CYTOTOXIC EVALUATION

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Keywords: cytotoxicity, biological evaluation.

New drugs, cosmetics, food additives, and so on go through extensive cytotoxicity testing before they are released for use by the public. This testing usually involves a large number of animal experiments. There is much pressure, both humane and economic, to perform at least part of cytotoxicity testing "in vitro".

The introduction of specialized cell lines and interactive organotypic cultures, and the continued use of long-established cultures, make this a reasonable proposition. The experiments carried out "in vitro" have the purpose of determining the potencial cytotoxicity of the compounds being studied, because the compounds can be used as pharmaceuticals and must be shown to be nontoxic.

The aim os the work was to evaluate the toxicity of proteins obtained from snake venoms. The method is based on the procedure originally developed by Borefreund and Puerner (1984) for the screening of cytotoxic agents, in general, over monolayer ceel. It is based on the quantitative assesment of surviving viable cells upon exposure to the toxic agent, by incubation with the supravital dye tetrazolium compound MTS, in a microtitration assay using Chinese hamster ovary cells culture (ATCC CHO K1). Cytotoxicity (CT50) was estimated, by curve interpolation, as the protein concentration resulting in 50% inhibition of MTS uptake, after ploting the mean percentage of surviving cells against the concentration of the protein.

A total of four proteins purified from snake venom, stored as dry powders at 20^0 C, were analysed: gyroxin (from Brazilian rattle snake venom); phospholipase A2 (from Brazilian rattle snake venom), and irradiated and non irradiated bothropstoxin (from Bothrops jararacussu venom). The cytotoxicity curves and CT50 obtained for each protein showed that the native bothropstoxin was the most cytotoxic compound.

A micrograph of the cultured CHO cells used in the assay is showed in (FIG.1).

In the (FIG.2) the graphic represents the comparison between the cytoxicity of irradiated and non irradiated bothropstoxin.

In despite of the variety of physiological activities present in those toxins, the results showed a good correlation between the "in vitro" and the "in vivo" tests.

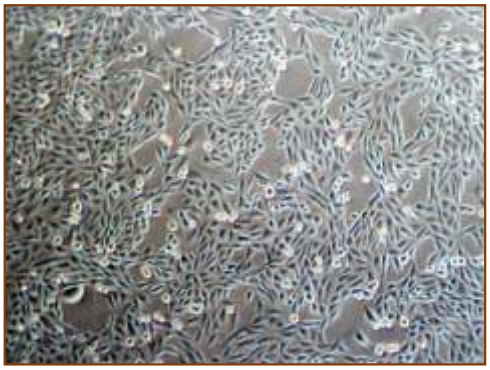

FIGURE 1 - Culture of Chinese hamster ovary cells (CHO) (magnification 100X).

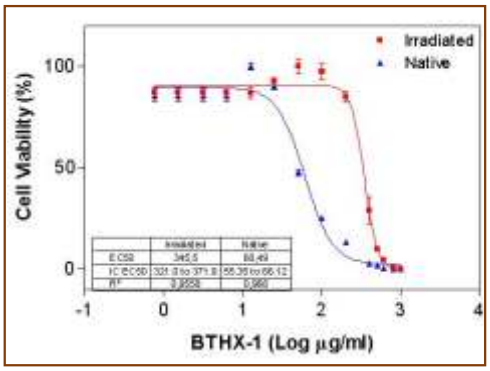

FIGURE *2* - Cytotoxicity test with CHO cells of native and irradiated bothropstoxin from *Bothrops jararacussu* venom.