GIARDIACIDAL ACTIVITY OF LEMON JUICE, VINIFER AND VINEGAR ON *GIARDIA INTESTINALIS* CYSTS

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Abstract. The giardiacidal efficacy of simple disinfecting materials, *ie* lemon juice, vinifer, and vinegar, for uncooked foods with *Giardia* cysts was investigated to help travelers in *Giardia*-endemic areas. The cysts were obtained from stools of individuals with *Giardia intestinalis* infection by modified sucrose gradient procedure. A pooled batch of $3x10^4$ /ml *Giardia* cysts was made from all specimens. The cysts were kept at 4˚C until use. Before each experiment, the number of cysts was determined by hemocytometer. Two sets of Eppendorf tubes were used for the experiments, one set at 4˚C and one at 24˚C. One thousand microliters each of lemon juice, vinifer, or vinegar was poured into each tube, and 1,000 µl of *Giardia* cysts were added. Variables were disinfectant materials, temperature, and time of exposure. Cyst viability 140 was determined by eosin inclusion procedure. Viability of at least 250 cysts in each tube at 0, 0.5, 1, 2 and 3 hours after the beginning of the experiments was determined. The mean giardiacidal activity at 4˚C after 3 hours for lemon juice, vinifer, and vinegar was 18.9, 12.8, and 28.4%, and at 24˚C, 28.3, 16.2, and 40.6%, respectively. In conclusion, the giardiacidal activity of vinegar was more than the other materials, and as exposure time and temperature increased, giardiacidal activity also increased; the highest giardiacidal activity of vinegar was at 3-hours exposure at 24˚C.

INTRODUCTION

Giardia intestinalis is a flagellated unicellular protozoan parasite of the small intestine (Thompson *et al*, 1994). It is an important cause of diarrhea and malabsorption in developed and developing countries (Sadjjadi and Tanideh, 2005). The presence of *Giardia intestinalis* in potable water supplies is a serious problem throughout the world (Jakubowski and Craun, 2002).

In certain areas of the world, water and vegetables contaminated with *G. intestinalis* cysts commonly cause travel-related giardiasis in tourists (Erdogrul and Sener, 2005). The main way to control the disease is through drug treatment and preventing cyst transmission to humans. Different physical and chemical methods have been used to separate or inactivate *Giardia* cysts (Jakubowski and Craun, 2002). Currently, giardiasis represents a major public health concern of water utilities in developing, and even developed, countries. *Giardia* cysts are resistant to conventional chlorination, and can persist and remain infective for extended periods in water or sewage. Fruits and vegetables, particularly those eaten raw and without peeling, have been demonstrated to be the vehicle for transmission of a range of parasites, including *Giardia* (Erdogrul and Sener, 2005). Different chemicals have been used to inactivate *Giardia* cysts, including chlorine and ozone. Some natural nutrients, *eg* garlic, have been used to inactivate *Giardia* cysts and treat giardiasis (Harris *et al*, 2000; Azadbakht *et al*, 2003). The use of edible materials to inactivate or kill *Giardia* cysts, especially for drinking water and vegetable salads, is of interest. To date, no study has been conducted assessing the *in vitro* giardiacidal effect of lemon juice, vinifer or vinegar, which are usually used in salads. Therefore, this study was conducted to investigate the *in vitro* giardiacidal efficacy of lemon juice, vinifer, and vinegar for uncooked foods with *Giardia* cysts, to help travelers in giardiasis-endemic areas, and to compare the giardiacidal effects of different disinfecting materials (lemon juice, vinifer, and vinegar) on *Giardia* cysts, according to exposure time and temperature.

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MATERIALS AND METHODS

Collecting and maintenance of *Giardia* cysts

The cysts were obtained from stools of individuals with *Giardia intestinalis* infection by modified sucrose gradient procedure (Bingham *et al*, 1979). Briefly, 5 grams of stool were treated with 10 ml distilled water followed by filtering through 4-fold gauze into a 15-ml conical tube. The tube was then centrifuged at 600-1,000 rpm for 10 minutes, and the supernatant discarded. The pellet was added to 10 ml of 2 M sucrose and centrifuged at 600*g* for 10 minutes. A

pooled batch of 3x10 4 /ml *Giardia* cysts was made from all specimens and kept at 4˚C until use. Before each experiment, the number of cysts was determined by hemocytometer.

***In vitro* experiments**

Two sets of Eppendorf tubes were used in the experiments, one set at 4˚C and one at 24˚C, 1,000 µl each of lemon juice, vinifer or vinegar was poured into each tube and 1,000 µl of pooled *Giardia* cysts added.

Viability test

Cyst viability was determined by eosin inclusion procedure, using 0.1 ml of 1:1,000 aqueous eosin solution and 0.1 ml of cyst suspension. Percentage eosin exclusion (unstained cysts/total cysts) was determined. The viability of at least 250 cysts in each tube at 0, 0.5, 1, 2 and 3 hours after the beginning of the experiment was determined. Living cysts exclude eosin and remain unstained. Stained cysts are nonviable.

Statistical analysis

The means± standard deviation of the inactivated cysts among the 250 cysts, counted in 4 groups (control, lemon juice, vinifer and vinegar) were analyzed by ANOVA. The least significant difference (LSD) between the group means were also determined.

RESULTS

The mean giardiacidal activities of lemon juice, vinifer and vinegar are shown in Tables 1 and 2. The results showed that giardiacidal activity at 4˚C after 3 hours for lemon juice, vinifer and vinegar was 18.9, 12.8, and 28.4%, and at 24˚C, 28.3, 16.2, and 40.6%, respectively. The ANOVA test showed that the differences between all groups and the control were significant ($p<0.05$). The LSD showed that vinegar was more effective in inactivating *Giardia* cysts, and this increased further at 24˚C.

DISCUSSION

Giardiasis is caused by the transmission of *Giardia* intestinalis cysts through water and food to humans. The main ways to control the disease are drug treatment (Sadjjadi *et al*, 2001) and prevention of cyst transmission to humans. Effective control of *Giardia* in water supplies can be achieved by applying multiple barriers, *ie*, source water protection, filtration, and disinfection. The proper management of human and animal waste can minimize contamination of source waters (Jakubowski and Craun, 2002). Fruits

Table 1
Comparison of giardiacidal activity of lemon juice, vinifer and vinegar on *Giardia intestinalis* cysts at 4º C.

Natural edible solutions	No. exp.	30 min exposure				One hour exposure				2 hours exposure				3 hours exposure			
		Mean cysts killed in 250 cysts	SD	LSD	% killed cysts	Mean cysts killed in 250 cysts	SD	LSD	% killed cysts	Mean cysts killed in 250 cysts	SD	LSD	% killed cysts	Mean cysts killed in 250 cysts	SD	LSD	% killed cysts
Control	22	15.4	6.38	2,4	6.16	16.59	6.67	2,4,	6.64	18.45	7.87	2,4	7.4	20.63	8.02	2,4	8.25
Lemon juice	34	21.97	7.42	4	8.18	31.94	11.83	4	12.2	39.35	13.69	4	15.67	48.05	15.42	4	18.9
Vinifer	16	15	6.55	2,4	6	20.37	9.6	2,4	8.15	25.93	11.61	2,4	10.38	32	14.6	2,4	12.8
Vinegar	22	33.9	18.11	1,2,3	13.56	47	21.54	1,2,3	18.8	59.54	22.14	1,2,3	22.62	71.18	28.38	1,2,3	28.4

Table 2
Comparison of giardiacidal activity of lemon juice, vinifer and vinegar on *Giardia intestinalis* cysts at 24° C.

Natural edible solutions	No. exp.	30 min exposure				One hour exposure				2 hours exposure				3 hours exposure			
		Mean cysts killed in 250 cysts	SD	LSD	% killed cysts	Mean cysts killed in 250 cysts	SD	LSD	% killed cysts	Mean cysts killed in 250 cysts	SD	LSD	% killed cysts	Mean cysts killed in 250 cysts	SD	LSD	% killed cysts
Control	22	15.63	6.57	2,4	6.25	17.63	7.41	2,4,	7.06	20.72	8.32	2,3	8.30	24.72	9.45	2,3,4	9.89
Lemon juice	34	26.26	6.98	4	10.50	39.17	11.61	4	15.67	52.05	13.99	4	20.82	70.88	24.99	4	28.35
Vinifer	16	15.81	7.84	2,4,	6.32	25.12	11.68	2,4	10.05	32.50	13.75	2,4	13	40.56	16.37	2,4	16.22
Vinegar	22	41.63	13.53	1,2,3	16.65	57.72	18.00	1,2,3	23.09	75.00	15.21	1,2,3	30.04	101.59	28.09	1,2,3	40.63

and vegetables, particularly those eaten raw and without peeling, have been shown to be vehicles for transmission of a range of parasites, including *Giardia* (Erdogrul and Sener, 2005).

Chemical disinfectants can also reduce cyst densities, but effectiveness can be affected by water temperature and pH, and residual disinfectant concentration and contact time, particles that can shield cysts from contact with the chemical and organic matter that can cause disinfectant demand (Jacubowski and Craun, 2002). A variety of plants and natural products has been examined for giardiacidal activity with mixed success (Lee, 1992; Lun *et al*, 1994; Soffar and Mokhtar, 1994; Harris *et al*, 2002; Azadbakht *et al*, 2003). The most effective natural product has been garlic, with differing efficacy (Soffar and Mokhtar, 1994; Harris *et al*, 2002; Azadbakht *et al*, 2003). Further development and refinement of the extract appears necessary before a highly effective anti-giardial treatment is derived from plants (Reynoldson, 2002).

Our experiments showed that the efficacious use of edible materials to inactivate *Giardia* cysts increased with temperature, so that at 24°C, inactivation increased.

Disinfection may be adequate to inactivate 99.9% of cysts, but the remainder may still be at infectious dose level (Jakuboswki and Craun, 2002). As *Giardia* cysts may come in contact with lettuce, parsley, cress, spinach, and strawberries (Erdogrul and Sener, 2005), and *Giardia* is more resistant to chlorination than bacteria (Korich *et al*, 1990), the need to inactivate *Giardia* cysts with other chemicals and edible materials, such as vinegar, which is used in salads, is of interest. Our experiments showed that vinegar had greater giardiacidal activity than other simple disinfectant material, and as time exposure and temperature increased, giardiacidal activity increased;, with the highest 3 hours' exposure and a temperature of 24°C.

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