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Homoeopathic *Viscum album* extract inhibits the growth of osteosarcoma cells

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ABSTRACT

Objectives: This study is aimed to evaluate the cytotoxic action of two homoeopathic medicines that are derived from *Viscum album* (VA) extract.

Materials and Methods: An osteosarcoma cell line was cultured in the presence of two homoeopathic VA preparations (VAD3 and VAD30) and cell viability was evaluated using MTT assay. The cell line U-2 OS was plated in two 96-well plates for 24 h with culture medium at 37.5°C and 5% CO_2. Subsequently, this medium was replaced by another one containing VAD3 and VAD30 separately in concentrations ranging from 10 to 100 µL/mL, as well as a control group (culture medium only). These plates were kept in culture for 48 h. MTT assay was performed to evaluate the percentage of viable cells. Subsequently, concentrations ranging from 1 to 10 µL/mL were tested. Results were compared to those of the control group and the mean half maximal inhibitory concentration (IC_{50}) was calculated.

Results: The MTT assay showed that it is possible to reduce 50% of the osteosarcoma cell population with low concentrations of the homeopathic VAD3 and VAD30 with IC_{50} of 6.62 µL/mL and 5.82 µL/mL, respectively.

Conclusion: This is a promising result that shows the action of VAD3 and VAD30 in the U-2 OS lineage of osteosarcoma cancer cells. This opens up the possibility of using this medicine in the treatment of these tumours; if not alone, at least in association with other medicines or techniques.

Keywords: Cancer, Cell viability, Homoeopathy

INTRODUCTION

Cancer remains a significant global health issue. While numerous advances have occurred in treatment methods, there is always space for a new clinical approach that can improve the patients' health and quality of life.[1,2] Osteosarcoma is not a common type of cancer despite being the most common primary osseous tumour. It is more prevalent in children and young adults between the ages of 10 and 30 years, but can occur in people of any age.[3-4] Osteosarcoma most frequently occurs in the long bones of the extremities; in rare cases, it can occur in other bones like the jaw and talus.[5,6]

For osteosarcoma, cure is achievable, but often requires aggressive surgical resection with amputation followed by chemotherapy. If a limb-salvage procedure is practicable, a course of multidrug chemotherapy precedes surgery to downstage the tumour, followed by wide resection of the bone and insertion of an endoprosthesis.[7,8]

Viscum album (VA), a hemiparasite plant that grows in deciduous trees, is one of the research subjects in the idea of screening therapeutic plants to discover effective anticancer agents.[9] Unfortunately, little is known about the screening of bioactive composites from parasitic plants and their possible active ingredients.[10]

In complementary medical streams, especially anthroposophical medicine, VA extracts are used to treat cancer. As VA extracts are being investigated for their anticancer effects and improvement regarding quality of life, it opens doors for new studies with the homoeopathic version of VA as well.[11-14]

For *in vitro* studies, the most used osteosarcoma cell line used is the U-2 OS, particularly because of their fast growth and high transfection efficiencies. The line was cultivated from the bone tissue of a 15-year-old girl with osteosarcoma.[15,16] U-2 OS is widely used as an osteoblastic model for their high adherence efficiency and unrestricted cell division. These properties distinguish them from normal osteoblasts and occur mostly because of gene mutations and a collection of chromosomal abnormalities. These abnormalities cause overexpression of various oncogenes and inactivation of tumour-suppressor genes or other components of their regulation pathways.[17,18] The aim of the present study was to evaluate the cytotoxic action of two homoeopathic medicines obtained from VA extract. The medicines used were VA ultradiluted in 10^{-3} (VAD3) and 10^{-30} (VAD30) potency. The primary objective was evaluating the behaviour of these medications in cell culture followed by evaluation of their cytotoxic action.

MATERIALS AND METHODS

Cell culture

The osteosarcoma cell line used in these tests, U-2 OS (ATCC® HTB-96™), was donated by the Biotechnology and Genomic Sciences laboratory from the Catholic University of Brasilia (purchased by the ATCC and grown according to the protocol). The cells were cultivated with Dulbecco's Modified Eagle Medium added with 10% of foetal bovine serum and 0.02% of amikacin (all from the Sigma-Aldrich® brand). The plates were incubated at 37.5°C, with CO_2 at 5% and saturated humidity.

Preparation of VAD3

For the preparation of the tested substances (VAD3 and VAD30 – COMPANY*), the Mother Tincture was the starting point. The Hahnemannian Decimal Method, as described in the Brazilian Homeopathic Pharmacopoeia, was used. One part of the active ingredient with 9 parts of the inert ingredient, using a sterile isotonic solution, was succeed 100 times, yielding VAD1 (1×10^{-1}). Then, 1 part of VAD1 was used with 9 parts of the inert ingredient and succeed 100 times, yielding VAD2 (1×10^{-2}). The successive dilution continued till VAD30 was obtained. These products were then packaged in 1.1 mL ampoules.

Cytotoxicity (MTT assay)

To evaluate the cytotoxicity of the dynamised drugs VAD3 and VAD30, all cells were cultured *in vitro* for 48 h in the following experimental groups: Control (cells with culture medium) and the homoeopathic medicines (VAD3 and VAD30, separately) in different concentrations (10 μL/mL, 12.1 μL/mL, 14.7 μL/mL, 17.8 μL/mL, 21.5 μL/mL, 26.1 μL/mL, 31.6 μL/mL, 38.3 μL/mL, 46.4 μL/mL, 56.2 μL/mL, 68.1 μL/mL, 82.5 μL/mL and 100 μL/mL of culture medium).

After 48 h of culture, the MTT colorimetric assay was performed. The culture medium with VAD3 or VAD30 was substituted with the MTT reagent. The plates were incubated for more 4 h, dimethyl sulfoxide was added to each well and homogenised for dilution of the formazan crystals. Absorbance reading was performed at 570 nm in a microplate spectrophotometer (Elisa Plate Reader DR-200B-BI, Kazuaki®, Wuxi, China) for the identification of the viable cells. Then, the percentage of viable cells was calculated in each group compared to the controls.

With the abovementioned results, it was not possible to determine the half maximal inhibitory concentration (IC_{50}) (concentration that inhibits the growth of 50% of the cells in culture), after which the test was repeated with lower concentrations of VAD3 and VAD30 (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 μL/mL). With these values, the cell viability and mean IC_{50} of VAD3 and VAD30 were calculated, making it possible to evaluate the action of the medicine in the cell culture.

Statistical analysis

The analysis of the results of the MTT assay was performed using the Graph Prism® 7.04 program with the Tukey test for multiple comparisons. The data were subjected to analysis of variance using the MIXED procedure of the SAS software (SAS University Edition), with repeated measurements overtime, to consider the self-correlation between sequential measurements. Differences between means were compared using the Tukey test.

RESULTS

The finding of the first test was that cells of U-2 OS that was in contact with the different concentrations of VAD3 and VAD30 (10–100 μL/mL) were not viable [Table 1]. The control groups (cell culture without the homeopathic medicines) presented no alteration of viability, which means that both medicines caused considerable cell death. Even the lowest concentration (10 μL/mL) caused an intense decrease

in cell viability and the IC_{50} could not be calculated. For this reason, lower concentrations ranging from 1 to 10 µL/mL (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µL/mL) were tested.

The MTT assay showed that the homoeopathic medicine was effective against the osteosarcoma cell lines tested. Using the new absorbance values, it was possible to calculate the cell viability and the IC_{50} for each cell line with the VAD3 and VAD30 [Table 2].

For the homoeopathic VAD3, as observed in [Figure 1], the absorbance of the MTT reaction gradually decreased as the concentration of VA increased, indicating a lower number of living cells.

There was no significant difference in the analysis between the control sample and the 1 µL/mL of VAD3 sample. With the other samples tested, all of them presented a significant difference in the cell population in comparison with the control ($P < 0.0001$). With this result, it was possible to calculate that the IC_{50} of the ultradiluted VAD3 in U-2 OS cells was 6.62 µL/mL.

With the ultradiluted VAD30 [Figure 2], after a 48 h incubation, the same pattern of absorbance was observed. There was no significant change with 1 µL/mL of VAD30 sample, but after 2 µL/mL, the significance in relation to the control sample was already greater ($P < 0.0001$). VAD30 had

Table 1: Descriptive data of cell viability of U-2 OS cell line after MTT in concentrations of 10–100 µL/mL with both VAD3 and VAD30.

Concentration (µL/mL)	% viable cells (VAD3)	% viable cells (VAD30)
0	100.00	100.00
10	25.14	22.5
12.1	21.37	20.68
14.7	22.88	18.42
17.8	17.52	16.11
21.5	17.00	15.42
26.1	10.16	12.04
31.6	8.43	8.28
38.3	7.75	8.63
46.4	7.14	7.44
56.2	6.99	7.50
68.1	6.83	7.48
82.5	6.90	7.89
100	7.33	7.53

Table 2: Descriptive data of cell viability of U-2 OS cell line after MTT in each concentration tested as well as the cytotoxicity value using the concentration response (IC_{50}) with both VAD3 and VAD30.

Concentration (µL/mL)	% viable cells (VAD3)	% viable cells (VAD30)
0	100.00	100.00
1	98.60	93.93
2	83.13	73.73
3	71.37	60.69
4	63.40	57.96
5	60.61	49.22
6	52.10	42.69
7	42.64	41.05
8	40.58	35.15
9	34.83	32.13
10	31.54	27.53
IC_{50}	6.62	5.82

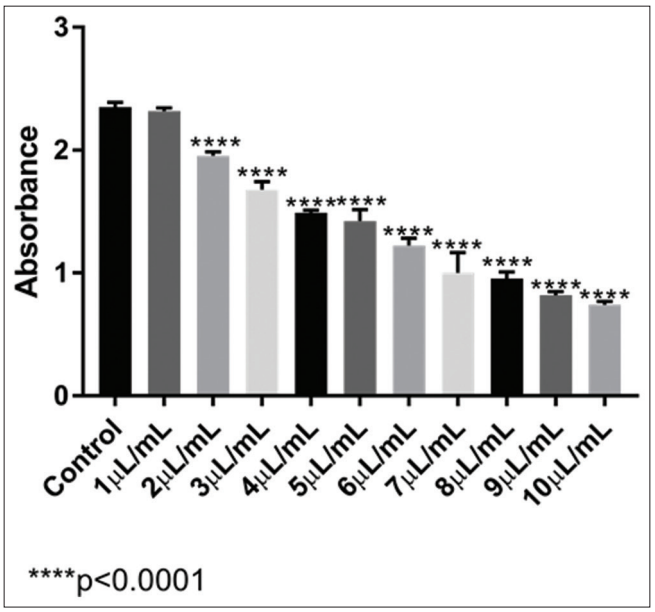

Figure 1: Absorbance levels obtained with the MTT test of VAD3 in U-2 OS cell line.

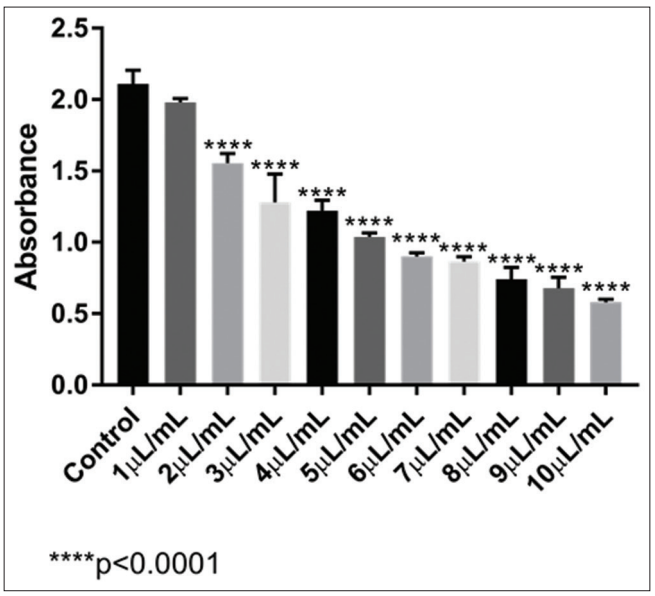

Figure 2: Absorbance levels obtained with the MTT test of VAD30 in the U-2 OS cell line.

an IC_{50} calculated result of 5.82 μL/mL, in which only 50% of the U-2 OS cell population survived; this implied that the cells were reduced significantly in a dose-dependent manner and the medication was extremely cytotoxic.

DISCUSSION

Homeopathy is considered an important supplementary therapeutic source in cases of cancer. Some *in vitro* validations have been made till date; for many years, the VA plant extract has been an object of scientific interest with its anticancer potential as well as its cardioprotective effects.[19-21]

The results obtained in the present study are promising as they show the potential action of the ultradiluted VA (VAD3 and VAD30) against osteosarcoma U-2 OS cell lineage. This finding corroborates the literature regarding the cytotoxic action of VA extract.[22] However, some studies have demonstrated side effects when VA extract is used in high dosages, including dose-dependent flu-like symptoms, fever, local reactions at the injection site and various mild unspecific effects.[13] As little is known about the homoeopathic version, this study joins with the few existing in the literature today to reverse this scenario. Considering *in vitro* studies are a field of increasing interest in science, as they can show the action of the medication in cellular level with specific and direct results, our study brings a new perspective for homoeopathic science.

The predicted potential of action of this ultradiluted VA is important because it can show an indicative of concentration range that can be effective and beneficial to the patients as well. Several compounds with pharmacological properties have been found in the VA plant, among which we highlight viscotoxin and phoratoxin (both producing bradyarrhythmia and decreasing myocardial contractility) and galactose-specific lecithin I, which increases the immune response and release endorphins. The effects of VA extracts against cancer cells have been demonstrated in several instances,[23,24] including an additive antitumor effect when used in combination with ionising radiation.[25,26] However, further studies are needed to assess their true role in cancer treatment, as our study was not designed to go so far. Other *in vitro* studies should be performed to evaluate the possibility of these extracts inducing cell apoptosis as well as to determine the genomic responses to treatment with homeopathic VA.

CONCLUSION

This study brings a promising result that shows the action of VAD3 and VAD30 in the U-2 OS lineage of osteosarcoma cancer cells. This opens up the possibility of using this medicine in the treatment of these tumours; if not alone, at least in association with other medicines or techniques.

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Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

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