Macedonian pharmaceutical bulletin, 68 (Suppl 2) 151 - 152 (2022)
ISSN: 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.068
Short communication

Garlic essential oil shows no antiviral effects on the replication of HSV-1 in the virus-infected VERO cells

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Introduction

Allium sativum L. has long been used in folk medicine in many countries to treat various ailments (Majewski et al., 2014; El-Saber Batiha et al., 2020). Garlic was also found to exert virucidal activity against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), parainfluenza virus type 3 (HPIV-3), vaccinia virus (VV), vesicular stomatitis virus (VSV), and human rhinovirus type 2 (HRV-2). Unfortunately, concentrations that were virucidal were also toxic to HeLa and VERO cells (Weber, 1992). The constituents responsible for the virucidal effect of garlic in the order of decreasing activity were found to be: ajoene, allicin, allyl methyl thiosulfinate, and methyl allyl thiosulfinate (Weber et al., 1992).

Fresh garlic bulbs extract was shown to inhibit influenza virus A/H1N1 propagated in MDCK cells, reducing hemagglutinin (HA) titer, CPE induction, and viral RNA (Mehrbod, 2009). Ajoene was shown to inhibit early events of viral replication, particularly virus adsorption, of HIV-1 (Walder et al., 1997). However, garlic and garlic supplements require special attention when used in HIV-infected patients, since serious interactions were reported with anti-HIV drugs, ex. saquinavir (James, 2001).

As it was mentioned above, garlic was found to exert virucidal activity against Herpesviruses, but little is known about the influence of garlic on the replication of those viruses inside the infected cells. That is why the purpose of our research was to evaluate the antiviral potential of garlic essential oil against HSV-1 replicating in VERO cells. The cytotoxicity was evaluated against VERO (ECACC, No. 84113001) cells and for comparison, the human hypopharyngeal cancer cell line FaDu (ATCC, HTB-43).

Materials and methods

Chemical characterization of extracts

The phytochemical profile of garlic essential oil (commercially available) was examined by means of gas chromatography-mass spectrometry, performed with a Shimadzu GC-2010 Plus coupled to a Shimadzu QP2010 Ultra mass spectrometer. A fused-silica capillary column ZB-5 MS (30 m, 0.25 mm i.d.) with a film thickness of 0.25 mm (Phenomenex) was used. GC-MS conditions followed Sawicki et al. (2018). The retention indices were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions. Compounds were identified using a computer-supported spectral library (http://webbook.nist.gov), mass spectra of reference compounds, as well as MS data from the literature.

Cytotoxicity determination

Cytotoxicity was evaluated using microculture tetrazolium-based assay (MTT) as described previously (Świątek et al., 2021). Data were analyzed using GraphPad Prism software, and the CC_{50} values (50% cytotoxic concentration) and non-toxic concentrations were evaluated.

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Antiviral experiments

Assessment of antiviral activity was based on the observation of the influence of tested samples in non-toxic concentration on the occurrence of cytopathic effect (CPE) in HSV-1 (ATCC, Cat. No. VR-260) infected VERO cells. Subsequently, plates with antiviral experiments were thrice frozen (-72°C) and thawed, and samples were collected for viral DNA isolation (QIAamp DNA Mini Kit, QIAGEN GmbH, Hilden, Germany). Afterwards, the viral titer was assessed using Real-Time PCR. The amplification was performed with HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne, Tartu, Estonia) and primers (UL54F – 5' CGCCAAGAAAATTTCATCGAG 3', UL54R – 5' ACATCTTGCACCACGCCAG 3') on the CFX96 thermal cycler (Bio-Rad Laboratories, Inc., California, USA). The quantitative analysis was performed using a calibration curve comprised of tenfold dilutions of HHV-1 DNA isolate, which were previously quantitatively analysed using IVD certified GeneProof Herpes Simplex Virus (HSV-1/2) PCR Kit (GeneProof a.s., Czech Republic) (Świątek et al., 2021).

Results and discussion

The main components of the studied essential oil were diallyl sulfides and methylated diallyl sulfides, which are typical volatiles of garlic tubers. As previously reported, garlic essential oils can differ in their main constituents depending on the origin and distillation process (Satyal et al., 2017), however, the qualitative profile obtained in this study is in agreement with literature data.

The studied garlic essential oil showed similar CC_{50} values on VERO and FaDu, with 57.87 ± 5,79 and 60.3 ± 4.14 µg/mL, respectively, indicating no anticancer selectivity. However, at 32 µg/mL the cellular viability of VERO cells was above 90%, whereas, for FaDu it was approx. 65%. At the concentration of 5.38 µg/mL garlic essential oil, the viability of FaDu cells was 90%. The HSV-1 infected VERO cells were incubated with garlic oil in decreasing concentrations: 30, 15, 10 and 5 µg/mL until the CPE was observed in the virus control (VC, infected, untreated cells). Subsequently, the CPE in the tested samples was compared with the VC. It was observed that garlic essential oil didn't inhibit the formation of CPE. This observation was supported by the Real-Time analysis which indicated that the HSV-1 viral load was comparable in the garlic essential oil-treated samples and the virus control.

Conclusion

The results of the study show that garlic oil shows high toxicity both toward normal and cancer cells. Moreover, garlic essential oil shows no antiviral effects on the replication of HSV-1 in the virus-infected VERO cells.

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