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In Vitro Cytotoxic Activity of Different Viscum Album Potencies in Glioma Cells

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ABSTRACT: The incidence of cancer has dramatically increased around the world. Among the different types, glioma (brain cancer) represents the majority of diagnosed brain tumors and is known to be a rapidly developing tumor with a high mortality rate. Therapies with viscum album have performed well in cancer treatments and have been the subject of many studies. This work evaluated the in vitro action of homeopathic medicines prepared with viscum album against glioma cells (KNS-42) and was possible to prove the cytotoxic activity of viscum album at D3, D6, D9, D12, and D30 potencies. This result broadens opportunities for homeopathy in cancer treatment as the primary therapy or as a complementary treatment.

KEYWORDS: Cancer, Viscum Album, Gliome, Homeopathy

I. INTRODUCTION

Cancer is a disease that has caused the death of thousands of people around the world. As per data from the World Health Organization, it is estimated that by 2030, 17 million people will die from cancer each year (Grech et al., 2020). In addition, according to the National Cancer Institute (Inca), it is expected that about 625,000 new cases will have been diagnosed in Brazil by the end of 2022 (Inca, 2019).

Among the various types of possible tumors, glioma (brain cancer) represents the majority of diagnosed brain tumors and is known to be a rapidly developing tumor with a high mortality rate (Molinaro et al. 2019).

Therefore, searching for innovative therapies is a promising way to assist with cancer treatments. Viscum album (VA) is a semiparasite plant used in oncology treatments for many years with excellent results. This plant has a particular biochemical composition and has been widely studied by the scientific community (Kim et al., 2020; Park et al., 2021). Its biochemical compounds were gradually discovered throughout the years and present two main activities: immunomodulation and cytotoxicity. Lectins, viscotoxins, flavonoids, phenolic acids, sterols, lignans, terpenoids, phenylpropanoids, alkaloids, and greasy acids are among the biochemical compounds found in this species (Bonamin et al., 2017).

In order to better elucidate the Viscum album activity, in vitro tests become important allies to evaluate its activity in tumor cells, with no need to use animals in such tests. The cells of interest are cultured in this case, and the medicine is added to evaluate its application potential. In this context, this study evaluated the in vitro action of different potencies of VA in KNS-42 cells (glioma tumor cells) by culturing these cells, treating them with VA, and assessing cell viability.

II. MATERIAL AND METHODS

Preparation Of Viscum Album Potencies

The Mother Tincture was used as the starting point to prepare the tested substances (Viscum album D3, D6, D9, D12, and D30). The Hahnemannian Decimal Method was used, as described in the Brazilian Homeopathic Pharmacopoeia. One part of the active ingredient was mixed with 9 parts of the inert ingredient, using a sterile isotonic solution, and succussed 100 times, yielding VA D1 (1×10−1). Then, 1 part of VA D1 was used with 9 parts of the inert ingredient and succussed 100 times, yielding VA D2 (1×10−2). The successive dilution continued until potencies were obtained. These products were then bottled in 1.1 mL ampoules.

Cytotoxicity Assay With 3-4,5-Dimethyl-Thiazol-2-Yl-2,5-Diphenyltetrazolium Bromide (MTT)

Human glioma cells (KNS-42) were obtained from a commercial bank and grown in 75 cm² culture bottles with Dulbecco's Modified Eagle Medium Medium with high glucose concentration (DMEM-HG) supplemented with an antibiotic. The culture bottles were incubated in an oven at 37 ºC, 5% CO_2, and the culture medium was changed every 48 hours until cells reached a confluence between 60-80%.

Subsequently, these cells were trypsinized and plated in 96-well plates at 10,000 cells per well. After 24 hours of incubation under the same conditions described above, these cells were treated using the Viscum album D3, D6, D9, D12, and D30 potencies, at a

concentration of 50 µL/mL. The control group was not submitted to treatment. The plates were incubated again for another 48h in an oven.

After the incubation time of the cell culture treated with the product, 100 µL of MTT (5 mg/mL) was added to each well. The plates were then incubated in an oven for additional 4 hours. Finally, MTT was removed from each of the wells, and DMSO (dimethyl sulfoxide) was added to dilute the formazan crystals. The viability reading and measurement were performed by measuring the absorbance at 570 nm in a spectrophotometer.

Statistical Analysis

Cell viability was calculated based on the absorbance obtained in the control group in the groups treated with Viscum album at each potency. Data were analyzed by Tukey's comparison test using GraphPad Prism® 7.04.

III. RESULTS

Cell Viability Of Glioma Cells After Treatment With Viscum Album D3, D6, D9, D12, And D30

After treatment of glioma cells with Viscum album at D3, D6, D9, D12, and D30 potencies, it was observed a cytotoxic effect of the 50 µL/mL concentration on the cells.

The comparison of the different potencies identified that medicines in the decimal potency (VAD3, VAD6, and VAD9) showed higher cytotoxic activity against the cells tested, as illustrated in Figure 1. The treated groups were compared with the control, showing viabilities of 35.9 ± 2.82 cell% for cells treated with VAD3, 31.22 ± 4.54% for VAD6, 44.52 ± 3.02% for VAD9, 62.68 ± 2.1% for VAD12, and 90.1 ± 4.52% for VAD30.

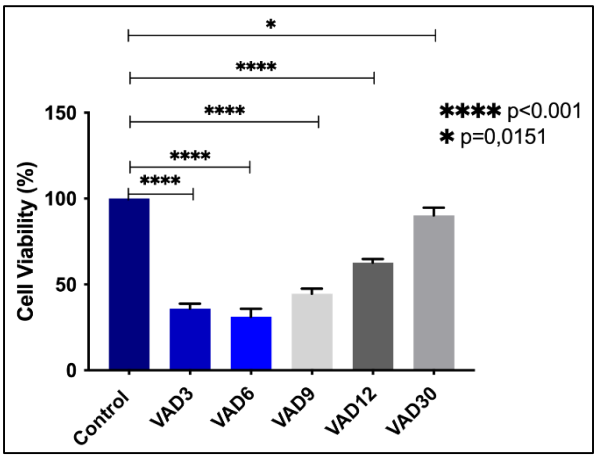

Figure 1 Analysis of KNS-42 (glioma) cell viability after treatment with Viscum album at D3, D6, D9, D12, and D30 potencies. The assays were performed in triplicates, and the bars indicate the standard deviation between the obtained values. The control was not subjected to any of the treatments.

IV. DISCUSSION

Some studies have been conducted to evaluate the Viscum album potential in cancer therapy, and it is possible to identify that it acts both as an inducer of apoptosis and as an immunomodulator (Steinborn et al., 2017).

In this study, Viscum album decimal potencies had a higher cytotoxic effect on glioma cells (KNS-42), while centesimal potencies acted differently, with no apoptosis promotion. As stated in other studies, this medicine can act as an immunomodulator (Steinborn et al., 2017), so this activity was possibly present in the most diluted potencies. The Viscum album chemical composition comprises lectins and viscotoxins, which may act as apoptosis-inducing agents, thus causing the cytotoxic effect, which may be essential in cancer treatment. In addition, the phenolic acids, phenylpropanoids, and flavonoids present in this plant may have antioxidant and anti-inflammatory activities (Nazaruk & Orlikowski, 2016).

This data can be corroborated by Valle et al. (2021), in which the Viscum album D3 action was evaluated on human breast cancer cells (MCF-7). In this research, it was possible to verify that the product can decrease cell viability as the concentration increases in the in vitro tests.

Another study by Menke et al. (2021) evaluated the action of Viscum album on medulloblastoma cell lines (Daoy and ONS-76) and non-tumor fibroblasts. The authors observed that tumor lines were more susceptible to treatment than non-tumor lines, showing reduced proliferation and induction of apoptosis.

The immunomodulatory action of the most diluted potencies of Viscum album may be related to the ability of this medicine to act on natural killer (NK) cells, as already identified in other studies (Braedel-Ruoff, 2010; Yoon et al., 2003). Another study also identified the induction of Th-1 response after treatment of tumors with Viscum album (da Silva Facina et al., 2014).

V. CONCLUSION

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In this work, it was possible to prove the cytotoxic activity of Viscum album at D3, D6, D9, D12, and D30 potencies in glioma cells. This result broadens opportunities for homeopathy in cancer treatment as the primary therapy or as a complementary treatment. Its benefits may range from improved quality of life to direct action on cancer cells, as evaluated in this in vitro study.

REFERENCES

1) Bonamin, L. V., de Carvalho, A. C., & Waisse, S. (2017). Viscum album (L.) in experimental animal tumors: A meta-analysis. Experimental and Therapeutic Medicine, 13(6), 2723–2740. https://doi.org/10.3892/etm.2017.4372
2) Braedel-Ruoff, S. (2010). Immunomodulatory Effects of Viscum album Extracts on Natural Killer Cells: Review of Clinical Trials. Forschende Komplementärmedizin / Research in Complementary Medicine, 17(2), 63–73. https://doi.org/10.1159/000288702
3) Da Silva Facina, A., Facina, G., Guerreiro da Silva, I. D. C., Aparecida Gonçalves, G., Augusto de Almeida, F., Aparecida Alves Corrêa de Noronha, S., Marcos Ribeiro de Noronha, S., & Uchiyama Nakamura, M. (2014). Viscum Album Modulates Apoptotic Related Genes in Melanoma Tumor of Mice. American Journal of Molecular Biology, 04(02), 49–58. https://doi.org/10.4236/ajmb.2014.42007
4) Grech, N., Dalli, T., Mizzi, S., Meilak, L., Calleja, N., & Zrinzo, A. (2020). Rising Incidence of Glioblastoma Multiforme in a Well-Defined Population. Cureus. https://doi.org/10.7759/cureus.8195
5) Kim, J. D., Choi, J. W., Park, H. O., Lee, C. E., Jang, I. S., Choi, J. Y., Kang, D. H., Jung, J. J., Yang, J. H., Moon, S. H., Byun, J. H., Kim, S. H., & Kim, J. W. (2020). Chemical pleurodesis with Viscum album L. extract for secondary spontaneous pneumothorax in elderly patients. Journal of Thoracic Disease, 12(10), 5440–5445. https://doi.org/10.21037/jtd-20-708
6) Menke, K., Schwermer, M., Eisenbraun, J., Schramm, A., & Zuzak, T. J. (2021). Anticancer Effects of Viscum album Fraxini Extract on Medulloblastoma Cells in vitro. Complementary Medicine Research, 28(1), 15–22. https://doi.org/10.1159/000507318
7) Nazaruk, J., & Orlikowski, P. (2016). Phytochemical profile and therapeutic potential of Viscum album L. Natural Product Research, 30(4), 373–385. https://doi.org/10.1080/14786419.2015.1022776
8) Park, Y. J., Mok, S. K., Kim, J. Y., Yun, S. S., & Park, S. C. (2021). Retroperitoneal Viscum album extract instillation in patients with a large amount of drainage after kidney transplantation. Annals of Surgical Treatment and Research, 101(6), 368. https://doi.org/10.4174/astr.2021.101.6.368
9) Steinborn, C., Klemd, A. M., Sanchez-Campillo, A.-S., Rieger, S., Scheffen, M., Sauer, B., Garcia-Käufer, M., Urech, K., Follo, M., Ücker, A., Kienle, G. S., Huber, R., & Gründemann, C. (2017). Viscum album neutralizes tumor-induced immunosuppression in a human in vitro cell model. PLOS ONE, 12(7), e0181553. https://doi.org/10.1371/journal.pone.0181553
10) Valle, A. C. V., Aguiar, L. R., Brunel, H. dos S. S., Malard, P. F., & Andrade, R. V. (2020). Homoeopathic Viscum album extract inhibits the growth of osteosarcoma cells. Journal of Integrated Standardized Homoeopathy, 3, 59. https://doi.org/10.25259/JISH_32_2020
11) Valle, A. C. V., Aguiar, L. R., Brunel, H. dos S. S., Malard, P. F., Villarroel, C. L. P., & Andrade, R. V. (2021). Homoeopathic Viscum album (10-3) is More Cytotoxic to in vitro Culture of Human Breast Cancer Cell Line than to Human Mesenchymal Stem Cells. International Journal of High Dilution Research - ISSN 1982-6206, 19(4), 25–34. https://doi.org/10.51910/ijhdr.v19i4.1036
12) Yoon, T. J., Yoo, Y. C., Kang, T. B., Song, S. K., Lee, K. B., Her, E., Song, K. S., & Kim, J. B. (2003). Antitumor activity of the Korean Mistletoe Lectin is attributed to activation of macrophages and NK cells. Archives of Pharmacal Research, 26(10), 861. https://doi.org/10.1007/BF02980033