EFFECT OF DYNAMIZED ETHYL ALCOHOL IN MICE INFECTED WITH *Trypanosoma cruzi*: CAN THIS DYNAMIZED SUBSTANCE REALLY BE CONSIDERED INERT TO INFECTED ANIMALS?

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ABSTRACT

In homeopathy, ethyl alcohol is considered an inert ingredient. However, dynamized ethyl alcohol is also described as homeopathic medicine and is called *Ethylicum*, indicating that ethyl alcohol cannot correctly be classified as ingredient inert. Thus, the aim of this study was evaluate the clinical and parasitological parameters of mice infected by *Trypanosoma cruzi* and treated with different ethyl alcohol dynamizations (1cH, 6cH and 30cH). Dynamized ethyl alcohol had biological effects on murine *T. cruzi* infection. The different dynamizations caused different effects. The effects were more beneficial in dynamization 1cH, which induced better clinical prognosis, with temperature maintenance, higher water and feed consumption, reflecting higher survival compared to CI. The treatment with dynamizations 6cH and 30cH worsened the clinical conditions of the animals. However, dynamization 6cH promoted greater survival compared to CI, not excluding the possibility of its influence on some other important markers of resistance, not evaluated in this study. Dynamized ethyl alcohol changes the natural evolution of murine infection with *T. cruzi* and cannot be considered as an inert ingredient in homeopathic preparations.

KEYWORDS: Inert ingredient, dynamized ethyl alcohol, animal model, homeopathy, *Trypanosoma cruzi*.

1. INTRODUCTION

Homeopathic medicines are obtained by through successive diluitions and sucussions, in which the active ingredients are diluted in inert ingredients[1]. Ethyl alcohol is considered an inert ingredient[1,2], and there are publications demonstrating that substance did not show significant effect[3,4], in several experimental model (in vivo and in vitro).

However, in homeopathic literature, dynamized ethyl alcohol is also described as homeopathic medicine and is called *Ethylicum*[5,6]. Among the symptoms described by healthy individuals who ingested *Ethylicum* are large liver, splenomegaly, cardiomegaly, circulatory disorders, cachexia, paralysis of extensor muscles, among others[5,6], indicating that these are the changes for which the medicinal product may be prescribed.

These events lead to the conclusion that ethyl alcohol cannot correctly be classified as ingredient inert in homeopathy and according to the symptoms described above, which are similar to the symptoms of Chagas disease, it can be a choice for the treatment of this disease.

Studies with humans and animals show that homeopathic medicines are capable of promoting clinical improvement in several pathologies[7-11]. The effect obtained can be either of the diluted active substance or of the inert ingredient in which it is diluted[12] or even of the mixed action of these components, indicating the need to carry out a rigorous methodological approach.

In this context, this study aimed to evaluate the effect

of different ethyl alcohol dynamizations on parasitological and clinical parameters in the murine model of acute infection with *T. cruzi*. This study is the side product of another research project that evaluated the effects of the dynamized *Atropa belladonna* in murine infection with *T. cruzi*. The different dynamizations of ethyl alcohol were considered the control groups in the study of the dynamized *Atropa belladonna*, however the control groups showed beneficial effects when statistically compared to the groups of dynamized *Atropa belladonna* and the group of infected and untreated animals (infection control)[13].

2. MATERIAL AND METHODS

Ethics approval

The research was conducted in the Chagas Disease Laboratory of the Universidade Estadual de Maringá under the Brazilian legislation for the use of animal experimentation (Federal Law nº 11,794, October 08, 2008). The study was approved by the Committee on Ethics in the Use of Animals (CEUA), registration nº 062/2014, respecting the Brazilian College of Animal Experimentation (COBEA).

Study design

Eighty Swiss mice, male, eight weeks old, weighing 39.6 ± 3.65g were used. Of the total, 68 animals were infected intraperitoneally with 1400 trypomastigotes of *T. cruzi*- Y strain[14] and divided into four groups (17 animals / group): one group with infected and untreated animals (CI – infection control); Three groups with animals treated with ethyl alcohol 1cH, 6cH and 30cH. The remaining 12 animals were nominated uninfected control (CNI), and used for comparison of clinical parameters.

The experiment was conducted as a blind, controlled and randomized assay. For each group, there were four experimental units, three of which contained four animals / cage and one with five animals. The experimental units used were placed in micro-acoustic cages (Alesco®, polysulfone cages). The animals were kept in a controlled temperature (22.7 ± 1.2 ºC), with light / dark cycle of 12 hours, *ad libitum* water and feed.

Dynamized ethyl alcohol 1cH, 6cH and 30cH were prepared as recommended by the Farmacopéia Homeopática Brasileira. Intermediate dilutions were prepared using ethyl alcohol (CereAlcool® Brazil) diluted to 70%. Final dilutions (1cH, 6cH and 30cH) were prepared in the same manner as the intermediate solutions, but diluted in sterile water (Sigma-Aldrich®), to be administered to the animals.

The animals were treated 2 days before infection and on days 2, 5 and 8 post-infection (p.i.). The medications were diluted in water (10 µl/ml) and offered *ad libitum* in a sterile amber bottle[15] for 16 consecutive hours (medications were available to the animals from 5:00 PM to 9:00 AM). This treatment scheme was chosen based on preliminary studies[16,17].

Evaluation of clinical and parasitological parameters

The animals were clinically evaluated using a standard schedule. For the analysis, the evaluation periods were divided into two phases: during treatment (1st day p.i. to 8th day p.i.) and after treatment (11th day p.i. to 25th day p.i.).

Body mass (g) was measured using a semi-analytical balance (BEL Engineering, Class Mark II). Body temperature (°C) was measured on the front region of the left hind leg because of its smaller amount of fur using an Icel thermometer (model no. TD-920.0387). Water and feed intake were measured for each experimental unit and divided by the number of animals in each unit. For each group the mean more or less standard deviation was obtained considering the experimental units.

The parasitemia was evaluated by daily counting of blood trypomastigotes, using the Brener[14] technique. The parasitemia curve was plotted using the mean parasitemia of each group. Based on the parasitemia curve, we determined the prepatent period, patent period, parasitemia peak, total parasitemia[15,16].

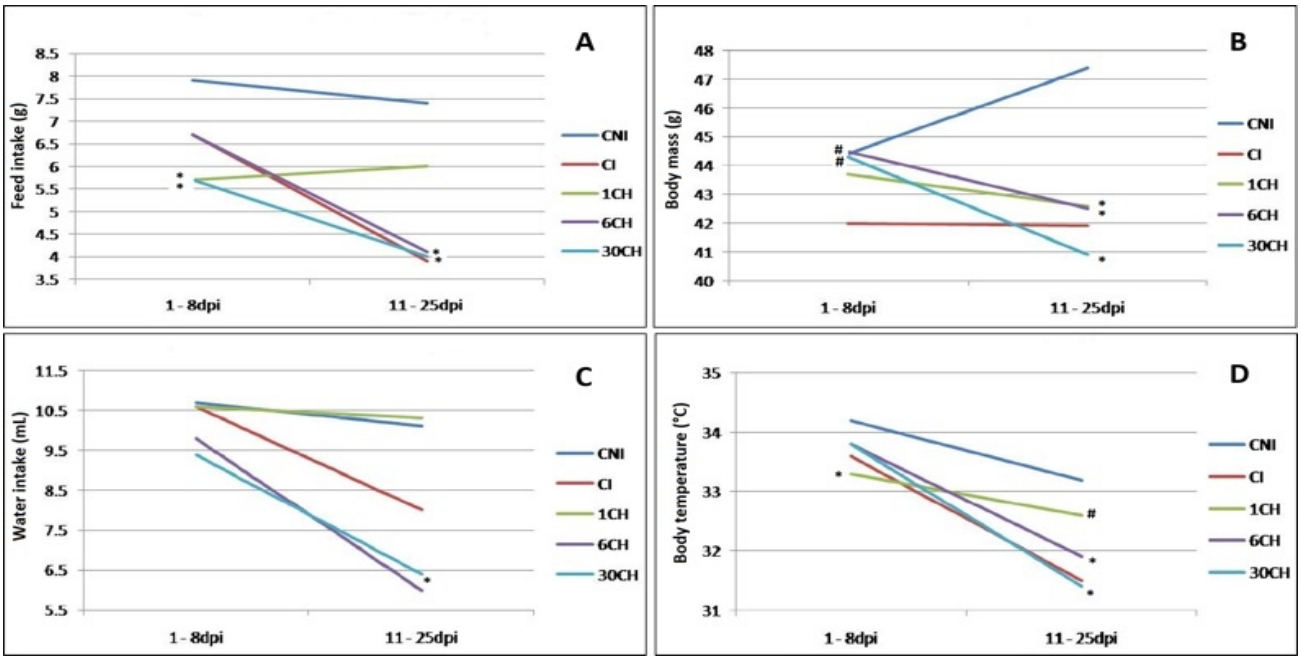
Survival was assessed up to 82 days p.i.; eight animals from each group were randomly assigned and used for evaluation of survival time and mortality. The survival time was obtained through the mean survival time of the surviving animals, in days, from the 1st day to the 82nd day p.i. The mortality rate was calculated by group, by the total of dead animals in relation to the initial total of animals of the group, until the 82nd day p.i.

The parasitological and clinical data were compared using BioEstat 5.0 software at a 5% level of significance. Variables with a normal distribution were compared using Student's t-test. Data with a nonparametric distribution were compared using the Mann-Whitney test. The log-rank test was used to compare survival curves and Z test to compare mortality.

3. RESULTS

Clinical parameters

In the first evaluated period (1–8 days p.i.), the animals treated with ethyl alcohol 1cH presented lower feed intake and lower temperature than the CNI ($p < 0.05$) and without significant difference in relation to the CI ($p >$



0.05). The animals treated with ethyl alcohol 6cH and 30cH presented higher weight when compared with CI ($p < 0.05$). In addition, the group treated with ethyl alcohol 30cH presented lower feed intake ($p < 0.05$) than the CNI (Figure 1).

Figure 1. Clinical parameters assessed in groups of mice infected with *T. cruzi* and submitted to treatment using different dynamizations of ethyl alcohol. **(A)** Feed intake (g); **(B)** Body mass (g); **(C)** Water intake (mL); **(D)** Body temperature (°C). Groups: CI - infection control; 1cH – animals treated with ethyl alcohol 1cH; 6cH - animals treated with ethyl alcohol 6cH; 30cH - animals treated with ethyl alcohol 30cH; *Statistical significance ($p < 0.05$) compared with the CNI group and # Statistical significance ($p < 0.05$) compared with the CI group.

After the treatment (11 – 25 days p.i.), a significant reduction in the weight of all the infected animals was observed compared with the CNI ($p < 0.05$). The group treated with ethyl alcohol 1cH had the best clinical values, with a clinical evolution similar to CNI ($p > 0.05$), except for weight. It is possible to observe the maintenance of treated with ethyl alcohol 1cH had the best clinical values, with a clinical evolution similar to CNI ($p > 0.05$), except for weight. It is possible to observe the maintenance of body temperature with a significant difference compared with CI ($p < 0.05$). Significant lower temperature was obtained in the other infected groups (CI, 6cH and 30cH), in relation to the CNI group ($p < 0.05$). The animals of the 6cH and 30cH groups presented lower feed intake ($p < 0.05$) than the CNI. The animals treated with ethyl alcohol 30cH consumed less water ($p < 0.05$) than the CNI animals (Figure 1).

Parasitological Parameters

No significant difference was observed in the parasitological parameters (Table 1). However, the animals treated with ethyl alcohol 1cH and 6cH presented higher survival (75% and 62.5%, respectively) than CI ($p < 0.05$), with expected survival of 13.4 and 12.8 days and lower mortality of 25.0% (2/8) and 37.5% (3/8), respectively (Table 2).

4. DISCUSSION

The results obtained in this study show that, even though it was considered an inert ingredient in homeopathy[1], dynamized ethyl alcohol was able to modify the course of murine infection with *T. cruzi*. Moreover, the dynamizations used (1cH, 6cH and 30cH) had different effects on infected animals.

The animals treated with ethyl alcohol 1cH showed, during the first period evaluated (1- 8 days p.i.) clinical worsening, with hypothermia and low feed consumption compared with uninfected animals (CNI - Healthy animals). However, in the post - treatment period (11 – 25 days p.i.), the animals showed a reestablishment of the clinical parameters (temperature, water and feed consumption), with clinical evolution similar to uninfected animals. The worsening on the first days of treatment (homeopathic aggravation)[18] especially in acute diseases, is expected from the intake of the appropriate highly diluted medicament in low dynamizations, due to its capacity to provoke an imbalance in the diseased and susceptible system, exacerbating the preexisting symptoms to force a reaction of the organism. However, this aggravation is momentary, and the organism can react by nullifying the imbalance caused by the sum of the actions of the disease and the medicine, rebalancing the organism[19]. As a consequence of the better clinical evolution, the animals in

the group treated with ethyl alcohol 1cH presented the highest survival, which represents treatment benefit, considering the sensitivity of the experimental model used for the Y strain of *T. cruzi*, whose mortality rate is high, unless some positive intervention is performed[15-17].

Table 1. Mean and standard deviation of the parasitological parameters evaluated in Swiss mice, male, 8 weeks-old, infected with *T. cruzi* and treated with different ethyl alcohol dynamizations.

Group	Prepatent period (days)	Patent period (days)	Parasitemia peak 8th day of infection (trypomastgotes/ mL)	Total parasitemia (trypomastigotes/ mL)	Parasitemia curve (trypomastigotes/ mL)
CI	5.2±1.4	13.9±1.9	(28.2±25.9) x10^5	(94.9±42.8) x 10^5	(5.6±11.7) x 10^5
1cH	5.8±1.5	12.1±1.6	(20.0±19.9) x10^5	(58.7±38.2) x 10^5	(3.3±7.9) x 10^5
6cH	5.4±1.6	13.6±1.6	(22.4±27.7) x10^5	(89.3±93.2) x 10^5	(4.9±10.9) x10^5
30cH	4.5±0.9	12.9±1.6	(44.3±28.1) x10^5	(118.1±42.8) x 10^5	(8.1±15.5) x10^5

CI - Control group infected with *T. cruzi*; 1cH, 6cH and 30cH - Group infected and treated with alcohol at dynamizations 1cH, 6cH and 30cH, respectively. *Statistical significance ($p < 0.05$), compared with the CI group.

Table 2. Mean and standard deviation of survival and mortality of Swiss mice, male, 8-week-old, infected with *T. cruzi* and treated with different ethyl alcohol dynamizations.

Groups	Survival (days)	Expected Survival (days)	Mortality (%)	Mortality (N/T)
CI	27.8±22.9	7.2	87.5	(7/8)
1cH	65.3±31.0*	13.4*	25.0*	(2/8)*
6cH	59.9±33.3*	12.8*	37.5	(3/8)
30cH	26.0±23.6	6.0	87.5	(7/8)

CI - Control group infected with *T. cruzi*; 1cH, 6cH and 30cH - Group infected and treated with alcohol at dynamizations 1cH, 6cH and 30cH, respectively. *Statistical significance ($p < 0.05$), compared with the CI group.

On the other hand, the animals treated with ethyl alcohol 6cH and 30cH presented worse clinical evolution after treatment (11 – 25p.i.). The animals treated with ethyl alcohol 6cH, even with worse clinical evolution and parasitological evolution similar to infected and untreated (CI) animals, had a higher survival, with five animals surviving until 82days p.i. Dynamization 6cH is also a low dynamization and is indicated for the treatment of acute infections[20] and, although it does not lead to the clinical improvement of the infected animals, it can not be ruled out the possibility of being able to influence some other important marker, that was not identified in this study, and that implied a greater survival in this model of acute infection. The animals treated with ethyl alcohol 30cH, presented worse clinical evolution and parasitological evolution / survival similar to infected and untreated (CI) animals. Dynamization 30cH is considered a high dynamization and is not suitable for the treatment of acute infections[18]. Furthermore, Sandri (2016)[13] in a complementary study to this one demonstrated that the treatment with ethyl alcohol 6cH was related to decreased inflammation in heart tissue with Th2 cytokine modulation; and the treatment with ethyl alcohol 30cH was related to increased inflammation in heart with Th1 cytokine modulation.

The findings express direct consequences of the effect of the different dynamizations used in this study on the equilibrium / imbalance of the physiology in the infected animals. Homeopathic medicines stimulate the body to reestablish its equilibrium state[21] but this only happens when the active ingredient, the dynamization and the therapeutic scheme are appropriate for the case, according to the principle of similarity[19]. Thus, as observed in this and other studies, acute infections, as is the case of murine infection with *T. cruzi* in this experimental model, should be treated with low dynamizations and higher frequency of doses[22]. This information may be relevant to understand the different effects caused by the dynamizations1cH, 6cH and 30cH of ethyl alcohol, using the same treatment scheme.

5. CONCLUSION

In summary, dynamized ethyl alcohol can not be considered an inert substance in this experimental model. In addition, the effect observed for active ingredients, in homeopathic medicines, diluted in ethyl alcohol should be analyzed with caution, which may have inbuilt effects of the ethyl alcohol or even the effect of the mixture of active ingredient and ethyl alcohol, requiring the inclusion of untreated controls in the experimental protocols where ethyl alcohol was used as control.

This study, in spite of showing the effect of the ethyl alcohol 1cH and 6cH in *T. cruzi* experimental infection,

through an extremely important marker that is the highest survival of the infected animals does not yet demonstrate exactly the mechanism of this effect. From these important results, new studies will be designed in an attempt to understand the mechanism of action of the ethyl alcohol 1cH and 6cH in *T. cruzi* experimental infection.

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