

## **Anti-giardial activity *Capparis spinosa* Linn leaf and fruit extracts on *Giardia lamblia* cysts**

### **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, anti-parasitic activity of hydroalcoholic extracts of *Capparis spinosa* Linn (*C. spinosa*) leaves and fruits with different concentrations (4- to 0.125 mg/ml) were assessed against human isolates of the *G. lamblia* cysts and incubated at 37°C. After staining Giardia cysts with 0.1% eosin, the lethal percentage and 50% lethal concentration (LC50) of fruit and leave *C. spinosa* L extracts and metronidazole on *G. lamblia* cysts was calculated after 3, 6, 24 and 48 hours.

**Results:** the lethal activity of fruit and leaf extracts of *C. spinosa* was different between all 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. LC50 values were  $0.38 \pm 0.02$  mg/ml for fruit extract,  $2.32 \pm 0.1$  mg/ml for leaf extract and  $0.53 \pm 0.03$  µg/ml for metronidazole after 48 hours.

**Conclusion:** *C. spinosa* can be effective in eliminating *Giardia* cyst for therapeutic application.

*Keywords:* *Giardia lamblia*, herbal extract, *Capparis spinosa* L, medicinal plants

### **1. INTRODUCTION**

*Giardia lamblia* is one of the most common pathogenic protozoa causing giardiasis. and its annual prevalence was estimated about 280 million people worldwide [1]. Giardia cysts were transmitted through the consumption of contaminated water and food [2]. In human, the parasite can cause a variety of symptoms, from 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 for the treatment of giardiasis. Some studies were reported lethal effect of *Zingiber officinale* and *Curcuma longa*, *Mentha x piperita* and *Cannabis sativaplants* against *G. lamblia* cyst or trophozoite forms was [5-7]. The caper bush, *Capparis spinosa*, belongs to the dicotyledon and apometalous 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, rutin, (phenolic and flavonoid compounds) 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 *Microsporium canis* and *Trichophyton violaceum* has been completely inhibited by the aqueous extract of *C. spinosa* [11]. other studies showed limited activity of gram-positive and gram-negative bacteria (*Helicobacter pylori*, *Escherichia coli*, *Bacillus cereus* and *Mycobacterium tuberculosis*) by *C. spinosa* [12, 13]. In addition, total polyphenols extracted 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 Giardia cysts in vitro.

## 2. MATERIAL AND METHODS

### 1.

#### 1.1. 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 a 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[15, 16].

#### 1.2. 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 shade at ambient temperature 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.

#### 1.3. 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).

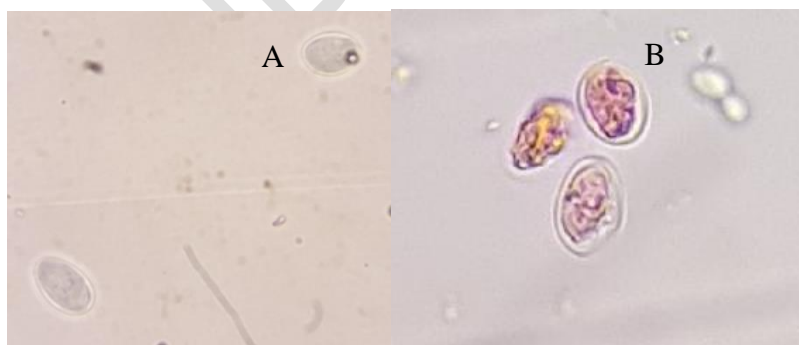


Image 1. it shows *G. lamblia* cysts after staining with 0.1% eosin. Panels A and B display live cysts (colorless) and dead cysts (pink) respectively.

#### 1.4. 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°C 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 a 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 ( $p < 0.005$ ). 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 mortality percentage (17.5%) was related to 1.25mg/ml concentration of *C. spinosa* leaf and fruit extracts (Table 1) .

The mean lethal effect of hydroalcoholic extracts of *C. spinosa* leaves and fruits on *G. lamblia* cysts increased over time. There was a statistically significant difference between lethal effect of each concentration of hydroalcoholic leaf or fruit extract of *C. spinosa* at 24 hours ( $P = 0.012$ ) and 48 hours ( $P < 0.001$ ). Also, standard drug of Amphotericin B induced a lethal efficacy on *G. lamblia* cysts in a dose – and time dependent response ( $P < 0.00$ ). LC50 values were  $0.38 \pm 0.02$ mg/ml for fruit extract,  $2.32 \pm 0.1$  mg/ml for leaf extract and  $0.53 \pm 0.03$  µg/ml for metronidazole after 48hours.

The highest levels of lethality (100%) of 4 mg/ml concentration of *C. spinosa* fruit extract on Giardia cyst was observed - after 48hand the lowest lethal activity (20.50%) of the same concentration was seen after three hours.

83% at 2 mg/ml concentration after 48 and 24 hours respectively. At all concentrations, the mean lethal effect hydroalcoholic extract of *C. spinosa* fruits and leaves as well as Amphotericin B increased over time ( $P < 0.0001$ ).

Comparison of difference of mean lethal activity of treated groups (metronidazole and fruit and leaf extracts ) exhibited a significant difference between all groups ( $P < 0.001$ ),

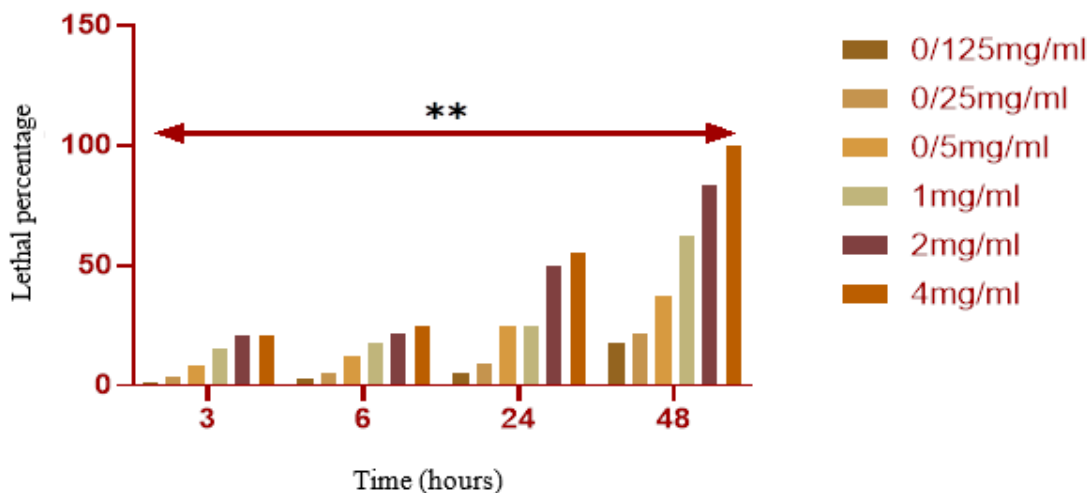


Figure 1. Lethal activity of different concentrations of *Capparis spinosa* fruit extract on Giardia cysts after 3,6, 24 and 48 hours. The asterisks (\*\*) show P value = 0.02.

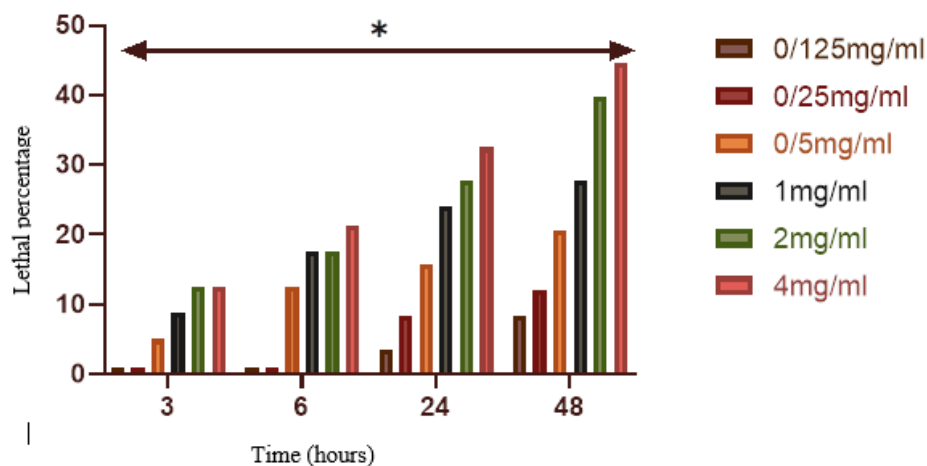


Figure 2. Lethal activity of different concentrations of *Capparis spinosa* leaf extract on *Giardia* cysts after 3, 6, 24 and 48 hours. The asterisk (\*) shows P value  $\leq 0.05$ . times.

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 a *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 a greater 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 extract 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 alone or in combination with other plant parts is applied for therapeutic goals against giardiasis. Also, subsequent research is recommended on *G. lamblia* trophozoite or other intestinal protozoa in laboratory animals. In addition, it is needed to investigate its toxicity on mammalian cells and to obtain appropriate concentrations for clinical administration.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.”

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