Hemolymph from *Podalia* sp (Lepdoptera) prevents apoptosis by enhancing actin depolymerization

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Key words: Anti-apoptotic, hemolymph, bioprospection

Introduction. Apoptosis has a central role in many cellular processes, including embryonic differentiation and development of some diseases like cancer and Alzheimer's. Molecules that interfere in the apoptotic process may be used in the biotechnology industry, specially in the development of products employed in cell culture. Thus, the discovery of new antiapoptotic proteins as well as the control and understanding of their action mechanisms are essential for further progress in this field. So, the objective of this study, is to identify the potential antiviral of a protein isolated from hemolymph of larvae of *Podalia sp* (*Lepidoptera: Megalopygidae*).

Figure 1 - *Podalia sp*

Methods. The hemolymph from larvae of *Podalia sp* was collected **(Fig.01)** and the citotoxicity/genotoxicity were evaluated in culture (up to 5%). The anti-apoptotic protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel filtration column system (Superdex 75) and further Fractionated using a Resource-Q ion exchange column system. The fractions and sub fractions obtained were tested for antiapoptotic activity in VERO cells. Samples were obtained daily and analyzed by epifluorescence microscopy after acridine orange and ethidium bromide stain **(Fig.02).**

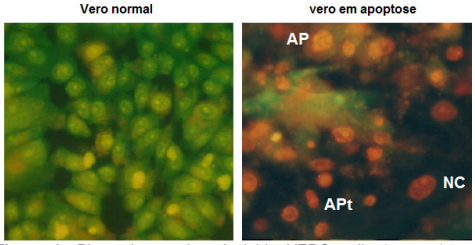

Figure 2: Photomicrography of viable VERO cells (green-v) or in the process of apoptosis initial (AP) or late apoptosis tPA) (in red). NC = VERO cell death by necrosis. Cells were stained with acridine orange and ethidium bromide and observed in an epifluorescence microscope (20x).

Results. Cytotoxicity and genotoxicity of *Podalia* sp. hemolymph was evaluated and no adverse effect was observed. This protein was capable to protect VERO cells against death induced by nutrients depletion as well as apoptosis induced by chemical agents Tert-butyl (50 to 200 µM) or Hydrogen Peroxide (50 to 2mM). Apoptosis was induced in VERO cells and the protective effect of hemolymph on the cytoskeleton was studied. For this, apoptosis was induced with 200µM of terbutil in cells treated and not treated with hemolymph of Podalia. After 24 hours the medium was removed from cells and the cells were incubated with FITC-conjugated **.** As can be observed at Fig 3, hemolymph of Podalia was able to avoid the lost of cytoskeletal structure, ahter 24 hours of apoptosis induction by 50µM of terbutil. The same was observed after H_2O_2 treatment**.**

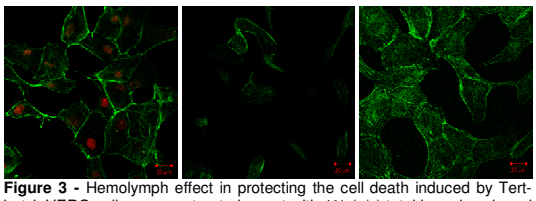

Figure 3 - Hemolymph effect in protecting the cell death induced by Tert-butyl. VERO cells were pretreated or not with 1% (v/v) total hemolymph and the cell death was induced with 50uM of Tert-butyl for 4 hours. Nuclei were stained in red by propidium iodide and cytoskeleton (F-actin) in green by phalloidin and observed in confocal microscope.

Conclusions. The hemolymph of *Podalia* sp contain components able to inhibit death by apoptosis inducet by nutrient depletion or chemical agents. Was observed that this component can act in cytoskeletal structure, increasing the cell viability acting to maintain the physiological and functional conditions of the cells. So, this product can be of high biotechnological importance to cell culture used for imunotherapic production as viral vaccines and recombinant products.

Acknowledgements. Supported by FAPESP 10/52434-6

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