Zincum metallicum controls tissue inflammation in mice infected by *Trypanosoma cruzi*

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Consider the organism as a network of adaptive systems can be the key to controlling various diseases. Homeopathic medicines have the ability to start a beneficial adaptive response to infectious diseases[1-3], and *Zincum metallicum* (ZN) is involved at several points in signal transduction pathways[4,5].

Objective: To evaluate the effect of *Zincum metallicum* on histopathology of animals infected by *Trypanosoma cruzi*.

Methodology: In a blind, cotrolled and randomized study, 36 swiss male mice, 56 days were distributed into groups: CNI - uninfection and untreated control; CI - infected and untreated control; 5cHZN - infected and treated with *Zincum metallicum* prepared in Lactose; 5cHLAC - infected and treated with Lactose. The infected animals were inoculated with 1400 blood trypomastigotes of *T. cruzi* - Y strain. The medicines were prepared according to the Homeopathic Pharmacopeia Brazilian[6] and provided *ad libitum*, 48 hours before and after the infection, followed by doses of 56/56 hours until the 9th day after infection[7,8]. Three animals from each experimental group were submitted to euthanasia for the removal of organs (heart, large intestine (taken 2 cm above the rectum),

skeletal muscle (taken from the right lower limb) and liver) in the times: T0 (before infection), T8 e T12 (8^{th} and 12^{th} days after infection). The collected organ was fixed in 4% paraformaldehyde for 24 hours and then processed for paraffin inclusion. Semi serial cuts of 5 micrometers thickness were made, in the range 10 cuts, and processed by Hematoxylin-Eosin staining (HE). Slides were observed under a microscope Olympus BX41 (Tokyo Japan) and images captured with Qcolor3 camera (Olympus) coupled to the microscope. For each organ were evaluated 20 microscopic fields/cutting with a 40X objective accounting for 120 fields/animal. It was quantified the number of amastigotes per nest and the inflammation percentage classified on each organ as absent, discrete and focal, discrete and dispersed, moderate and dispersed, moderate and focal.

Results: Histopathological analysis is presented in Figures 1 and 2. On the 8^{th} days of infection there is no relevant difference in the number of amastigotes per nest between the infected groups, but on the 12^{th} day we can already see significant differences on *Zincum metallicum* treated group which evolves most beneficially. The data show that infection (CI) evolves with increasing number of amastigotes per nest in the heart and liver ($p<0.05$), considering T12, in relation to others infected groups. The 5cHZN group displayed the lowest tissue parasitism ($p<0.05$), in the heart, the organ with the bigger tropism rate, resulting in less inflammation ($p<0.05$), which also happens in other organs for this group. The 5cHLAC group displays less number of amastigotes per nest in the T12 than CI in the heart and liver ($p<0.05$), and higher number of amastigotes than CI in the intestine ($p<0.05$). The 5cHLAC group displays higher number of amastigotes than 5cHZN in the heart, intestine and muscle ($p<0.05$). The data related the inflammation, showed up higher in all organs for such treatment (Figure 2).

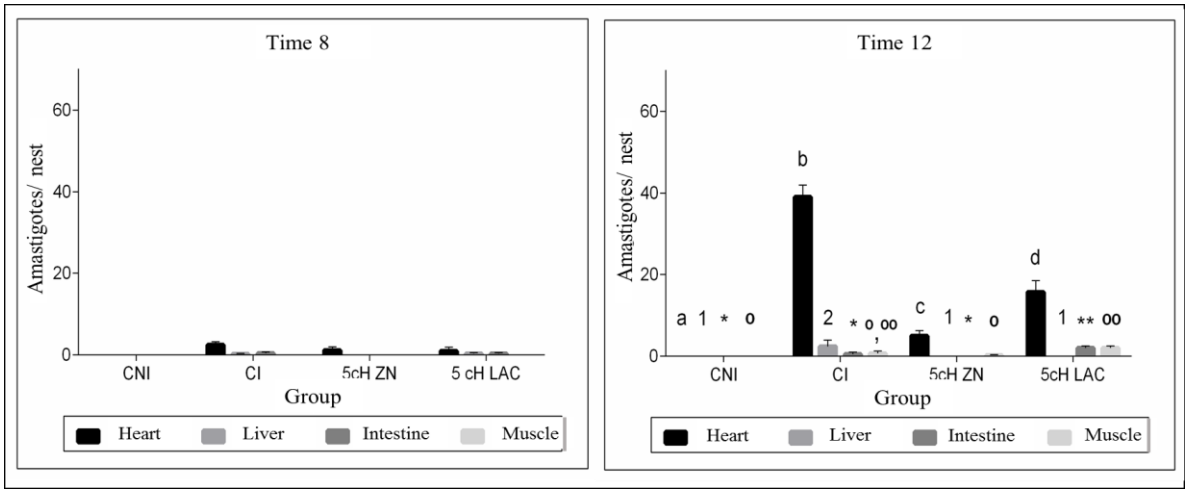

Figure 1. Mean and standard deviation of amastigotes per nest in swiss male mice, 56 days

and infected by *Trypanosoma cruzi*. Group: CNI - uninfected and untreated control; CI - infected and untreated control; 5cH ZN - infected and treated with *Zincum metallicum*; 5cH LAC - infected and treated with Lactose. Different symbols represent statistical difference ($p<0.05$), Letters: heart; Numbers: Liver; *: Intestine and; º: Skeletal muscle. Time 8 (8^{th} day of infection) and Time 12 (12^{th} day of infection).

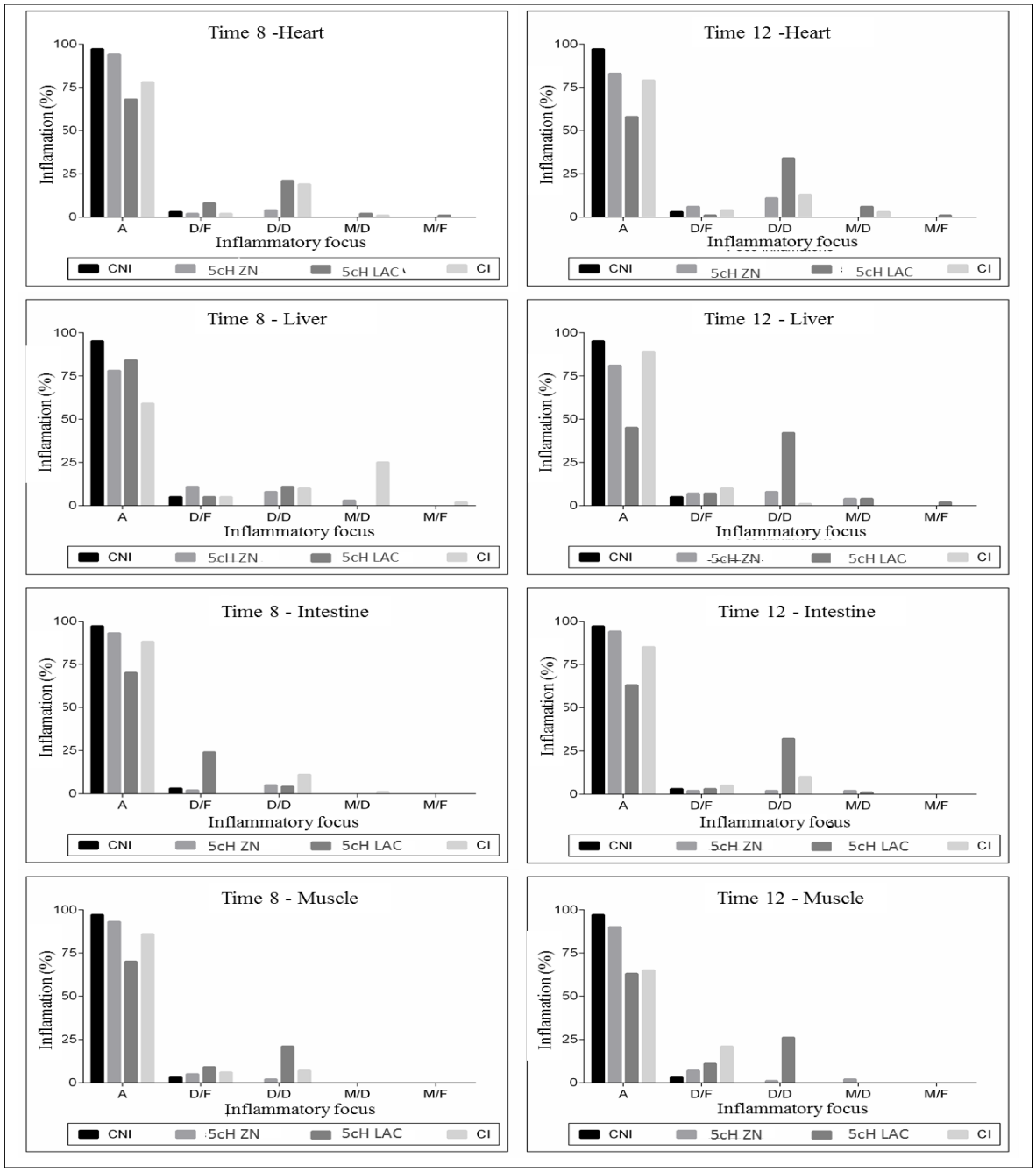

Figure 2. Percentage of inflammation in organs (heart, liver, intestine and muscle) of the swiss males mice, 56 days, infected by *Trypanosoma cruzi*. CNI - uninfected control and untreated; CI - infected and untreated control; 5cHZN - infected and treated with *Zincum metallicum*; 5cHLAC - infected and treated with Lactose. Time 8 (8th day of infection), Time 12 (12th day of infection). Inflammation: A - absence; D / F - Discrete and focal; D / D - Discrete and dispersed; M / D - moderate and dispersed; M / F - moderate and focal.

Discussion: Considering that zinc modulates the function of several regulatory proteins and is associated with a variety of cellular activities and cell signaling[4,5], treatment with *Zincum metallicum* possibly induced an adaptive response in these animals by modulating the body's response to infection, so as to contain the damage caused by the parasite. This was not observed for 5cHLAC group, which shows a decrease in the number of amastigotes per nest in the heart (highest tropism organ), but increased inflammation in relation to CI, showing that probably there was not a sufficient answer to controlling damage, impairing animal.

Conclusion: Treatment with *Zincum metallicum* 5cH altered the course of murine *T. cruzi* infection, modulated the inflammatory response, interfering with the biological system adaptive towards of animal health reestablishment.

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