https://doi.org/10.31925/farmacia.2021.6.16 **ORIGINAL ARTICLE**

ANTI-*GIARDIA* ACTIVITY OF *TANACETUM VULGARE* FLOWERS EXTRACT ON INFECTED MICE

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Abstract

The *Giardia duodenalis* infection is considered one of the common pathologies caused by flagellated protozoa in humans, and medicinal plants are important sources of antiprotozoal agents with fewer side effects. The aim of this study was to evaluate the anti-*Giardia* activity of *Tanacetum vulgare* L. (*Asteraceae*), common tansy flowers hydroalcoholic extract. The experiment was conducted on mice infected with *G. muris*, using metronidazole and Giardiplant® (a commercial product) for comparison. After 5 days of treatment, the *G. muris* trophozoites count in the small intestine was significantly decreased ($p < 0.01$) in the group treated with *T. vulgare* tincture, compared to the group treated with metronidazole. The obtained results have shown that *T. vulgare* flowers extract has *in vivo* anti-*Giardia* effects and it can be considered a promising natural alternative for the treatment of giardiasis.

Rezumat

Infecția cu *Giardia duodenalis* este una din cele mai frecvente infecții cu protozoare flagelate la om, iar plantele medicinale reprezintă surse importante pentru obținerea de agenți antiprotozoare cu mai puține efecte secundare. Scopul acestui studiu a fost evaluarea activității anti-*Giardia* a extractului hidroalcoolic din flori de *Tanacetum vulgare* L. (*Asteraceae*), vetrice. Experimentul s-a realizat pe șoareci infectați cu *G. muris*, folosind metronidazol și Giardiplant® (un produs comercial) pentru comparație. După 5 zile de tratament, tinctura de *T. vulgare* a determinat scăderea semnificativă a numărului de trofozoiți de *G. muris* din intestinul subțire ($p < 0,01$), efect comparabil cu al metronidazolului. Rezultatele obținute au arătat că extractul din flori de *T. vulgare* are acțiune anti-*Giardia in vivo* și poate fi considerat o alternativă naturală promițătoare pentru tratamentul giardiozei.

Keywords: *Tanacetum vulgare*, tansy flowers, mice, anti-*Giardia* activity

Introduction

Giardia is a genus of flagellated protozoan parasites for which six species are currently recognised, including *Giardia agilis* in amphibians, *Giardia ardeae* and *Giardia psittaci* in birds, *Giardia muris* and *Giardia microti* in rodents and *Giardia duodenalis* in mammals [12]. *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*) is the only species found in humans, although it has also been reported in domestic, farmed and wild animals [21]. The current sub-classification of *G. duodenalis* indicates the heterogeneity of the organism that consists of eight (A-H) assemblages with human infections are caused by assemblages A and B; these can also infect other mammalian hosts with demonstrated potential for zoonotic transmission [12, 14]. As the recognised etiological agent of giardiasis in people, *G. duodenalis* is responsible for gastro-intestinal infections with severity ranging from asymptomatic to watery diarrhoea, associated with abdominal pain, malabsorption and body weight loss. Giardiasis is regarded as a parasitic disease of great epidemiological and clinical importance, due to its high prevalence and pathogenicity in animals as well as in humans. Although the incidence of giardiasis across the world has diminished in the last decade, a high number of cases are still reported, involving high risk of transmission and important costs. Among the drugs recommended for the treatment of giardiasis, metronidazole is the first choice, followed by others such as tinidazole, albendazole and furazolidone [1, 11]. These medications could be associated with drug resistance and potential risks of mutagenicity and carcinogenicity, as well as multiple undesirable side effects (e.g., metallic taste, headache, dry mouth, glossitis)

[1, 11]. Finding alternative solutions becomes imperative and in this regard, plants from several genres: *Artemisia, Allium, Curcuma, Cinnamomum, Mentha, Origanum, Punica, Trigonella, Satureja, Tanacetum, Zingiber* etc. have been studied and recommended as safe alternatives [2, 5-10, 17, 23, 24, 27-29]. Species of the genus *Tanacetum* have been used as medicinal plants for more than 2000 years [15]. *Tanacetum vulgare* L. (*Asteraceae* family), common tansy, is traditionally used to treat intestinal worms, rheumatism, digestive problems, in many countries, including Romania [18, 19, 26]. The flowers contain phenolic compounds (luteolin, apigenin, chlorogenic and caffeic acids), essential oil (with thujone, cineole), carotenoids, sesquiterpene lactones (eudesmanolides, parthenolide) [3, 15, 16, 20, 30]. Several studies have evaluated certain therapeutic properties, such as anti-inflammatory, antioxidant, antimicrobial, antiviral and antitumor activity [3, 4, 15]. To our knowledge, *T. vulgare* flowers extract has not been studied before for anti-*Giardia* activity, however it is empirical used as anthelmintic drug [5]. Therefore, the aim of the present study was to evaluate the *in vivo* anti-*Giardia* activity of tansy tincture. The study of the phytochemical composition and the analysis of the polyphenolic and volatile compounds from *T. vulgare* flowers were previously performed [15, 16].

Materials and Methods

Plant material, extraction procedure

The flowers of *T. vulgare* were collected during the blossom period (June, 2017) from the spontaneous flora of Transylvania, Cluj County, Romania. A specimen of these plants is deposited at the Department of Pharmacognosy, Faculty of Pharmacy Cluj-Napoca, Romania, (Voucher no. 26). The tincture (1:10) was prepared as follows: the plant material powder was extracted with 70% ethanol, using a simple maceration process, according to the method described in the Romanian Pharmacopoeia [31].

Experimental design and animals

The anti-*Giardia* activity was evaluated after the administration of three medicinal products: tincture of *T. vulgare*, Giardiplant® (Plafar SA, Romania) and metronidazole, respectively. Giardiplant® is a commercial phytotherapeutic product (containing *Calendula officinalis* hydro-alcoholic extract, *T. vulgare* hydro-alcoholic extract and *Thymi aetheroleum*), commonly used in giardiasis. In order to ensure the physical stability of the extractive products, *T. vulgare* tincture and the commercial product Giardiplant® were diluted with a micellar dispersion of 1% Tween 80, corresponding to obtain suitable volumes for administration. Metronidazole (chosen as antiprotozoal drug control) was administered as a suspension in 1% methylcellulose mucilage. The tested products were administrated by oral gavage in a single dose of 0.2 mL/mouse/day, each administration containing the established dose of treatment: 0.08 g *T. vulgare* tincture, 0.16 g Giardiplant® and 0.016 g metronidazole, respectively [22, 25, 27, 29]. Sixty male Swiss albino mice, free from any intestinal parasitic infection, aged 3 weeks and weighing 20 ± 5 g each (at the beginning of the experiment) were used. The animals were housed in a temperature and light-controlled room (21°C, a 12 h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. The animals were divided into 6 groups, 10 mice in each group: Group 1 - positive control, infected; Group 2 - negative control, uninfected; Group 3 – infected, received a saline solution (0.9% NaCl), placebo; Group 4 - infected, received *T. vulgare* tincture (0.08 g/mouse/day); Group 5 - infected, received Giardiplant® (0.16 g/mouse/day); Group 6 - infected, received a suspension of metronidazole (0.016 g/mouse/day) [25].

Animals' infection, confirmation of the infection and treatment

The mice from groups 1, 3, 4, 5 and 6 were intragastrically infected with 1 x 10^5 *Giardia muris* cysts/mL. The infectious material was obtained from 10 sacrificed mice, already infected with *G. muris* (from the Laboratory Animal Facility of UASVM Cluj-Napoca, Romania), which was mixed with 0.9% NaCl solution. The infection of mice was confirmed by intestinal examination, using a direct microscopic method (200x magnification), to reveal *Giardia* trophozoites [25]. Subsequently, fresh faecal pellets were collected and examined every 24 h to prove the experimental infection, using the Blagg method with some modifications, to reveal *Giardia* cysts [25]. Throughout the experiment, the mice were kept under the same conditions, in terms of maintenance status (the bedding was replaced every 2 days) and nutrition, and their clinical status was also constantly monitored [2, 25, 29].

Prior to treatment, on the eighth day after infection and on the tenth day after infection, respectively, control sacrifices were performed from each group, with one mouse *per* group being sacrificed. The duodenal mucosa was scraped in 0.9% NaCl solution, and the viability and number of parasites were monitored by direct microscopic examination method (200x magnification). The intensity of infection was evaluated by counting the identified parasites in microscopic fields. The count was done on 10 microscopic fields, and then the average/field was determined [25]. After 8 days, the groups 3, 4, 5 and 6 received the treatments presented above. Faecal samples were collected at the beginning of the experiment (day 1), at the beginning of treatment (day 8), after 3 days of treatment (day 10) and at the end of treatment (day 12), then analysed by the Blagg method with some modifications, to reveal *Giardia* cysts [25]. The treatment duration was 5 days. On the sixth day (day 13 of the experiment), all mice were sacrificed to evaluate the number of *G. muris* vegetative forms (trophozoites) in the small intestine. The efficacy of the

therapy was assessed by direct microscopic examination (200x magnification) of duodenal scraping. Attachment of *Giardia spp.* trophozoites to enterocytes is essential for colonization of the small intestine and it is considered a prerequisite for parasite-induced enterocyte dysfunction and clinical disease. The duodenum of each mouse was removed and placed in a Petri dish containing 1 mL 0.9% NaCl solution. This dish was vortexed to release the trophozoites from the intestinal wall. Trophozoites were counted using a haemocytometer, in ten fields, with 20x microscopic lens [13, 25].

Ethical considerations

The mice were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare from Romania and all procedures were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. According to the 43/2014 national law for the protection of animals used for scientific purposes, the project was approved by the Commission for Bioethics and Research Ethics of UASVM and also, efforts were made to minimize animal suffering and to reduce the number of used animals.

Statistical analysis

T-test was used for comparison between control and treated groups. The p-value < 0.05 was considered as significant difference between groups. Data were displayed as mean ± SD.

Results and Discussion

The results of investigating the *in vivo* effect of *T. vulgare* extract on mice experimentally infected with *G. muris* are shown in Table I and Figure 1. Metronidazole was used as antigiardial drug and Giardiplant®, a commercial product, was chosen only for comparison, due to its content in *T. vulgare* extract.

Table I

Giardia muris trophozoites count for the studied groups

Groups	Trophozoites count average/field		
	Day 8 (1st day of treatment)	**Day 10 (3rd day of treatment)**	**Day 13 (after 5 days of treatment)**
1 - infected, without treatment (positive control, Pc)	54.9 ± 2.10	55.2 ± 0.80	55.22 ± 0.87
2 - uninfected (negative control, Nc)	0	0	0
3 - infected, saline treatment (placebo, P)	51.8 ± 1.20	50.8 ± 0.20	51.63 ± 0.77
4 - infected, *Tanacetum vulgare* extract treatment (TvE)	51.6 ± 1.40	10.4 ± 0.40	2.11 ± 0.38^{a}
5 - infected, Giardiplant® treatment (G)	39.8 ± 2.20	14.1 ± 0.10	$6.08 \pm 0.15^{b,e}$
6 - infected, metronidazole treatment (M)	28.6 ± 1.20	14.4 ± 0.90	$2.45 \pm 0.68^{c,d}$

The values represent the average of three determinations ± SD. [a]p < 0.01 (group 1 *versus* group 4), [b]p < 0.01 (group 1 *versus* group 5), [c]p < 0.01 (group 1 *versus* group 6), [d]p > 0.05 (group 4 *versus* group 6), [e]p < 0.01 (group 4 *versus* group 5).

Thus, on the 3rd day of treatment, the *T. vulgare* tincture showed significant activity against flagellates ($p < 0.01$), with the *G. muris* trophozoites number decreasing from 51.6 ± 1.40 to 10.4 ± 0.40 trophozoites/field, while metronidazole and Giardiplant® produced a decrease of up to about 14 (Table I, Figure 1). After 5 days of treatment, *T. vulgare* tincture greatly reduced the count of trophozoites of *G. muris* in the duodenum (2.11 ± 0.38 trophozoites/field), comparable with metronidazole (2.45 ± 0.68), and more effective than Giardiplant® (6.08 ± 0.15, $p < 0.01$). The *T. vulgare* tincture could affect the attachment of trophozoites, leading to their sliding and disintegration from the intestinal mucosa [13]. Our tincture exhibited a significant reduction in the number of trophozoites recovered from the intestine, compared to the positive control (group 1; $p < 0.01$). However, there were no significant differences between *T. vulgare* extract treated group (group 4) and Metronidazole treated group (group 6) ($p > 0.05$).

According to Table I, significant differences ($p < 0.01$) were observed between the positive group (group 1) and Giardiplant® group (group 5), and metronidazole group (group 6), respectively.

Previous studies have evaluated the anti-*Giardia* activity for hydro-alcoholic extracts of *Rosmarinus officinalis* [27], *Mentha x piperita* [28], *Origanum vulgare* [6], known for their rich polyphenolic content. High concentrations of some phenolic compounds, such as phenolic acids (gallic, ellagic, caffeic, p-coumaric, vanillic acid) or flavonoids (quercetin), were related with the giardicidal activity of pomegranate peel methanolic extract [29]. Another research showed that higher levels of phenolic compounds (chlorogenic acid, rosmarinic acid, flavonoids) from *Origanum vulgare* extract could be responsible for anti-*Giardia* activity, by interacting with cysts membrane [6].

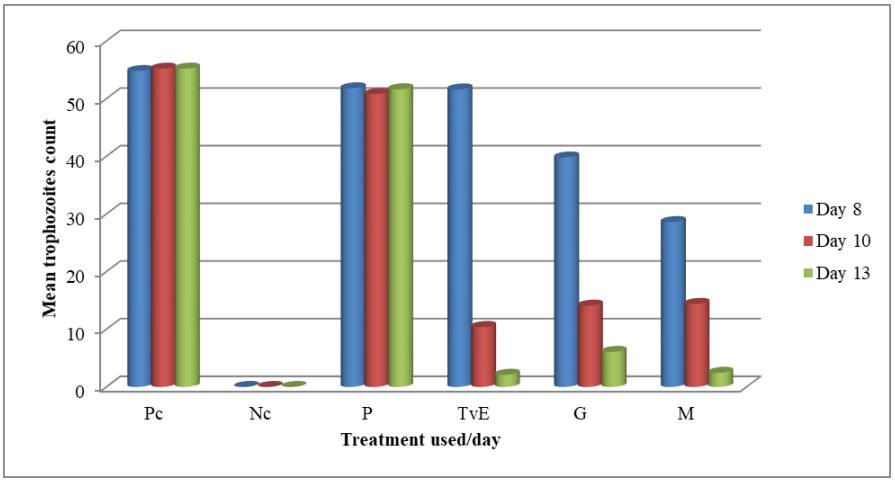

Figure 1.
Giardia muris trophozoites count for control, infected and treated groups in the 8th, 10th and 13th days
Pc = positive control, Nc = negative control, P = placebo, TvE = *T. vulgare* extract treatment,
G = Giardiplant® treatment, M = metronidazole treatment

The phenolic compounds identified in our previous research on *T. vulgare* flowers (phenolic acid derivatives: chlorogenic, p-coumaric, ferulic acids and flavonoids: quercitrin, hyperoside, rutin, isoquercitrin, quercetin, apigenin, luteolin) [15], as well as the terpenes, could be involved in the efficacy of treating the infection caused by *Giardia spp*. Through this first study *in vivo* that used the experimental infection with *G. muris* as a model, the potential therapeutic effect of *T. vulgare* tincture in giardiasis illness is sustained, as a promising alternative to the antiprotozoal drugs commonly administered.

Conclusions

To our knowledge, this is the first study to evaluate and demonstrate the anti-*Giardia* effect of the *T. vulgare* tincture in mice experimentally infected with *G. muris*. Our results provide relevant scientific proof of the *in vivo* efficacy of tansy flowers tincture in the treatment of infections caused by *G. muris*, comparable with Metronidazole, but without its adverse reactions. The *T. vulgare* extract can be considered a promising alternative to anti-*Giardia* drugs recommended for the treatment of giardiasis, and this study may direct further research concerning its efficacy against other flagellated parasites.

Conflict of interest

The authors declare no conflict of interest.

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