Title: Biochemical characterization and in vitro cytotoxic effect of a polipeptide and protein mix obtained from the Rophalurus junceus venom.

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Introduction

Pharmacological evaluation of extracts of organisms and their isolation is an essential aspect in the process of drug discovery and development.
In this study we carried out an in vitro evaluation of a mixture of polipéptídidos and proteins derived from the venom of *Rophalurus junceus*, popularly known as blue scorpion.
To perform this work, venom fluid extracts of Rophalurus junceus was obtained from Labiofam laboratories in the province of Camaguey. Biochemical characterization of fluid extract was carried out using a high-pressure purification system (HPLC) and electrophoresis on a polyacrylamide gel.
To measure the cellular cytotoxicity, a metabolic damage staining method with neutral red was used.
As a result it was found that the fluid extract of Rophalurus junceus contains at least 13 fractions, within which about 50% consist of polypeptides of less than 14 kD. Evaluation of the in vitro cytotoxic effect of the fluid extract in prostate cancer line Dunning R3327-G, revealed that the dose of 100ug produced the greatest inhibition of tumor growth, while in the model of murine myeloma P3-X63 / AG8 / 653, a greater cytotoxic effect was observed at doses from 1 to 10 ug.

Methods

<u>Collection and preparation of the toxin</u>.
Venom biochemical characterization. (Protein Electrophoresis Method of Laemly, high pressure liquid Chromatography (CM-Sephdex C25).
<u>Cell lines and culture conditions:</u>
Myeloma P3X63 / AG8 / 653.
Rat Prostate tumor cancer Dunning R3327-G.
<u>In vitro cytotoxicity assays</u>:
Growth kinetics assay (dye exclusion method with Trypan blue dye); metabolic damage Test (neutral red).
Selected doses were 1, 10, 100, and 200 micrograms per milliliter.
As a positive control the cytostatic 5 fluorouracil was used at a concentration of 5 micrograms per milliliter.
As negative control culture medium lacking the toxin, was used where the volume occupied in the former case was substituted by poison 1xph7.2 PBS.
The tests were conducted for 72 hours as established in the literature, performing cell counts every 24 hours with the help of Neubauer chamber.

Results

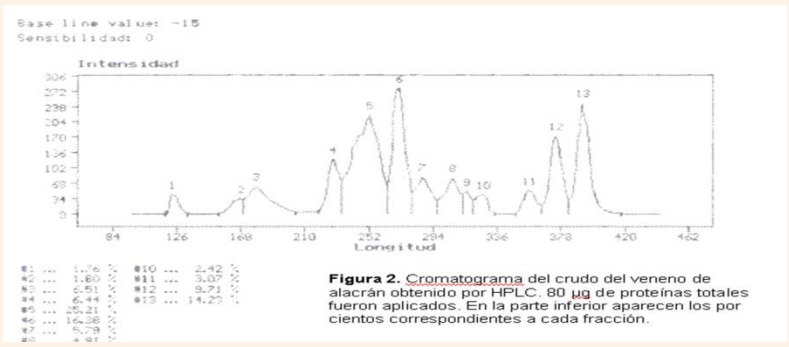
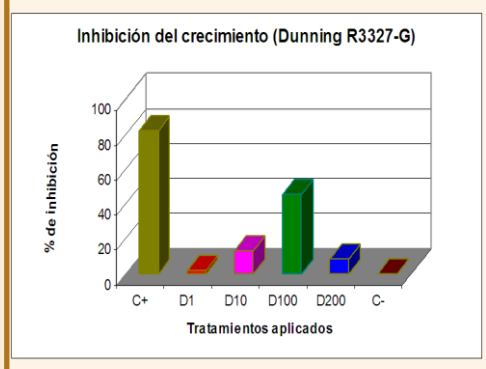


Figura 2. Cromatograma del crudo del veneno de alacrán obtenido por HPLC. 80 µg de proteínas totales fueron aplicados. En la parte inferior aparecen los por cientos correspondientes a cada fracción.

Figure 1. Discontinuous reduced protein electrophoresis (SDS-PAGE 15%).
Line 1: Molecular weight markers. Albumin-67KDa, 43KDa-Ovalbumin, 14,5KDa-lysozyme.
Line 2: Raw scorpion venom back to salting out with 70% ammonium sulfate.



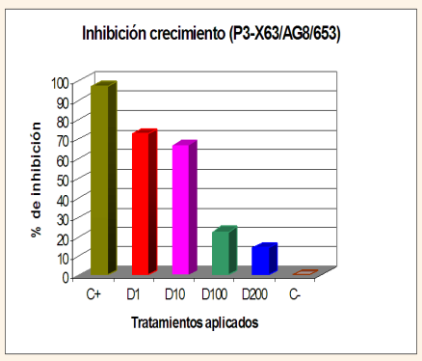


Fig. 3.Cell cytotoxicity test. using a mixture of polypeptides and proteins derived from scorpion venom junceus Rophalurusin in cell ines (P3-X63 / AG8 / 653 and Dunning R3327-G) from the ATCC.
a) The doses of 1 and 10 ug / mL caused significant tumor growth inhibition (72.3 and 66.7% respectively) in murine myelomas, however higher doses (100 and 200ug) caused a very modest effect.
b) Effect of different fractions from scorpion venom Rophalurus junceuss in the the hormone-sensitive prostate cancer cell line Dunning R3327. As can obsseve, only concentrations of 100 ug / mL. produced cell growth inhibition

Conclusions

In conclusion, we can state that the extract poison Rophalurus junceus contains a mixture of polypeptides and proteins responsible for its cytotoxic effect which varies between cell lines especially according to its concentration