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CRAMBE'S HIDDEN WEAPON: 2S ALBUMIN'S POTENTIAL IN TARGETING TRYPANOSOMA CRUZI

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Abstract

Plant storage proteins play a vital role in the growth and maintenance of seedlings, and their significance extends beyond basic plant survival. In mature seeds, a substantial portion of proteins, ranging from 80% to 90%, consists of distinct groups of storage proteins. These proteins not only serve as a source of nutrition but also possess remarkable properties, including antinutritional, antimicrobial, and even anti-cancer activities. Among these multifaceted plant compounds, a subgroup known as plant antimicrobial peptides has garnered considerable attention. These peptides are classified into various families, such as thionins, defensins, and cyclotides.

While the antinutritional properties of some storage proteins serve as a defense mechanism for plants, they also have significant implications for human health. Thionins derived from wheat isoforms and PTH1 defensin from potatoes have demonstrated leishmanicidal activities, showcasing their potential as therapeutic agents. Similarly, certain plant antimicrobial peptides, particularly α- and β-defensins and cathelicidin, have exhibited action against African Trypanosomes, including Trypanosoma brucei and subspecies.

Intriguingly, the repertoire of plant-derived antimicrobial and leishmanicidal peptides is extensive, but there is a notable gap in our understanding of the potential leishmanicidal activity of 2S albumin, a class of small proteins with a conserved pattern of cysteines linked by disulfide bridges. This study aims to address this gap by investigating the impact of 2S albumins derived from crambe seeds on Trypanosoma cruzi, the protozoan responsible for Chagas disease. Typically, 2S albumins are characterized by their small size (12–15 kDa) and conserved cysteine pattern, making them increasingly intriguing subjects for clinical research.

Introduction

Plant storage proteins, essential for the growth and maintenance of seedlings, are crucial to plant survival. In mature seeds, approximately 80–90% of proteins are composed of a few groups of storage proteins [1]. Depending on requirements, some seed storage proteins can also act as defensive plant molecules. They have antinutritional, antimicrobial, and anti-cancer properties [2]. The plant antimicrobial peptides comprise a large

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group of bioactive products that have been further classified into several distinct families, including thionins, defensins, and cyclotides [3]. The leishmanicidal activity attributed to thionins from wheat [*Triticum aestivum*] isoforms and PTH1 defensin from potato [*Solanum tuberosum*] were observed by [4]. The action of AMP, particularly the α- and β-defensins and cathelicidin, on African Trypanosomes (*T bruci* and subspecies) were also described by [5]. The plant trypanocidal activity was also observed for no proteic compounds. Sousa et al. observed that polysaccharides extracted from *Genipa americana* leaves have an antiparasitic, epimastigote, trypomastigote, and amastigote forms of T. cruzi [6]. Although a list of peptides with antimicrobial and leishmanicidal activity has been described, there are no reports on the action of 2S albumin against protozoa. Now we describe the effect of 2S albumins from crambe seeds on *T. cruzzi*. Usually, 2S albumin are small proteins (12–15 kDa) with a conserved pattern of eight cysteines in different polypeptide chains linked by two disulfide bridges [6]. These proteins are becoming of increasing interest in clinical studies [7]. Parasitic diseases have high incidences and negative economic impacts in developing countries. Chagas disease, or American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan *Trypanosoma cruzi*, which is transmitted when the feces of an infected triatomine vector enter a bite site or pass through an intact mucous membrane of the mammalian host. Still, transmission can occasionally occur by blood transfusion, organ transplantation, laboratory accident, congenitally, or orally [8].

Human Chagas disease is a tropical illness that affects 6-8 million people worldwide, causing approximately 8000 deaths in 2015 [9]. Chagas disease is currently treated with antiparasitic drugs such as benznidazole (BNZ). However, nifurtimox and BNZ could be promoted severe adverse effects after prolonged courses of treatment. The benefits of these drugs have not been demonstrated for the chronic phase of the disease [10]. The development of effective medication for all stages of the disease is urgently needed to control Chagas disease.

Crambe abyssinica [Hochst] is an oilseed crop belonging to the Brassicaceae family and is native to the Mediterranean region. It tends to be a promissory crop because of its high tolerance to drought and frost, short annual cycle, and high oil content of the seeds [11]. The oil extracted from the seeds, rich in erucic acid, has many industrial uses as face creams and a high potential for biodiesel production [11]. The fruits are used in traditional medicine to treat snake bites (http://www.prota.org). Crambe is a new crop, so few data on seed proteins are available. This study aimed to evaluate the toxic effects of the protein extracts of crambe seeds, in particular, isolated 2S albumin, against *T. cruzi* and LLC-MK2 cells and thus identify potential biotechnological applications for these proteins

Methodology

Biological material

*C. abyssinica*seedsweresuppliedbythe Fundação MS para a Pesquisa e Difusão de Tecnologias Agropecuárias, Maracaju, Mato Grosso do Sul, Brazil. Epimastigotesof*T. cruzi*(DM28 strain)andthecellline LLCMK2 weremantainedby Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brazil.

Protein extraction and characterization

Fractions were obtained as described [12] with minor modifications. The seeds were ground and immersed in 0.2 mol/L sodium phosphate pH 7.2 [1:5 [w/v]] for 3 h. The crude extract was centrifuged (15 000 × *g* at 4 °C for 10 min), and the supernatant was recentrifuged under the same conditions. The resulting supernatant was frozen at -20 °C until needed.

Gel-filtration chromatography

The crude extract [1 mL containing 10 mg of protein] was fractioned in a Sephadex G-50 [Sigma-Aldrich] gel-filtration column. The proteins were eluted with 0,1% trifluoroacetic acid (TFA; Sigma-Aldrich) at a flow rate

of 1 mL/min. The absorbance of the eluted proteins was monitored at 280 nm and 220 nm. The eluted fraction with molecular weights of 5-20 kDa [FCrF5-20] was freeze-dried for biological assay.

SDS-PAGE and Western analysis

The FCrF5-20 was submitted to SDS-PAGE - 12.5%. The proteins resolved by SDS-PAGE 12.5% were transferred onto nitrocellulose or PVDF membrane using 25 mM Tris, 192 mM glycine, and 20 % MeOH pH 8.3. Polyclonal mice antibody against 2S albumin from *Ricinus communis* was diluted at 1:500 for immunoblotting experiments. The immunoreactive band was developed using protein A-peroxidase (Sigma) and 3,3'diaminobenzidine (DAB) from BioRad as a substrate.

N-terminal sequence

After separation by SDS-PAGE, the protein transferred onto PVDF membrane was subjected to Edman degradation using a PPSQ-33A Protein Sequencer Shimadzu Scientific Instruments, Inc. [Columbia, Maryland, USA] to determine N-terminal partial amino acid sequence. The polypeptide sequence obtained was submitted to automatic alignment using a BLAST [Basic Local Alignment Search Tool] for sequence similarity search.

Host-cell maintenance

LLC-MK2 [*Macaca mulatta* kidney epithelial cell] cells were maintained in RPMI 1640 medium supplemented with 5% FBS [fetal bovine serum, Gibco] in sterile plastic flasks. The bottles were incubated at 37 °C in 5% CO2, and the culture medium was changed every 48 h. The monolayer cell cultures were washed three times with phosphate-buffered saline [PBS] and treated with trypsin. The suspended cells were transferred to new flasks or sterile 24-well plates [3 × 104 cells per well] with coverslips for 24 h to allow adhesion before infection with parasites [13].

Parasite maintenance

*T. cruzi*e pimastigotes were cultivated in 5 mL of liver infusion tryptose (LIT) medium supplemented with 10% FBS and 4% hemin at 28 °C. An aliquot of 1mL parasites in the exponential growth phase was added to 4 mL of new medium every 5 d. *Trypomastigotes were obtained with the acclimatization of the epimastigotes in RPMI 1650 medium at 37* ℃ for 72 h [13,14].

Determination of cytotoxic activity [2S albumin] in LLC-MK2 cells

The plated LLC-MK2 cells were incubated with various concentrations (50, 100, 500, 1000 µg/mL) of FCrF5-20, identified as 2S albumin by N-terminal partial sequence, and maintained at 37 °C for 24 h in a humidified 5% CO2 atmosphere to determine its cytotoxic effect. The cells were then quantified by counting the number of cells per visual field by light microscopy using the 40x objective [14]. Controls without the protein fraction were treated under the same conditions. All assays were performed in triplicates.

Cytotoxicity assays of the 2S albumin fraction with T. cruzi epimastigotes

Epimastigotes in the exponential growth phase were counted in a Neubauer chamber using a light microscope (Zeiss Axioinvert 135, 20× objective), and the cell number was adjusted to 1.8 × 106/mL. Aliquots of the 2S albumin were diluted in dimethyl sulphoxide and added to the LIT medium [final concentration of dimethyl sulphoxide was 1.5%], sterilized by filtration [Millex-GV 0.22 µm, Millipore], added to epimastigotes, and incubated at 28 °C in a humidified 5% CO2 atmosphere. After 24 and 48 h, samples were quantified in a Neubauer chamber. The assays were performed in triplicate [15].

The activity of the fractions on T. cruzi amastigotes

Host cells were plated over coverslips or culture flasks as described above for 24 h and were infected at a ratio of 10 parasites [trypomastigotes] per cell and incubated at 37 °C for 48 h to allow the establishment of the infection. The 2S albumin (50, 100, 500, and 1000 µg/mL) was added to the cells and further incubated at 37 °C and 5% CO2 for 24 h [13]

Morphological and amastigotes number analysis by light microscopy

InfectedLLC-MK2 samples were washed three times with PBS, fixed in Bouin's solution (19:1 picric acid: acetic acid, v/v) for 5 min, and washed four times with PBS. The cells were then stained with Giemsa stain (10%, v/v) at room temperature for six h. The coverslips containing the cells were dehydrated in decreasing concentrations of an acetone-xylene solution and mounted on histological slides using Entellan. After the incubation period, epimastigotes were centrifuged at 900 × *g* for 10 min and washed with PBS, pH 7.2, at room temperature**.** They were then fixed in a solution containing 4% paraformaldehyde in PBS and stained with Giemsa stain [10% v/v] at room temperature for two h.Aliquots of 100 µL were spread on microscope slides, dried at 37 °C, and examined under a light microscope equipped with a 40× objective. Images were obtained using an Olympus DP72 camera and processed using Cellˆ F [13].

Ultrastructural analyses by transmission electron microscopy

Uninfected and infected LLC-MK2 cells in culture flasks and epimastigotes were treated with 2S albumin at 500 µM for 24 h and then processed for transmission electron microscopy. Epimastigotes were centrifuged at 900 × *g* for 10 minutes, washed in PBS for 10 minutes, and recentrifuged under the same conditions. Uninfected and infected LLC-MK2 cells were mechanically removed with a cell scraper and centrifuges. The Pellet of the cells was fixed with 4% formaldehyde, 1% glutaraldehyde, 0.2 M sodium cacodylate buffer, and 5% sucrose at room temperature for one h. The cells were washed and post-fixed with a solution containing 2% OsO4, 0.8% potassium ferrocyanide, and 5 mM CaCl2 in 0.1 M cacodylate buffer, pH 7.2. Samples were washed in sodium cacodylate buffer and dehydrated in an increasing concentration series of acetone. The dehydrated samples were incubated in a solution of 100% acetone-EPON resin in proportions of 2:1, 1:1, 1:2, and pure EPON resin for six h each step.The samples were then embedded in EPON resin and polymerized at 60 ºC for 48 h. Ultrathin sections were obtained using an ultramicrotome (Reichert Ultracuts Leica Instruments®). The cells were stained in the dark with 5% aqueous uranyl acetate for 20 min, lead citrate for 5 min, and subsequently observed and photographed with a Jeol 1400 plus transmission electron.

Results

Protein purification and characterization

Three protein fractions of crambe seeds extract were obtained by gel-filtration chromatography, Sephadex-G50, as shown in Figure. 1A: a protein with high molecular weight, F1 (MW> 20kDa); F2, fraction range protein 5<MW<20 denoted FCrF5-20, and peptide fraction (F3). The Fraction FcrF5-20 presented trypanocidal activity and was characterized.

FcrF2-20 was characterized by SDS-PAGE, immunodetection, and N-terminal sequence. The band, MW<10kDa, observed by SDS-PAGE, transferred to the PVDF membrane, was immunoreactive with anti-2S albumin from castor seeds (Figure 1B, C). The partial N-terminal sequence of this protein determined by the Edman sequence was PQGPQQIPLLQQCCNALHQAPLLVV.

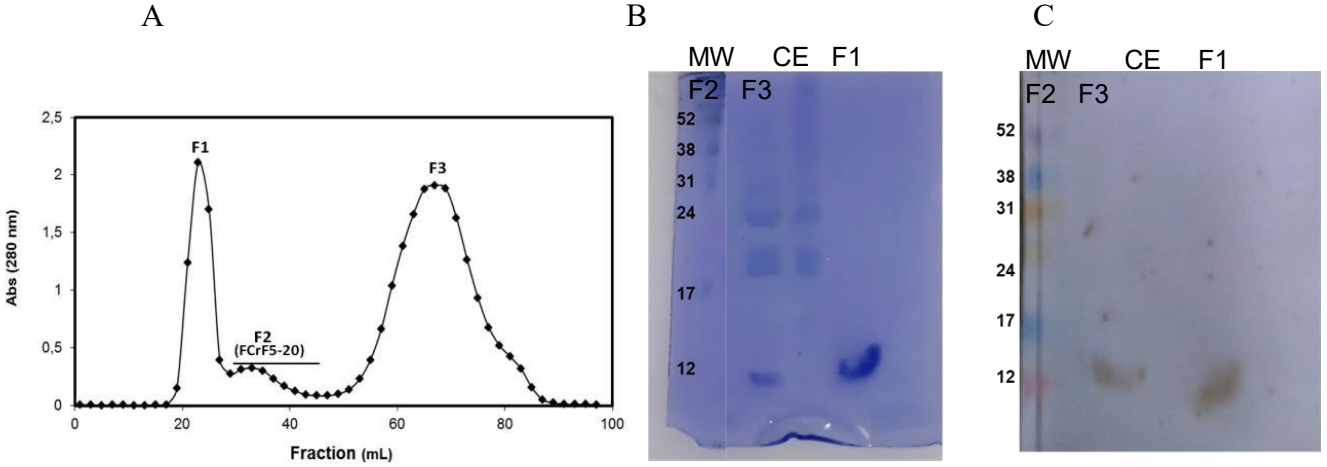

Figure 1 – Protein purification and characterization

A) Gel Filtration Chromatography. 500 µL of the crambe extract (10 mg of protein) was applied to a Sephadex G-50 column (45 x 1.0 cm) equilibrated in 0.1 % TFA. The flow rate was 42 mL/h;

B) Electrophoretic (12 % SDS-PAGE) analysis stained by coomassie brilliant Blue R-250: Lines 1,2 and 3 are crude extract, F1 and F2, respectively. C) Western blot analysis, proteins were probed with 500-fold diluted immune serum against 2S albumin from *Ricinus communis*.

The Partial N-terminal sequence of isolated crambe protein FcrF5-20 showed 100 % of identity with napin large chain L2A, allergen sin a 1.0104 [Sinapis alba], a 2S albumin and 2S seed storage protein 3 (Capsella rubella) and other 2S albumins (Figure 2). A pattern of two cysteine residues (CC) crucial to the 3D structure of 2S albumin was conserved. Bold letters indicate conserved cysteine residues (Figure 2 bold letters).

Crambe . . .. . 1 PQGPQQRPLLQQ**CC**NELHQEEPL 23

AAB37416.1 .... 1 PQSPQQRPLLQQ**CC**NELHQEEPL 23

AAS68184.1 .... 20 PQGPQQRPLLQQ**CC**NELHQEEPL 43

XP_013688210.1 92 PQGPQQRPLLQQ**CC**NELHQEEPL 115

P80208.1 38 PQGPQQRPLLQQ**CC**NELHQEEPL 61

P38057.1 40 PQGPQQRPLLQQ**CC**NELHQEEPL 63

AAA63471.1 61 PQGPQQRPLLQQ**CC**NELHQEEP 83

AAB37414.1 2 QGPQQRPLLQQ**CC**NELHQEEPL 24

CAA46785.1 93 QGPQQRPLLQQ**CC**NELHQEEPL 115

CAA46784.1 93 QGPQQRPLLQQ**CC**NELHQEEAL 115

CAA46782.1 93 QGPQQPPLLQQ**CC**NELHQEEPL 115

CAA46783.1 92 PQGPQQRPLLLQ**CC**NELDQEEPL 115

XP_018469298.1 92 PQRPPLLQQ**CC**NELHQEEPL 111

AAB22712.1 1 PQGPQQRPLLQQ**CC**NNLLQ 20

FiXP_006284992.1gure 2. Blast alignment: of isolated Crambe Protein. 87 QRPLLQQ **CC**NELQEEEP 104

Query: N-terminal sequence of isolated crambe protein. AAB37416.1, napin large chain L2A; AAS68184.1, napin 1.7S [Brassica napus var. napus]; XP_013688210.1,napin-2 [Brassica napus]; CAA62909.1, allergen sin a 1.0104 [Sinapis alba], a 2S albumin; P80208.1, Napin-3; 1.7S storage protein;P38057.1, RecName: Full=Trypsin inhibitor; large chain; AAA63471.1, storage protein [Raphanus sativus]; CAA46785.1,2S storage protein [Brassica juncea]; CAA46784.1, 2S storage protein [Brassica nigra];

46782.1; 2S storage protein [Brassica rapa]; CAA46783.1, 2S storage protein [Brassica oleracea]; XP_018469298.1,PREDICTED: napin-like [Raphanus sativus]; AAB22712.1,2S storage albumin large subunit=antifungal protein [Raphanus sativus; XP_006284992.1, 2S seed storage protein 3 [Capsella rubella]. Bold letters indicate conserved cysteine residues.

Biological evaluation Effect of 2S albumin on the LLC-MK2 host cells

The LLC-MK2 host cells viability was not affected under our assay conditions (Figure 3A). The number of the cells was not modified by the treatment with 50 to 1000 of FcrF5-20. By light microscopy analysis, untreated cells (Figure 3B) and cells treated with 100-500 µg/mL of 2S albumin for 24 h (Figure3C and 3D) had similar morphologies, spread over the substrate and typical cytoplasmic structure. However, some cells treated with 1000 µg/mL of 2S albumin were elongated, with condensed cytoplasm and retracted nuclei (Figure 3E).

The ultrastructure of the host cells presented in Figure 3F and 3G, respectively, showed that control cells and cells treated with 500 µg/mL of 2S albumin for 24 h had intact membranes and regular intracellular organization, confirming that treatment with 500 ug/mL of 2S albumin was not toxic to the host cells and did not cause morphological modifications.

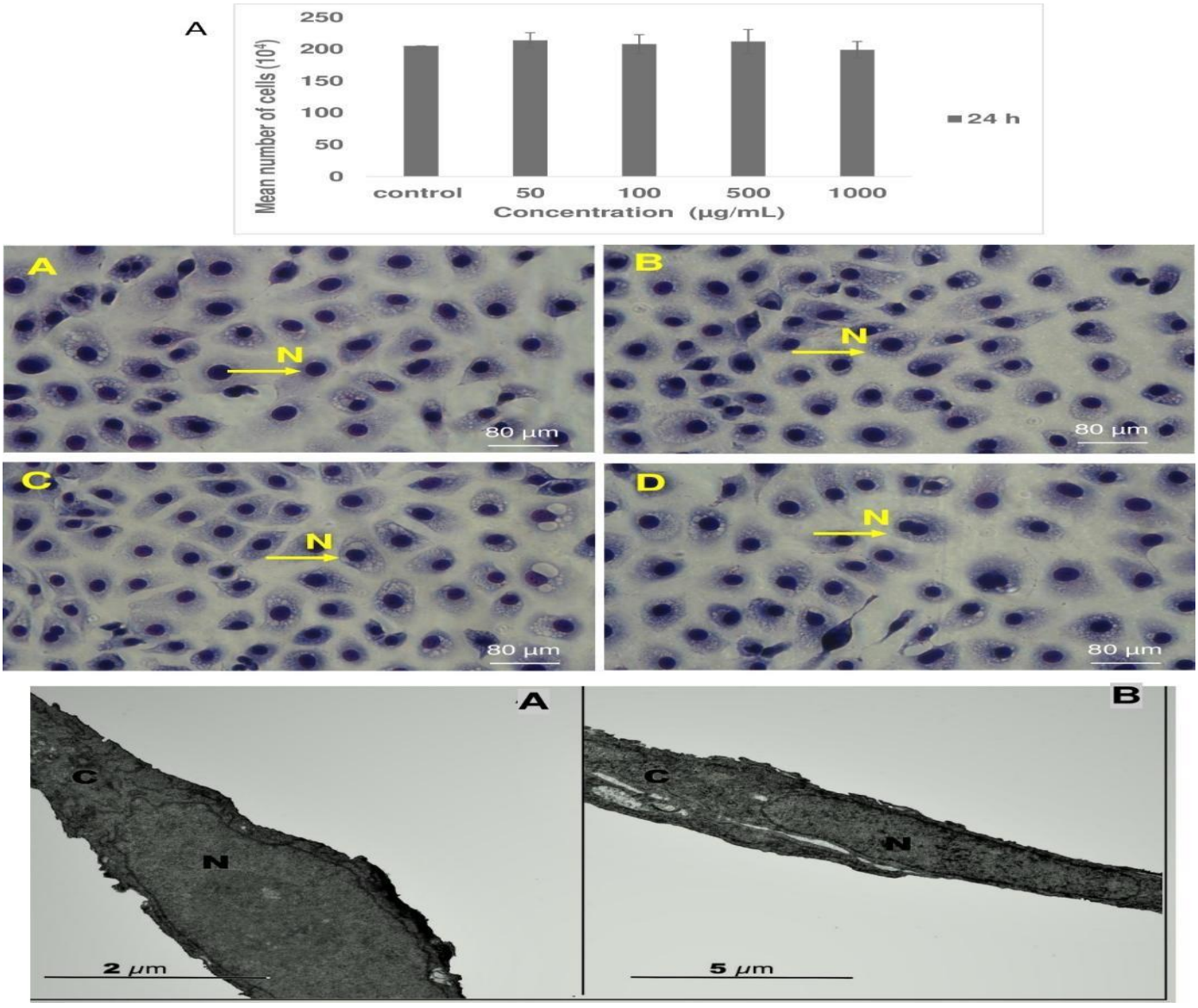

Figure 3. Toxicity assay of 2S albumin on LLC-MK2 cells

(A) Effect of the 2S albumin fraction at different concentrations (100, 500, and 1000 µg/mL) on the viability of LLC-MK2 cells after 24 h incubation. Mean the number of cells per visual field.

(B) Light microscopy - Morphology of the cells treated with 2S albumin for 24 h. (B) Untreated cells. (C) Cells treated with 100 µg/mL. (D) Cells treated with 500 µg/mL. (E) Cells treated with 1000 µg/mL. Arrows indicate condensed cytoplasms and retracted nuclei. N, nucleus. Scale bars = 80 µm.

Transmission electron microscopy - Ultrastructural analysis of LLC-MK2 cells after treatment with the 2S albumin. (F) Untreated cells. (G) Cells were treated with 500 µg/mL for 24 h. C, cytoplasm; N, nucleus. Vertical bars represent standard deviation.

Effects of crambe 2S albumin on T. cruzi epimastigotes

The anti-trypanosomal activity of 2S albumin against *T. cruzi* epimastigotes' proliferation was evaluated at various concentrations (10, 50, 100, 200, and 500 µg/mL) for 24 and 48 h. The toxic effects were similar for the concentrations ranging from 10 to 200 µg/mL after 24 and 48 h of incubation (~30% of parasites eliminated). Interestingly, 2S albumin, at 500 µg/mL, produced a much more potent inhibition, 60% after 24 h and 70% after 48 h of incubation (Figure 4).

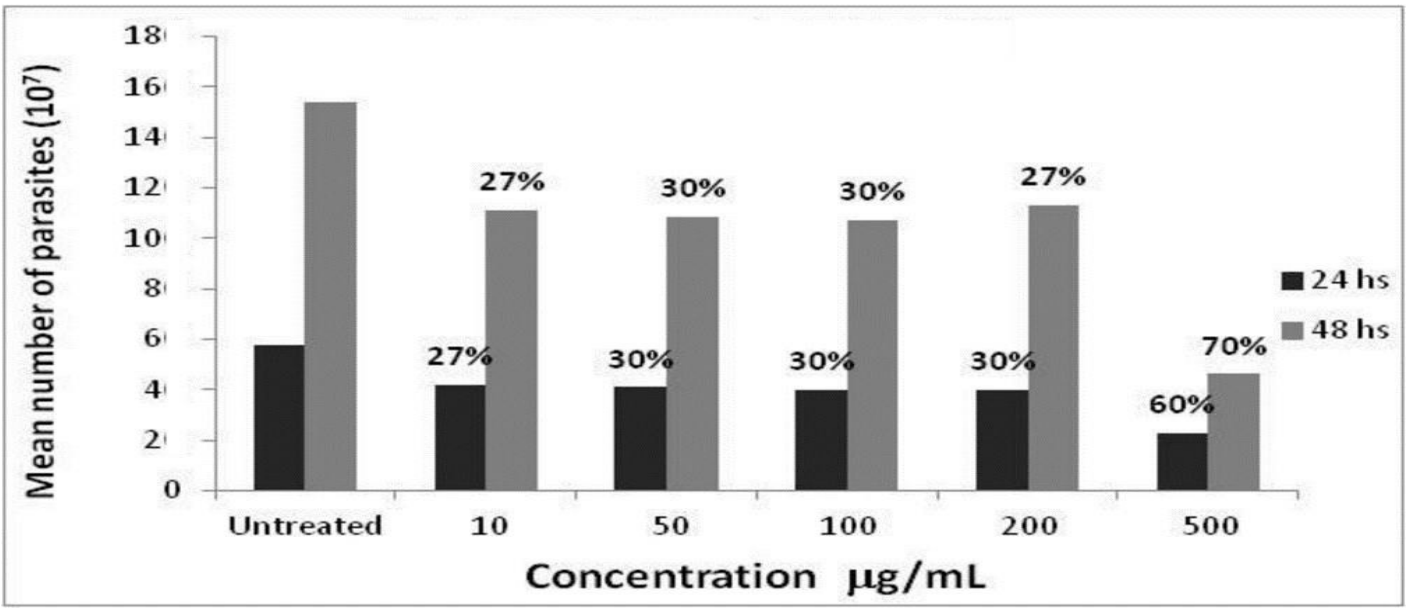

Figure 4. Effect of 2S albumin from crambe on T. cruzi epimastigotes

Antitrypanosomal effects against *T. cruzi* epimastigotes at different 2S albumin concentrations after 24 and 48 h incubation. The percentages above the bars indicate the degree of parasite elimination relative to the control. Mean the number of parasites per mL. Vertical bars represent standard deviation.

Structural and ultrastructural analyses of epimastigotes exposed to crambe 2S albumin

Morphological and ultrastructural analyses indicated that untreated epimastigotes had typical morphology, with flagella and elongated bodies (Figure 5A). However, around 30% of the remaining epimastigotes treated with 500 µg/mL of 2S albumin for 24 h presented condensed cytoplasm and flagellar loss as indicated by white arrows in Figure5B.

Control epimastigotes analyzed by transmission electron microscopy showed normal ultrastructure with an elongated body with typical nuclei, mitochondria, flagellar pockets, and kinetoplasts (Figure. 5C). Epimastigotes treated with 500 µg/mL of 2S albumin for 24 h, though, had altered kinetoplasts, large metabolic vesicles, multivesicular bodies (clue of autophagic vacuoles) (Figure. 5D), intense mitochondrial swelling, and multinucleation (Figure. 5E).

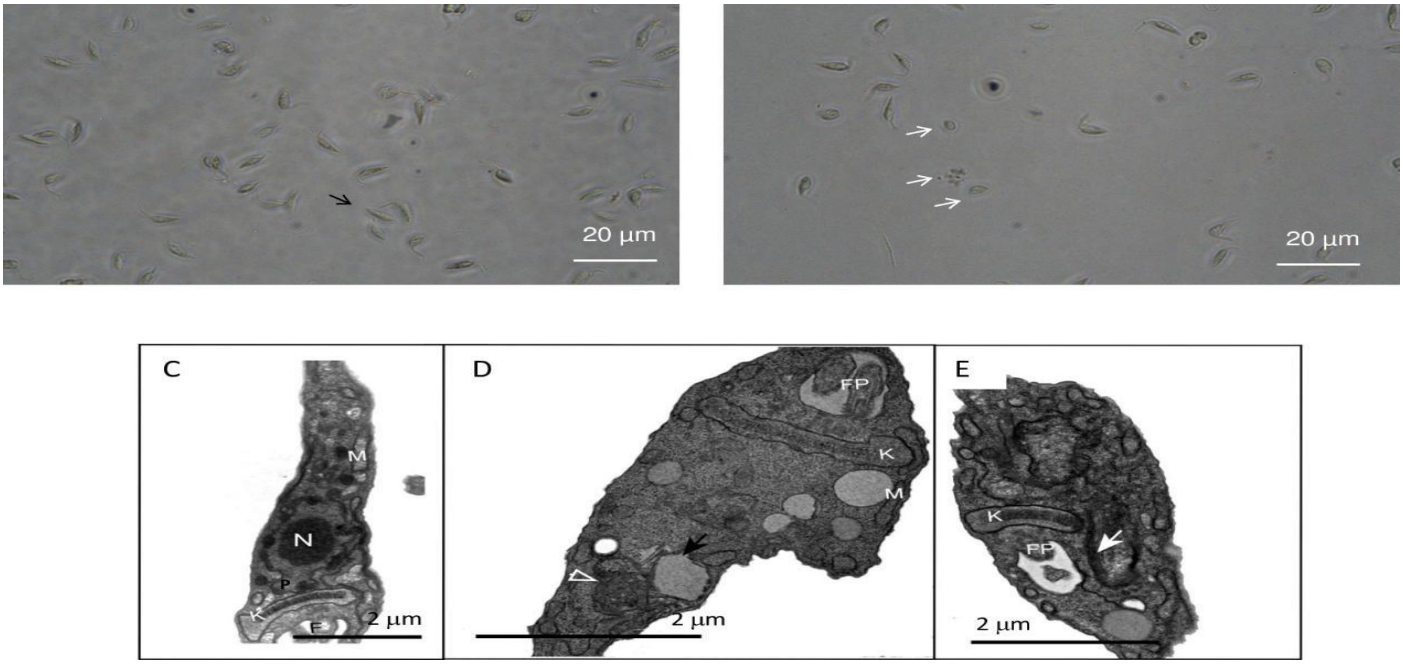

Figure 5. Morphology of *T. cruzi* epimastigotes treated with **crambe** 2S albumin.
(A) Untreated epimastigotes. Black arrows indicate viable parasite; B) Epimastigotes treated with 500 µg/mL for 24 h.; white arrows indicate morphologic alterations on the parasite. Scale bars = 20 µm. Ultrastructural effects of the 2S albumin on *T. cruzi* epimastigotes; (C) Untreated epimastigotes. (D, E) Epimastigotes were treated with 500 µg/mL for 24 h. The black arrow indicates large metabolic vesicles, and the white arrow indicates multivesicular bodies. N, nucleus; FP, flagellar pocket; M, mitochondrion; K, kinetoplast.

Morphological and ultrastructural analyses of amastigotes treated with crambe-2S albumin

We also evaluated the toxicity of crambe 2S albumin against intracellular amastigotes at various concentrations. Light microscopy revealed that untreated cells presented many intracellular amastigotes spread on the cytoplasm (Figure. 6A). The number of infected cells decreased dose-dependent after 24 h of incubation with 2S albumin, suggesting the elimination of intracellular parasites (Figure 6 B-D). Another observed effect was gathering cytoplasmatic amastigotes into the vacuole-like structure, as shown in (Figure. 6 B – white circle). The morphology of the intravacuolar parasites changed, becoming rounded and smaller (Figure. 6B-C). 2S albumin had negligible cytotoxic effects on the host cells. The mean number of infected cells (Figure. 6E black bars) decreased, and the mean number of uninfected cells increased [from 48 to 82%] (Figure 6E). The number of intracellular amastigotes decreased by about 50% (380 × 106 to 200 × 106) after treatment with 2S albumin.

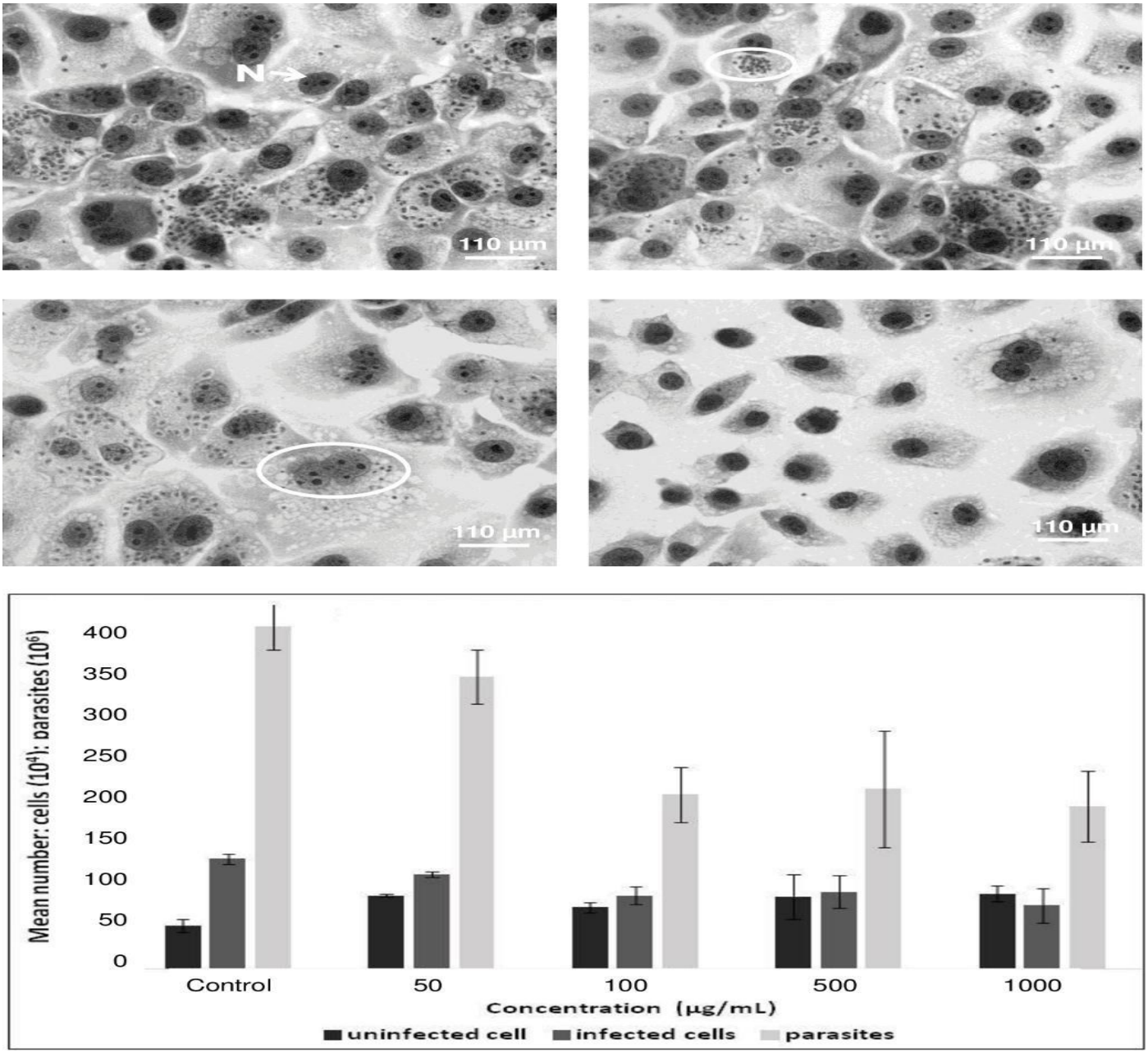

Figure 6. Morphological analyses

(A-D) Light microscopy of infected host cells after treatment with different crambe 2S albumin concentrations. (A) Control. (B) Cells treated with 50 µg/mL. (C) Cells treated with 500 µg/mL. (D) Cells treated with 1000 µg/mL. White oval indicates crowding and rounded shape of amastigotes: N, nucleus. Arrowheads indicate intracellular parasites. Scale bars = 110 µm. (E) Effects of different concentrations (50, 100, 500, and 1000 µg/mL) of 2S albumin on amastigote number in infected LLC-MK2 cells treated for 24 h (mean cell number × 104 per visual field). Vertical bars represent standard deviation.

Untreated infected cells and intracellular amastigotes had preserved typical morphology (Figure. 7A). In contrast, the parasites' morphology in infected cells treated with 2S albumin changed (Figure. 7B). The intense vacuolization, including degraded material, indicated the parasite's destruction, demonstrating the high degree of antiparasitic activity of the 2S albumin protein. Our results demonstrated that crambe 2S albumin changed the morphology of *T. cruzi* epimastigotes, which had very condensed cytoplasms, followed by their fragmentation and death. The ultrastructural analysis of epimastigotes indicated vesicular, nuclear, and kinetoplast alterations, mitochondrial swelling, and multinucleation (Figure 7).

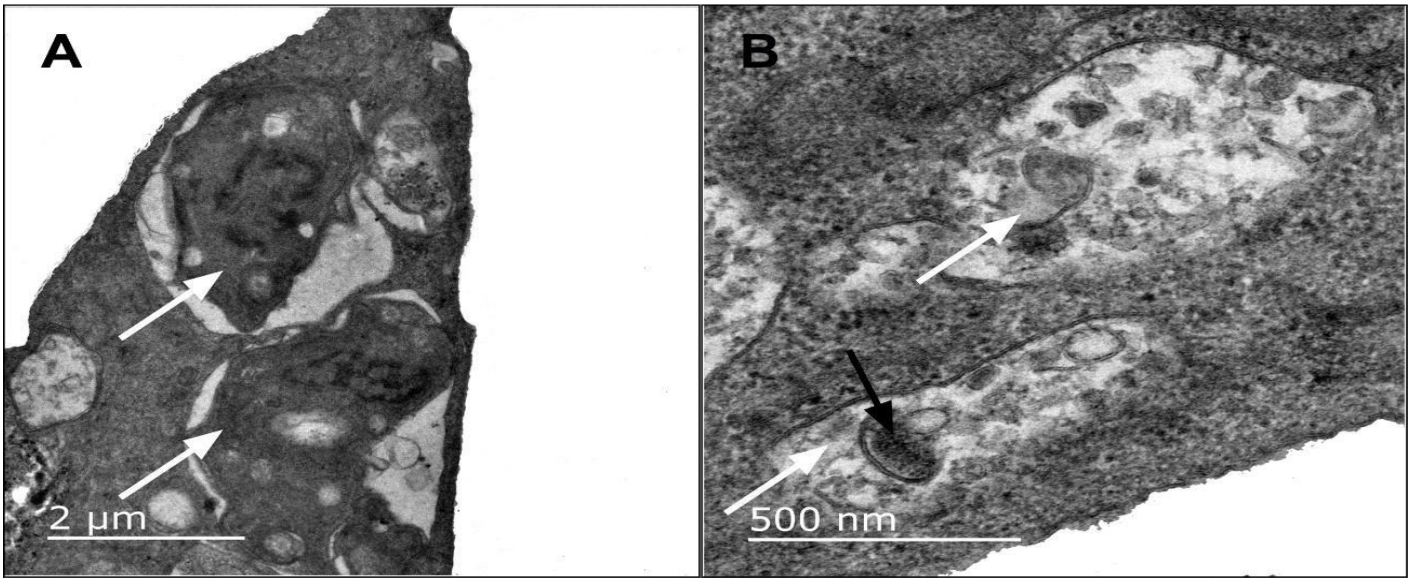

Figure. 7**.** Ultrastructural analysis of the effect of 2S albumin on the intracellular forms of *T. cruzi*.
(A) Control. (B) Infected cells treated with 500 µg/mL for 24 h. The black arrow indicates degraded parasites, and the white arrows indicate intracellular parasites.

Discussion

Neglected diseases such as Chagas-disease affect developing countries; thus, the scientific development of new low-cost and efficient drugs is needed. *C. abyssinica*is a promising new oilseed crop because of the specific properties of its oil and the cake produced since it has high levels of protein, nearly 40%. An important protein present in these seeds, the 2S albumin with trypanocidal activity was characterized. 2S albumins are seed storage proteins (SSPs), belonging to the Prolamine family, consisting of two polypeptide chains held together by well conserved disulfide-bridges, supporting the stability of temperature, pH, and proteolysis (16). The identified Nterminal sequence showed more than 90% identity with the large-chain of 2S albumins from several sources, maintaining the conserved position of amino acid residues "CC", crucial for 3D structure.

The use of 2S albumins as antibacterial agents has been proposed in an attempt to inhibit and control the growth of pathogenic bacteria in humans [17]. Gram-positive bacteria such as Pseudomonas aeruginosa, which cause respiratory infections, had their growth inhibited by “napin-like” 2S albumin (18). Bioinformatics tools and the expression of mutated proteins, as well as proposals for antimicrobial peptides derived from these molecules, have aroused great interest (19,20). De Melo 2014 et al.(15) reported that defensins from *Phaseolus vulgaris* inhibited 70% of *L. amazonensis* proliferation after 24 h of incubation. These proteins promoted the loss of cytoplasmic material, fragmentation, and the formation of multiple cytoplasmic vacuoles. Fardin et al. 2016 (21) demonstrated that 2S albumin isolated from *Bertholletia excels* induced cell membranes' permeabilization and eliminated 91% of the parasites *Leishmania amazonensis* after 48 h of incubation. These results suggest that these proteins act during a specific developmental stage of the infection, on the interaction between parasites and host cells, on the ability to transform into the proliferative form, to divide, to infect other host cells, or on the competence of the parasite to avoid the defensive mechanisms of the host cells. These processes probably involved different receptors in the membranes of the parasites and host cells and other protein classes, enzymes, and enzymatic cofactors, as described by (22,23).

Together, light microscopy and ultrastructural analysis reveal substantial morphological changes in the intracellular parasites that caused them to lose their typical shape, become rounded, and disappear from the host cytoplasm. A curious event involved in parasite elimination was gathering the amastigotes within a vacuole-like structure, as observed in figure 6B. This event should be further investigated because it can indicate a route of parasite elimination as autophagy. Modifications in intravacuolar parasites, such as changes in shape and appearance, suggest an antiparasitic effect against amastigotes. It leads to the lysis of the parasite

(Figure. 7), [24,25]. These proteins' actions on infected host cells and the parasitic life cycle inhibition can be targeted for new drug development.

Conclusion

Potential new drugs with trypanocidal activity have been surveyed amongst natural products, mainly plant compounds.

The antimicrobial activity of 2S albumin may be related to their ability: to permeate the membrane, indicating damage, promoting acidification of the medium, and oxidative stress resulting in morphological and ultrastructural alterations on cells. Our results suggest an opportunity to develop new drugs with therapeutic potential for treating human Chagas disease, but additional studies are needed to clarify their action mechanisms.

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