Antiviral activity of *Carica papaya*leaf extract against experimental infections with dengue virus-2,-3 and -4

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Introduction and Objectives: There are no specific anti-dengue anti-viral drugs available at present. Plant derived compounds are an important option for development of new drugs and *Carica papaya* is a traditionally used medicinal plant. Antiviral activity of *Carica papaya* extract against experimental DENV-1 infection was published previously (SLJID 2021 (Suppl 1)).The objective of this study was to determine the anti-dengue viral activity of *Carica papaya*leaf extract against experimental (dengue virus) DENV-2, DENV-3, and DENV-4 infections.

Methods:*C. papaya* leaf extract was prepared in two-fold dilutions from neat to 1/1024 in normal saline. As a first step, the cytopathic effects were determined to select a minimum toxic concentration of *C. papaya* leaf extract in C6/36 cells. Then,24-well cell culture plates containing C6/36 cells infected with DENV-2, DENV-3 and DENV-4 separately weretreated with *C. papaya* leaf extracts at selected concentrations. After 24 hours post infection, cells were harvested with the supernatant for testing. Viral RNA was extracted (QIAGEN, Germany) from DENV-infected untreated control and four infected treated samples with *C. papaya* leaf extracts. Viral RNA extracts from DENV-2, DENV-3 and DENV-4 experiments were subjected to qRT-PCR. The analytical system based negative control, positive control and four standard dilutions of positive control in replicates were used to quantify DENVRNA using the Dengue generic real time RT-PCR (Liferiver, China).

Results: Based on themorphological changes or the cytopathic effects (CPE), less cytotoxicity in cells was observed from 1/16-1/512 dilution of *C. papaya* leaf extracts. Concentrations above 1/16 were toxic to C6/36 cells because it causes cell rupturing with high CPE.Thus, 1/32-1/256dilutions were selected for treating the experimental DENV infections.qRT-PCR resultsshowed DENV RNA in all 4 standards controls (SD1-10^7, SD2-10^6, SD3-10^5, SD4-10^4DENV copies/mL), infected untreated controls (DENV-2=$6x10^6$, DENV-3=$6.9x10^6$, DENV-4=$4x10^6$copies/mL). qRT-PCR did not showDENV RNA in negative controls and showed 0/<50 copies/mLDENV RNA in treated samples with 1/32-1/256 concentrations of *C.papaya* leaf extracts.

Conclusions:*C. papaya* leaf extractsinhibited experimental DENV-2, DENV-3 and DENV-4 infections. These promising findings are the early steps in producing scientific evidence for the anti-dengueviral activity of *C. papaya* leaf extracts.

Keywords: Dengue, Antiviral activity, C. papaya leaf extract, DENV-2, DENV-3, DENV-4

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