J Parasit Dis (Jan-Mar 2016) 40(1):88–92
DOI 10.1007/s12639-014-0453-3

ORIGINAL ARTICLE

Survey on efficacy of chloroformic extract of *Artemisia annua* against *Giardia lamblia* trophozoite and cyst in vitro

Shirzad Golami · Bahman Rahimi-Esboei · Parisa Mousavi · Zahra Marhaba · Mohammad Reza Youssefi · Mohammad Taghi Rahimi

Received: 19 July 2013 / Accepted: 20 February 2014 / Published online: 12 March 2014

Abstract Giardiasis is a parasitic cosmopolitan disease that the rate of infection in developing countries is considerable. This infection directly is associated with poor hygienic conditions, poor water quality control, and overcrowding. Reinfection and drug resistance are two major problems in endemic areas. Recently, researchers are concentrating on herbal drugs as a proper solution. Therefore, the objective of the present study was to survey on efficacy of chloroformic extract of *Artemisia annua* against *Giardia lamblia* trophozoite and cyst in vitro. *G. lamblia* cysts were prepared from faces of giardiasis patients from different hospitals of Mazandaran Medical University. Four concentrations (1, 10, 50 and 100 mg/ml) of chloroformic extract of *A. annua* were utilized for 1, 5, 30, 60 and 180 min. Viability of *G. lamblia* cysts was confirmed by 0.1 % Eosin staining. Cyst and trophozoite contact (intermix) of *G. lamblia* with extract of *A. annua* with variant concentrations (1, 10, 50 and 100 mg/ml) after 1 and 180 min caused following cyst and trophozoite elimination rates: (67, 69, 71 and 73 %), (65, 67, 67 and 72 %), (94, 96, 97 and 99 %) and (100, 100, 100 and 100 %), respectively. Authors from the current investigation draw a conclusion that chloroformic extract of *A. annua* has the ability to eliminate *G. lamblia* cysts and trophozoites in vitro.

Keywords Plant extract · Parasite · Antiprotozoal activity · Intestinal parasites · *Giardia lamblia* · In vitro

S. Golami · M. T. Rahimi (✉)
Department of Parasitology and Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
e-mail: rahimimt@gmail.com

B. Rahimi-Esboei · P. Mousavi
Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Z. Marhaba
Department of Medical Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

M. R. Youssefi
Departments of Veterinary Parasitology, Islamic Azad University, Babol Branch, Babol, Iran

Introduction

Giardiasis or beaver fever is a parasitic intestinal disease and its causative agent is *Giardia intestinalis*. This flagellate protozoon has a wide range of mammalian hosts besides humans, thus making it very difficult to eradicate (Larry and Janovy 2006; Choubisa et al. 2012). The prevalence rate of giardiasis in developing countries is 20–30 % (Sayyari et al. 2005) and *G. intestinalis* in the United State and Jamaica is the most common intestinal parasite (Kappus 1994; Lindo et al. 1998). A range of clinical syndromes may occur, with gastrointestinal syndromes being the most prevalent such as mal absorption syndrome (diarrhea, abdominal pain, asthenia and weight loss) and extra intestinal symptoms, such as fever, maculopapular rash, pulmonary infiltrates, lymphadenopathy, polyarthritis and urticarial (Larry and Janovy 2006). Failures of remedy have been reported with whole of the commonly used antigiardial drugs and drug resistance to all available drugs, has been proven in the laboratory (Wright et al. 2003).

Within the last decade and so, isolated bioactive compounds from plants were utilized against a wide range of

microorganisms particularly parasites. Among these plants, *Artemisia annua* has an undeniable allotment and a plenty of researches were carried out in order to study on impact of this immensely precious plant (Bone and Morgan 1992). The species *A. annua* also is known as Sweet Wormwood, Sweet Annie or Annual Wormwood. This highly aromatic herbaceous plant belongs to the family Asteraceae and is a common type of wormwood that in temperate Asia is considered native, but naturalized all over the world (Bailey and Bailey 1976; Simon et al. 1984). It was used since A.D. 341 by Chinese herbalists who exploited it for the treatment of malaria fever which is considered as dreadful disease (Hilen and White 1993: Mahdavi et al. 2013).

Ridley and Hudson (1998) described that *artemisinin* ruins the cells of parasitic organisms by either the production of highly reactive oxygen-based free radicals or electrophilic intermediates. It has been proven that the impact of *artemisinin* is equally mediated through disruption of membrane potential by interacting with the electron transport chain in the mitochondrial membrane, as a result of free radical damage and dysfunctional mitochondria (Li et al. 2005). Jorge et al. (2010) stated that the flavonoids present in *A. annua* leaves have been associated to repression of CYP450 enzymes which is responsible for altering the absorption and metabolism of *artemisinin* in body, but also have been connected to a beneficial immunomodulatory activity in subjects afflicted with parasitic and chronic diseases. Consumption of extracts and the essential oil of *A. annua* as an anti-parasitic drug in herbal therapies received particular attention and the following studies were performed and they indicates that this plant is effective and active against trypanosomiasis or "sleeping sickness" (Mishina et al. 2007), schistosomiasis (Utzinger et al. 2001), toxoplasmosis (Oliverira et al. 2009), leishmaniasis (Chawla and Madhubala 2010), cryptosporidiosis (Arab et al. 2006), coccidiosis (Brisibe et al. 2008). Furthermore, Basic compound of artemisinin which is an antimalarial drug was extracted from *A. annua* (Oliverira et al. 2009).

Although *A. annua* is widely distributed in Mazandaran province there is not adequate and documented information of influence on this herb against *G. lamblia* cysts and trophozoites. The present survey was conducted to survey on efficacy of chloroformic extract of *A. annua* against *G. lamblia* trophozoite and cyst in vitro.

Materials and methods

Giardia lamblia cysts were collected from faces of giardiasis patients from different hospitals of Mazandaran Medical University. All specimens were processed immediately after arrival, ordinarily within 48 h after excretion. By the sucrose flotation method a highly purified cyst and trophozoite suspension was achieved. Purified cysts were resuspended in distilled water and stored at 4 °C for a maximum of 3 days prior to use. The method of Bingham et al. (1979) was used for excystations. Initial experiments demonstrated that survival of most of the primary cultures could be favorably affected by the addition of bile and by increasing the serum component to 20 % (Laarman et al. 1986). Therefore the excystation medium used consisted of filter-sterilized TYI-S-33 culture medium, with bovine bile added (Keister 1983) supplemented with 20 % heat inactivated fetal calf serum. Screw capped borosilicate glass culture tubes containing 8 ml of the medium, supplemented with penicillin (500 mg/ml) and streptomycin (500 mg/ml), and was incubated at 37 °C in a slant.

Artemisia annua were dried under shade, and powdered mechanically. To obtain the chloroformic extract, 70 g of dry powder was added to 350 ml of pure chloroform and mixed gradually for 60 min using a magnetic stirrer. The obtained solution was left at room temperature for 24 h. The solution was stirred again and filtered and then the solvent was removed by evaporation in a rotating evaporator. The remaining semisolid material was freeze-dried. The obtained filtrate (5.5 g) was placed into a sterile glass container and stored at 4 °C for further use. In the current research, four concentrations (1, 10, 50 and 100 mg/ml) of the *A. annua* extract were applied for 5, 10, 30, 60 and 180 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 incubated at 37 °C for 5, 10, 30, 60 and 180 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 viability of were determined by counting 500 cysts. Non treated cysts were considered a control group in each experiment. The experiments were performed in triplicate. In the present study eosin stain with the concentration of 0.1 % (1 g of eosin powder in 1,000 ml distilled water) was used to check the viability of the cysts. The cysts with no absorbed dye were considered potentially viable otherwise they were recorded as dead (Fig. 1).

Statistical analysis was performed by means of one-way ANOVA (analysis of variance) considering a level of significance of 95 % ($P < 0.05$), with SPSS software.

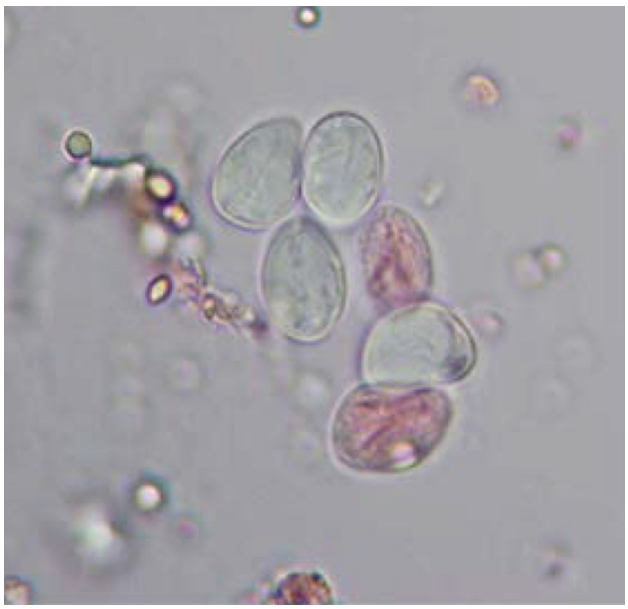

Fig. 1 Viable cysts with no absorbed dye and dead cysts with absorbed dye

Results

Table 1 illustrates recovered results of using the following different concentrations and time exposure of chloroformic extract of *A. annua* against *G. lamblia* cyst and trophozoite respectively: (1, 10, 50 and 100 mg/ml) (5, 10, 30, 60 and 180 min). Intermix of cysts with the extract of *A. annua* with dilution of 1, 10, 50 and 100 mg/ml after 180 min caused the following cyst and trophozoite demolishing rates: (94, 96, 97 and 99 %) and (100, 100, 100 and 100 %), respectively; whereas mentioned concentrations after 1 min just led to (67, 69, 71 and 73 %) and (65, 67, 67 and 72 %) of cyst and trophozoite elimination, respectively. On the whole of examination cyst elimination by increase of dilution without change of time raised except of 30 min by consumption of 100 mg/ml that was just 78 % compared to 82 % in the similar time but different concentration (50 mg/ml). In addition, Charts 1 and 2 depicts recovered results of using different concentrations and time exposure of chloroformic extract of *A. annua* against *G. lamblia* cyst and trophozoite.

Discussion

Recently there is a high tendency among researchers to solve drug resistance which is a major problem in endemic areas against parasitic diseases particularly giardiasis by using herbal therapies (Rahimi-Esboei et al. 2012). In a review of the literatures, following reports were found concerning effect of herbal remedies on giardiasis:

On the screen of some methanolic extracts of Mexican plants which are used traditionally in treatment of gastrointestinal disorders it was concluded that *Sennavillosa*, *Dorsteniacontrajeva* and *Rutachalepensis* were effective against *G. lamblia* with IC50 <38 mg/ml (Calzada et al. 2006). In another study, testing of two antimalarial drugs (*Mefloquine* and *Artesunate*) on *G. lamblia* in experimentally infected hamsters shown that the number of *G. lamblia* cysts was significantly reduced (William and Ramzy 2008). In addition to, antiprotozoal activity of *A. absinthium* has been proven versus *Naegleria fowleri* (Mendiola et al. 1991) and *G. lamblia* (Gurra et al. 2001). In the earlier study on laboratory rats, aqueous extract of *Artemisia* spp., (4 g/kg) was active against intestinal flagellate *G. lamblia* (Shanawa 1995). The residents of North East of Mexico Previously utilized an infusion of leaves from *A. ludoviciana* as a therapeutic drug (antidiarrhoeal). The authors presumed that the aqueous acetone, hexane and methanol leaf extracts of mature plants were found to have an active antiprotozoal role in vitro against *Entamoeba histolytica* and *G. lamblia* (Salvador et al. 2005).

According to our results not only concentration of chloroformic extract of *A. annua* for destroying of *G. lamblia* cyst and trophozoite plays prominent role but also time is another undeniable factor. Because increasing of time has an effective impact regarding rate of cyst and trophozoite elimination under laboratory conditions. By considering that in the present study a variant dilution (from 1 to 100 mg/ml) and time (from 1 to 180 min) were applied and cyst and trophozoite elimination rate at least

Table 1 Effect of different concentrations and time exposure of chloroformic extract of *A. annua* on *G. lamblia* cyst and trophozoite in vitro

Time exposure (min)	1 mg/ml (%)		10 mg/ml (%)		50 mg/ml (%)		100 mg/ml (%)		Control (%)	
	Cyst	Trophozoite	Cyst	Trophozoite	Cyst	Trophozoite	Cyst	Trophozoite	Cyst	Trophozoite
1	67	65	69	67	71	67	73	72	3	9
5	69	78	75	79	77	84	79	86	3	9
30	75	83	79	87	82	88	87	92	5	9
60	77	89	79	92	84	96	86	100	7	11
180	94	100	96	100	97	100	99	100	9	11

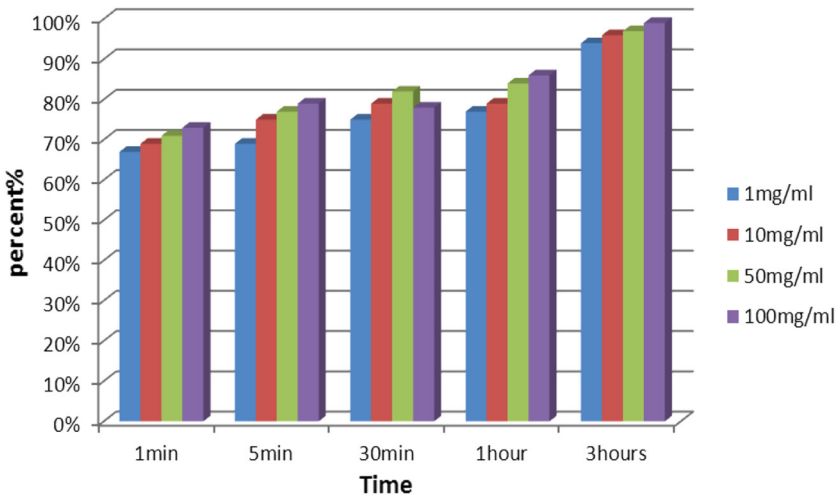

Chart 1 The effect of different concentrations and time exposure of chloroformic extract of *A. annua* on *G. lamblia* cyst in vitro

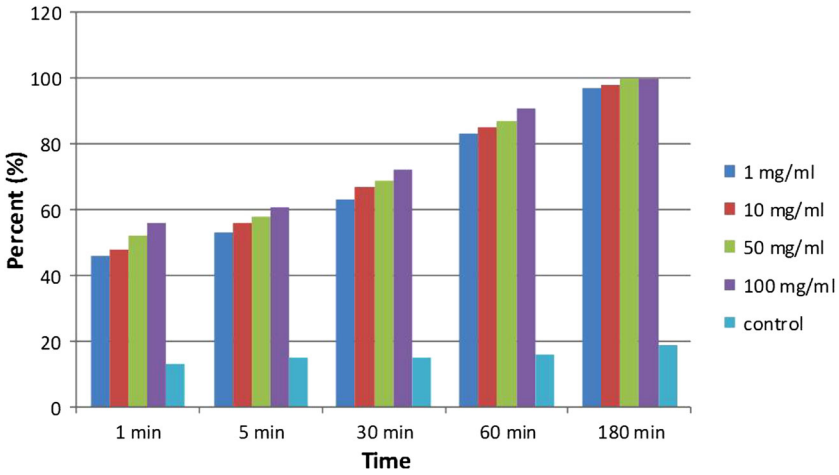

Chart 2 Effect of different concentrations and time exposure of chloroformic extract of *A. annua* on *G. lamblia* trophozoite in vitro

and at most were (67 and 99 %) and (65 and 100 %), respectively. It seems reasonable to assume that the trophozoite is more susceptible to chloroformic extract of *A. annua* in comparison with cyst and the results of the current investigation correspond closely to this fact; as we observed the elimination of whole of trophozoites (100 %) in time exposure of 180 min in mentioned different concentrations.

Owing to successful outcomes of the current study, chloroformic extract of *A. annua* can be considered as an alternative choice for patients who are engaging in either acute phase that excretes trophozoites or chronic phase that excretes cysts of Giardia as well.

Though there is not any reports regarding significant adverse or side effects of toxicity of *A. annua* in patients who were treated with therapeutic dosages, it is noteworthy that some cautions are advisable for consumption of *A. annua*: it can initially cause a worsening of symptoms, allergic reactions and some intestinal irritation. Moreover, *A. annua* should not be used during pregnancy (Vries and Dient 1996). Therefore, from this study it could be concluded that chloroformic extract of *A. annua* has the capability to destroy *G. lamblia* cysts and trophozoites. Hence, this is a value in endemic areas where people are prone to develop drug resistance and prevalently use antigiardiasis drugs. Further trials are demanded in order to assess the influence of *A. annua* in vivo both prescribing monotherapy and taking as a complementary treatment particularly in endemic areas.

Acknowledgments The authors are grateful to express their deep gratitude to Mazandaran University of Medical Science and Young Researchers Club of Islamic Azad University, Babol Branch.

References

Arab HA, Rahbari S, Rassouli A, Moslemi MH, Khosravirad F (2006) Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. Trop Anim Health Prod 38:497–503

Bailey LH, Bailey EZ (1976) Hortus third. MacMillan Publ Co, New York, pp 145–146

Bingham AK, Jarroll EL Jr, Meyer EA, Radulescu S (1979) *Giardia* sp.: physical factors of excystation in vitro, and excystation

versus eosin exclusion as determinants of viability. Exp Parasitol 47:284–291

Bone K, Morgan M (1992) Clinical applications of ayurvedic and Chinese herbs: monographs for the Western herbal practitioner. Phytotherapy Press, Warwick, pp 7–12

Brisibe EA, Umoren UE, Owai PU, Brisibe F (2008) Dietary inclusion of dried *Artemisia annua* leaves for management of coccidiosis and growth enhancement in chickens. Afr J Biol 7:4083–4092

Calzada F, Mulia LY, Aguilarb A (2006) In vitro susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharm 108:367–370

Chawla B, Madhubala R (2010) Drug targets in Leishmania. J Parasit Dis 34:1–13

Choubisa SL, Jaroli VJ, Choubisa P, Mogra N (2012) Intestinal parasitic infection in Bhil tribe of Rajasthan. India J Parasit Dis 36:143–148

Gurra M, Torres D, Martinez L (2001) Validación del uso tradicional de plantas medicinales cultivadas en Cuba. Rev Cubana Plant Med 2:48–51

Hilen TT, White NJ (1993) Qinghaosu. Lancet 341:603–608

Jorge FS, Ferreira L, Devanand LL, Saki T, Heyerick A (2010) Flavonoids from *Artemisia annua* as Antioxidants and Their Potential Synergism with Artemisinin against Malaria and Cancer. Molecules 15:3135–3170

Kappus KD (1994) Intestinal parasitism in the United States: update on a continuing problem. Am J Hyg Trop med 50:705–713

Keister DB (1983) Axenic culture of *Giardia lamblia* in TYI-S-33 medium supplemented with bile. Trans R Soc Trop Med Hyg 77:487–488

Laarman JJ, Hautus M, Abdillahi H (1986) Isolation of stocks of *Giardia lamblia* from stools and their use in the determination of specific immunoglobulins in patient sera. Trop Geo Med 38:320

Larry SR, Janovy J (2006) Foundations of parasitology, 7th edn. McmGraw, Hill companies, New York 590

Li W, Mo W, Shen D, Sun L, Wang J, Lu S, Gitschier JM, Zhou B (2005) Yeast model uncovers dual roles of mitochondria in the action of artemisinin. PLoS Gen 1:0329–0334

Lindo JF, Levy VA, Baum MK, Palmer CJ (1998) Epidemiology of giardiasis and cryptosporidiosis in Jamaica. Am J Hyg Trop med 59:717–721

Mahdavi SA, Raeesi A, Faraji L, Youssefi MR, Rahimi MT (2013) A case of misdiagnose of malaria infection. Asian Pac J Trop Biomed 3:748–750

Mendiola J, Bousa M, Perez N, Hernandez H, Torres D (1991) Extracts of *Artemisia abrotanum* and *Artemisia absinthium* inhibit growth of *Naegleria fowleri* in vitro. Trans R Soc Trop Med Hyg 85:78–79

Mishina YV, Krishina S, Haynes RK, Meade JC (2007) Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense* in vitro growth. Antimicrob Agents Chemother 51:1852–1854

Oliverira TC, Silva D, Rostkoaska C, Bela SR, Ferro E, Magalhaes PM (2009) *Toxoplasma gondii*: effects of *Artemisia annua* L. on susceptibility to infection in experimental models in vitro and in vivo. Exp Parasitol 122:233–241

Rahimi-Esboei B, Gholami SH, Azadbakht M, Ziaei H (2012) Effect of Hydroalcholic extract of *Artemisia annua* on cysts of *Giardia lamblia* in Invitro. J Mazand Univ Med Sci 22:72–80

Ridley RG, Hudson N (1998) Oxidative stress and antimalarial drugs. Curr Biol 8:346–349

Salvador SF, Guerra MCR, Cardenas BDM, Villarreal JV, Trevino LV (2005) In vitro antiprotozoal activity of the leaves of *Artemisia ludoviciana*. Fitoterapia 76:466–468

Sayyari AA, Imanzadeh F, Bagheri Yazdi SA, Karami H, Yaghoobi M (2005) Prevalence of intestinal parasitic infections in the Islamic Republic of Iran. East Med Health J 11:377

Shanawa BH (1995) Biological and immunological studies on *Giardia lamblia* (Stiles, 1915). Coll Sci 102:34–37

Simon JE, Chadwick AF, Craker LE (1984) Herbs: an indexed bibliography, 1971–1980. The scientific literature on selected herbs, and aromatic and medicinal plants of the temperate zone. Archon Books, Hamden

Utzinger T, Shuhua X, Keiser J, Minggan C, Jiang Z, Tanner M (2001) Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. Curr Med Chem 8:1841–1859

Vries PJ, Dient TK (1996) Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 52:818–836

William S, Ramzy F (2008) Testing two antimalarial drugs on *Giardia lamblia* in experimentally infected Hamsters. Re J Med Sci 41:1–6

Wright JM, Dunnla P, Upcroft JA (2003) Efficacy of antigiardial drugs. Expert Opin Drug Safe 2:529–541