ORIGINAL ARTICLE

GIARDICIDAL EFFECT OF BLACK TEA (*CAMELLIA SINENSIS*) IN THE TREATMENT OF *GIARDIA DUODENALIS* IN SWISS MICE

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Highlights: (1) Black tea enhances the giardicidal effect of metronidazole through synergistic action. (2) *Giardia duodenalis* shows increasing resistance to conventional metronidazole therapy (3) Black tea emerges as a promising phytotherapeutic alternative for giardiasis treatment. (4) The giardicidal activity of black tea is dose-dependent.

PRE-PROOF
(as accepted)
This is a preliminary, unedited version of a manuscript accepted for publication in Revista Contexto & Saúde. As a service to our readers, we are making this initial version of the manuscript available as accepted. The article will still undergo revision, formatting, and author approval before being published in its final form.

http://dx.doi.org/10.21527/2176-7114.2025.50.15395

How to cite:
Romera LI, Colli CM, dos Santos AGA, de Melo G de NA, Ferrari A, Gomes ML. Giardicidal effect of black tea (Camellia sinensis) in the treatment of Giardia duodenalis in swiss mice. Rev. Contexto & Saúde. 2025;25(50):e15395

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GIARDICIDAL EFFECT OF BLACK TEA (*CAMELLIA SINENSIS*) IN THE TREATMENT OF *GIARDIA DUODENALIS* IN SWISS MICE

ABSTRACT

This study evaluated the *in vivo* giardicidal potential of black tea (*Camellia sinensis*) in male Swiss mice experimentally infected with 10^4 cysts of *Giardia duodenalis* assemblage BIV. The animals were assigned to nine groups and treated with filtered water (GC+), metronidazole (MT, 500 mg/kg/day), black tea (CP) at 100, 150, or 200 mg/kg/day, or combinations of CP with MT. A negative control group (GC−) included uninfected animals receiving filtered water. Treatments were administered for seven days, and infection status was assessed through parasitological analysis and Polymerase Chain Reaction (PCR). CP at 200 mg/kg induced complete elimination of *G. duodenalis*, while doses of 100 and 150 mg/kg reduced parasite load from high to medium and low levels, respectively. MT alone did not result in cure but reduced parasite burden. The combination of MT with any CP dose achieved full parasite clearance, indicating a synergistic effect. These results demonstrate a dose-dependent giardicidal effect of black tea and suggest that its combination with metronidazole enhances treatment efficacy. The findings reinforce the therapeutic potential of phytotherapeutics as effective, low-cost adjuncts in the management of giardiasis. From a health promotion perspective, the identification of natural antiparasitic compounds supports the development of accessible and sustainable treatment strategies. This is particularly relevant in resource-limited settings, where conventional therapies may be less accessible or poorly tolerated. Further research, including clinical trials and mechanistic studies, is warranted to explore the broader applicability of these combinations in human populations.

Keywords: Dose-Response Relationship, Drug; Drug Resistance, Microbial; Drug Synergism; Metronidazole; Phytotherapy; Polymerase Chain Reaction.

1.Introduction

Giardia duodenalis (synonyms: *Giardia lamblia* and *Giardia intestinalis*), the aetiological agent of giardiasis is a eukaryotic, unicellular, flagellated microorganism. It infects the upper intestinal tract of animals and humans[1]. In humans, it is transmitted mainly through accidental ingestion of cysts in contaminated water and food. It occurs in two evolutionary forms: the cyst, which is infective and highly resistant to the environment, and the trophozoite, which colonises the intestinal mucosa and causes disease[2].

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Giardiasis has a global distribution and is one of the most common parasitic diseases worldwide[3]. It is considered a leading cause of diarrhoea and the second leading cause of waterborne gastroenteritis[4], with an estimated annual transmission affecting over 200 million people[3]. It occurs in both developed and developing countries, being more prevalent among children living in tropical regions and under unsanitary conditions[5]. The disease may be asymptomatic or symptomatic, presenting with chronic diarrhoea and intestinal malabsorption, which can lead to impaired growth as well as intellectual and cognitive developmental delays in children[6].

In humans, the main drugs available for the treatment of giardiasis are nitroimidazoles such as metronidazole, albendazole, secnidazole and tinidazole, with efficacy ranging from 80% to 100%[7], and nitazoxanide. These pharmacological agents have different modes of action and involve a variety of cytotoxic labelling and processing mechanisms that affect the cellular structure of trophozoites[7].

Metronidazole has been recommended as the drug of first choice for the treatment of giardiasis[8], but like most of the drugs used, it is often ineffective at targeting key cellular processes such as trophozoite proliferation and encystation. leading to drug resistance in chronic cases[9]. In addition, it has adverse effects such as metallic aftertaste, gastrointestinal disturbances, nausea, headache, leukopenia[10], neurotoxic effects, ataxia, convulsions and dizziness, which may lead to treatment discontinuation[8].

The revealed limitations associated with treatment duration, depending on the drug used, and the genetic diversity of the parasite, have led us to seek alternative strategies and new compounds for the treatment of giardiasis, in order to improve efficacy and reduce adverse effects. Phytotherapy is increasingly being used as an alternative to allopathic drugs. For many centuries, medicinal plants have been used in different parts of the world for the treatment of various diseases[11].

In recent years, medicinal plants have been increasingly utilised, owing to a growing number of studies demonstrating more reliable, cost-effective, and efficacious outcomes. Giardicidal[12,13] and anthelmintic[14] effects have been reported for phytogenic compounds, including *Camellia sinensis*, due to its bioactive constituents, which confer both pharmacological and physiological properties[15].

Black tea, which is obtained by roasting the leaves of *C. sinensis*, family Teaceae, is one of the most widely consumed teas in the world[16]. During the production of black tea, catechins are oxidised by polyphenol oxidase to produce various polyphenols, such as oligomers –theafavins, and polymers – polymeric black tea polyphenols/thearubigins[16,17]. Studies have shown that these compounds have beneficial effects in preventing diarrhoea, controlling gastrointestinal disorders, and antibacterial activity[18], with no apparent side effects. In addition, they possess regulatory activities in the intestinal microbiome, inducing the proliferation of beneficial bacteria that constitute an efficient defence mechanism of the innate immune system[19] and inhibit colonisation by pathogens[20]. The potential of these natural compounds to prevent or mitigate infection supports the development of integrative strategies aligned with the principles of health promotion, especially in regions with limited access to conventional treatments[21].

A prior study[13] showed that black tea at 100 mg/kg reduced parasite load but failed to clear the infection, revealing a dose-limited efficacy. Building upon these findings, the present research introduces a novel dose–response approach and evaluates the unexplored potential of black tea in combination with metronidazole to enhance antiparasitic outcomes.

Given the need for alternative therapies for giardiasis that offer improved efficacy and fewer side effects than currently available pharmacological options, and considering the promising preliminary results of black tea, the present study aimed to evaluate the giardicidal potential of black tea, both alone and in combination with metronidazole, at higher concentrations than those previously tested. Using a Swiss mouse model infected with *G. duodenalis* assemblage BIV, we investigated whether this phytotherapeutic approach could enhance antiparasitic efficacy and provide a basis for integrative therapeutic strategies.

2. Materials and Methods

2.1. Ethical Aspects

This study was approved by the Ethics Committee on Animal Use of the State University of Maringá, Paraná, Brazil (CEUA/UEM – process number 1360080620), and all guidelines of the Brazilian Society for Laboratory Animal Science (Sociedade Brasileira de Ciências em Animais de Laboratório – SBCAL) were followed. The ethical standards adhered to in this

study also encompassed the ARRIVE 2.0 guidelines, which are widely recognised and promote transparency and reproducibility in research. Both frameworks share the same fundamental principles of ethics, transparency, and scientific rigour, all of which were upheld throughout this study.

2.2. Animals

Non-isogenic (90) male Swiss mice (*Mus musculus*), 21 days old, from the Central Animal Facility of the State University of Maringá (UEM) were used in this study. The experiments were conducted between January and June 2023, with each repetition (performed in duplicate) lasting an average of 30 days. The study was conducted in a blinded, controlled, and randomised manner, with an equal number of animals in each group.

The animals were housed in the vivarium of the Laboratory of Environmental and Food Parasitology of the University of Maringá (LPAA/UEM) and underwent a seven-day acclimatisation period before the start of the experiments. They were housed in Alesco® microacclimated polysulfone cages (20 cm wide x 32 cm long x 21 cm high) in a climate-controlled animal house (22.7 ± 1.2°C) with a 12-hour light/dark cycle. Filtered water and food (Nuvital® Nuvilab Cr-1) were provided *ad libitum*. All materials used, including the sieve, were sterilised in a wet autoclave at 121°C for 20 minutes.

2.3. Inoculum and infection with *G. duodenalis* cysts

G. duodenalis cysts were isolated from freshly collected human stool samples, genotyped as BIV by PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) and stored at -20°C at LPAA/UEM, with the approval of the Standing Committee on Ethics in Research with Humans (COPEP/UEM - 439/2009).

The Trypan Blue test[22] was used to determine the viability of the cysts used to infect the animals. The 0.4% dye was added in a 1:1 ratio to a previously quantified cyst concentrate. Viable cysts were quantified in a volume of 0.4 µl in a Neubauer chamber. Counting was carried out in the four lateral quadrants. The final result was calculated using the following equation: (A×2,5×2), where A = number of cysts counted on the grids; 2.5 = Neubauer chamber correction factor; 2 = dilution factor.

The inoculum was prepared with 0.85% saline and contained 10^4 viable *G. duodenalis* cysts[23] in a volume of 300 µl/animal. A gavage tube was used for intragastric administration.

After receiving the *G. duodenalis* cyst inoculum, animals were monitored daily by parasitological examination and quantification of cysts excreted in faeces.

2.4. Treatments preparation

The black tea was commercially acquired from Fazenda Amaya, located in the Mantiqueira Valley, São Paulo, Brazil. Amaya Chás is recognised for producing premium-quality teas, cultivated in acidic soil near the Ribeira de Iguape River, in a region with an ideal climate and soil for *Camellia sinensis*. Metronidazole, in oral suspension form, used in this study was commercially acquired from a dispensing pharmacy and manufactured by a pharmaceutical company regulated by the Brazilian Health Regulatory Agency (ANVISA).

The black tea was prepared daily, according to Almeida et al.[13], with modifications, in which 2 g of dried herb was added to a beaker containing 100 ml of distilled water, obtaining a concentration of 20 mg/ml (2 mg in every 0.1 ml). The system was covered and maintained at 92°C for 15 minutes with magnetic stirring (400 rpm). After cooling, the contents were filtered through standardised filter paper, and the volume was corrected in a volumetric flask.

The doses used in this study were selected based on the work of Almeida et al.[13], who observed that a dose of 100 mg/kg of black tea only partially reduced the parasite load in Swiss mice infected with *Giardia duodenalis* (assemblage BIV). Therefore, this dosage was adopted as the starting point for the experiments and was increased to 150 and 200 mg/kg in order to investigate potential dose-dependent effects. As the dosages of 100, 150, and 200 mg/kg were adjusted according to the animals' weight, the mice were weighed daily to ensure accurate dose administration. Different volumes were administered depending on the dosage and the weight of each animal, with a maximum volume of 500 µl. The dosage and administration of metronidazole were based on the studies by Almeida et al.[12], Almeida et al.[13], and Bezagio et al.[24], who used 500 mg/kg for the treatment of *Giardia* spp.

2.5. Experimental Groups and Treatment Protocols

For each of the two independently conducted experiments, nine experimental groups were used: GC+ (Positive control group – infected and untreated animals); GCP1, GCP2, and GCP3 (Black

tea groups – infected and treated with black tea); GMT (Metronidazole group – infected and treated); GCM1, GCM2, and GCM3 (Black tea and metronidazole groups – infected and treated with combinations of black tea and metronidazole); and GC− (Negative control group – uninfected and untreated animals). Each group consisted of five mice. The groups, drugs, and treatment protocols are described in **Table 1**. The drugs were administered both separately and in combination to optimise treatment outcomes and to explore potential synergism between them.

Regarding the timing of treatment initiation, the choice was based on the pre-patency period, defined as the interval between infection and the onset of cyst shedding in the faeces. Romera et al.[23] reported a pre-patency period of six days. In addition to the information available in the literature, daily parasitological monitoring was performed to ensure that treatment commenced only when the animals exhibited a high parasite load. Treatment began on day eight post-infection (dpi), and parasite load was monitored daily until day 15 dpi, which corresponded to the day after treatment completion.

Table 1. Experimental groups and treatment protocols for the administration of *Giardia duodenalis* cyst-infected male Swiss mice.

Groups	No. of Animals	Treatment Protocols**
[1]**GC+**	5	Filtered Water 1×day/7 days
[1]**GCP1**	5	100 mg/kg Black Tea* (*Camellia sinensis*) 1×day/7 days
[1]**GCP2**	5	150 mg/kg Black Tea* (*Camellia sinensis*) 1×day/7 days
[1]**GCP3**	5	200 mg/kg Black Tea* (*Camellia sinensis*) 1×day/7 days
[1]**GMT**	5	500 mg/kg Metronidazole 1×day/7 days
[1]**GCM1**	5	100 mg/kg Black Tea* (*Camellia sinensis*) + 500 mg/kg Metronidazole 1×day/7 days
[1]**GCM2**	5	150 mg/kg Black Tea* (*Camellia sinensis*) + 500 mg/kg Metronidazole 1×day/7 days
[1]**GCM3**	5	200 mg/kg Black Tea* (*Camellia sinensis*) + 500 mg/kg Metronidazole 1×day/7 days
[2]**GC-**	5	Filtered Water 1×day/7 days

GC+: Positive control group - Infected and untreated animals; GCP1, GCP2, and GCP3: Black tea groups - Infected and treated with black tea; GMT: Metronidazole group - Infected and treated with metronidazole animals according to protocol by Bezagio et al.[24]. GCM1, GCM2, and GCM3: Black tea and metronidazole groups - Animals infected and treated with combinations of black tea and metronidazole. GC-: Negative control group - Uninfected and untreated animals.

Source: author's own elaboration

2.6. Parasitological evaluation before and after treatment

Faecal material (0.5 g) was pooled as a pool from each group and stored in preservative-free polypropylene bottles. Samples were prepared according to the method of Faust et al.[26] and analysed microscopically. Analyses were performed daily from the first day of infection until 15 dpi, which was the day after the treatment ended.

Cyst quantification was performed according to the semi-quantitative method established by Uda-Shimoda et al.[27] In the semi-quantitative analysis of the parasitological stool test, smears were inspected on 22 × 22 mm glass slides, and the number of cysts in each microscope field was counted using a 20× objective. Samples with up to 1 cyst, 1–2 cysts, and more than 2 cysts per field were considered to contain low, medium, and high numbers of cysts, respectively.

2.7. Molecular testing before and after treatment

A pool of stool samples (1 g) was collected from each group and processed using the modified Ritchie method[28]. DNA was extracted using the PureLink PCR Purification® Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations and Uda-Shimoda et al.[27] The Glutamate Dehydrogenase (GDH) gene was amplified using the Semi-Nested Polymerase Chain Reaction (PCR) method with modifications proposed by Colli et al.[29] for the identification of *G. duodenalis*. DNA was amplified by semi-nested (PCR) using external forward primer GDHeF (5'TCAACGTYAAYCGYGGYTTCCGT3'), internal forward primer GDHiF (5'CAGTACAACTCYGCTCTCGG3'), and reverse primer GDHiR (5'GTTRTCCTTGCACATCTCC3'), generating an approximately 432-base pair (bp) fragment of the GDH. Each amplification reaction was performed in a final volume of 11 µL, containing buffer 10× (200 mmol/L Tris-HCl pH 8.4, 500 mmol/L KCl, 1.5 mmol/L MgCl2, 0.5 U of Platinum Taq DNA Polymerase (Invitrogen), 200 µmol/L of deoxyribonucleotide triphosphates, 2 pmol of each primer, sterile Milli-Q water, and 2 µL of total DNA. The conditions for amplification were: denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 45 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 5 min, in the first and second reactions. The amplified products were visualized in 4.5% polyacrylamide gels, silver stained, and digitally recorded.

2.8. Total and differential Leukocyte count

Total and differential leukocyte counts were performed at 7 dpi, the day before the start of treatment, and at 15 dpi, one day after the end of treatment. Animals were immobilised in a restraining frame for tail vein puncture (in accordance with the NC3Rs standards of the National Centre for the Replacement, Refinement and Reduction of Animals in Research).

For the total count, 10 µL of blood was collected from each animal individually. The collected volume was added to 190 µL of Turk's fluid (1:20 dilution) and counted in a Neubauer chamber in triplicate. The final result was calculated using the following equation: $(WBC \times 20 \times 10)/4$, where WBC = mean white blood cell count; 20 = dilution factor; 10 = conversion factor to microlitres; 4 = conversion factor to 1 µL (mm^3) of blood[30].

For the differential leukocyte count, a blood smear was prepared on a glass slide and stained using the May–Grünwald–Giemsa method. The count was conducted on 100 leukocytes, which were differentiated according to their morphological types (segmented neutrophils, eosinophils, basophils, lymphocytes, and monocytes). Differential leukocyte counts were performed at both 7 and 15 dpi. The values were expressed per µL of blood and referred to the absolute number.

2.9. Monitoring of Clinical Parameters

During the infection and treatment periods, the animals were monitored for quantitative (weight, water and food consumption, and excreta elimination) and qualitative (fur and faecal appearance) clinical aspects, in accordance with the parameters established by Bezagio et al.[24].

2.10. Euthanasia, Necropsy, and Evaluation of Intestinal Scrape

The day after the end of treatment (15th dpi), the mice were anaesthetised in a saturated vapour chamber with inhaled isoflurane (CAS 26675-46-7) and euthanised by craniocervical dislocation. The small intestines of all animals were removed by opening the peritoneal cavity with a median incision and rinsed with 0.85% saline. The intestines were cut lengthwise, immersed in 25 ml of saline and scraped with a glass slide[13]. A portion of the fresh sample was examined by direct microscopy for *Giardia* trophozoites in the intestinal mucosa.

2.11. Cure criterion

Animals that were negative by both parasitological and molecular assays and did not show trophozoites on microscopic examination of intestinal scrapings at 15 dpi, the day after the end of treatment, were considered cured.

2.12. Statistical analysis

Statistical tests were defined according to data distribution using the Shapiro-Wilk test. Data with a normal distribution was compared between the groups by two-way analysis of variance (ANOVA) followed by Tukey's test, and for those with a free distribution, Kruskal-Wallis was used. A significance level of 5% ($p < 0.05$) was used for all tests.

3. Results

At 7 days post-infection (dpi), the number of cysts excreted by animals in all groups was high, with more than 50 cysts per field at 20× microscopic magnification. Amplification of an approximately 432 bp fragment of the GDH gene (**Figure 1A**) confirmed infection in animals in all groups (**Table 1**) that received *G. duodenalis* cysts.

At 15 dpi, the day after the end of treatment, animals in the GC+, GCP1, GCP2, and GMT groups continued to excrete cysts in their faeces, whereas animals in the GCP3, GCM1, GCM2, and GCM3 groups were considered cured. The GC+ group exhibited a high parasite load (>50 cysts per field), the GCP1 group showed a moderate load (1–2 cysts per field), and the GCP2 and GMT groups presented a low load (0–1 cyst per field) (**Figure 2**). In contrast, no faecal cysts were observed in animals from the GCP3, GCM1, GCM2, and GCM3 groups during parasitological examination. These negative findings were further confirmed by molecular analysis and by the absence of trophozoites in intestinal scrapings. The same results were observed in the negative control group (GC−) (**Figure 1B**).

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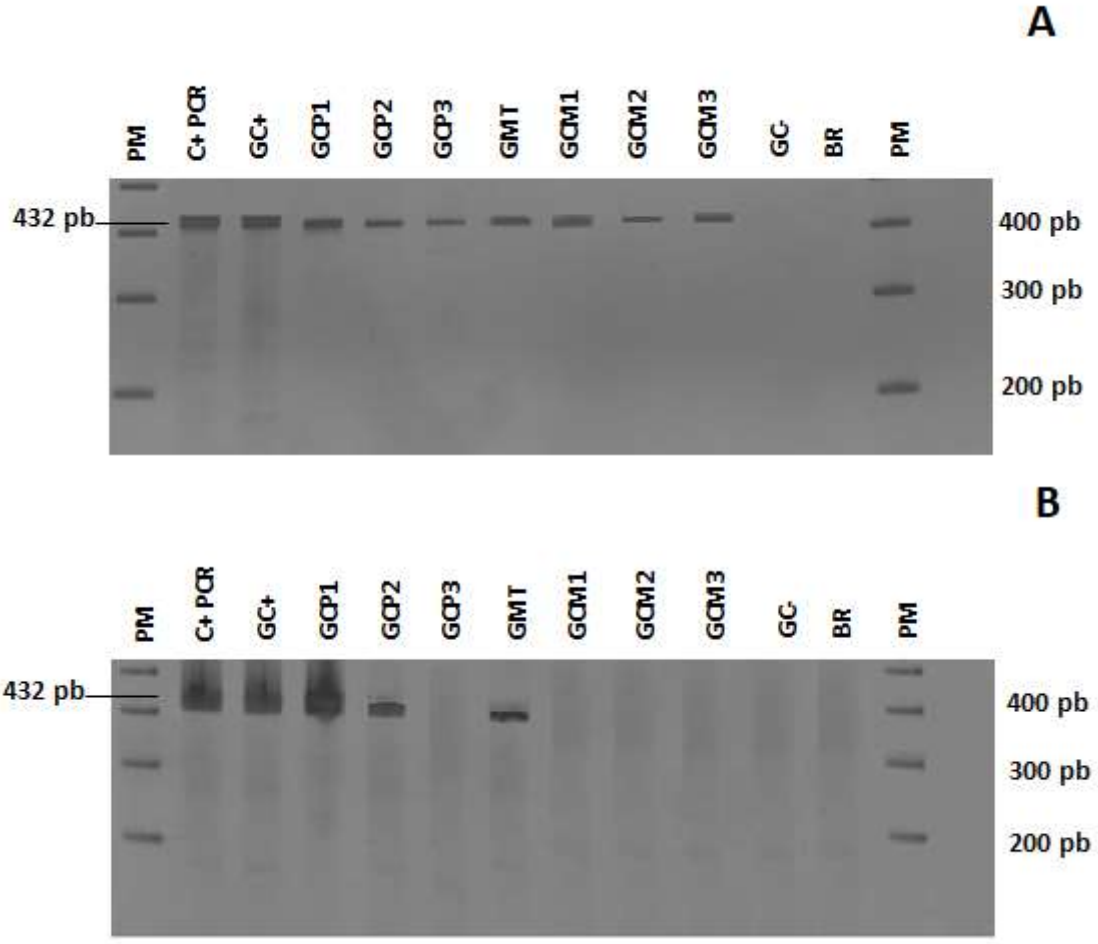

Figure 1. Representative amplification gel showing a fragment of approximately 432 bp of the Glutamate Dehydrogenase (GDH) gene in stool samples from male Swiss mice infected with *G. duodenalis* cysts, visualized on a 4.5% polyacrylamide gel stained with silver nitrate.

PM: 100 bp DNA Ladder molecular weight; C+ PCR: Polymerase Chain Reaction (PCR) positive control, DNA extracted from *G. duodenalis*; GC+: Infection positive control; GCP1: Black tea treated group 100 mg/kg/day; GCP2: Black tea treated group 150 mg/kg/day; GCP3: Black tea treated group 200 mg/kg/day; GMT: Group treated with 500 mg/kg/day metronidazole; GCM1: Group treated with black tea 100 mg/kg/day in combination with metronidazole; GCM2: Group treated with black tea 150 mg/kg/day in combination with metronidazole; GCM3: Group treated with black tea 200 mg/kg/day in combination with metronidazole; GC-: Infection Negative control; BR: PCR Negative Control, all reagents except DNA.
Samples were collected on dpi 7, before the start of treatment, to confirm infection (A); Samples were collected on dpi 15, the day after the end of treatment (B).

Source: developed by the authors.

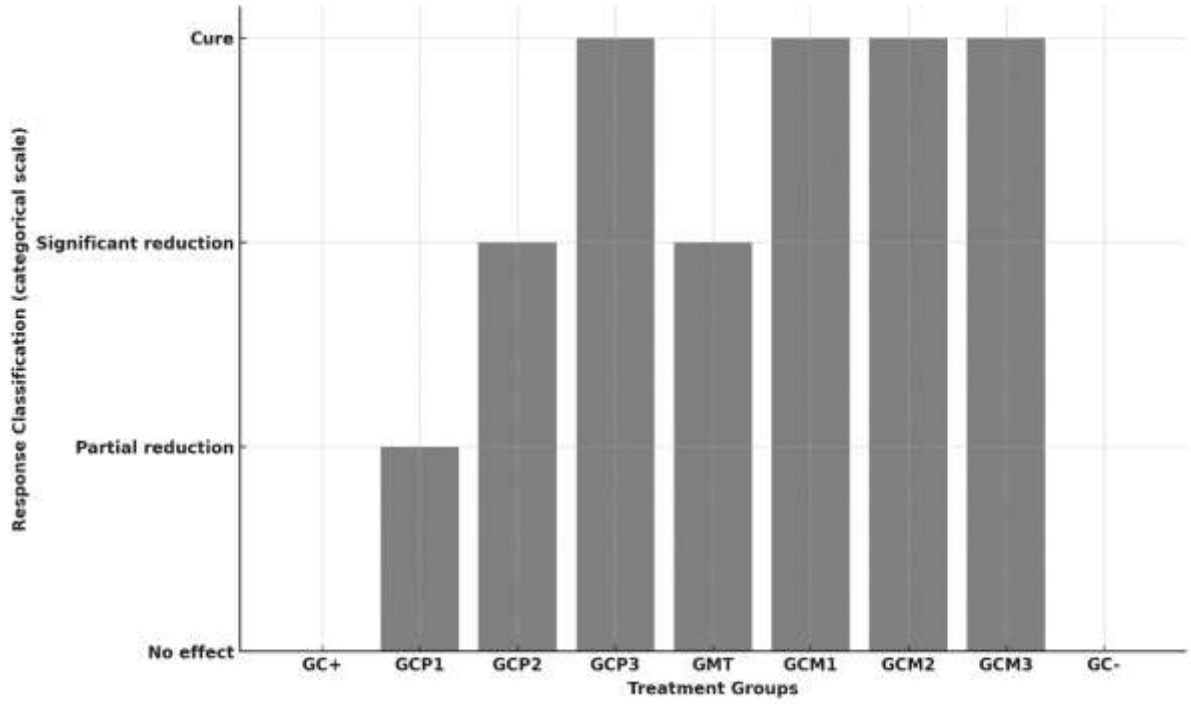

Figure 2. Dose-response relationship between treatment and faecal cyst elimination in male Swiss mice infected with *Giardia duodenalis*.

Partial reduction = refers to a decrease in parasite load from high (>50 cysts/field) to moderate levels (1–2 cysts/field); Significant reduction = refers to a decrease in parasite load from high (>50 cysts/field) to low levels (0–1 cysts/field).
GC+: Infection positive control; GCP1: Black tea treated group 100 mg/kg/day; GCP2: Black tea treated group 150 mg/kg/day; GCP3: Black tea treated group 200 mg/kg/day; GMT: Group treated with 500 mg/kg/day metronidazole; GCM1: Group treated with black tea 100 mg/kg/day in combination with metronidazole; GCM2: Group treated with black tea 150 mg/kg/day in combination with metronidazole; GCM3: Group treated with black tea 200 mg/kg/day in combination with metronidazole; GC-: Infection Negative control;

Source: developed by the authors

A fresh examination of the intestinal scrapings revealed the absence of *Giardia* trophozoites in animals from the GCP3, GCM1, GCM2, GCM3, and GC- groups, which correspond to those treated with 200 mg/kg/day of black tea (CP), the combination of CP and metronidazole (MT), and the negative control group, respectively. The GCP1 group presented 1–2 trophozoites per

field, while the GCP2 and GMT groups exhibited up to 1 trophozoite per field. The GC+ group showed a high parasite burden, with more than 40 trophozoites per field.

Total leukocyte count results at 7 dpi indicated leukocytosis in peripheral blood samples from infected animals when compared to non-infected ones. By 15 dpi, a reduction in leukocyte numbers was observed in the treated groups (GCP1, GCP2, GCP3, GMT, GCM1, GCM2, and GCM3) compared to the positive control group (GC+), as presented in **Table 2**.

Table 2. Total Leukocyte counts in blood samples obtained from the tail vein of male Swiss mice infected with *G. duodenalis* cysts before and after treatment.

Groups		**7th dpi[1]**	**15th dpi[2]**	
GC+	Range	6.80 – 14.70	9.25 – 11.50	$\times 10^3/mm^3$
	Mean ± SD	11.28 ± 3.63	10.20 ± 1.18	
GCP1	Range	13.20 – 15.80	4.80 – 6.55	$\times 10^3/mm^3$
	Mean ± SD	14.70 ± 1.34	5.54 ± 0.67	
GCP2	Range	6.20 – 11.35	1.90 – 5.10	$\times 10^3/mm^3$
	Mean ± SD	8.76 ± 2.40	3.46 ± 1.46	
GCP3	Range	6.15 – 10.45	2.15 – 6.25	$\times 10^3/mm^3$
	Mean ± SD	7.85 ± 1.99	4.53 ± 2.08	
GMT	Range	14.70 – 19.90	7.50 – 11.90	$\times 10^3/mm^3$
	Mean ± SD	17.39 ± 2.25	9.71 ± 1.58	
GCM1	Range	5.40 – 10.55	2.00 – 5.60	$\times 10^3/mm^3$
	Mean ± SD	7.15 ± 2.94	3.96 ± 1.29	
GCM2	Range	12.35 – 16.85	3.65 – 6.70	$\times 10^3/mm^3$
	Mean ± SD	14.70 ± 2.19	4.94 ± 1.19	
GCM3	Range	10.65 – 15.00	2.55 – 5.40	$\times 10^3/mm^3$
	Mean ± SD	11.82 ± 2.12	3.98 ± 1.28	
GC-	Range	2.75 – 5.95	2.00 – 4.95	$\times 10^3/mm^3$
	Mean ± SD	3.80 ± 1.24	3.26 ± 1.19	

Values are expressed as range (minimum–maximum) and mean ± standard deviation (SD).
[1]The 7th day post-infection (dpi) corresponds to the time point at which the parasite load in infected animals was classified as high, according to the reference standards of Uda-Shimoda et al.[27]
[2]The 15th dpi refers to the day on which the animals were euthanised, i.e., the day following the end of treatment.
The unit of measurement used was µl (or mm^3) of blood.

Source: author's own elaboration

At 7 dpi, an increase in eosinophils was observed in the infected groups (GC+, GCP1, GCP2, GCP3, GMT, GCM1, GCM2, and GCM3) compared to the uninfected group (GC−) ($p = 0.0001$). At 15 dpi, the treated groups (GCP1, GCP2, GCP3, GMT, GCM1, GCM2, and GCM3) exhibited lower levels of eosinophils than GC+ ($p = 0.001$).

Regarding the clinical parameters evaluated (**Table 3**) throughout the treatment, animals in the GC+ group consumed more water and food and excreted more faeces compared to the other experimental groups, in addition to showing a weight reduction. Groups GCP1, GCP2, and GMT did not differ, but consumed more water and food, excreted more faeces, and experienced lower weight gain compared to groups GCP3, GCM1, GCM2, GCM3, and GC-. Animals in the GC− group ate, drank, excreted fewer faeces, and had greater weight gain compared to animals in all other groups.

All the animals in the GMT group showed bristly hair and apparent irritability, which was assessed observationally and qualitatively during handling and not quantitatively as part of the experimental measurements.

Table 3. Comparison of quantitative clinical parameters of male Swiss mice infected with *G. duodenalis* and evaluated according to the experimental groups.

Parameters	Groups	Number	Mean	Standard deviation	*p*-value	Tukey's test
Water consumption (ml)	GC+	10	13.28	0.21	0,00010*	GC+ differs from all others groups; GCP1differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-; GMT differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-; GCP2 differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-.
	GCP1	10	12.02	0.47		
	GCP2	10	11.98	0.13		
	GCP3	10	10.85	0.82		
	GMT	10	12.09	0.51		
	GCM1	10	10.15	0.18		
	GCM2	10	10.08	0.23		
	GCM3	10	10.76	0.12		
	GC-	10	9.13	0.18		
Feed consumption (g)	GC+	10	8.74	0.09	0.00001*	GC+ differs from all others groups; GCP1differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-; GMT differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-; GCP2 differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-.
	GCP1	10	7.52	0.22		
	GCP2	10	7.49	0.15		
	GCP3	10	6.53	0.63		
	GMT	10	7.19	0.70		
	GCM1	10	6.48	0.55		
	GCM2	10	6.36	0.21		
	GCM3	10	6.56	0.06		
	GC-	10	5.75	0.16		
Volume of excreta (g)	GC+	10	8.85	0.11	0.00001*	GC+ differs from all others groups; GCP1differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-; GMT differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-; GCP2 differs from GCP3, GMT, GCM1, GCM2, GCM3 and GC-.
	GCP1	10	7.98	0.25		
	GCP2	10	8.00	0.62		
	GCP3	10	7.61	0.28		
	GMT	10	8.02	0.33		
	GCM1	10	7.43	0.52		
	GCM2	10	7.22	0.40		
	GCM3	10	7.36	0.32		
	GC-	10	6.92	0.09		

Weight (g)	GC+	10	31.48	3.89	0.00879*	GC- differs from all others groups; GC+ differs from all others groups.
	GCP1	10	34.13	2.08		
	GCP2	10	34.94	3.25		
	GCP3	10	35.89	4.00		
	GMT	10	34.26	4.11		
	GCM1	10	36.09	3.98		
	GCM2	10	36.71	5.24		
	GCM3	10	36.00	5.07		
	GC-	10	37.15	4.29		

GC+: Positive Control group - Infected and untreated animals; GCP1, GCP2, and GCP3: Black tea groups - Infected and black tea treated animals; GMT: Metronidazole group - Infected and metronidazole treated animals; GCM1, GCM2, GCM3: Black tea and metronidazole groups - Infected and black tea and metronidazole treated animals; GC-: Negative control Group - Uninfected and untreated animals.
*Significant Kruskal-Wallis test at 5% significance level.

Source: author's own elaboration

4. Discussion

The present study demonstrated the therapeutic effect of black tea (*Camellia sinensis*) in mice infected with *G. duodenalis* assemblage BIV. In isolation, black tea (200 mg/kg) eliminated the parasite, showing a dose-dependent giardicidal effect. The combination of black tea at all doses tested (100 mg/kg, 150 mg/kg, and 200 mg/kg) with metronidazole (500 mg/kg) resulted in complete cure, suggesting a synergistic effect between these two compounds.

Black tea is one of the most widely consumed teas worldwide[16], known for its high concentrations of flavonoids. These compounds have beneficial effects in preventing diarrhoea, controlling gastrointestinal disorders, and displaying antibacterial activity[18], with no apparent side effects. Previous studies[12,13] have also shown that natural products rich in flavonoids, with anti-inflammatory and antioxidant actions, exhibit potential anti-*Giardia* effects within a short period (5–7 days), either alone or in combination. These compounds are effective in reducing parasite load and alleviating the pathophysiology of giardiasis, indicating that they influence the infection process. Black tea may exert a direct action on the parasite or modify the intestinal environment by regulating the microflora, thereby promoting the proliferation of beneficial bacteria that form an efficient defence mechanism in the innate immune system[19], inhibiting pathogen colonisation and consequently eliminating the parasite[20]. In addition, even without a

detailed analysis of the main constituents of black tea, our laboratory's experience in two independent experiments[12,13], as well as the results of the present study, demonstrated the anti-*Giardia* activity of natural products rich in flavonoids, used either alone or in combination, suggesting that these components may have been responsible for the observed effects.

Almeida et al.[13] demonstrated the efficacy of black tea in reducing the parasite load in Swiss mice infected with 10^3 *G. duodenalis* cysts when treated with 100 mg/kg black tea for 5 days. Consistent with these findings, the animals in our study also showed a reduction in parasite load when treated for 7 days with doses of 100 and 150 mg/kg/day. Specifically, group CP1 showed a reduction from a high to a moderate load (1-2 cysts/objective field at 20× magnification), while group GCP2 showed a decrease from high to low (0-1 cysts/objective field at 20×), indicating that the effect of black tea on parasite load, when used alone, is also dose-dependent. Although metronidazole is recommended as first-line treatment, animals treated with metronidazole alone (GMT) were not cured, only showing a reduction in parasite load from high to low (0-1 cysts/objective field at 20×). Likewise, animals treated with 150 mg/kg/day of black tea alone (GCP2) showed similar results. This can be attributed to the ineffectiveness of the drug in impacting cellular processes in trophozoites, such as proliferation and conversion into infective cysts, and to the resistance of the parasite to the drug[9]. However, when metronidazole was combined with black tea, a synergistic effect was observed. The cytotoxic effect of metronidazole and the mechanisms affecting the cellular structure of trophozoites may have been enhanced by black tea, either through its direct action on the parasite or by regulating the microflora via the proliferation of beneficial bacteria, thereby forming an efficient defence mechanism within the innate immune system. Furthermore, the flavonoids present in black tea may modulate oxidative and inflammatory processes involved in *Giardia* infection, providing a basis for future research along these lines. Even at the lowest dose of black tea tested (100 mg/kg/day), the combination led to complete cure, suggesting the potential for reduced doses of metronidazole, which could offer a safer treatment with fewer side effects.

The increase in total leukocyte count at 7 dpi indicates that an inflammatory response is occurring, as this is a natural defence mechanism of the organism against infection. Anemia and leukocytosis are common hematological changes in parasitic infections[31]. At 15 dpi, in the cured groups (GCP3, GCM1, GCM2, and GCM3), leukocyte levels returned to the baseline observed in the negative control group (GC-). Infected and untreated animals (GC+) maintained

a high number of leukocytes, while animals in the GCP1, GCP2, and GMT groups, which showed reduced parasite load, also exhibited a reduction in total leukocytes. This suggests that leukocyte levels in peripheral blood are directly related to parasite burden.

In addition to leukocytosis, eosinophilia was observed in infected animals at both 7 and 15 dpi. Eosinophilia is a change typically associated with infections caused by helminths[32,33]. However, our study corroborates previous findings showing a strong correlation between eosinophilia and *G. duodenalis* infection in both animal and human studies[34,35]. Eosinophils play a key role in exocytosing granules containing enzymatic components, particularly Major Basic Protein (MBP), which contribute to parasite death[36]. While eosinophilia is commonly linked to helminth infections, in giardiasis, the mechanisms underlying this response remain unclear. The increased eosinophil population observed in infected mice could be attributed to tissue inflammation caused by the parasite in the intestinal mucosa, as well as alterations in humoral and cellular immune responses[36,37].

A comparison of clinical parameters between experimental groups revealed significant differences in water and food consumption, faecal excretion, and animal weight. These findings align with previous studies by Bezagio et al.[24] and Romera et al.[23], who observed similar effects in Swiss mice infected with *G. muris* and *G. duodenalis*, respectively. Higher parasite burdens were associated with increased food and water intake, as well as greater excretion, with a concomitant reduction in animal weight. This suggests that *G. duodenalis* infection alters the physiology of the host, which is consistent with similar observations in humans, particularly children. More pronounced changes in these parameters, seen in the GC+ animals and those with reduced parasite load (GCP1, GCP2, and GMT), are likely due to the pathogenic effects of the parasite. Trophozoites adhere to the mucosal surface, forming a physical barrier that impairs nutrient and water absorption. This effect can be exacerbated by intestinal microvillus injury or atrophy[38], leading to reduced enzyme activity[35,39]. In an attempt to compensate for the nutritional deficiency, animals increase their water and food intake, resulting in higher faecal excretion due to impaired absorption. Changes in the composition of the intestinal microbiota, along with lesions in the host's intestinal epithelium and increased mucus production in mice infected with the AII and BIV assemblages of *G. duodenalis*[38], further highlight the pathogenic impact of the parasite.

Phytotherapeutics have increasingly gained attention as a viable alternative for the treatment of various health conditions, particularly in resource-limited settings. Their use stands out not only for their therapeutic potential but also for their accessibility and low cost, characteristics that make these substances attractive, especially in developing countries. Moreover, many phytotherapeutics are derived from plants with a long history of traditional use, which strengthens confidence in their efficacy and safety.

The use of such treatments can be a valuable strategy to expand access to effective therapies, particularly in regions where conventional pharmacological treatments are financially inaccessible to a large portion of the population. In this context, the research and development of phytotherapeutics is crucial to ensuring sustainable and economically viable therapeutic options capable of meeting the growing demand for affordable and effective treatments.

Some limitations of this study should be pointed out to guide future research. For the results of this work to be applicable in clinical practice, human trials are needed. Furthermore, a detailed phytochemical characterisation should be conducted to identify the specific bioactive compounds responsible for the observed effects. Although preliminary histological analyses were initiated in selected groups (animals treated with black tea at 200 mg/kg and with 100 mg/kg combined with 500 mg/kg metronidazole, as well as respective controls), a comprehensive histological evaluation involving all treatment groups is warranted. These preliminary observations suggest potential alterations in intestinal architecture following infection and treatment. Expanding histological analysis to all groups would provide a more complete understanding of tissue alterations and the therapeutic potential of black tea and its combinations.

5. Conclusions

In Swiss mice experimentally infected with *G. duodenalis* BIV, black tea (*C. sinensis*) demonstrated a significant giardicidal effect, both when administered alone at 200 mg/kg and in combination with metronidazole at all tested doses. These findings highlight the potential of phytotherapeutic agents as adjunct or alternative treatments for parasitic diseases, offering safer and more accessible therapeutic strategies. From a public health perspective, particularly in vulnerable settings, this study supports the use of affordable, evidence-based interventions derived from widely available natural sources. It also contributes to the development of

innovative approaches that expand access to care and promote self-managed health actions within communities.

Further studies, including clinical trials and investigations into the mechanisms by which these compounds exert their effects, are needed to confirm the broader applicability and safety of these combinations in human populations.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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Submitted: December 13, 2023

Accepted: May 22, 2025

Published: August 22, 2025

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All the authors approved the final version of the text.	
Conflict of interest:	There is no conflict of interest.
Financing:	No financing.
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Editor: Eliane Roseli Winkelmann. PhD

Editor-in-chief: Adriane Cristina Bernat Kolankiewicz. PhD