DETERMINATION OF CYTOCHROME C AS A PARAMETER OF CYTOTOXICITY TO CHINESE HAMSTER OVARY (CHO) CELLS TREATED WITH SAPONINS

Diego A. Trujillo-Roldán[1], Fernando Orozco-Sanchez[1], Juan Lopez[1], Norma A. Valdez-Cruz[2], Mauricio A. Trujillo-Roldán[2].

1. Bioprocesos y Biotecnología Animal, Universidad Nacional de Colombia-Sede Medellín.

2. Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

datrujil@gmail.com

Key words: Cytotoxicity, Cytochrome c, Permeabilization, Spectrophotometry

Introduction. Triterpene saponins are glycosidic plant substances of which have been reported proapoptotic activities with action mechanisms on cell membranes (1). Saponins permeabilize biomembranes allowing the output of the metalloprotein mitochondrial cytochrome c to the extracellular space (2). In this work, reduced cytochrome c was determined as an indicator of the decrease of cell viability and its measurement in the culture medium (turbid medium) is made by a novel and selective spectrophotometric technique. This determination is compared with typical viability assays as MTT an trypan blue.

Methods. A novel spectrophotometric technique was used, this modulates the optical signal (FFM), obtaining direct measurements of concentration of reduced cytochrome c from 10ug/ml to 100ug/ml (0.8nmol/ml to 10nmol/ml). Furthermore, membrane permeabilization of human red blood cells and CHO-K1 and Jurkat cells membranes was assayed by the action of the triterpenoid saponin Quillaja Bark. Saponin cytotoxic activity was also determined in two cell lines by trypan blue and MTT bioassays.

Results. Cytotoxic activity was determined by *Qillaja Bark* saponins (S7900 and S2149 Sigma) in CHO-K1 cells by trypan blue bioassay and for MTT. The initial viability of each culture treated was assumed as 100% for comparison terms. After a saponin treatment of 24 h, a decrease in viability was observed for each evaluation at different saponin concentration (fig 1). Moreover, the half maximal inhibitory concentration (IC50) was 14ug/ml using MTT.

On the other hand, dispersion, absorption and modulation of the optical signal were used, in order to characterized the spectrum of cytochrome c and CHO-K1. This allow the obtaining of a ratio of the number of CHO-K1 cells with respect to reduced cytochrome c from 10000 cell/ml with 0.5nmol/ml of reduced cytochrome c until $0.9X10^6$ cells/ml with 7.2nmol/ml of reduced cytochrome c. As can be seen in fig 1, the determination of cytochrome c is inversely proportional to MTT and trypan blue bioassays.

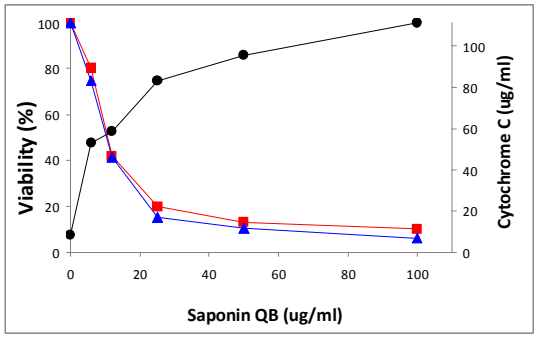

Fig.1 Relationship between the quantification by cythochrome C (*dots*), MTT (squares) and trypan blue (triangles).

Conclusions. A novel spectrophotometric method can be used as a cytotoxic bioassay, contributing to the known battery of bioassays. The determination of cytochrome c is inversely proportional to MTT and trypan blue bioassays.

Acknowledgements. Fellow and laboratory access on the Universidad Nacional de Colombia. Prof. Mario Arias-Zabala and Prof. Victor Lemeshko. Conacyt 178528, 181895, 104951-Z and PAPPIT-UNAM 209113, 210013.

References. **1.** Appaix F, Minatchy M, Riva-Lavieille C, Olivares J, Antonsson B, Saks VA (2000). (BBA) 1457 175-181. **2.** Arias M, Orduz S, Lemeshko VV (2009). (BBA) 1788 532-537.