Original Article

Viscum album homeopathic tinctures: phytochemical profile and antiproliferative activity.

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Abstract

Background: The mistletoe *Viscum album* L. (Santalaceae) is a semi-parasitic plant that grows on different host trees. Although *V. album* antitumor activity is mainly associated with aqueous preparations, recently we showed antitumoral effects of homeopathic *V. album* tinctures containing also the ethanolic soluble compounds [1].

Aims: to analyze the phytochemical profile of different *Viscum album* homeopathic tinctures as well as their in vitro effects in tumoral cells.

Methodology: The *Viscum album* samples (leaves, stems and berries) were collected in 2016 and 2017 (summer), and in 2018 (winter) at different locations in Switzerland. After harvest, they were immediately submitted to ethanolic extraction (45% V/V) using homeopathic methodology [2, 3]. The following mother tinctures (MT) were prepared: *V. album* ssp. *album* growing on *Malus domestica*, *Quercus* sp. and *Ulmus* sp., *V. album* ssp. *austriacum* from *Pinus sylvestris*, and *V. album* ssp. *abietis* from *Abies alba*. The homeopathic dynamizations (1-3dH; 6, 12, 30dH) were prepared with the respective MT. The phytochemical profiles were analysed by High Performance Liquid Chromatography tandem Mass Spectroscopy, HPLC and Thin Layer Chromatography. MT prepared in 2016 were also submitted to the Pfeiffer's circular chromatography (PCC) [4]. The proliferation assay was performed by WST-1 [5] after incubation of tumoral cells (Yoshida and Molt-4) with non-dynamized (0.5 to 0.05% v/v) and dynamized MT. Apoptosis/necrosis was measured by flow cytometer (FACS) using Annexin V-FITC [5], and the cellular morphological aspects were analysed by light microscope.

Results and Discussion: The chemical analyses of MT identified the presence of phenolic acids, flavonoids, lignans, as principal compounds. Besides, the HPLC indicated higher viscotoxin concentration in the summer harvest of *Abies alba*, *Malus domestica* and *Quercus* (897, 475, 219 µg/mL), respectively. The PCC methodology permitted the MT differentiation. The higher levels of cellular mortality were attribute to *Abies alba*, *Malus domestica* and *Quercus* (p<0.05, ANOVA with Dunnett post hoc test), and it is might be related to their phytochemical profile as well as their higher viscotoxin contents. The dynamization process removed the toxicity of all MT, except after

incubation with these same MT (1dH and 2 dH). FACS analyses showed necrotic events with intensive cellular lysis, registered by optical microscopy.

Conclusion: The *Viscum album* mother tinctures presented promising antitumoral potential. The loss of cytotoxicity effects after dynamization could be related to denaturation reactions induced by the shaking process. To test this hypothesis, chemical analyses will be performed with 1 and 2dH, in future investigations. Stability assessments of *Viscum album* MT will be done in order to guarantee the reproducible pharmaceutical parameters.

Keywords: Mistletoe (*Viscum album* L.), cancer, homeopathy, phytochemistry, cytotoxicity.

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