

A comparative evaluation between anti Giardia effect of Artemisia, Punica granatum and Mebendazole in native dogs

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ABSTRACT: Aims: Mebendazole applied in veterinary medicine for Giardia treatment but it has interaction with other drugs such as metronidazole and side effects such as stomach pain and diarrhea, so our aim is to replace artemisia and punica granatum as herbal medicines of Iran.

Research method: In recent study, 20 native dog with mean weight 30kg selected in 4 groups, randomly from 2 sex. First group (control) took 5mg/kg mebendazole daily as orally for 5 days. Second group (treatment) received the artemisia autoclaved powder orally as 5mg/kg one time in the day for 5 days. Third group (treatment) received punica granatum autoclaved powder as 5mg/kg in 5 days daily, orally. Forth group received all of three above drug for that period time.

Findings: The dogs of first and forth groups had: Excessive diarrhea as mebendazole side effect and no GI giardiasis. But second and third groups had no side effects and no GI giardiasis, two.

Discussion and Conclusion: In current report, both artemisia and punica granatum as herbal medicine had no side effects in comparison to mebendazole so we suggested replace them in dogs as herbal anti-Giardia drug because is safe, potent and available.

Keywords: Mebendazole, Artemisia, Punica granatum, anti-Giardia, dog

INTRODUCTION

Giardiasis is a parasite disease, caused by a protozoan called *Giardi duodenalis*, which shares similar morphological characteristics with other species such as *G. lamblia* and *G. intestinal*, for which the same pathogen has been considered (Bowman, 2009. Payne, 2009. Lane, 2002); this protozoan affects many domestic and wild species (WHO, 1996). In the canine and feline species, it is described as *G. intestinalis* (Heredia, 2015), previously described as *Giardia canis*; this protozoan affects not only animals but also man (Minetti, 2015).

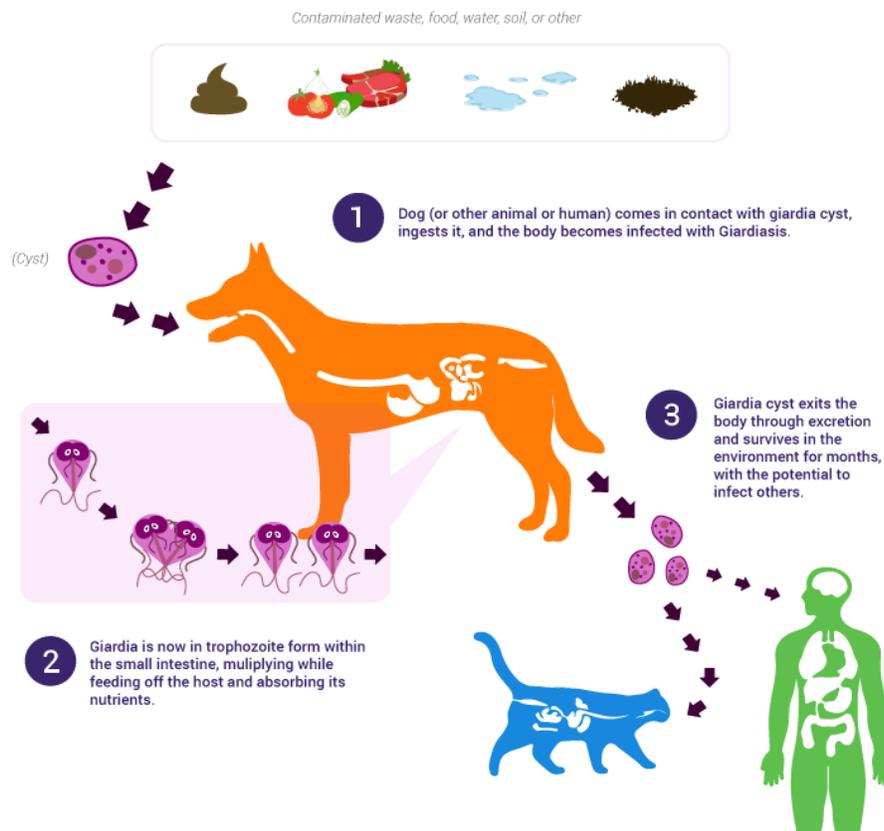


Figure 1: Giardia life cycle in dogs and cats and human.

The benzimidazoles are a group of drugs that have frequently been used for the treatment of parasitic nematodes mainly, although their effectiveness in the control of cestodes and trematodes has been evident. Its capacity to eliminate not only the adult forms but also the ovoposition has been the treatment of gastrointestinal parasites in many animal species (Murphy, 2016), including humans, where this group is of greater importance in the control of giardiasis (Durigan, 2014). Nowadays, benzimidazoles are considered as the standard drugs for the control of canine and feline giardiasis (Bondi, 2014), not only because of its effectiveness, which is 100% (Sprong, 2009), but also because they require fewer days of treatment as well as a longer half-life, which facilitates their administration especially in small animals that mebendazole is one of them. Mebendazole is a broad-spectrum anti-worm drug that is effective in Roundworms, echinococcus and tapeworms in dogs and cats (Kayde, 2004. Bowman, 2010), but unfortunately, this chemical drugs such as mebendazole has many GI side effects in small animal like dog such as stomach pain and diarrhea, Acute necrosis hepatic lip hepatitis and lethal hepatitis in subsequent dogs mebendazole administration has been reported (Boothe, 2001). Acute and chronic intoxication of this drug in oral form, in humans and many animal species have been observed (Iappin, 2003, 2004, 2006).

The metabolism of mebendazole in dogs is how that the drug is mainly excreted in the feces (about 90%). It is excreted in the urine unchanged and very small amounts (about 1%) up to 4 days after administration (WHO, 2003).

If a dog under the administration of mebendazole develops nausea, vomiting, anorexia or diarrhea, the drug should be discontinued immediately. Co-administration with other drugs that are with the system Cytochrome P450 enzymes may compete, may causes a decrease in mebendazole metabolism. By the time Long-term treatments, measurement of serum enzyme concentrations are recommended to adjust the dose of the drug if necessary

(Lappin, 2003), so our aim is to replace artemisia granatum as herbal medicines of Iran because they have no side effects on the body and are safe, cheap and available.

Protozoan diseases significantly endanger the health of human and animal communities and cause great economic damage to human and animal husbandry communities each year. The use of herbal compounds has a long history and today many societies have turned to the use of plant products as a treatment for many diseases, including parasitic diseases, instead of using chemical compounds. Consumption of plant-derived antiparasitic compounds reduces the prevalence of many chronic diseases (Calixto, 2000). Artemisia is one of the valuable plants with healing properties such as anti-parasitic properties, especially anti-protozoan.

Artemisia is a plant of the genus Plantae of the order Asterales of the family Asteraceae under the family Asteroideae of the genus Anthemideae and the genus artemisia. In Persian, this plant is called artemisia, wormwood and rice bran. Artemisia grows in temperate climates from both hemispheres, but is more common in arid and semi-arid habitats (Watson, 2002).

There are several species of artemisia, with between 200-400 worldwide and 33 species in Iran. Most of them have bitter and fragrant leaves caused by trinoids and lactone and also have more or less similar medicinal properties.

So far, extensive studies have been conducted on the chemical composition of different species of artemisia. Among the species of this genus that are cultivated in different countries and have medicinal importance can be *A.pontica*, *A. maritima*, *A.kurramensis*, *A.dracunculus*, *A.ciniformis*, *A.absinthium*, *A.abrotanum*, *A. vulgaris* (Kordali, 2005), *A.kulbadica* (Rahimi, 2014), *A.santolina*, *A. annua*, *A. turanica* (Emami, 2008), *A. aucheri boiss* (Rostami, 2009), *A. seiberi* (Dalimi, 2013), *A.roxburghiana* (Karabegović, 2011) And *A.scoparia* (Afshar, 1990).

The result of these studies is the discovery of new and unique compounds in plants of this genus. In general, the chemical compounds in artemisia can be classified into monoterpenes, sesquiterpenes and especially sesquiterpene-lactones, flavonoids, phenylpropanoids, polystylenes and coumarins (Schwabe, 2005).

Giardia lamblia causes giardiasis in humans, dogs, cats and cattle. This protozoan is located in the duodenum (human and dog), jejunum and ileum (cat). Major lesions in giardiasis include destruction of intestinal villi structure with villi shortening, occurrence of inflammatory foci in crypts and lamina propria, and impaired fat digestion. Giardiasis causes fatty, bloodless diarrhea and weight loss. Treatment of giardiasis with artemisia annua hydroalcoholic extract eliminates the protozoan cystic stage of *Giardia lamblia* and increasing the concentration of hydroalcoholic extract increases its lethality on *Giardia* cysts (Rahimi, 2012).

Pomegranate (*Punica granatum*) is a plant that has been used in folk medicine for the treatment of various human and animal diseases (Moneim, 2012). The constituents of pomegranate include high levels of hydrolyzable tannins (punicalins and punicalagins), ellagic acid (a component of ellagitannins), and gallic acid (a component of gallotannins), with well-known antioxidant and antimicrobial activities (Reddy, 2007). Additionally, pomegranate is widespread in the Mediterranean region and is well known for its anti-inflammatory effect due to its high content of antioxidants (Moneim, 2012). Furthermore, pomegranate is a potential antifungal and it is casually used in the treatment of *Trichophyton rubrum* (Foss, 2014). Pomegranate exhibits several other pharmacological activities, including anthelmintic effects on various intestinal trematodes, nematodes, and cestodes in addition to its anti-amoebic, antimalarial, and anticoccidial effects (El-Kady, 2021).

In one study, it was aimed to investigate the therapeutic utility of pomegranate in *G. lamblia* infections. For that purpose, pomegranate peel ethanolic extract was tested for its effectiveness in eliminating the parasite and ameliorating the disease in a rat model of giardiasis and pomegranate extract effectively killed *G. lamblia* cysts (El-Kady, 2021). By attention to above, we decided to comparative pomegranate autoclaved powered by artemisia autoclaved powder and mebendazole in giardiasis treatment in native dogs in Tehran province and we suggest that our work is the first comparative work on these two above mentioned herbal drugs in Iran and maybe in the world because we cannot take any similar comparative work like this in the literature. Our zero hypothesis is that the both pomegranate and artemisia has no side effects on dogs in giardiasis treatment duration as herbal drugs against mebendazole tablets as chemical drug and they could use in giardiasis treatment in dogs as safe, cheap and available drugs instead of mebendazole and our first hypothesis is against the zero one.

Research method

In recent study, 20 native dog with mean weight 30 kg selected in 4 groups, randomly from 2 sex. First group (control) took 5mg/kg mebendazole tablet daily as orally for 5 days. Second group (treatment) received the artemisia autoclaved powder orally as 5mg/kg one time in the day for 5 days. Third group (treatment) received Punica granatum autoclaved powder as 5mg/kg in 5 days daily, orally. Forth group received all of three above drug for that period time. After 5 days Giardia sampling was done by stool sampling.

Sample collection and Purification of Giardia lamblia cysts

Stool samples infected with Giardia cyst with daily referral to Central Laboratory of Tehran and Laboratory Medical diagnosis of healing located in Tehran city. Existence of abundant Giardia cysts with proliferation. Direct and microscopic examination of samples by experienced microscopist confirmed. In order to obtain Maximum live cysts. Isolation and purification of cysts in Samples were collected on the same day. Stool samples with Distilled water was thoroughly mixed and passed through four layers of gas to separate large particles. The filtered solution put in a laboratory tube, again and it centrifuged in $800 \times g$ for 5 minutes. Drain the supernatant and re-precipitate with distilled water and it centrifuged in $800 \times g$ for 5 minutes. The resulting precipitate was mixed with 20 ml of distilled water and divided evenly into four tubes of 5 ml. The contents of each tube slowly added on the 3 ml of cold 85 percent sucrose with a pasteurizer pipette in to the 15 ml centrifugal with coned bottom tubes, so that two distinct phases were formed. The tubes were centrifuged in $600 \times g$ for 10 minutes, in which 4 layers were finally formed into them. The layer between water and sucrose (containing a large number of Giardia cysts) was carefully and patiently collected with the help of Pasteur pipette. In most purification methods, bacterial contamination is inevitable due to the entry of bacteria. To solve this problem in the present study, the method of Alvarado et al. was used (Alvarado et al., 2006). In this way, the collected layer is mixed with distilled water in proportion to 20 times its volume and then it smoothed using a vacuum-filtration system, by filtering 5 micron cellulose acetate membranes (Sartorius, Germany) .

Due to the small size, the bacteria passed through the filter pores and the remaining cysts on the filter surface were washed with 5 ml of distilled water and collected in a sterile petri dish. The fluid containing the cyst was transferred to sterile tubes. Number of cysts by homocytometry Neobar slides (Neobar slides by homocytometry method) are counted and their number in milliliters was calculated.

Number of cysts per 1 ml = average number of cysts counted in 4 squares for white blood cell \times Diluted factor $\times 10^4$.

The fluid contains the cyst was maintained until the test time at a temperature of 4 Celsius.

Artemisia and pomegranate was dried and powdered and autoclaved for 2 minutes in oven in laboratory condition, too.

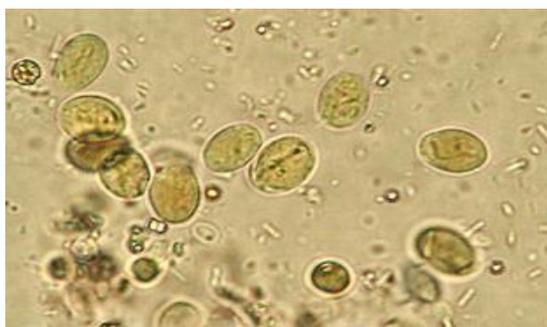


Figure 2: Cysts of *Giardia* are present in the feces imaged by Optical microscope; 100x magnification.

Data analysis method

In order to compare the means in different groups used to one-way analysis of variances (ANOVA) and independent t-test and to examine the relationship the variables were Spearman correlation coefficient was used.

Data analyzed using SPSS 14 software and GraphPad Prism 5 used to draw graphs. In all cases, $p \leq 0.05$ was considered as a significant level.

Findings (Results and Discussion)

The dogs of first (control group took 5mg/kg mebendazole tablets daily as orally for 5 days) and forth (received all of three above drugs (mebendazole tablets and autoclaved powder of Artemisia and punica granatum orally as dose 5mg/kg from each drug one time in the day for duration 5 days) groups had: Excessive diarrhea as mebendazole side effect and no giardia parasite in their stool on stool sampling, daily for 5 days. But second ((treatment) received the Artemisia autoclaved powder orally as 5mg/kg one time in the day for 5 days) and third ((treatment) received Punica granatum autoclaved powder as 5mg/kg in 5 days daily, orally) groups had no side effects and no GI giardiasis, two. This is the first research work in this comparative herbal drugs as powder so don't have any same results but before some ethanolic research has done in Iran as alone in laboratory not comparative so most research must be done for comparison. All vital signs came as the table 1 as below.

Table 1- Comparative study of changes in vital signs during 5 days of daily use of drugs

(5 dog in each group) (In all 4 groups Giardiasis had been treated)

Group 1 (Mebendazole)	Group 2 (Atemisia)	Group 3 (Punica granatum)	Group 4 (3 drugs)
Day 1 no sign	Day 1 no sign	Day 1 no sign	Day 1 no sign
Day 2 no sign	Day 2 no sign	Day 2 no sign	Day 2 no sign
Day 3 stomach pain	Day 3 no sign	Day 3 no sign	Day 3 stomach pain
Day 4 stomach pain and diarrhea	Day 4 no sign	Day 4 no sign	Day 4 diarrhea
Day 5 diarrhea	Day 5 no sign	Day 5 no sign	Day 5 diarrhea

Conclusions

In current report, both Artemisia and Punica granatum as herbal medicines had no side effects in comparison to mebendazole tablets as chemical drug, so we suggested after applying the same our research in different countries, replace them in dogs as herbal anti- Giardia drug because is safe, potent and available.

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