



# Antigiardial effect of *Anethum graveolens* aqueous extract in children

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## ABSTRACT

**Background:** *Giardia lamblia* is the most common intestinal parasite of humans identified worldwide. In spite of metronidazole (Met) is the most commonly used drug for the treatment of giardiasis in humans, low patient compliance and side-effects, especially in children encourage efforts to look for new and safe agent; many plants used in folk medicine thought to have anti-giardial effect, *Anethum graveolens* (AG) (dill) is an annual herb cultivated in Iraq used both as a medicinal agent and as food spice. The aim of this study was to investigate the effect of aqueous extract (AE) of AG leave in the treatment of giardiasis, compared with that of standard drug Met. **Patients and Methods:** A prospective randomized clinical trial was carried out on 28 pediatric patients of both sexes with age of < 1 year ranging from 3 to 11 months, who attend to outpatient private clinic in Baghdad for a period of 6 months from June 2013 to December 2013, Patients participate in this study were allocated into two groups: Group A composed of 14 patients treated with Met 15 mg/kg 3 times a day for 5 days. Group B composed of 14 patients treated with AGAE 1 ml 3 times a day for 5 days. Stool samples were collected at 0 time before administration of treatment, after 5 days and after 14 days from starting the treatments to check the efficacy of treatment. **Results:** Administration of AGAE results in a significant decrease in incidence of *G. lamblia* after 5 days of treatment indicating the efficacy of AGAE in the treatment of giardiasis a result that is comparable to that of Met. **Conclusion:** This study showed that pediatric patients with giardiasis may benefit from 5 days treatment with AGAE administered as 1 ml 3 times daily, the improvement in the symptom with this herbal agent was comparable to the standard pharmacological agent Met; results showed that AG is safe and tolerable over treatment course.

**KEY WORDS:** *Anethum graveolens*, giardiasis, herbal medicine, metronidazole

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## INTRODUCTION

*Giardia lamblia* (also known as *Giardia intestinalis* and *Giardia duodenalis*) is the most common intestinal parasite of humans identified worldwide [1]. This flagellated protozoan causes a generally self-limited clinical illness (i.e. giardiasis) typically characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption; asymptomatic infection also occurs frequently [2].

*Giardia* infection is transmitted through the fecal-oral route and results from the ingestion of *Giardia* cysts through the consumption of fecally contaminated food or water or through person-to-person (or, to a lesser extent, animal-to-person) transmission [3]. The cysts are infectious immediately upon being excreted in feces. The infectious dose is low; ingestion of 10 cysts has been reported to cause infection [4]. The prevalence of infection is commonly between 2% and 5% in the developed world and 20-30% in the developing and underdeveloped countries [5]. Existing chemotherapy protocols recommend that patients should be treated if the parasite is found, irrespective of the presence or absence of acute symptoms [6]. However, some investigators question the

usefulness of chemotherapy in infected people in endemic areas due to the extremely high rate of reinfection, as high as 90% in some studies [7]. Treatment preferences vary among clinicians and in different locations. The most widely used treatment protocols employ metronidazole (Met) given 3 times/day for 3-5 days [8]. Met is typically administered in doses of 250 mg 3 times a day for 5-7 days for adults and 15 mg/kg 3 times a day for 5-7 days in children. In recent years, therapeutic failure of Met, the first-line drug of choice in giardiasis in humans, has increasingly been reported from all around the world [9]. Met is prescribed widely for a wide range of nonparasitic infectious diseases. Low compliance of patients with the current Met therapy protocols, the emergence of the Met-resistant strains of the parasite and other pathogens, and rapid reinfection of treated patients in the endemic areas are additional reasons for considering alternative therapies [10]. Poor adherence to the prescribed frequency and duration of Met, especially in children less than 1 year, in addition to common adverse reactions frequently reported with Met include metallic taste, nausea, vomiting, diarrhea, and epigastric discomfort represent important causes for finding safer drugs with less toxicity and more effective therapeutic properties with low incidence of side-effects.

Research in herbal medicine has increased in the world as an alternative solution to health problems; furthermore, side-effects of drugs in current use shift the orientation toward herbal medicine, especially high percent of people thought that using of herbal medicine is free of side-effect [11].

*Anethum graveolens* (AG) Umbelliferae, known as dill, is an annual herb growing in the Mediterranean region, Europe, Central and Southern Asia; the plant is used both as a medicinal agent and as food spice. Dill has been used traditionally for gastrointestinal disturbances such as flatulence, indigestion, and colic [12]. In Iraq, dill was used as food spice and in Gripe Water for children as antifatulent. The active constituents of AG have been classified as flavonoids, phenolic compounds, and essential oils, the phytochemical screening of plant showed that leaves, stems, and roots were rich in tannins, terpenoids, cardiac glycosides and flavonoids [13-15]. Pharmacological effects of AG include antibacterial [16], antifungal [17], antispasmodic [18], antisecretory, and mucosal protective effects [19].

The aim of this study was to investigate the effect of aqueous extract (AE) of AG leave in the treatment of giardiasis, compared with that of standard drug Met.

## PATIENTS AND METHODS

A prospective randomized clinical trial was carried out on 28 pediatric patients of both sexes with age of <1 year ranging from 3 to 11 months, who attend to outpatient private clinic in Baghdad for a period of 6 months from June 2013 to December 2013; the study was approved by Scientific and Ethical Committee in Alkindy College of Medicine/University of Baghdad, an informed consent was taken from all the parents. To be included in the study, a child had mono-infection with *G. lamblia* proven by microscopic examination of fecal sample, with diarrhea and abdominal pain; the exclusion criteria were known history of sensitivity to Met, those receiving any antiparasitic or antibiotic chemotherapy within 3 weeks and patients having disease other than giardiasis. Patients participate in this study were allocated into two groups:

- Group A: Composed of 14 patients treated with Met 15 mg/kg 3 times a day for 5 days
- Group B: Composed of 14 patients treated with AGAE 1 ml 3 times a day for 5 days.

History was taking from parents; a special form was used to record clinical signs and symptoms before starting treatment and at the end of course, a physical examination and weight measurement was carried out for each child.

The evaluation of efficacy of the chemotherapy was based on parasitological response to therapy assessed by the same laboratory tests that were done initially. Parents of each child were asked to provide three fecal samples on day 0, 5, and 14 after treatment. Furthermore, they were encouraged to return to the clinic at any time, if they considered that his or her child was ill. A child was only considered to be cured, if no *Giardia* trophozoites or cysts could be found in any of the three fecal specimens.

## AGAE Preparation

AG was obtained from local market; it is approved by Medicinal Plant Center-Baghdad, Iraq. Aqueous extraction was performed by adding 200 ml of water to 20 g of AG dried leaves then boiling for 10 min; wait for the solution to become cold; the extract filtered and leaved for evaporation until 100 ml.

Minitab software package was utilized for statistical analysis; results expressed as mean ± standard deviation; paired Student's *t*-test was used to evaluate significant changes;  $P \leq 0.05$  considered being significant.

## RESULTS

The results of this study showed that the incidence of *G. lamblia* was 100% in the stool samples of all pediatric patients before treatment, administration of Met to the group A decreased significantly  $P \leq 0.05$  the incidence percent to 7.14% after 5 days of treatment, while after 14 days stool examination revealed nil incidence of parasite Figure 1; on the other hand, treatment with AGAE 1 ml 3 times a day for 5 days reduce significantly  $P \leq 0.05$  the incidence percent in Group B to 14.28% where only two samples contain the parasite, and after 14 days the percent was zero, indicating the efficacy of AGAE in the treatment of giardiasis compared to Met, Figure 1.

Figure 2 showed that treatment with Met significantly  $P \leq 0.05$  decrease the frequency of bowel motion in pediatric patients by 118.93% at day 1 of treatment, while at day 2 after starting treatment the frequency of bowel motion reduced by 54.35% compared with day 1, and at days 3, 4, and 5 the frequency of bowel motion become normal, Figure 2; in Group B, administration of AGAE 1 ml 3 times daily reduce the frequency of bowel motion significantly  $P \leq 0.05$  at the first treatment day by 82.24%, while at 2nd day after starting treatment the reduction percent was 87.18% compared to day 1, at days 3, 4, and 5 the frequency of bowel motion return to normal, Figure 2; these data again indicating the efficacy of AGAE in the treatment of giardiasis compared to Met.

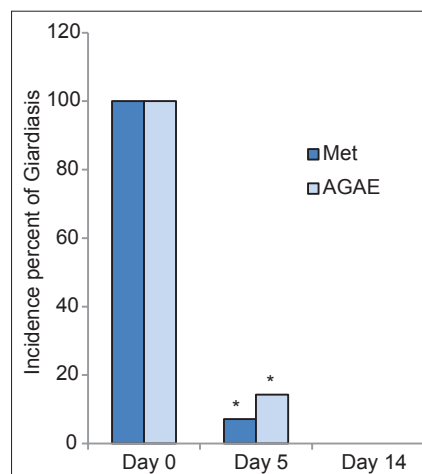
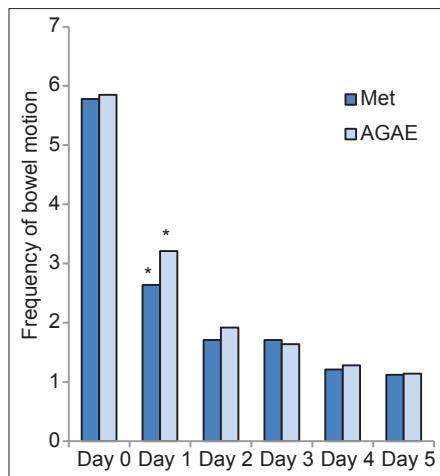


Figure 1: Antigiardial effect of *Anethum graveolens* aqueous extract compared to metronidazole in pediatric patients



**Figure 2:** Frequency of bowel motion in pediatric patients during 5 days treatment with *Anethum graveolens* aqueous extract compared to metronidazole, results represents mean value

## DISCUSSION

Because of the treatment failure and the adverse effects of medications used to treat giardiasis, many patients consider conventional treatment to be disappointing and often turn to complementary therapies. The choice to use natural rather than chemical therapies is attractive because many patients assume that natural products are safe and that they do not cause adverse effects, this have provided a continuous stimulus to search for other therapeutic alternatives [20].

Many natural materials have been shown to have anti-giardial activity, the anti-giardial activity of the phenol-rich essential oils of several plants had been examined, and their activity was evaluated based on the change in parasite growth, cell viability, adherence, and morphology [21]. Another *in vitro* study suggests that the crude extracts of *Citrullus lanatus* Var. were active against *G. lamblia* and may be recommended as a new source for the treatment of giardiasis [22]. Anthony *et al.* confirmed that berry polyphenols can influence *Giardia* survival *in vitro* and suggests that ellagitannins are most effective. Tannin-rich preparations may also have efficacious effects on diarrheal symptoms often associated with *Giardia* infection. Unlike many plant sources of anti-giardial agents, berries are a natural and palatable foodstuff and therefore have few issues with toxicity, side-effects or acceptance [23]. Rahimi *et al.* Studied the anti-giardial activity of *Sambucus ebulus* *in vitro*, he reported that there is excellent anti-giardial of methanolic extract of *S. ebulus* *in vitro* against cyst of *G. lamblia* [24]. On the other hand, Al-masoudi studied the anti-giardial activity of *Zingiber officinale* in combination with honey *in vivo*, she examined the effects of watery extracts against *G. lamblia* on the basis of killed trophozoite number, using experimental infections of *G. lamblia* in balb/c mice; she reported that extract of *Z. officinale* was more active specially when mixed with honey, so the percentage of dead trophozoite reach to 97.7% [25].

In this clinical trial, the anti-giardial effect of AGAE was examined in pediatric patients; the results showed that the administration

of 1 ml of the AE 3 times a day cause a significant reduction in the survival incidence of giardial trophozoite in stool samples a result that is comparable with that obtained from administration of the standard drug Met. There was no definite mechanism documented so far by which AG exert its effect; many studies had been shown multiple antimicrobial effects for this spice. It has been reported that AEs of AG showed a broad-spectrum antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri* and *Salmonella typhi* [26]. The higher activity of extract can be explained on the basis of the chemical structure of their major constituents such as dillapiole and anethole, which have aromatic nucleus containing polar functional group that is known to form hydrogen bonds with active sites of the target enzyme [27]. Furthermore, it has been reported that compounds of dill when added to insecticides have increased the effectiveness of insecticides. Essential oil of AG is used as repellent and toxic to growing larvae and adults of *Tribolium castaneum*, wheat flour insect pest. In doses of 60 minims, anethole is a fairly potent vermicide for hookworm. All these activities against diverse microorganisms beside the safety profile of AG as edible herb encourage the use of its AE in the treatment of giardiasis. Another important point is the multi pharmacologic effects of AG like mucosal protective, antisecretory, antioxidant activity and the potent relaxant effect of contractions induced by a variety of spasmogens in rat ileum, which supports the use of dill in traditional medicine for gastrointestinal disorders [28]. These properties of dill may contribute effectively in rationalize the use of this herb in the treatment of giardiasis where many signs and symptoms like frequent bowel motion, abdominal pain and flatulence may be relieved in addition to its main use against giardiasis. However, determination of the exact mechanism by which dill exerts its anti-giardial effect needs further deep investigation on molecular level, while large scale clinical trials with large sample size and multicenter studies are needed to clarify its role in the treatment of giardiasis. Another limitation in this study is the difficulties in monitoring the incidence of side-effects that may occur with Met such as headache, nausea, metallic taste, and abdominal pain compared to that when using AGAE since it was impossible to document such subjective side-effects in pediatric patients under 1 year of age, although according to pediatrics' mother the episodes of crying in pediatric patients on AGAE were less much than that on Met (data not shown) which may explained depending on the spasmolytic and antifatulent properties of dill herb.

## CONCLUSION

This study showed that pediatric patients with giardiasis may benefit from 5 days treatment with AGAE administered as 1 ml 3 times daily, the improvement in the symptom with this herbal agent was comparable to the standard pharmacological agent Met; results showed that AG is safe and tolerable over treatment course.

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