



Effect of *Valeriana officinalis* hydroalcoholic extract on *Giardia lamblia* cysts

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ABSTRACT

Introduction: *Giardia lamblia* is an important and prevalent parasitic cause of diarrhea and gastroenteritis. Regarding the significance of giardiasis treatment particularly by medicinal plants and *G. lamblia* resistance to chemical drugs, this study was conducted to study in vitro effect of *Valeriana officinalis* hydroalcoholic extract on *G. lamblia* cysts.

Methods: In this experimental, laboratory study the hydroalcoholic extract of *V. officinalis* at concentrations of 12.5, 25, 50, 100, and 200 mg/mL was applied on *G. lamblia* cysts. The findings were compared with controls.

Results: Mean results of the effect of *V. officinalis* hydroalcoholic extract at different concentrations on *G. lamblia* cysts after 1, 6 and 24 hours demonstrated that the extract at all concentrations caused a notable decrease in alive cysts, with more intensive effect at 100 and 200 mg/mL concentrations and 100% fatality after 1 hour. As the extract concentration decreased, the speed of *G. lamblia* cysts inhibition declined.

Conclusion: *V. officinalis* hydroalcoholic extract might be recommended as an effective compound for removing *G. lamblia* protozoan cysts, although further studies are needed to show this effect on human.

Implication for health policy/practice/research/medical education:

Valeriana officinalis might be used as an effective compound for removing *Giardia lamblia* protozoan cysts. Separation and recognition of the effective compound(s) is recommended.

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Introduction

Giardia lamblia is an important and prevalent parasitic cause of diarrhea and gastroenteritis and giardiasis is a significant health problem worldwide. *G. lamblia* is known as the most common human intestinal protozoa especially in temperate regions, and exists in animals including birds, amphibians, rodents, some mammals, and human (1). In Iran, *G. lamblia* prevalence has been reported 10.9%, the highest contribution in intestinal parasitic infections nationwide (2).

This protozoan parasite is a main factor for epidemic diarrhea due to contaminated food and water. The prevalence of *G. lamblia* infection differs by age, geographic area, health conditions, and water situation. *G. lamblia* exists in 2 forms, trophozoite and cyst, and eating 100 or more cysts results in infection. Parasitic cyst is excreted through

feces and causes infection. Cyst takes some days to some months to be excreted through feces. The children of under 10 years comprise the population at the highest risk of infection. However, the patients of any ages could present with mild diarrhea, flatulence, loss of appetite, crampy abdominal pain, epigastric tenderness, fatty stools, and malabsorption (3,4).

A variety of drugs is currently being taken for treatment of giardiasis, including metronidazole, albendazole, quinacrine, furazolidone, all of which lead to adverse effects particularly in children and women (5,6). Incidence of resistance to these drugs has been already demonstrated for *G. lamblia* and hence the research has been seeking to find compounds with fewer or no side effects (7,8).

In this regard, human beings have long used medicinal plants to treat diseases, which has been developed and

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evolved throughout centuries. By World Health Organization (WHO) report, over 80% of global population, particularly in developing countries and remote areas where health and safety facilities are lacking use medicinal plants to meet their basic healthcare needs (9). This wide welcome could be due to a number of reasons including fewer side effects, less cost of medicinal herbs, patients' and higher tendency thanks to traditional medicine recommendations, and use of these drugs by past generations as well as further agreement with normal physiological functions of the human body (10).

Of medicinal plants, cat grass (*Valeriana officinalis*) is a herbaceous perennial plant of the family Valerianaceae. *V. officinalis* root and rhizome contain valuable active essential substances and iridoid compounds called valepotriates. Valepotriates are a group of volatile compounds with sedative property. This plant has a variety of species containing other compounds such as alkaloids, flavonoids, tannins, gums, resins, and mucilage. In medicine this plant is taken as sedatives and analgesics as well as for the treatment of muscle cramps, irritable bowel syndrome, bloating, and headaches caused by stress and anxiety. Also it works for removing parasites and helminths, fixing constant hiccups, and treating stomachache of neural origin and liver diseases (11,12).

No study has been yet conducted to investigate *V. officinalis* effect on *G. lamblia* specifically. Therefore the present study was conducted to study the effect of *V. officinalis* on *G. lamblia* cysts in comparison to metronidazole and furazolidone.

Materials and Methods

The present experimental, laboratory study was conducted in 2014-2015 in laboratories of Department of Parasitology and Medical Plants Research Center of Shahrekord University of Medical Sciences.

Preparation of hydroalcoholic *V. officinalis* extract by maceration

Firstly, unusable parts of the plant were set aside, cleaned, shadow-dried in special trays at room temperature, pulverized by an electric mill and then passed through a sieve no. 10. The obtained powder was mixed with ethanol 70% at 1/3 ratio and extracted per maceration for 72 hours. Then vacuum distillation process was run to remove all alcohol in the solution using rotary vacuum evaporator and the obtained extract was left to be used for later tests (13). Notably, dimethyl sulfoxide was used for further dissolution of *V. officinalis* extract.

Preparation of *G. lamblia* cysts

G. lamblia cysts were isolated by sucrose density gradient. Firstly 10-15 mL of distilled water was added to fresh stool samples containing the appropriate number of cysts in a disposable glass for preparation of a suspension and then the suspension was refined by a layer of wet gauze fabric. The tubes were centrifuged for 5 minutes and then the upper layers were set aside. This procedure was run in trip-

licate. Then 3 mL 0.85 sucrose solution and afterwards 3 mL of the prepared stool suspension were introduced into the tubes. Next, the tubes were centrifuged in 4°C refrigerator for 10 minutes. As a result, four layers per tube were formed in a way that *G. lamblia* cysts were compressed as a ring-shaped cloud in the middle layer.

The contents of cloud layer were closely taken out by a Pasteur pipette and transferred to another tube. Then, 3-5 mL distilled water was added to the samples and the tubes were centrifuged at 600 g for 5 minutes to isolate sucrose solution. Afterwards, the upper liquid was set aside and the obtained sedimentation was centrifuged at 600 g for 5 minutes. This procedure was run in duplicate. At the end the upper layer was discarded, the sedimentation containing the cysts was mixed with 1 mL distilled water, and the suspension was transferred to the microtubes and frozen at -20°C for later tests after they were covered with a parafilm (14).

Testing

Firstly *G. lamblia* cysts were tested with *V. officinalis* hydroalcoholic extract at a wide range of concentrations in a pilot study for the best fatality effect and 12.5, 25, 500, 100, and 200 mg/mL concentrations were adopted.

For tests, eight microtubes were placed into a special rack and 100 µL of the extract under study was poured into the first tube and 50 µL normal saline was introduced into the second to fifth tubes by a 100 µL sampler. Hence the extract concentration in the first tube was 200 mg/mL. Then 50 µL of the extract in the first tube was introduced into the second tube and mixed well with normal saline. The procedure was similarly run till the fifth tube and therefore the extract concentration in the first to fifth tube was 200, 100, 50, 25, and 12.5 mg/mL, respectively

In the next step, 50 µL of *G. lamblia* cysts suspension containing 5×10^4 cysts, counted by Theobar lam, was introduced into all the eight microtubes and then 100 µL dimethyl sulfoxide, instead of the extract, was poured into the sixth microtube as negative control and 100 µL metronidazole and furazolidone poured into the seventh and eighth microtube, respectively, as positive controls. Subsequently, the microtubes were incubated at 37°C for 1, 6, and 24 hours for provision of better temperature and time conditions for the extract's effect on *G. lamblia* cysts. Afterwards, eosin (0.1%) staining was used to investigate the fatality effect of the extract at different concentrations on *G. lamblia* cysts (15). To enhance the precision of the findings, any tests of the extract effect on the cysts were done in triplicate.

To select the appropriate concentration of metronidazole and furazolidone as positive control, these two drugs at different solution concentrations were exposed to *G. lamblia* cysts in different microtubes in a pilot study and then incubated under the conditions already described and the effectiveness of this solution on *G. lamblia* was tested in triplicate. Metronidazole at a concentration of 5 mg/mL with 40% fatality effect and furazolidone at a concentration of 25.6 mg/mL with 30% fatality effect had the great-

est effect on cysts after 24 hours and hence they were selected as the desired concentrations for comparison with *V. officinalis* extract at different concentrations in each test.

For data analysis, descriptive statistics such as frequency, percentage, mean and standard deviation, and analytical statistics such as Repeated Measures analysis of variance (ANOVA) and Probit analysis in SPSS 20 were used. Level of significance was considered 0.05.

Results

Mean results of three tests of the effect of *V. officinalis* hydroalcoholic extract at different concentrations on *G. lamblia* cysts after 1, 6 and 24 hours demonstrated that the extract at all concentrations caused a remarkable decrease in alive cysts, with further effect at 100 and 200 mg/mL concentrations and a rapid, 100% fatality effect. As the extract concentration decreased, the speed of *G. lamblia* cysts inhibition declined so that the fatality effect was derived 84.00% and 69.3% at 25 and 12.5 mg/mL concentrations, respectively. Furthermore, with increasing time of exposure for each concentration, the fatality percentage in *G. lamblia* cysts increased at concentrations of 50, 25 and 12.5 mg/mL, so that mean fatality effect after 1 hour was derived 87.3%, 69.3%, and 54.7%, respectively, while it increased to 96.7%, 84.0%, and 69.3%, respectively, after 24 hours.

On the other hand, comparison of the effect of *V. officinalis* hydroalcoholic extract at 5 concentrations on *G. lamblia* cysts at different intervals with that of metronidazole and furazolidone as positive controls indicated that mean fatality effect of metronidazole and furazolidone after 24 hours was 47.33% and 30.00%, respectively. Therefore, *V. officinalis* hydroalcoholic extract, even at the smallest concentration, exerted a significantly higher fatality effect than these two drugs with pharmacologic concentration of 5 and 6.25 mg/mL respectively ($P < 0.001$). This represents the high effect of *V. officinalis* extract on *G. lamblia* cysts (Table 1).

Figure 1 illustrates the effect trend of *V. officinalis* hydroalcoholic extract at different concentrations on *G. lamblia* cysts in comparison to metronidazole and furazolidone in triplicate.

Discussion

This study was conducted to study in vitro effect of *V. officinalis* hydroalcoholic extract on *G. lamblia* cysts. The anti parasitic effects of *V. officinalis* have not been yet investigated satisfactorily; however, its antifungal and antioxidant properties have been examined in some studies such as Saatchi et al study. Saatchi et al study demonstrated that alcoholic extract of *V. officinalis* and *Melissa officinalis* exhibited high antimicrobial and antioxidant activities and inhibited the growth of 10 fungi (16). In the present study *V. officinalis* hydroalcoholic extract had pronounced anti parasitic properties on *G. lamblia* protozoa.

Many studies have investigated medicinal plants effects on *G. lamblia* cysts. Rahimi-Esboei et al (17) studied in

Table 1. Mean fatality percentage of *Valeriana officinalis* hydroalcoholic extract at different concentrations on *Giardia lamblia* cysts in triplicate

Extract concentration (mg/mL)	Mean fatality effect at different intervals		
	1 h	6 h	24 h
200	100.0%	100.0%	100.0%
100	100.0%	100.0%	100.0%
50	87.3%	93.3%	96.7%
25	69.3%	75.3%	84.0%
12.5	54.7%	61.3%	69.3%
Positive control (metronidazole)	23.3%	33.3%	47.3%
Positive control (furazolidone)	22.0%	22.0%	30.0%
Negative control	0.0%	0.0%	0.0%

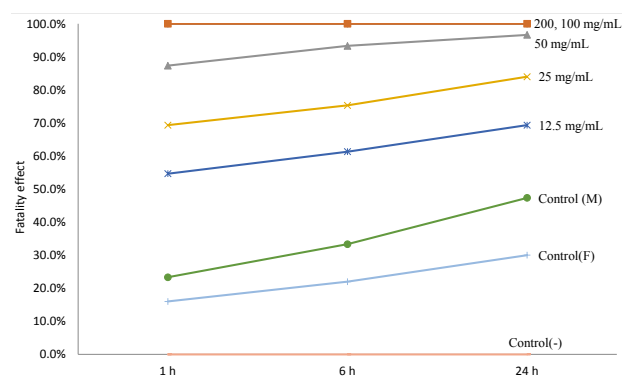


Figure 1. Effect trend of *Valeriana officinalis* hydroalcoholic extract at different concentrations on *Giardia lamblia* cysts at intervals under study (in triplicate).

vitro effect of hydroalcoholic leek extract on *G. lamblia* cysts and found that the extract at 100 and 50 mg/mL concentrations after 24 hours had the highest (99% and 96%) fatality effect (respectively) on *G. lamblia* cysts. Similarly, *V. officinalis* hydroalcoholic extract at 100 and 50 mg/mL concentrations, in the present study, had the highest (100% and 96.67%) fatality effect (after 1 hour and 24 hours, respectively) on *G. lamblia* cysts.

In Shahabi et al study (18), copticum extract at 200 mg/mL concentration caused the death of all *G. lamblia* cysts after 1 hour, consistent with our findings of *V. officinalis* extract at 100 mg/mL in the present study.

In Safarnejad et al study (19), savory methanol extract at 200 mg/mL concentration exerted an 84.3% fatality effect on *G. lamblia* cysts after 1 hour, smaller than the fatality effect of *V. officinalis* in the present study.

Conclusion

Overall the findings of the present study indicated that *V. officinalis* hydroalcoholic extract had a high fatality effect on *G. lamblia* cysts and therefore could be recommended as an effective compound for removing *G. lamblia* protozoan cysts. However further research is needed for clinical application of *V. officinalis* extract, including study of effective chemicals in *V. officinalis* extract and controlled trial of laboratory animals and, if the property of interest

was demonstrated, humans.

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Authors' contributions

All contributed to the conception of the work, approval of the final version of the manuscript, and agreed for all aspects of the work. BKh conducted the study, contributed in preparation of the first draft and revising the final draft. FD contributed to statistical analysis.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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