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ORIGINAL PAPER

Highly diluted medication reduces tissue parasitism and inflammation in mice infected by *Trypanosoma cruzi*

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Aim: **To evaluate the effects of *Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* 13cH in mice infected by *Trypanosoma cruzi*.**
Materials and methods: **In a blind, controlled, randomized study, 102 male Swiss mice, 8 weeks old, were inoculated with 1400 trypomastigotes of the Y strain of *T. cruzi* and distributed into the following groups: Cl (treated with 7% hydroalcoholic solution), Ca (treated with *Kalium causticum* 13cH), Co (treated with *Conium maculatum* 13cH), and Ly (treated with *Lycopodium clavatum* 13cH). The treatments were performed 48 h before and 48, 96, and 144 h after infection. The medication was repertorized and prepared in 13cH, according to Brazilian Homeopathic Pharmacopoeia. The following parameters were evaluated: infectivity, prepatent period, parasitemia peak, total parasitemia, tissue tropism, inflammatory infiltrate, and survival. Statistical analysis was conduced considering 5% of significance.**
Results: **The prepatent period was greater in the Ly group than in the Cl group ($p = 0.02$). The number of trypomastigotes on the 8th day after infection was lower in the Ca group than in the Cl group ($p < 0.05$). Total parasitemia was significantly lower in the Ca, Co, and Ly groups than in the Cl group. On the 12th day after infection, the Ca, Co, and Ly groups had fewer nests and amastigotes/nest in the heart than the Cl group ($p < 0.05$). Decreases in the number of nests and amastigotes in the intestine were observed in the Ly group compared with the Cl group ($p < 0.05$). In the liver (day 12), Ly significantly prevented the formation of inflammatory foci compared with the other groups. In skeletal muscle, Co and Ly decreased the formation of inflammatory foci compared with Cl ($p < 0.05$). Ly afforded greater animal survival compared with Cl, Ca, and Co ($p < 0.05$). The animals in the Co group died prematurely compared with the Cl group ($p = 0.03$).**
Conclusions: **Ly with 13cH potency had significantly more benefits in the treatment of mice infected with *T. cruzi*, reducing the number of blood parasites, amastigote nests in tissue, and the number of amastigotes per nest and increasing animal survival.** *Homeopathy* (2016) **105**, 186–193.

Keywords: *Trypanosoma cruzi*; Homeopathy; Chagas disease

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Introduction

Trypanosoma cruzi is the etiologic agent of Chagas disease, which is disseminated throughout the America.[1–3] An estimated 10 million people are infected, mainly in Latin America, where 25 million are at potential risk of acquiring the disease.[4] Chagas disease has reached countries like the United States and Canada and even extended to Europe.[4] In Brazil, three million people are in the chronic phase of the disease.[5] Tissue parasitism by *T. cruzi* in vertebrate hosts causes an inflammatory process, with the development of an immune response in an attempt to destroy the parasite.[1,6]

The available medications for the etiological treatment of Chagas disease include benznidazole and nifurtimox.[7] Both of these present low efficacy and significant side effects, often leading patients to cease treatment.[8–10] The search for new therapeutic approaches with fewer side effects and better treatment efficacy has been a major challenge.

Alternative treatment approaches have included moderate exercise[11–14] and the use of highly diluted medications.[15–18] Homeopathy is an interesting option. Medications that are "constitutional" have been prescribed according to the individual physical and behavioral characteristics of patients, considering their present and former status and future trends.[19] Although studies have been performed with highly diluted medications in murine models that are experimentally infected with *T. cruzi*,[15–17] the effects of homeopathic medications that are prescribed according to the physical and psychological characteristics of Swiss mice ("constitutional" medications) have not yet been studied. Thus, the present study evaluated the effects of *Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* as "constitutional" medications in Swiss mice on blood and tissue parasitism and in the inflammatory *foci* formation.

Objectives

To evaluate the effect of *Kalium causticum*, *Conium maculatum, Lycopodium clavatum* in boosting 13cH (dilution $1{:}10^{26}$), inoculated with *T. cruzi* mice.

Material and methods

Ethics

The study was approved by the Ethics Committee on the Use of Animals in Research (CEAE), Universidade Estadual de Maringá (registration no. 054/2011).

Animals

Swiss male mice (n = 102), 8 weeks old, were obtained from the Central Bioterium, at Universidade Estadual de Maringá. The animals were intraperitoneally infected with 1400 blood trypomastigotes of the Y strain of *T. cruzi*. The study was conducted in two stages: (1) parasitological analysis and (2) histological analysis. For parasitological analysis, 42 animals were used. For histological analysis, 60 animals were used.

Experimental design

The experiment was conduced as a blind, controlled, randomized assay. The animals were maintained in a controlled environment with a 12 h/12 h light/dark cycle and received food and water *ad libitum*.

Experimental groups

The animals were allocated to the following groups: CI (infected control; treated with 7% hydroalcoholic solution; i.e., the vehicle preparation for the other medications; $n = 26$), Ca (treated with homeopathic *Kalium Causticum* 13cH; $1{:}10^{26}$ dilution; $n = 25$), Co (treated with homeopathic *Conium maculatum* 13cH; $1{:}10^{26}$ dilution; $n = 26$), and Ly (treated with homeopathic *Lycopodium clavatum* 13cH; $1{:}10^{26}$ dilution; $n = 25$). The animals were divided into these different groups so that the mean weight in each group was not significantly different.

For both the parasitological and histopathological analyses, fragments of heart, spleen, liver, large intestine (distal colon), and skeletal muscle (posterior thigh) were analyzed.

Medication selection

For medication selection, three homeopaths (one of whom was a veterinarian) made numerous observations of healthy animals. Using Lince Expert System Software (Albuquerque, NM, USA), they evaluated the behavioral, social, mental, and physiological characteristics of the mice, including need for company, fear, conscientious, shy, small amount of water, diminished vision, keen hearing, and heightened sense of smell. Using these characteristics were then selected the medicines *Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* were then selected. The medications were used in 13cH potency at a $1{:}10^{26}$ dilution according to the recommendation of an homeopathic veterinarian because *T. cruzi* is an acute infection in small animals.

Medication manufacturing: The medications were produced from mother tinctures (all from Deutsche Homöopathie-Union, Karlsruhe, Germany) and prepared in 70% grain alcohol (Agro-Industrial Tarumã Ltda, São Pedro do Turvo, Brazil) until 12cH[20] dynamization was reached and then 7% alcohol until 13cH was reached. Mechanical dynamization (AUTIC Dinamizador, Denise Model, Campinas, Brazil) was used. The steps for the preparation of the medications followed the techniques recommended by Farmacopéia Homeopática Brasileira (2011).

Treatment schedule: The treatments were performed according to Falkowski.[21] The medications were administered 48 h before inoculation and 48, 96, and 144 h after infection, for a total of four administrations. The medications were diluted in water (1 mL/100 mL). The animals had *ad libitum* access to the water in an amber water dispenser for 16 h. The control group received only 7% hydroalcoholic solution (i.e., an inert ingredient used in medication preparation), which was also diluted 1 mL/100 mL water. The choice of this treatment regimen was based on our previous studies, in which we found that treatment before infection provided protection against the infective organism,[17] and continuous

treatment after parasite contact controlled parasitemia and increased the prepatent period.[18]

Evaluation of parasitological parameters

Ten to 11 animals were analyzed per group. Parasitemia was assessed using the technique of Brener.[22] Parasite count was performed daily beginning on the 3rd day after infection until the animals died or until three consecutive zeroes were obtained. Parasitemia curves were generated using the mean parasitemia of the animals for each group. The following parameters were evaluated: infectivity, prepatent period (the time in days between inoculation and the first day of positive fresh blood test), parasite peak (the largest number of parasites observed in the group), and total parasitemia (mean sum of daily parasitemia for each animal).

Evaluation of histopathological parameters

Fifteen animals were evaluated per group. The animals were anesthetized with 90 mg/kg ketamine + 13 mg/kg xylazine, i.p., for organ collection on days 0, 5, 8, and 12 after infection. Fragments of the heart, liver, spleen, intestine, and skeletal muscle were collected and fixed in 10% formalin for 24 h. The samples were dehydrated, diaphonized, and embedded in paraffin. Histological semi-serial sections were cut at a 5 μm thickness using a Slee Mainz Microtome (CUT 5062 model, Mainz, Germany) at intervals of 20 μm and stained with hematoxylin-eosin. Four sections were obtained for each organ, and 40 microscopic fields (40× objective) were counted in each section. After evaluation, images were captured with a photonic trinocular microscope (Olympus CX31, Minato-ku, Japan) coupled to a high-definition digital camera.

Histopathological parameters included inflammatory infiltrate and tissue tropism (i.e., the number of nests and number of amastigotes/nest). Inflammatory foci were classified according to intensity and distribution as the following: (1) intensity according to the number of inflammatory cells observed (absent, 0−9 cells; discreet, 10−25 cells; moderate, 26−50 cells, and intense, >50 cells)[23,24]; and (2) distribution of inflammatory infiltrate in each field (focal, a single focus in the visual field; multifocal, more than one focus in the visual field; diffuse, inflammatory cells diffusely distributed in the visual field).[23]

Survival evaluation

Survival was computed up to 90 days after inoculation. The mean survival time of the animals in each group was compared using the Mann−Whitney test.

Statistical analysis

The data distribution was verified using the D'Agostino Pearson and Shapiro−Wilk tests. The data are expressed as mean ± standard deviation and compared using the Mann−Whitney test for comparisons between the control group and each treatment group. The data were analyzed using BioEstat 5.0 software with a 5% level of significance.

Results

Parasitological parameters

The parasitemia curve showed a characteristic profile of the Y strain of *T. cruzi* for all experimental groups. Figure 1 shows the mean parasitemia curves for each group. The prepatent period was 23.4% higher in the Ly group compared with the CI group ($p = 0.02$). The number of trypomastigotes on the 8th day after infection was 33.0% ($p = 0.04$), 34.3% ($p = 0.02$), and 28.9% ($p = 0.04$) lower in the Ca, Co, and Ly groups compared with the CI group. On the 9th day, the number of parasites was 36.5% ($p = 0.03$), 61.8% ($p < 0.01$), and 38.5% ($p = 0.02$) lower in the Ca, Co, and Ly groups compared with the CI group. Total parasitemia was 38.3% ($p = 0.18$), 47.1% ($p = 0.03$), and 37.4% ($p = 0.20$) lower in the Ca, Co, and Ly groups compared with the CI group (Table 1).

Histopathological parameters

On the 5th and 8th days after infection, no significant differences were observed between groups in the number of amastigote nests or number of amastigotes/nest in the evaluated organs. On the 12th day after infection, the number of nests and amastigotes/nest in the heart was significantly lower in treated animals compared with the CI group (Figure 2). In the Ca, Co, and Ly groups, the number of nests decreased 64.9% ($p <0.01$), 50.7% ($p = 0.03$), and 82.5% ($p < 0.01$), respectively, compared to CI group. The number of amastigotes/nest decreased 74.9% ($p < 0.01$), 98.5% ($p < 0.01$), and 86.1% ($p < 0.01$), respectively, to Ca, Co, and Ly groups, compared with the CI group (Table 2). No significant difference was found between the Ca, Co, and Ly groups.

In skeletal muscle, no significant difference was found between groups. In the intestine on the 12th day after infection, the number of nests and amastigotes/nest decreased by 93.6% ($p = 0.03$) and 92.8% ($p = 0.03$) in the Ly group compared with the CI group. In the Ly group, the number of nests and amastigotes/nest significantly decreased by 92.3% ($p = 0.02$) and 95.5% ($p = 0.03$) compared with the Ca group (Table 2).

On days 0, 5, and 8 after infection, no differences were observed between groups in the number or distribution of inflammatory *foci* in the different organs ($p > 0.05$).

On the 12th day after infection, no significant difference was found between groups in the heart or intestine. The liver presented fewer inflammatory foci in the Ly group compared with the other groups (Ly × Ca, $p < 0.01$; Ly × Co, $p = 0.01$; Ly × CI, $p = 0.01$). The decrease was 39.5% compared with the CI group. Liver inflammation during the assessment period was predominantly focal in all of the groups (Figure 3).

In muscle on the 12th day after infection, the Co and Ly groups exhibited 62.5% (Co × CI, $p < 0.01$) and 43.8% (Ly × CI, $p = 0.05$) decreases in inflammatory foci. Inflammation was predominantly focal in all of the groups and lower in the Ly group (Figure 4). This group presented an absence of multifocal inflammation. Comparisons of the effects of Co and Ca in skeletal muscle revealed that

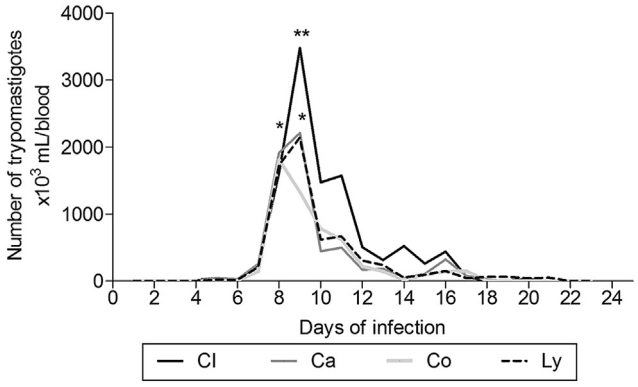

Figure 1 Mean parasitemia curve in male Swiss mice, 8 weeks old, that were inoculated with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (Cl group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group). *$p < 0.05$, **$p < 0.01$, statistically significant difference (Mann Whitney test).

the formation of inflammatory foci decreased by 62.5% in the Co group (Co × Ca, $p = 0.01$; Table 3).

Animal survival

The survival analysis showed that Ly (50.1 ± 37.3) afforded a longer survival time compared with the CI group (26.8 ± 25.6 days; $p = 0.02$). Survival time was also longer in the Ly group than in the Ca group (17.4 ± 2.1 days; $p < 0.01$) and Co group (15.4 ± 1.9 days; $p < 0.01$). The Co group presented early death compared with the CI group ($p = 0.03$; Figure 5). No significant difference in survival time was observed between the Ca and CI groups or between the Co and Ca groups.

Discussion

The present study evaluated the effects of constitutional homeopathic medications (*Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* 13cH; $1{:}10^{26}$) on parasitological and histopathological aspects in healthy

Table 1 Parasitological parameters evaluated in male Swiss mice, 8 weeks old, that were infected with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (Cl group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

Groups	*Infectivity (%)*	*Prepatent period (days)*	*Peak parasites (trypomastigotes/mL × 10^5)*	*Number of trypomastigotes/mL × 10^5- 9th dpi*	*Parasitemia total (trypomastigotes/mL × 10^5)*
Cl	100	4.7 ± 1.0^{a}	33.3 ± 10.3^{a}	34.8 ± 11.9^{a}	81.3 ± 47.5^{a}
Ca	100	5.1 ± 0.6	22.3 ± 10.8^{b}	22.1 ± 8.4^{bc}	50.2 ± 28.8
Co	100	5.5 ± 1.4	21.9 ± 14.3^{b}	13.3 ± 7.5^{bd}	43.0 ± 27.8^{b}
Ly	100	5.8 ± 1.1^{b}	23.7 ± 7.7^{b}	21.4 ± 8.1^{b}	50.9 ± 19.2

The data are expressed as mean ± standard deviation. Different letters (a, b, c, and d) in the same column represent a statistically significant difference ($p < 0.05$; Mann–Whitney test)

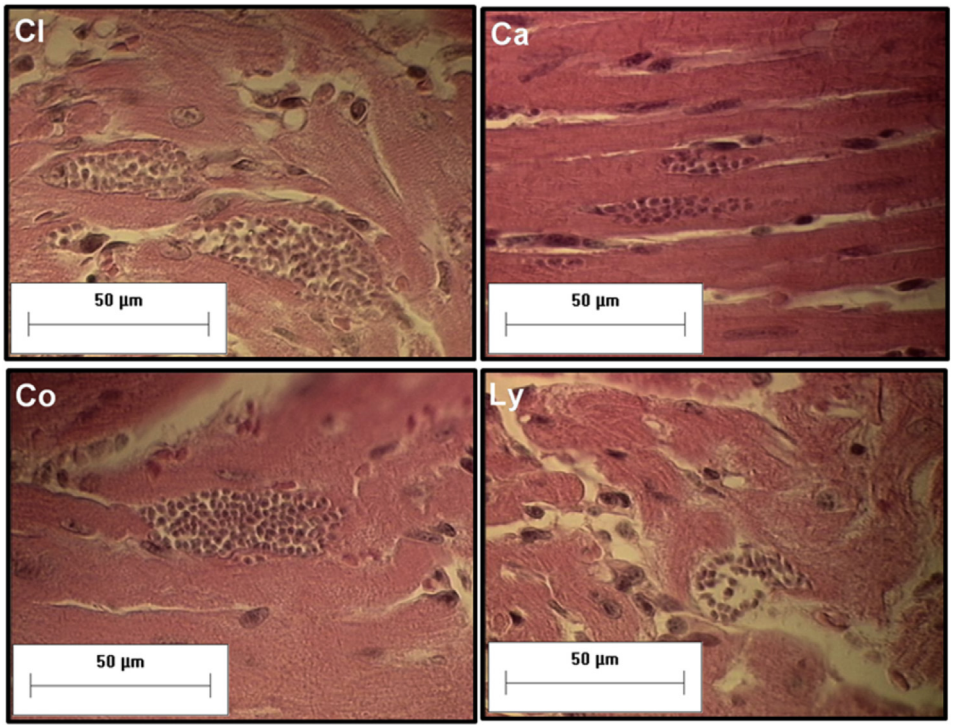

Figure 2 Amastigote nests in the heart in male Swiss mice, 8 weeks old, that were inoculated with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (Cl group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

Table 2 Tissue parasitism on the 12th day after infection in male Swiss mice, 8 weeks old, that were infected with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (CI group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

Groups	*Heart*		*Intestine*	
	Nest	*Amastigote nest*	*Nest*	*Amastigote nest*
CI	15.4 ± 12.2^{a}	557.2 ± 444.7^{a}	0.9 ± 1.3^{a}	7.8 ± 11.4^{a}
Ca	5.4 ± 6.7^{b}	139.8 ± 195.7^{b}	1.3 ± 1.7^{ac}	12.1 ± 17.9^{ac}
Co	7.6 ± 8.5^{b}	201.0 ± 198.8^{b}	0.6 ± 1.0^{a}	5.4 ± 9.9^{a}
Ly	2.7 ± 3.2^{b}	77.4 ± 80.2^{b}	0.1 ± 0.3^{bd}	0.6 ± 2.3^{bd}

The data are expressed as mean ± standard deviation. Different letters (a, b, c, and d) in the same column represent a statistically significant difference ($p < 0.05$; Mann–Whitney test). dai, days after infection

mice infected with *T. cruzi*. A repertory of assessment of healthy mice was used to determine the medications that provide the best conditions for fighting acute infection by *T. cruzi*. If left untreated, the present experimental protocol of *T. cruzi* infection leads to death.[17,18]

The parasitological parameters showed that Ca, Co, and Ly altered the course of infection compared with CI. A previous study also found that the prepatent period was significantly longer in animals that were infected with the Y strain[25] of *T. cruzi* and treated with Ly compared with an infected and untreated control group. This increase in the prepatent period is beneficial to infected animals. Previous studies conducted by our group showed that an increase in the prepatent period is highly correlated with positive treatment results in controlling *T. cruzi* infection in mice.[18]

Another observed benefit was the significant decrease in the parasite peak on the 8th and 9th days after infection and decrease in total parasitemia in the Ly, Ca, and Co groups compared with the CI group. Comparisons between the treatment groups on the 9th day after infection showed that the Co group exhibited a larger decrease in the number of trypomastigotes compared with the Ca group. In murine models, such as Swiss mice, that are more susceptible to infection by *T. cruzi*, specifically the Y strain, morbidity and mortality are generally directly related to parasite load.[17,26] The significant decrease in parasite load caused by a particular medication is one indication of the medication's beneficial effect. However, although a significant decrease in total parasitemia was observed in the Co group compared with the CI group, the animals in this group died early, with a significant difference compared with the CI group. This result indicates that other factors affect morbidity and mortality in addition to parasite load.

When evaluating histopathological parameters on the 5th and 8th days after infection, no significant difference was observed between groups with regard to the number of amastigote nests and amastigotes/nest in the evaluated organs. Parasitemia reflects tissue parasitism.[27] Parallel to finding no significant difference in the number of amastigote nests and amastigotes/nest, the parasite peak was significantly lower in animals that were treated with constitutional homeopathic medications. This result suggests a trypanocidal action when considering that parasites were released from nests on the 8th day after infection in animals in the Co group and 9th day after infection in animals in the Ca, Co, and Ly groups. The homeopathic medications did

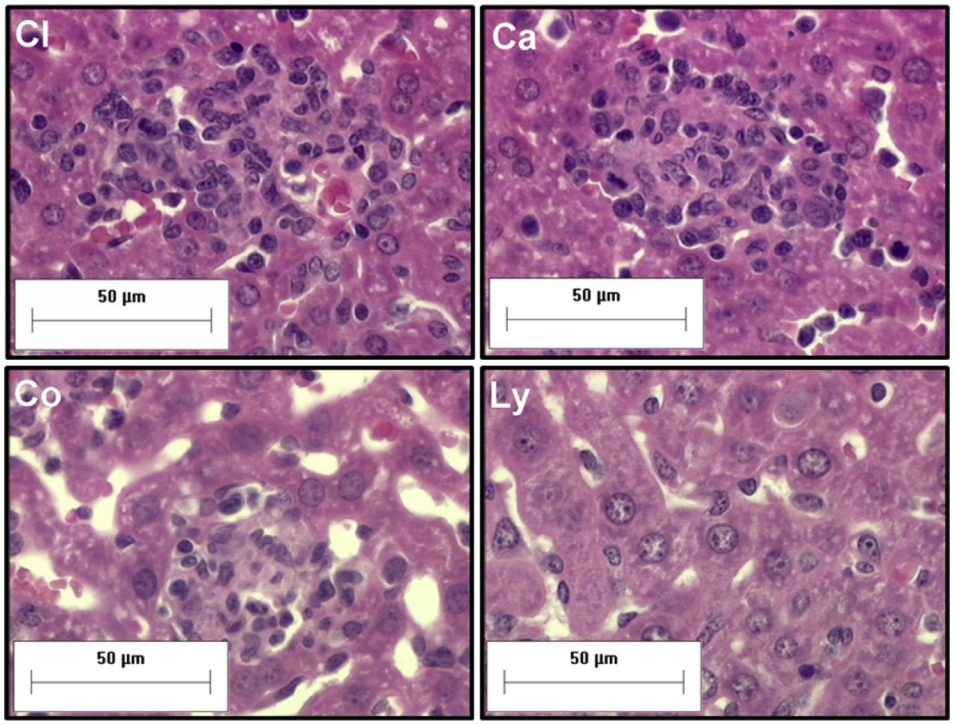

Figure 3 Inflammatory foci in the liver in male Swiss mice, 8 weeks old, that were inoculated with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (CI group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

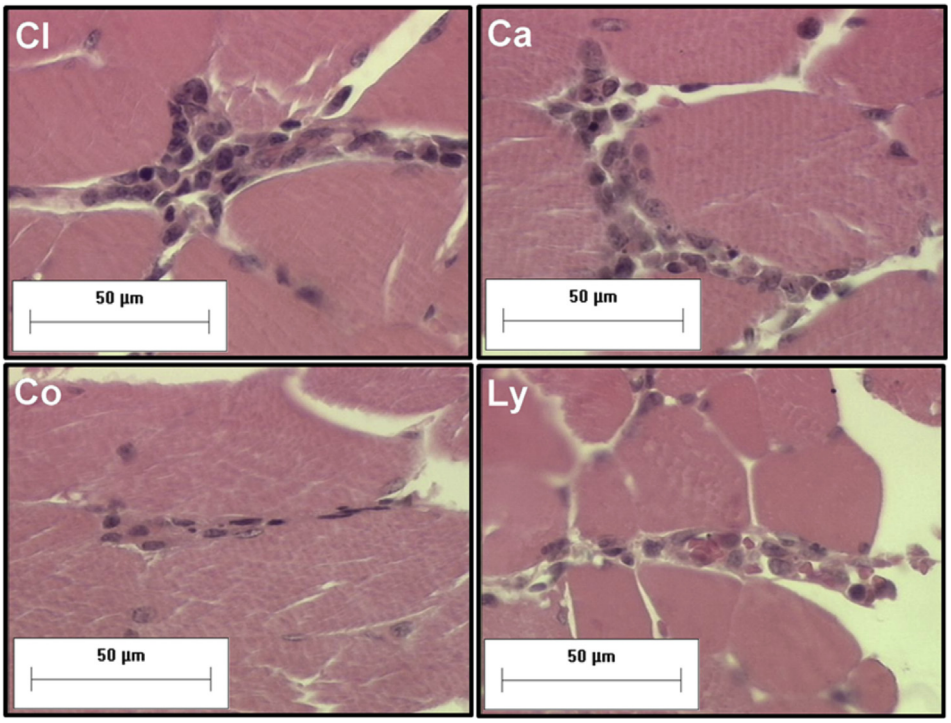

Figure 4 Inflammatory foci in skeletal muscle in male Swiss mice, 8 weeks old, that were inoculated with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (CI group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

not exert trypanocidal action, and other mechanisms may have been activated by the organism to modulate infection by controlling parasitemia.[28] Apoptosis, a mechanism by which programmed cell death occurs, is related to the maintenance of tissue homeostasis.[29–31] Sandri et al.[28] showed that treatment with a highly diluted medication prepared from *T. cruzi* significantly increased the rate of apoptosis in mice infected with this parasite, concomitant with an increase in animal survival. These results are consistent with the present study, in which we observed significant reductions of the number of amastigote nests and amastigotes/nest in the heart on the 12th day after infection in animals that were treated with homeopathic medications compared with animals in the CI group, and Ly presented the best effects. The heart is seriously affected by *T. cruzi* infection, with the destruction of neurons in the autonomic nervous system that results in functional loss of the organ and affects the formation and conduction of nerve impulses. This can lead to death, especially in species that are more sensitive to infection.[32,33] A previous study evaluated a treatment regimen with Canova medication in mice infected with the Y strain of *T. cruzi* and found changes in parasite tissue tropism, including an increase in cardiac parasitism and a worsening of the infection.[16] In the spleen and liver, no significant differences in these parameters were observed between groups.

In the digestive system, *T. cruzi* promotes the destruction of neurons in the enteric nervous system,[14] with the presence of inflammation throughout the entire intestinal circumference[14] that results in an impairment of peristalsis.[1] In the present study, animals treated with Ly exhibited significant reductions of the number of amastigote nests and amastigotes/nest in the intestine compared with the CI group, showing that this medication was important for the activation of regulatory mechanisms, the maintenance of intestinal integrity, and a better course of the infection in animals in this group. The Ly group also exhibited significant decreases in the number of nests and amastigotes/nest compared with the Ca group. These results are consistent with recent data from our laboratory. Massini[34] showed that Ly administered before and during *T. cruzi* infection provided anatomical and clinical improvements in treated Wistar rats, which had a better prognosis.

With regard to inflammation, we observed a decrease in the number of inflammatory foci on the 12th day after

Table 3 Inflammatory infiltrate assessed on the 12th day after infection in male Swiss mice, 8 weeks old, that were infected with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (CI group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

Groups	*Liver*	*Múscle*
CI	5.3 ± 1.8^{a}	1.6 ± 1.0^{a}
Ca	5.4 ± 1.3^{a}	1.6 ± 1.1^{c}
Co	5.0 ± 1.3^{a}	0.6 ± 0.7^{bd}
Ly	3.8 ± 1.3^{b}	0.9 ± 1.0^{b}

The data are expressed as mean ± standard deviation. Different letters (a, b, c, and d) in the same column represent a statistically significant difference ($p < 0.05$; Mann–Whitney test). dai, days after infection

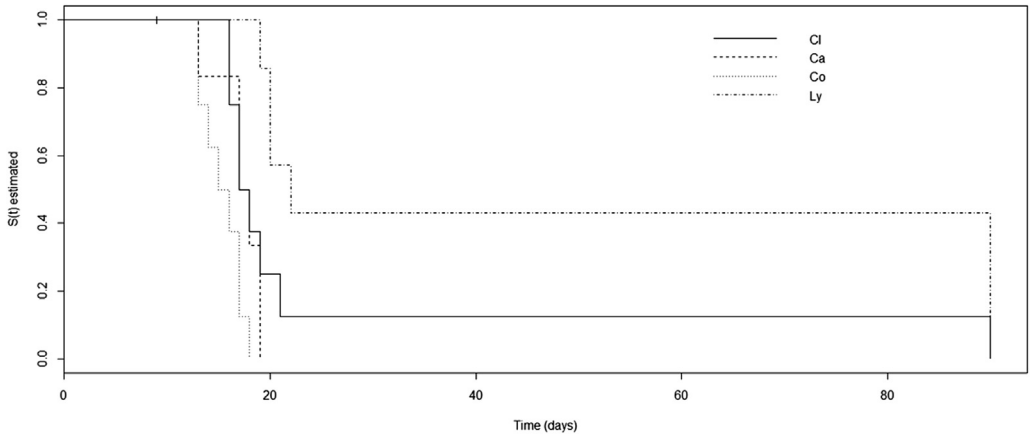

Figure 5 Analysis of survival in male Swiss mice that were infected with 1400 blood trypomastigotes of the Y strain of *T. cruzi* and treated with 7% hydroalcoholic solution (CI group) and the constitutional medications *Kalium causticum* 13CH (Ca group), *Conium maculatum* 13CH (Co group), and *Lycopodium clavatum* 13CH (Ly group).

infection. When analyzing liver inflammation, the Ly group presented significantly lower inflammation than all of the other groups. Chagas disease is characterized by a generalized inflammatory process.[35] A recent study showed that reductions of parasitemia and mortality were associated with a lower degree of inflammation[36] in animals infected with *T. cruzi* and treated with Curcumin during the acute phase of the disease. In the present study, both the Ly and Co groups exhibited a decrease in the formation of an inflammatory process in skeletal muscle compared with the CI group.

Furthermore, Ly and Co also had better efficacy than Ca. Although the animals that received Co presented the lowest number of trypomastigotes, total parasitemia, parasite peak, and nests in the heart and intestine during treatment compared with the CI group, Co promoted early death compared with all of the other groups. This was likely attributable to the fact that *Conium maculatum* extract is toxic to the heart,[37] one of the primary organs affected by this parasite. Immunomodulatory mechanisms that are triggered by highly diluted substances may have failed to balance the toxic action of the compound.

The decrease in inflammation supports the hypothesis that immunoregulatory mechanisms were activated to control parasitism in the blood and organs. Further studies should investigate the mechanisms that participate in this immunoregulation, including evaluations of apoptosis[28] and inflammatory mediators.[38]

With regard to mortality, Ly presented the best performance in increasing animal survival compared with the CI group and other treatment groups. These results indicate that "constitutional" medications that were administered in the present treatment regimen altered the response pattern of infected animals. Camandaroba[39] assessed mortality in Swiss mice that were intraperitoneally infected with the Colombian strain of *T. cruzi* and reported the death of all of the infected animals up to the 14th day after infection. The Swiss mouse is an animal model that is highly sensitive to *T. cruzi* infection, including both the Y strain[14,17,18,28] and other strains.[38] An increase in welfare, as shown in the present study, attests to the potential of using "constitutional" medications as an effective treatment approach in the clinical management of *T. cruzi* infection.

Conclusion

All of the constitutional homeopathic medications with 13cH dynamization studied herein reduced the parasite peak and total parasitemia. Ly decreased the number of inflammatory foci and their dispersion, with an increase in survival. Ly provided the most benefits in mice infected with *T. cruzi*, indicating that the use of constitutional medication may be a good strategy for fighting *T. cruzi* infection. These data may contribute to changes in management strategies in individuals with Chagas disease.

Conflict of interest

The authors declare no conflict of interests.

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