

ULTRASTRUCTURAL STUDY OF *Trypanosoma cruzi* TREATED WITH BRAZILIAN GREEN PROPOLIS

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Propolis is a natural resinous substance collected by honey bees from buds and exudates of plants to be used as a protective barrier in the beehive. It is used in folk medicine for treatment of different disorders and during the last decades an increasing number of studies about its composition, biological activity and therapeutic uses have been published [1]. For more than 15 years our group has been studying the effect of propolis on *T. cruzi*. The composition of this resin is variable and complex depending on the botanic sources available to the bees [2]. In temperate zones the main sources of propolis are poplar trees and the bioactive compounds comprise mostly flavonoids. On the other hand, in tropical zones, with totally distinct vegetation, these compounds are usually present in small amounts. In Brazilian samples, several new compounds with microbicidal and cytotoxic activities have been already identified [1]. In a recent report we have shown that in spite of the striking differences between the composition of Bulgarian (**Et-Blg**) and Brazilian (**Et-Bra**) ethanolic extracts, both were active against trypomastigotes of *T. cruzi* [3], and induced in epimastigotes intracellular disorganization. **Et-Bra** was extracted from a sample of Brazilian green propolis, which has as botanical source the *Baccharis dracunculifolia*. Green propolis presents a fairly amount of flavonoids and recently has become the object of many scientific papers and also a valued exportation product, especially for Japan.

In this work we performed an ultrastructural study of epimastigote forms (Y strain) treated with 50 to 300 µg/mL of **Et-Bra** for 24 h at 28°C. Control and treated parasites were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, washed and post-fixed in 1% OsO₄ in the same buffer plus 0.8% potassium ferricyanide and 2.5 mM CaCl₂, dehydrated in crescent concentrations of acetone and embedded in Epoxy resin. The material was examined by transmission electron microscopy (TEM) (Zeiss EM10C microscope), and by scanning electron microscopy (SEM) (Zeiss DSM 940 microscope). For detection of acidic compartments in epimastigotes we incubated the parasites with acridine orange and the analysis was performed by flow cytometry using a Becton Dickinson FACSCalibur with Cell Quest program and by fluorescence microscopy (Zeiss Axioplan).

The treatment of epimastigotes with **Et-Bra** led to atypical morphology of reservosomes with increase of its volume and of the number of lipids droplets; to dilatation of the Golgi complex cisternae, and to mitochondrial swelling, with scarcity of matrix and crystae and also, the presence of membranous structures inside the organelle (Figure 1). At higher doses, the extract caused intense cytoplasmatic vacuolization. SEM corroborated morphological alterations observed by TEM. The decrease of acridine orange fluorescence was dose-dependent and statistically significant in comparison with control parasites (Figure 2). This result associated with the reservosome disorganization observed by TEM in epimastigotes suggests that this organelle is a target for **Et-Bra**. These data encourage us to continue our study with propolis, investigating cellular targets in the parasite and toxicity to animals.

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References:

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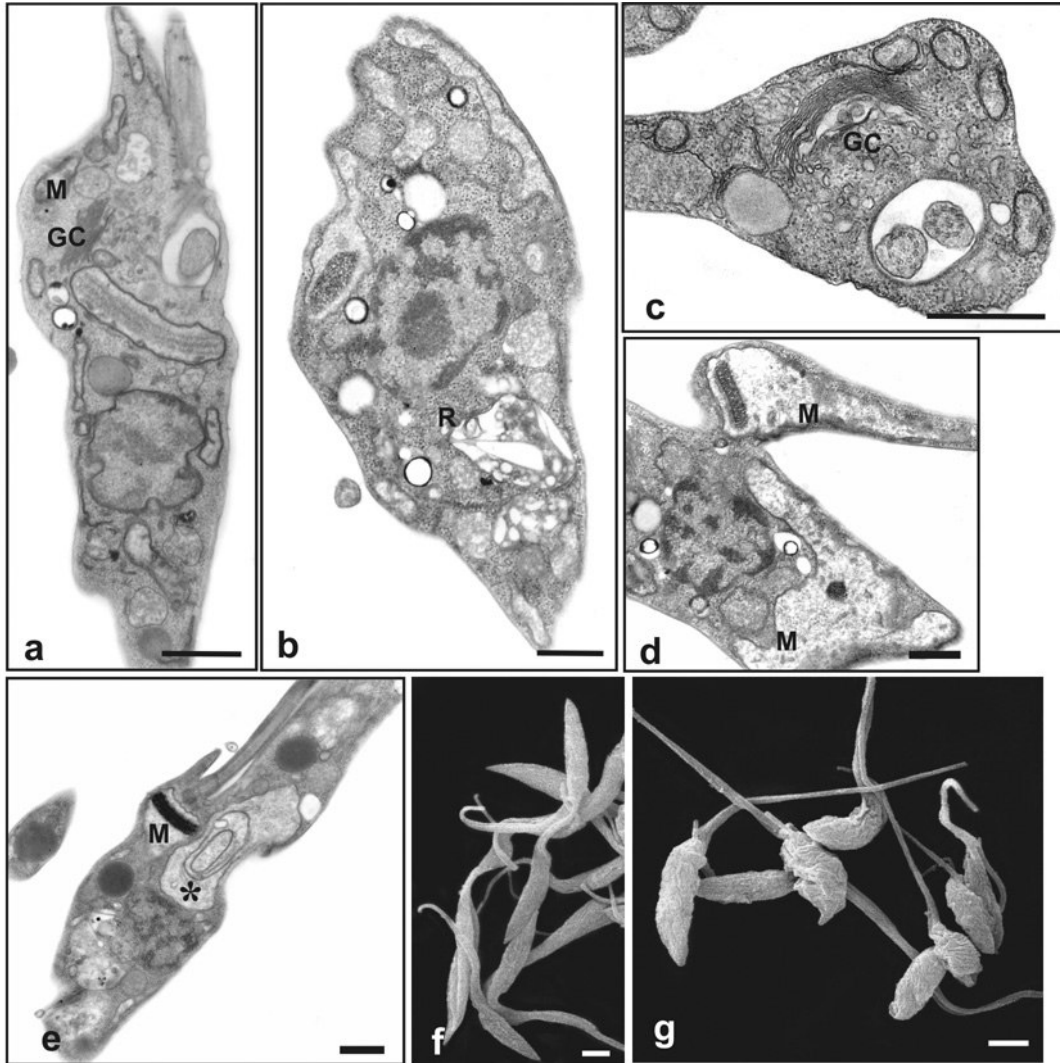


Figure 1 - TEM epimastigotes (a-e). (a) Control cells. Parasites with normal aspect of mitochondria (**M**) and Golgi complex (**GC**). (b) Treated parasites with 50 µg/mL **Et-Bra** for 24 h, showed atypical morphology of reservosomes (**R**) with increase of its volume and of the number of lipids droplets reducing the proteic matrix. (c) 100 µg/mL **Et-Bra** induced dilatation of the Golgi complex (**GC**) cisternae with structural disorganization of the organelle, (d) with 100 µg/mL **Et-Bra**, parasites showed damage at the mitochondria (**M**) with swelling of the organelle. (e) 300 µg/mL **Et-Bra** induced swelling of the mitochondria (**M**) and membranous structures inside the organelle (*). Bars = 0,5 µm. SEM (f-g). (f) Control cells, with normal morphology. (g) parasites treated with 250 µg/mL **Et-Bra** showed morphological alterations. Bars = 4 µm

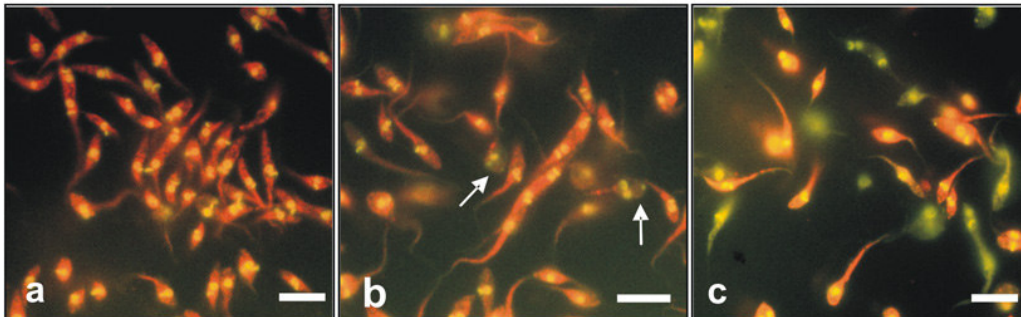


Figure 2 - Fluorescence microscopy of epimastigotes labelled with acridine orange. (a) Control; (b) Treatment with 100 µg/mL **Et-Bra**/24 h, showing decrease in the acridine orange fluorescence (arrow); (c) treatment with 300 µg/mL/24h **Et-Bra** induced a more pronounced decrease of the lanelling. Bars = 10 µm