ANTIVIRAL ACTIVITY OF VESTITOL AGAINST CHIKUNGUNYA VIRUS: *IN VITRO* AND *IN SILICO* STUDIES

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ABSTRACT

Background: Chikungunya virus (CHIKV) belongs to the *Alphavirus* genus and the *Togaviridae* family. It is transmitted primarily by mosquitoes of the *Aedes* genus, notably *Ae. aegypti* and *Ae. albopictus*. CHIKV has a positive-sense single-stranded RNA (+ssRNA) genome of 11.8 kb, encoding a structural polyprotein that includes a capsid protein (C) and envelope proteins (E1, E2, E3 and 6K), along with four nonstructural proteins (nsP1, nsP2, nsP3 and nsP4). This virus causes Chikungunya fever (CHIKF), a self-limited disease characterized mainly by joint and muscle pain. Despite the substantial impact of CHIKV infection on global public health, no antivirals have been approved to date. Vestitol, an isoflavonoid found in various natural sources, is known for its anti-inflammatory and antimicrobial properties. **Objectives**: This work aims to investigate the antiviral activity of vestitol against CHIKV via both *in vitro* and *in silico* assays. **Methods**: Vero E6 cells were infected with CHIKV at an MOI of 0.001 and treated with vestitol at 100 µM. After 36 hours of treatment, intracellular flow cytometry was performed with an anti-CHIKV monoclonal antibody and anti-mouse IgG/Alexa Fluor 488. The cell supernatants were collected, and viral RNA (vRNA) was obtained for absolute quantification via RT–qPCR. A standard curve with eight points, ranging from 7.7×10^8 copies/µL to 7.7×10^1 copies/µL, was generated. All the data were analyzed by converting cycle threshold (Ct) values into vRNA copy numbers. The qPCR standard curve presented a slope of −3.8 and a coefficient of correlation of 0.987%. Molecular docking was performed with AutoDock Vina software, utilizing three-dimensional structures of CHIKV molecular targets deposited in the Protein Data Bank (PDB). **Results and discussion**: After 36 hours of treatment with vestitol, a significant reduction in the percentage of CHIKV-positive cells was detected by intracellular flow cytometry. Additionally, vestitol treatment reduced the number of vRNA copies in the cellular supernatant from 3.6×10^6 copy number/µL to 8.3×10^3 copy number/µL ($p \leq 0.001$). An *in silico* analysis of the possible interactions of vestitol with 12 molecular targets of CHIKV available in the PDB was performed. The molecular docking revealed that vestitol interacts with the CHIKV nsP3 protein, with an affinity energy value of -8.7 kcal/mol, and that it interacts with Gly112 via two hydrogen bonds and Val113 and Tyr114 via hydrophobic interactions. **Conclusion**: In summary, vestitol showed promising antiviral activity against CHIKV *in vitro*. *In silico* molecular docking analysis suggested that vestitol targets the nsP3 protein. These findings require further studies to elucidate the vestitol's antiviral mechanism of action.

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