

## Cytotoxic effects of *Carica papaya* crude leaf extracts on Vero cells and C6/36 cells prior to testing antiviral activity of the extracts against dengue viral infections

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**Introduction and Objectives:** Plant-based compounds are an important option in developing new anti-viral drugs. They contain a variety of secondary metabolites with a wide range of biological activities. *Carica papaya* has anti-microbial properties. CytoTox 96® (Promega, USA) is a colorimetric assay that quantitatively measures lactate dehydrogenase (LDH) released upon cell lysis. The objective of this study was to identify the *in vitro* cytotoxicity of *C. papaya* leaf extracts against a normal African green monkey kidney epithelial cell line (Vero cells) and C6/36 *Aedes albopictus* cells prior to conducting antiviral studies against established dengue viral infections.

**Methods:** *C. papaya* leaves were ground, and the fresh, neat extract was diluted in normal saline to prepare a two-fold dilution series. Two 96 well assay plates were prepared separately with Vero cells and C6/36 cells. The assay was set up using CytoTox 96® (Promega-USA) kit manufacturer's instructions which included the following controls, 1. Negative control-without Vero/ C6/36 cells, 2. Vehicle control-untreated cells, 3. Positive control-lysis solution with four replicates. *C. papaya* extract was added into the test wells at different concentrations and one plate was incubated for 5 hours at 37 °C and the next plate was incubated for 24 hours at 37 °C. The absorbance data were measured using a standard 96-well plate reader (Labtech LT-4500, Singapore) and percentage cytotoxicity was calculated for each of the concentrations tested.

**Results:** The cytotoxicity driven colour intensity and absorbance values decreased with the decreasing concentrations of *C. papaya* leaf extract. The percentage cytotoxicity against Vero cells for dilutions of 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024 were 102.25, 80.41, 82.90, 82.10, 83.94, 56.13, 4.64, 1.89, -10.50, respectively for the 5-hour incubation and 152.32, 134.84, 130.59, 133.11, 121.75, 76.93, 19.12, 2.67, 7.26, respectively for the 24 hour incubation. The percentage cytotoxicity against C6/36 cells were 85.55, 38.81, 33.13, 31.11, 27.36, 18.09, 14.37, 11.71 respectively for the 5 hour incubation and 233.77, 126.85, 90.64, 52.22, 36.54, 22.86, 20.20, 15.49, 12.94 respectively for the 24 hour incubation.

**Conclusions:** High concentrations of *C. papaya* leaf extracts showed high level of cell lysis. A high concentration of the extract was cytotoxic to both Vero and C6/36 cells. Knowing the cytotoxicity of leaf extracts helps to select the minimum toxic concentrations for antiviral testing of the extract.

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