml concentration for 24 hours was used as an effective compound to eliminate *Giardia* cysts [16]. In the present study, 4 mg/ml concentration of *C. spinosa* fruit demonstrated the highest lethal activity compared to other concentrations as well as a greater lethal effect was observed at 4 mg/ml concentration of the exract after 48hours than 24 hours. Therefore, our results were in agreement with the study of Rahimi et al. so that increasing concentration and time have a significant effect on eliminating the *Giardia* cysts.

4. CONCLUSION

Based on the results of this study, *C. spinosa* can be effective in eliminating Giardia cyst at concentrations of 2 and 4 mg/ml after 24 and 48 hours. It is suggested that *C. spinosa* extracts may be considered as candidates to eliminate environmental cysts, which would be extremely important to treat potential sources of contamination. *Giardia* cysts are known to be resistant to chlorine and common disinfectants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been examined and approved by the medical research ethics committee in Iran (Approval ID: IR.MEDILAM.REC.1397.047).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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