

Original Article

The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*

Denise Lessa Aleixo^{1*}, Mateus Joacir Benvenutti¹, Katiucha Rebeca Jennifer Lopes Lera², Larissa Ciupa¹, Fabiana Nabarro Ferraz¹, Silvana Marques de Araújo¹

¹Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Maringá - Paraná, Brazil.

²Departamento de Medicina Veterinária, Faculdades Ingá – Maringá – Paraná, Brazil.

Emails: *deniselessaaleixo@gmail.com, <http://lattes.cnpq.br/3751803485429742>;

mateusbenvenu@hotmail.com, <http://lattes.cnpq.br/6062381232091700>;

katiucha.llera@hotmail.com, <http://lattes.cnpq.br/8954528497195279>;

ciupalarissa@gmail.com, <http://lattes.cnpq.br/4843511543957270>;

fabiana.nabarro@gmail.com, <http://lattes.cnpq.br/2547950454827640>;

smaraujo@uem.br, <http://lattes.cnpq.br/0914668307033301>

Abstract

Although several diseases are treated by toxic drugs, their side effects may hamper adherence to the therapy. The aim of this study is to evaluate the effect of the association of ponderal benznidazole (BZ) with its ultra-high diluted (UHD) formula on clinical and parasitological parameters of mice infected by *Trypanosoma cruzi* (*T. cruzi*). 24 non-isogenic Swiss mice were divided into groups: CI - infected animals treated with 7% alcohol; BZp - infected animals treated with BZ (500 mg/ kg) from the beginning of infection; BZp+d - infected animals treated with ponderal BZ and with UHD BZ, which started to be administered four days after the beginning of treatment with ponderal BZ; CNI - group of non-treated and non-infected animals. The UHD medicine was prepared according to Phamacopoeia until 30x. The different treatment schedules were statistically compared through parasitological and clinical parameters. The group BZp+d displayed more favorable clinical evolution than the group BZp, with improvement of mass gain, feed conversion and water intake, presenting data approximated to CNI group. The significant increase of the body temperature of BZp+d group indicates an activation of the immune system which was not observed in the other groups. Moreover, the anti-parasitic effect of the ponderal drug was maintained in all parasitological parameters of this group. By reducing the side effects and maintaining the action of the ponderal drug, the combination of toxic drugs with their UHD formula could be considered a way of improving efficacy of the treatment.

Keywords: *Trypanosoma cruzi*; ultra-high dilution; homeopathy; benznidazole; parasitemia; toxic symptoms



Cite as: Aleixo DL*, Benvenutti MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*. *Int J High Dilution Res.* 2015;14(3): 10-19

Introduction

Toxic effects are observed in a variety of patients using drugs in ponderal doses (Ammassari *et al*, 2001), mainly in long term treatments (Nittayananta *et al*, 2010; MM, 2010; Castro *et al*, 2006; SS, 2010), what certainly influences the adherence to the therapy. Some authors defend that complementary therapeutic methods that use diluted doses of the intoxicating substance might decrease the adverse effects of the conventional treatments (Neto, 2006; Mundhenke, 2010).

Our group has been using the murine model of *Trypanosoma cruzi* (*T. cruzi*) infection to evaluate the effects of diluted treatments (WHO, 2013). This model is widely known with published data that might contribute to the understanding of the action of drugs. Besides this, it allows the assessment of a variety of parameters that are also observed in the human infection (WHO, 2013; Araújo-Jorge, 2000).

The Chagas disease affects millions of people worldwide (WHO, 2013), meanwhile, Benznidazole (BZ) and Nifurtimox are the only available drugs for etiologic treatment. The therapy using BZ has better effect on the acute phase of infection and limited action on the chronic phase (Guedes *et al*, 2011). However, researchers have reported significant adverse effects, either in human or in animals, such as anorexia, weight loss and decreased water intake, which affect the general health status of an individuals under this therapy, hindering their perseverance to

treatment (Guedes *et al*, 2011; LS, 1987; Rodrigues *et al*, 2002; Cancado, 2002).

Protocols of experimental use of benznidazole recommend 100mg/kg/animal in murine models and a recent study by our group has proved that the association of ponderal BZ, in this recommended dose, with its diluted formula, might be a successful way to decrease its side effects (Benvenuti *et al*, 2013). Besides this, once the effect of BZ is dose-dependent, the possibility of increasing its dose and the time of treatment would provide an improvement of therapy efficacy (Guedes *et al*, 2010). However, as it is dose-dependent, we took a dose five times greater and used the ultra-high dilution (UHD) of benznidazole to see if it would reduce the adverse effects of the drug, especially with this very high dose to administer.

Under this perspective, the aim of this study is to evaluate the effect of the association of ponderal benznidazole, in a dose of 500mg/kg/animal, with its diluted formula on clinical and parasitological parameters of mice infected by *T. cruzi* in prospect of increasing the anti-parasitic effect of BZ, minimizing its side effects.

Materials and methods

Ethics

The research was developed in the Experimental Laboratory of the State University of Maringá under the Brazilian legislation for the use of animal experimentation (Federal Law number 6.638 /1979). The study was approved by the Committee of Ethics and Animal Experimentation (CEEA-UEM), protocol



Cite as: Aleixo DL*, Benvenuti MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*. *Int J High Dilution Res.* 2015;14(3): 10-19

05/2006, respecting the norms of the Brazilian College of Animal Experimentation (COBEA), which is an institution associated to The International Council for Laboratory Animal Science.

Study design

The study utilized 24 non-isogenic Swiss male mice (6 animals per group), at eight weeks of age, weighing $41.87\text{g} \pm 4.23$. The amount of animals used in this experiment was decided considering other animal studies with *Mus musculus* (Eckelman *et al*, 2007; Scheibe, 2008; Damy, 2010), murine infection by *T. cruzi* (Araújo-Jorge, 2000) and following the principle of reduction of the number of animals in experimental procedures, recommended by Russel & Burch *et al*, 1958.

Infected animals were intraperitoneally inoculated with 1400 blood trypomastigotes of *T. cruzi* Y strain (Brenner, 1962) and were divided into three groups according to treatment: CI – animals treated with 7% alcohol; BZp – animals treated with BZ (500 mg/kg/day); BZp+d – animals treated with association of ponderal BZ and with UHD BZ. Another group of non-infected animals served as a control group.

The experiments were performed twice, as a blind, controlled and randomized trial. The animals were divided in pairs (experimental units) into separate cages without external contamination (ALLESCO®). The cages were covered with wood shavings, which was preheated in an oven. The mean initial weight of mice in each group was statistically equal and animals were kept

in a climate-controlled vivarium, under controlled temperature ($22.7 \pm 1.2^\circ\text{C}$), light/dark cycles of 12 hours, with treated water and feed offered *ad libitum*.

Experimental procedures

Medicines utilized

1) Benznidazole in ponderal dose: N-benzyl-2-nitro-1-imidazolacetamide produced by LAFEPE (Pharmaceutical Laboratory of Pernambuco) in tablets, dissolved in gum arabic and distilled water. It was administered daily, once a day, at 11:30 a.m., by gavage in a concentration of 500mg/kg/animal/day and in a volume of 0.2 mL/day/animal.

2) Highly diluted Benznidazole: The dilution of the medicine followed the protocol for insoluble drugs recommended by the Brazilian Homeopathic Pharmacopoeia (Farmacopéia Homeopática Brasileira, 2011). One entire tablet of Benznidazole was triturated in lactose, according to the decimal scale (one part of active ingredient to nine parts of vehicle) insoluble compounds until 6x dilution ($1:10^6$), and thereafter solubilized up to $1:10^{30}$ dilution (30x potency according to Pharmacopoeia) in 7% alcohol, using mechanical dynamizer (DENISE - AUTIC®). The medicines were administered daily, once a day, at 11:30 a.m., by gavage in a volume of 0.2 mL/day/animal after the administration of the ponderal drug. In order to orally administer the medications, reusable cannula (one for each medicine) designed for oral gavage procedures in laboratory animals was used.

Treatment with ponderal BZ immediately started after detection of



Cite as: Aleixo DL*, Benvenuto MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*. *Int J High Dilution Res.* 2015;14(3): 10-19

infection in all treated groups. In BZp+d group, the diluted medicine was administered four days after the beginning of treatment with the ponderal drug. Treatment was given for 20 consecutive days, and the groups were treated following the order: CI, BZp and BZp+d. This schedule of treatment was chosen based on preliminary studies by our group (Benvenuti *et al*, 2013; Aleixo *et al*, 2012; Ferraz *et al*, 2011).

Evaluation of parasitological parameters

Parasitemia – evaluated by daily counting of blood trypomastigotes, using the technique of Brener, 1962, from the day of infection until endpoints were noticed or within 30 days after infection in those mice who did not present endpoints. The curve of parasitemia was designed using the daily average parasitemia of each group. From the parasitemia curve, the following parameters were evaluated: total parasitemia (P_{total}) - average of sum of the daily parasitemia for each mouse; parasitemia on the 8th day of infection ($P_{8th\ day}$) - mean of the parasitemia level observed in each animal of each group on the 8th day of infection; patent period (PP) – mean period in which parasitemia can be detected in each group.

Evaluation of clinical parameters

Clinical parameters were daily evaluated and the groups were randomly assessed according to the protocol recommended by Falkowski *et al*, 2012. Body temperature was measured on the front region of the left hind leg due to smaller amount of hair (Icel Thermometer, Model TD-920.0387). Feed and water intake

were measured for each experimental unit and divided by two (number of animals per unit). Body weight was measured with semi analytical balance (Balance BEL®).

Hypothermia (drop of more than 4 degrees of the body temperature), weight loss (loss of more than 15 % of the body weight) and anasarca were considered endpoints for humane euthanasia considering the expertise of our group in conducting experimentation with the murine model of *T. cruzi* infection (Russel *et al*, 1959; Brener, 1962; Benvenuti *et al*, 2013). In cases that animals did not present such traits, the survival was evaluated up to the 120th day after infection, when so they were also euthanized by deep anesthesia using ketamine 50mg/kg, and xylazine 10 mg/kg, intraperitoneally, according protocols of CEEA. None animals died naturally by not meeting the established clinical criteria.

Animals

This experiment utilized non-isogenic Swiss male mice (*Mus musculus*) at eight weeks of age, weighing $41.87g \pm 4.23$. The animals were provided by the Central Animal vivarium of the State University of Maringá, where they used to be monitored by a veterinarian. They were a conventional lineage with intestinal and ecto parasites control. At the sectoral vivarium, one week prior to the beginning of the experiment, the animals were submitted to Amitraz bath (concentration of 1:250) in order to eliminate ectoparasites. Animals were daily monitored by researchers, who



Cite as: Aleixo DL*, Benvenuti MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with Trypanosoma cruzi. *Int J High Dilution Res.* 2015;14(3): 10-19

carried out welfare assessments during the whole experiment.

Statistical analysis

All the parasitological and clinical data were statistically compared amongst the groups by the Statistica 8.0 program. Kruskal-Wallis test (ANOVA followed by multiple comparisons of mean ranks for all groups) was used since not all analyzed parameters displayed a normal distribution. This test is used to evaluate the degree of association between samples and its use might highlight significant differences amongst the treatments tested.

Results and Discussion

This research shows the effects of the treatment with diluted BZ, associated with BZ in higher concentration (500 mg/Kg/day/animal) than that proposed by the literature (100 mg/kg/day/animal) on parasitological and clinical parameters of mice infected by *T. cruzi*.

Parasitological parameters

The parasitological parameters are shown in table - I. Treated groups (BZp and BZp+d) presented lower outcomes of P_{total} ($p=0.025$), PP ($p=0.003$) and $P_{8th\ day}$ ($p=0.025$ and 0.003 , respectively) when compared to control group of infection (CI). When compared with themselves, treated groups (BZp and BZp+d) did not show statistical difference in these parameters. The animals from BZp and BZp+d groups survived throughout all the study period without presenting endpoints (120 days), differently from the control group of infection in which such markers started to appear, in average, on the 18th day ($p\leq 0.001$).

These data prove that the diluted BZ did not interfere on the effect of the ponderal drug. Instead of that, the action of ponderal BZ was increased in this experiment once its dose is higher in this experiment (500 mg/Kg/day/animal) and its effect is dependent of dose.

Group	$P_{total}(x10^6)$	$P_{8th\ day}(x10^6)$	PP (days)	SURVIVAL (days)
CI	14.37±7.57 ^a	2.00±1.19 ^a	12.17±0.75 ^a	18.17±5.30 ^a
BZp	4.64±4.55 ^b	0.55±0.55 ^b	5.33±0.82 ^b	120 ^b
BZp+d	4.81±3.94 ^b	0.11±0.10 ^b	4.83±1.94 ^b	120 ^b

Table - I: Mean and standard deviation of the parasitological parameters observed in Swiss male mice, 8 weeks of age, intraperitoneally infected with 1400 blood trypomastigotes of *T. cruzi* Y strain, subjected to treatment using highly diluted or BZ 500 mg/kg/animal. Statistical comparison between values is displayed in the column.

*Different letters in a column means significant difference ($p\leq 0.05$). P_{total} = sum of the daily mean of parasitemia levels for each mouse; $P_{8th\ day}$ - mean of the highest parasitemia level observed in the 8th day of infection each group; PP = patent period considered the time in which parasitemia can be detected.



Cite as: Aleixo DL*, Benvenuti MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benzimidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with Trypanosoma cruzi. *Int J High Dilution Res.* 2015;14(3): 10-19

Clinical parameters

Clinical parameters were assessed every two days: temperature, weight, water

intake and feed intake for each animal of each experimental group. The data are presented in table - II .

Group	WATER (mL)	WEIGHT (g)	FEED (g)	TEMPERATURE (°C)
CI	20.74±1.44 ^a	36.21±4.45 ^a	8.36±4.02 ^a	32.65±1.36 ^{ab}
CNI	23.12±2.85 ^b	49.08±5.31 ^b	16.69±3.8 ^b	32.76±0.99 ^a
BZp	18.46±6.57 ^a	44.6±3.42 ^c	14.34±3.57 ^c	32.97±1.07 ^{ab}
BZp+d	23.16±3.61 ^b	46.1±4.21 ^d	14.88±3.45 ^c	33.21±0.94 ^b

Table - II: Mean and standard deviation for clinical parameters assessed in Swiss male mice, at 8 weeks of age, intraperitoneally infected with 1400 blood trypomastigotes of *T. cruzi* Y strain, treated with ponderal BZ or with the association of ponderal BZ and its diluted formula.

* Different letters in a column means significant difference (p<0.05).

First, the BZp+d group presented greater water intake compared with the group treated only with BZp (p<0.001). This increase was observed in other studies by our group (Benvenuti *et al*, 2013). However, how the water consumption might influence the course of animal infections is an issue not yet elucidated in the scientific community, since research that correlates the water intake and infectious diseases are scarce. Concerning this criterion, it was observed that the BZp+d and CNI groups exhibited similar water intake (p=0.919), as well as BZp and CI groups (p=0.362). Such result suggests that the utilization of BZ associated with its diluted formula tends to take the organism of the infected mouse to a similar condition of healthy mouse. On the other hand, the use of ponderal BZ does not modify this criterion, leaving the mice of this group in a similar condition of infected mice without treatment.

Regarding to the feed intake, it was not possible to notice an increase of consumption in BZp+d group, when compared to BZp group (p=0.161) as it was observed in our previous study (Eckelman *et al*, 2007). However, if we compare the final weight of these two groups, the group treated with the association of HD and ponderal BZ gained significantly more weight than the group treated only with the ponderal drug (p<0.001). Other animal studies have correlated the use of diluted drugs with mass gain (Monteiro da Silva *et al*, 2011; Soto *et al*, 2012) and improvement of feed conversion (de Lira *et al*), demonstrating that these medicines might interfere with the harnessing of the food ingested. In animals infected by *T. cruzi* and treated with homeopathic medicines, the increase of the colon length (Massini, 2013) and the preservation of gut neural population (Brustolin, 2014) were seen as attempts of the organism to balance the damage caused by the parasite in the intestine.



Cite as: Aleixo DL*, Benvenuti MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with Trypanosoma cruzi. *Int J High Dilution Res.* 2015;14(3): 10-19

Such anatomic and functional adaptability reveals the pursuit of the body to retake the digestive and absorptive functions (Hwang *et al*, 2002; das Chagas *et al*, 2011) and might explain the mass gain despite the low feed intake.

Considering the temperature, the BZp+d group displayed the highest average, being statistically different only from CNI group ($p=0.01$). However, the other infected groups (BZp and CI) did not present temperature statistically different from CNI ($p=0.275$ and 0.243 , respectively). Such outcome points that only the diluted medicine was able to significantly elevate the temperature of the infected mice. Overall, some research indicates that elevations in body temperature are beneficial to the host immune response by increasing the leukocyte mobility (Bryant *et al*, 1966), the production of interferon (Postic *et al*, 1966) and antibody (Ipsen, 1952); by enhancing either the lymphocyte response to mitogen and antigen or the bactericidal capacity of PMN leukocytes (Roberts *et al*, 1977). These data indicate that the diluted medication somehow contributed to the activation of the mice's immune response.

The improvement in the harnessing of food, just like the greater water intake of BZp+d group, indicates that the UHD medicine contributed to the clinical improvement of the mice treated with the association of ponderal BZ and its diluted formula. Such data is even more impressive if we consider that the dose of ponderal BZ utilized in this experiment is five times higher than the recommended dose. Even with such a significant

increase in dose, the mice of BZP+d group presented clinical improvement. Moreover, the increase in the average temperature of this group points what other studies have already shown: UHD medicines act, though by yet unknown mechanisms, in modulating the host immune response (Brustolin, 2014; Chabel *et al*, 2009; Aleixo *et al*, 2013) improving the overall status of treated organisms; this fact, coupled with the increment in the parasitocidal effect of BZ, might increase the chances of curing the infection.

Conclusion

The clinical improvement of BZp+d group indicates a decrease of the toxic effects of the ponderal drug without losing the parasitocidal effect, what demonstrates that it is possible to increase the dose of BZ, incrementing its efficacy without causing discomforts to the patient. Furthermore, the significant increase of temperature of this group suggests that the diluted medicine acts by enhancing the defense systems of the infected organism.

Although these results are simple they open up a range of possibilities for a variety of toxic treatments. Therefore, these results suggest that the association of toxic drugs with their diluted formulas could be applied in other therapies that are also dependent on dose and whose side effects hinder the adherence to treatment.

Acknowledgments

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), CNPQ



Cite as: Aleixo DL*, Benvenuto MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benzimidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*. *Int J High Dilution Res.* 2015;14(3): 10-19

and Fundação Araucária for financial support.

Conflict of interest statement

None declared.

References

[Aleixo DL, Ferraz FN, Ferreira EC, de Lana M et al. Highly diluted medication reduces parasitemia and improves experimental infection. BMC Res Notes. 2012; 5: 352. DOI: 10.1186/1756-0500-5-352.](#)

[Aleixo DL, da Veiga FK, Ciupa L, Portocarrero AR et al. Highly diluted medication modifies parasitological, immunological and histopathological parameters of Trypanosoma cruzi infection. Int J High Dil Res 2013;12\(44\):107-8.](#)

[Ammassari A, Murri R, Pezzotti P, Trotta MP et al. Self-reported symptoms and medication side effects influence adherence to highly active antiretroviral therapy in persons with HIV infection. JAIDS Journal of Acquired Immune Deficiency Syndromes, 2001;28 \(5\):445-9.](#)

Araújo-Jorge TC, 2000. Modelos experimentais para o estudo in vivo da doença de Chagas: Camundongo, In: Araújo-Jorge TC, Castro SL (Eds.), Doença de Chagas: Manual de experimentação animal.

Benvenuto MJ, Ferraz FN, Veiga FK, Falkowski GJ et al., 2013 Medicamento Homeopático diminui efeitos adversos do tratamento na infecção murina por *T. cruzi*. I Congresso médico da

Universidade Estadual de Maringá, Maringá.

[Brenner Z, 1962. Therapeutic activity and criterion of cure on mice experimentally infected with Trypanosoma cruzi. Rev Inst Med Trop Sao Paulo, 4:389-96.](#)

Brustolin CF, 2014. *Lycopodium clavatum* e *Phosphorus* modulam a resposta de citocinas, protegem neurônios mientéricos e diminuem a inflamação em ratos *Wistar* infectados pelo *T. cruzi*. Maringá - Paraná: Universidade Estadual de Maringá.

[Bryant RE, DesPrez RM, VanWay MH, Rogers DE. Studies on human leukocyte motility I. Effects of alterations in pH, electrolyte concentration, and phagocytosis on leukocyte migration, adhesiveness, and aggregation. The Journal of experimental medicine 1966;124\(3\):483-99.](#)

[Cancado JR, 2002. Long term evaluation of etiological treatment of chagas disease with benznidazole. Rev Inst Med Trop Sao Paulo, 44\(1\):29-37.](#)

Castro JA, de Meca MM, Bartel LC. Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis). Hum Exp Toxicol 2006;25(8):471-9.

Chabel JC, Van Onselen VJ, da Graça Morais M, Neto IMC ET AL. Efeito de um complexo homeopático " Homeobase Convert H®" em ovinos sob condições de restrição alimentar. Brazilian Journal of Veterinary Research and Animal Science 2009;46(5):412-23.

OPEN ACCESS

Cite as: Aleixo DL*, Benvenuto MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benznidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with Trypanosoma cruzi. *Int J High Dilution Res.* 2015;14(3): 10-19

Damy SB, Camargo RS, Chammas R, Figueiredo LFPd. Aspectos fundamentais da experimentação animal-aplicações em cirurgia experimental. Revista da Associação Médica Brasileira 2010; 56(1):103-11.

de Lira AD, de Medeiros SC. A homeopatia no controle de parasitoses de peixes the homeopathy in the control of fish parasites.

Eckelman WC, Kilbourn MR, Joyal JL, Labiris R et al. Justifying the number of animals for each experiment. Nuclear medicine and biology 2007;34(3):229-32.

Falkowski GJS, Sandri PF, Tiyo R, Aleixo DL et al. Parameters for evaluation of clinical trial in mice infected with *Trypanosoma cruzi*. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2012; 64(6):1539-46.

Farmacopéia Homeopática Brasileira 2011. In: ANVISA ANdVS, editor. 3ª edição ed. Brasília DF: Agência Nacional de Vigilância Sanitária.

Ferraz FN, Simone GK, do Nascimento A, de Melo CS et al. Different forms of administration of biotherapy 7dH in mice experimentally. Homeopathy 2011; 100(4):237-43.

das Chagas Neto FA, Barreto ARF, Francisco V, Muglia JEJ et al. Avaliação e seguimento de pacientes adultos com síndrome do intestino curto pelo exame contrastado de trânsito intestinal. Radiologia Brasileira 2011; 44(3): 188-91.

Guedes PM, Silva GK, Gutierrez FR, Silva JS. Current status of Chagas disease chemotherapy. Expert Rev Anti Infect Ther 2011; 9(5):609-20.

Hwang ST, Shulman RJ. Update on management and treatment of short gut. Clinics in perinatology 2002;29(1):181-94.

Ipsen J. The effect of environmental temperature on the immune response of mice to tetanus toxoid. The Journal of Immunology 1997; 69(3):273-83.

LS F, Z B. Susceptibility and natural resistance of *Trypanosoma cruzi* strains to drugs used. Trans R Soc Trop Med Hyg 1987; 81(5):755-9.

Massini PF, 2013. *Rattus norvegicus* infectados com *Trypanosoma cruzi*: efeitos clínicos do tratamento com *Lycopodium clavatum* e *Phosphorus* ultradiluídos. Maringá - Paraná: Universidade Estadual de Maringá.

MM S-A. Role of carnitine in cancer chemotherapy-induced multiple organ toxicity. Saudi Pharm J. 2010;18(4):195-206.

Monteiro da Silva SL, Arcanjo AHM, Pinto LdR, Rosa GP et al. Gain of mass between two profiles of treatments to pigs with Self-Organizing Factors. International Journal of High Dilution Research 2011; 10(36):271-82.

Mundhenke M. Vertigo: A Common Problem in Clinical Practice. *Biomedical* 2011; 500(2):22-3.



Cite as: Aleixo DL*, Benvenuto MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benzimidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*. *Int J High Dilution Res.* 2015;14(3): 10-19

Neto RMB, 2006. Bases da Homeopatia. UNICAMP: Liga da Homeopatia.

Nittayananta W, Talungchit S, Jaruratanasirikul S, Silpapojakul K, et al. Effects of long-term use of HAART on oral health status of HIV-infected subjects. *Journal of oral pathology & medicine* 2010; 39(5):397-406.

Postic B, DeAngelis C, Breinig MK, Ho M. Effect of temperature on the induction of interferons by endotoxin and virus. *Journal of bacteriology* 1952; 91(3):1277-81.

Roberts NJ, Steigbigel RT. Hyperthermia and human leukocyte functions: effects on response of lymphocytes to mitogen and antigen and bactericidal capacity of monocytes and neutrophils. *Infection and immunity* 1977; 18(3):673-9.

Rodrigues J, de Castro SL. A critical review on Chagas disease chemotherapy. *Mem Inst Oswaldo Cruz* 2002; 97(1):3-24.

Received: April 22 2015, Accepted: August 15 2015.

Russell WMS, Burch RL, Hume CW, 1959. The principles of humane experimental technique.

Scheibe PO. Number of samples—hypothesis testing. *Nuclear medicine and biology* 2008; 35(1):3-9.

S S, J C. Antimony toxicity. *Int J Environ Res Public Health* 2010; 7(12):4267-77.

Soto FR, Vuaden ER, Melville PA, Moreno AM et al. Evaluation of homeopathic and biotherapeutic treatments in a swine farm to control *Escherichia coli* infection: a long term study. *International Journal of High Dilution Research* 2012; 11(40):136-7.

WHO. Chagas disease (American Trypanosomiasis). *Fact Sheet* 2013 [cited 2014 July 16].

© International Journal of High Dilution Research.
Not for commercial purposes.



OPEN  ACCESS

Cite as: Aleixo DL*, Benvenuti MJ, Lera KRJL, Ciupa L, Ferraz FN, de Araújo SM. The Association of Ponderal Benzimidazole with its Ultra-high Diluted Formula Reduces the Toxic Effects and Allows Increasing of Dose in Dose-dependent Protocol in Mice Infected with *Trypanosoma cruzi*. *Int J High Dilution Res.* 2015;14(3): 10-19