



## Letter to the Editor

### Inhibition of Human Herpes Virus Type 2 Replication by Water Extract from *Nepeta nuda* L.

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Herpes simplex virus type 2 (HSV-2), a human pathogen, is a member of the large family of Herpesviridae. HSV-2 infection is usually transmitted sexually and can cause recurrent, painful genital ulcers. In neonates the infection is associated with significant morbidity and mortality. Moreover, HSV-2 infection increases the risk of human immunodeficiency virus (HIV) acquisition. There are about 11 licensed antiherpetic drugs for treatment of herpesvirus infections (De Clercq *et al.*, 2006). The most commonly used ones are the nucleoside analogs acyclovir, its derivatives and cidofovir (Elion, 1993). Unfortunately, continuous therapy leads to a selection of resistant strains. (Piret *et al.*, 2011). This requires development of new antivirals directed against other viral targets. Moreover, the toxicity associated with some antivirals limits their use, and therefore less toxic and more effective drugs are needed. For these reasons special attention is focused on compounds of natural origin. Plant extracts have complex chemical structures and lower cytotoxicity, hence the occurrence of strains resistant to their action is delayed.

The genus *Nepeta* (*Lamiaceae*) comprises about 250 species distributed in the central and southern parts of Europe, Asia and the Middle East. *Nepeta* species are widely used in folk medicine because of their antispasmodic, expectorant, diuretic, antiseptic, antitussive, antiasthmatic and febrifuge activities. (Zargari, 1990; Newall *et al.*, 1996; Baser *et al.*, 2000).

We have studied the antiviral activity of a water extract from *Nepeta nuda* L. derived from *in vivo* propagated plants. The cytotoxicity was tested on Madin Darby Bovine Kidney (MDBK) cell line. The maximal nontoxic concentration (MNC) and the cytotoxic concentration (CC50) of the extract

were determined by a colorimetric method (MTT assays) (Mosmann, 1983). The results were measured at 48 hour and 72 hour after the addition of the extract. The maximal nontoxic concentration (MNC) of the extract determined at 48 hour was  $\approx 4$  mg/ml, and the cytotoxic concentration (CC50) was  $\approx 8$  mg/ml. The results obtained for MNC and CC50 at 72 hour after the addition of the extract were 2 mg/ml and 4.5 mg/ml, respectively. To determine the antiviral activity of the extract against HSV-2 strain BA, a modification of MTT assays at low MOI was used (Takeuchi *et al.*, 1991) (effect expressed as % of protection). As a long-term experiment (results are measured 5-6 days p.i.), MNC values measured at 72 hour after the extract addition were used. The water extract from *Nepeta nuda* significantly inhibited the replication of HSV-2. The protection rate is up to 70% (IC50 is  $\approx 0.75$  mg/ml). We also conducted a yield reduction assay at high MOI (Souza *et al.* 2008). As long as this experiment is terminated at the 24<sup>th</sup> hour, MNC measured at the second day can be used (effect expressed as % of inhibition). Inhibition yield production reached  $\approx 98$  % at 4.5 mg/ml. There was almost no activity at 2 mg/ml. Further we tested the direct inactivating effect of the extract against the extracellular form of HSV-2. The extract did not show any change in the virus titer.

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