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LS-1-P-1502 PHOTOTOXICITY OF CRAJIRU EXTRACT (Arrabidaea chica) INCORPORATED IN POLYMERIC NANOEMULSION AGAINST MURINE MAMMARY ADENOCARCINOMA CELLS (4T1)

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Photodynamic therapy (PDT) is an alternative therapy to cure a lot of diseases, including cancer. A new set of photosensitizing (PS) drugs arising from natural plants, as vegetable oils and extracts have been tested in cancer therapy. However, most of these PS drugs present some difficulties for clinical use and in this context, polymeric nanoemulsions, kinetically stable systems, have several potential as drug carrier systems. The aim of this study was to evaluate the possible photodamage of the chloroform extract of aerial parts of the Amazon plant - crajiru (Arrabidaea chica) - incorporated in polymeric nanoemulsion (NanoECr) in PDT against murine mammary adenocarcinoma cells (4T1) in vitro. By dynamic light scattering measurements, NanoECr had an average hydrodynamic diameter of 370.5 ± 264.31 nm, and presented characteristics of a stable formulation. After incubation of 4T1 cells with different NanoECr concentrations and times, in the absence of irradiation (in the dark), non-toxic concentration (54 µg/mL) was determined by MTT and microscopy techniques and the maximum time of interaction between NanoECr and 4T1 cells (15 minutes) was determined by confocal microscopy (Figure 1). Associating 54 µg/mL of NanoECr for 15 minutes of incubation and irradiation using a laser of 670 nm wavelength at different energy doses, cell death occurred when the cells were irradiated with energy ranging from 8.57 J/cm2 to 85.7 J/cm2 and only at the maximum energy dose of 85.7 J/cm2 used in this experiment occurred 100% of cell death. Evaluating the type of cell death, cells irradiated at an energy dose of 25.7 J/cm2 had death by apoptosis, visualized by intense cytoplasmic staining of the apoptotic bodies with acridine orange in confocal microscopy (Figure 2) and the presence of blebs and mitochondria and endoplasmic reticulum damage observed in Transmission Electron Microscopy (TEM - (Figure 3). At an energy dose of 85.7 J/cm2, necrosis was the type of cellular death occurred noted by intense staining of cells cytoplasm in red (ethidium bromide) in confocal microscopy and cell membrane damage with leakage of cellular contents and presence of vacuoles inside the cells observed in TEM (Figure 3). At Scanning electron microscopy (SEM), in both energy doses (25.7 J/cm2 and 85.7 J/cm2), disruption/disintegration of the plasma membrane with the presence of numerous perforations in its structure and detachment of the cells from the substrate were observed (Figure 4). Damage was not observed when cells were incubated with NanoECr in the absence of irradiation or irradiated without NanoECr incubation. We can conclude that the chloroform extract of the aerial parts of crajiru incorporated in polymeric nanoemulsion is a potential PS formulation for use in PDT.

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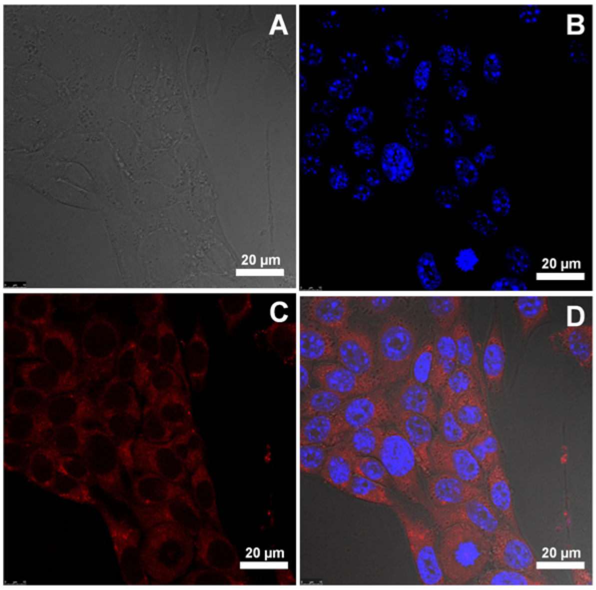

Fig. 1: Confocal micrographs of 4T1 cells exposure in vitro to NanoECr for 15 minutes in the dark. A) Phase contrast, B) Nucleus cell stained with DAPI (blue), C) NanoECr fluorescence in cell cytoplasm (red), D) Overlay of A, B and C.

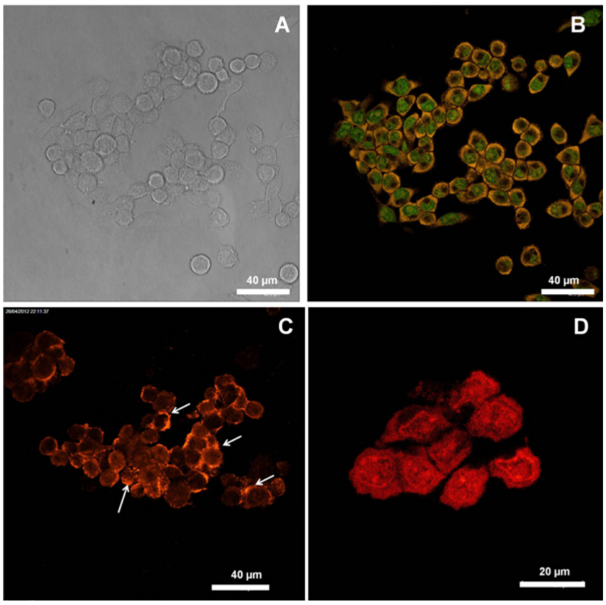

Fig. 2: Confocal micrographs of 4T1 cells stained with acridine orange and ethidium bromide after PDT. A) Control - Bright field, B) Control – Fluorescence, C) PDT 25.7 J/cm^2. Blebs in plasma membrane represented by intense orange staining (arrows) indicating apoptosis, D) PDT 85.7 J/cm^2. Cells stained in red indicating cell death by necrosis.

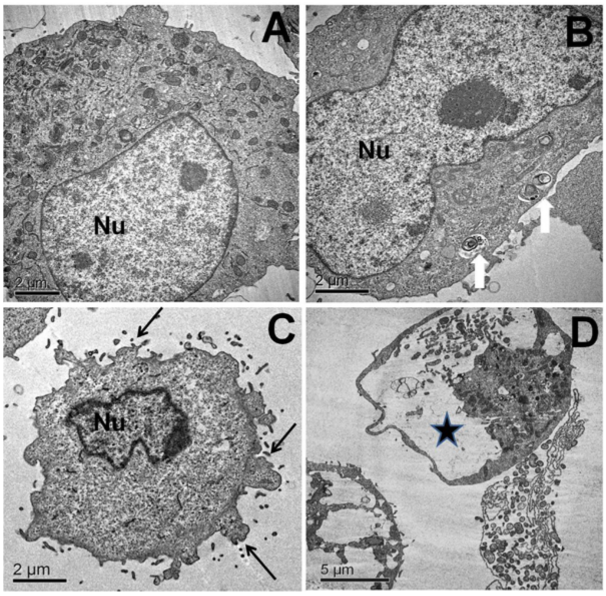

Fig. 3: TEM Electron micrographs of 4T1 cells. A) Control, B) Cell incubated with 54µg/mL of NanoECr in the dark. White arrows indicate amorphous vesicles, C) PDT 25.7 J/cm^2. Black arrows indicate blebs in plasma membrane, D) PDT 85.7 J/cm^2. Note the release of cytoplasmic contents and vacuoles (star) inside cells. Nu = cell nucleus.

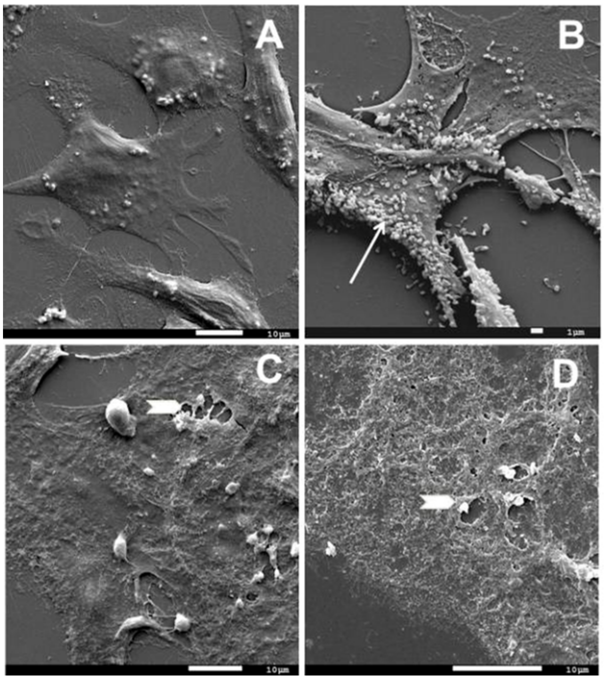

Fig. 4: SEM micrographs of 4T1 cells. A) Control, B) Cell incubated with 54µg/mL of NanoECr in the dark. Note the presence of diffuse nanostructures in the membrane (arrow), C) PDT 25.7 J/cm^2, D) PDT 85.7 J/cm^2. Note the presence of perforations in plasma membrane (arrows). In D, total disintegration of the cell.