



Research Article

Volume 28 Issue 2 - March 2025
DOI: 10.19080/CTOIJ.2025.28.556240

Cancer Ther Oncol Int J

Copyright © All rights are reserved by Razvan Rentea MD

The Mother Tincture as well as a High Dilution of proprietary Mistletoe (*Viscum album* L.) extracts Inhibit the Proliferation of the Prostate Cancer Cell line LNCaP, while Stimulating the Cell Proliferation of the Normal Human Prostate Cell Line RWPE: A Pilot Study



Razvan Rentea^{1*}, Mark Kamsler² and Malory Mueller³

Lilly Kolisko Institute for Anthroposophic Medicine, 1005 Richards Road, Hartland, WI, USA

Submission: March 13, 2025; **Published:** March 25, 2025

Corresponding author: Razvan Rentea MD, Lilly Kolisko Institute for Anthroposophic Medicine, 1005 Richards Road, Hartland, Wisconsin, 53029, USA, Email: ross.rentea@truebotanica.com

Abstract

Conventional treatments for advanced prostate cancer frequently entail major side effects. These drive patients to seek adjuvant therapies that are more tolerable. Pharmaceutically prepared extracts of mistletoe (*Viscum album* L.) are very popular and well established as complementary, adjuvant therapies to many cancer interventions, yet studies with mistletoe in prostate cancer are lacking. We have demonstrated that mother tinctures as well as the D23 dilution of proprietary extracts of several sub-species of *Viscum album* L. can inhibit the in vitro proliferation of the prostate cancer line LNCaP. On the other hand, the growth of the normal prostate cell line RWPE is stimulated. This points towards the potential safe clinical use of these extracts. We also demonstrate that apoptosis is induced by the high dilution D23. Being able to use high dilutions of this therapeutic plant would have significant advantages both for therapeutic purposes as well as for botanical environmental preservation.

Introduction

Prostate cancer is the second most prevalent cancer in men after skin cancer [1]. The relative survival rate at 5 years is about 98%. However, the treatment with surgery, radiation therapy or medication for advanced stages, while often highly effective, is frequently accompanied by serious side effects, like nausea, fatigue, pain, depression, or a sense of lost “masculinity” when androgen depletion is necessary [2]. The desire to mitigate some of these side effects, or to avoid some of these treatments altogether has caused the use of complementary therapeutic modalities to gain increasing interest. Patients are interested in several aspects: reduction of the side effects of conventional medicine, strengthening their immune system and enhancing quality of life both during and after conventional treatments, but also receiving assurance that those complementary treatments also have at

least a minimum of anti-tumoral effect. Among many researchers Abrams [3], Hann [4] Auerbach [5] have discussed complementary therapies in prostate cancer and Wilkinson [6] provided a critical review of complementary therapies in prostate cancer. In the last few years, several studies have specifically shown that nutritional and other substances of natural plant origin have shown promise against prostate cancer cells. Naming only a few here: Nakayama et al, showed that curcumin combined with the 5-alpha reductase inhibitor dutasteride inhibited LNCaP Prostate Cancer Cells [7]. Moga found beneficial effects of anti-proliferative plant derived extracts against prostate and cervical cell lines. [8] Stanislawski et al determined that intake of certain minerals, like iron, zinc and selenium can modulate the protective effect of isoflavonoids over prostate cancer cells [9] and Abouhamra [10] showed the inhibiting effect of Tongkat Ali on LNCaP cells. Of note also is the

fact that substances at high and ultra-high dilutions were shown to be able to inhibit proliferation of prostate cancer cells. Among others, Tembugade [11] showed the potential of Lachesis 200C to inhibit the prostate cell line PC-3. McLaughlin [12] assessed the antiproliferative effects of homeopathic preparations of *Sabal serrulata*, *Thuja occidentalis*, and *Conium maculatum*, in vivo, on nude mouse xenografts, and in vitro, on PC-3 and DU-145 human prostate cancer and other cancer cell lines. Dos Santos [13] and Witt [14] reviewed fourteen studies showing the inhibitory effects of high homeopathic dilutions on prostate cancer lines. Evidence in support of gene modulation by Condurango 30c and *Hydrastis canadensis* 30C was presented by Khuda-Bukhsh [15].

However, not all experiments provided positive results. Jonas [16] et al. found that the selected homeopathic remedies for their study had no direct cellular anticancer effects but nevertheless appeared to significantly slow the progression of cancer and reduce cancer incidence and mortality in Copenhagen rats injected with MAT-LyLu prostate cancer cells. Tangapazham [17] could find no alteration of growth or gene expression in prostate cells. Prominent among complementary treatments are therapies with extracts of mistletoe (*Viscum album* L.). The mistletoe “drugs” are used for their potential qualities: anti-tumor effects, ability to improve quality of life and minimal side effects. They are manufactured through so-called anthroposophical pharmaceutical processes, meaning the combining of the summer and winter harvested mistletoe and uniting the two harvests through high centrifugation [18,19]. They are administered largely in injectable form and have been very popular in Europe for over one hundred years and are slowly spreading to other countries. Multiple high-quality studies have demonstrated that these mistletoe therapies enhance treatment tolerability, improve survival of disease, lower fatigue, etc. [20].

The German social health insurance covers the prescription of these standardized mistletoe extracts when those are prescribed as palliative cancer treatments with the aim of improving quality of life [20-22]. Clinical studies with mistletoe regarding colon, breast, lung, and dermal cancers have also shown promise for anti-tumor effects [23,24]. However, we were not able to find any controlled clinical studies with mistletoe on prostate cancer- outside of real-world based case observations. These are mentioned only in the anthroposophical literature [25,26]. In the last decades, the mechanism of action of the mistletoe extracts has been intensely investigated in the laboratory to better understand their overall pharmaceutical effects and potential specific anti-tumor activity. An increasing number of in vitro studies have shown that both concentrated mistletoe extracts as well as their ultra-high dilutions have significant anti-proliferative activity. Mechanisms of action mentioned are: cell cycle inhibition, generation of reactive oxygen species (ROS), disruption of mitochondrial function, activation of apoptotic pathways, and modulation of key signaling molecules involved in cell growth and survival. These experiments were

done on a variety of cell lines-colon, breast, bladder, glioma, melanoma, prostate, etc. [27-29]. As we have reviewed before [30] comparisons between different experiments are made difficult since the bioactive compounds (principally mistletoe lectins, but also viscotoxins, flavonoids, and phytosterols) vary depending on which host tree the plant had grown on as well as the specific manufacturing procedure.

We have found several studies involving high diluted mistletoe extracts. Viana Valle showed the effect of *Viscum* 3C and 30C on mesenchymal stem cells [31] and at a different time her group demonstrated effects on osteosarcoma cells with high dilutions at D3 and D30 [32]. Rentea [20] et al. showed both stimulating and inhibiting activity in a range above the D24 dilution of the *Viscum acer* and *Viscum crataegus*. We have only been able to find two in vitro experiments performed specifically to show the influence of mistletoe on prostate cancer cells. Juengel et al found that the proliferation of prostate tumor cells PC3, DU145 and LNCaP was significantly diminished by the mistletoe type *Iscucin Tiliae*®. [33] Also, in a paper published in 2021, Boyapati demonstrated that the mistletoe drug Helixor M® has an inhibitory effect on cell proliferation and survival of the androgen receptor (AR) positive (LNCaP, LAPC4 and VCaP) and AR negative (PC3 and DU-145) cell lines. Their data indicates that LNCaP and LAPC4 cells were most sensitive to Helixor M®, while PC3, DU-145, and VCaP cells were relatively resistant to treatment [34]. Based on the above information, lack of experimentation with mistletoe on prostate cancer cell lines and lack of sufficient data on whether or not ultra-high dilution of mistletoe can inhibit prostate cancer lines, we considered it an urgent need to study the effect of high and ultra-high dilutions of mistletoe on the LNCaP androgen dependent prostate cancer cells. Moreover, for the first time we also compare the behavior of these preparations on the normal prostate cell line RWPE vs the LNCaP line.

Materials and Methods

Mistletoe Extraction and Potentization

The mother tincture (Ø) of each mistletoe was obtained through a proprietary method of extraction. The mistletoe was harvested from each host tree at two different opposing seasons (e.g., summer and winter). After the separate extraction of each seasonal harvest, the summer harvested extraction was allowed to drip by gravity downwards into the centrifugally rotating extract from the winter season. The resultant final mother tincture was designated “*Viscum Kolisko...*(host tree name)” (Appendix A). This final mother tincture contained the extracted contents of 200mg dry mistletoe per ml. The “potency”, a dilution prepared in “homeopathic” style, was designated as D1, and was made by 1:10 dilution using 1 ml of the mother tincture and 9 ml sterile diH₂O, succussed for two minutes, followed by one minute of rest. The D2 “potency” was made by 1:10 dilution of D1 as before and this method was repeated to D30.

Cell Culture

LNCAp clone FGC, a human prostate cancer cell line (ATCC, CRL-1740), was typically seeded at 20% confluency in 75cm² flasks, and grown to approximately 80% confluency at 37°C, 5% CO₂, which typically took four days. The medium used was RPMI-1640 (ATCC, 30-2001) supplemented with 10% Fetal Bovine Serum (FBS; ATCC, 30-2020) and 1% penicillin streptomycin (ATCC, 30-2300). Once confluent, the medium was removed, and the cells rinsed with Dulbecco's Phosphate Buffered Saline 1X (D-PBS) (ATCC, 30-2200). The cell monolayer was then harvested by adding 3 mL 0.25% Trypsin, 0.53 mM EDTA (ATCC, 30-2101) to the flask and incubated at room temperature for 5 minutes, followed by 6 mL fresh medium. The suspension was transferred to a 15-ml centrifuge tube, and sedimented at 130 x g for 7 minutes. The supernatant was removed, and the pellet resuspended in fresh media. The countess 3 (Invitrogen, AMQAX2000) was used to count cell inoculum and to check viability.

RWPE-1, a normal human prostate cell line (ATCC, CRL-3607), was typically seeded at 20% confluency in 75cm² flasks, and grown to approximately 80% confluency at 37°C, 5% CO₂, which typically took three days. The medium used was Keratinocyte Serum Free Medium (Gibco, 17005-042) supplemented with 0.05 mg/ml bovine pituitary extract (BPE) (Gibco, 13028-014) and 5 ng/ml human recombinant epidermal growth factor (EGF) (Gibco, 10450-013). Once confluent, the medium was removed, and the cells rinsed with D-PBS. The cell monolayer was then harvested by adding 3 mL 1:1 0.25% Trypsin, 0.53 mM EDTA and D-PBS to the flask and incubated at 37°C and 5% CO₂ for 5 minutes, followed by 6 mL soybean trypsin inhibitor (ATCC, 30-2104). The suspension was transferred to a 15-ml centrifuge tube, and sedimented at 130 x g for 7 minutes. The supernatant was removed, and the pellet resuspended in fresh media. The countess 3 (Invitrogen, AMQAX2000) was used to count cell inoculum and to check viability.

Cell Viability Assay

Cells were plated in white, 96-well plates (Costar, CLS3917) at a density of 1 x 10⁴ cells per well, and a volume of 90 µl per well. The controls contained 10 µl diH₂O (0% and 100% controls) and the experimental wells contained 10 µl of the appropriate potency (Ø-D30), with four replicates per treatment or control. Plates were left for 1 hour at room temperature, before subsequent incubation at 37°C and 5% CO₂, to produce uniform cell distribution on the growth surface and reduce edge effect. Cell growth was assessed using the CellTiter-Glo luminescent cell growth assay (Promega, G9243), using 100 µl of reagent per well according to the manufacturer's instructions. The luminescence was measured in a BioTek, Synergy LX plate reader. Cell growth was assessed for 0% control wells after an initial 18 hours of incubation, while sample and 100% control wells were assessed after an additional 48 hours of growth.

Apoptosis and Necrosis Assay

Cells were plated in white, 96-well plates (Costar, CLS3917) at a density of 2 x 10⁵ cells per well, and a volume of 50 µl per well and the plates were left at room temperature for 1 hour. The controls contained 50 µl diH₂O, or 100 µl of media (containing no cells), and the experimental wells contained 50 µl of the appropriate potency (Ø-D6, D23-D30), with four replicates per control or experimental. The RealTime-Glo Annexin V Apoptosis and Necrosis Assay (Promega, JA1012) was used to determine apoptosis and necrosis according to the manufacturer's instructions using 100 µl of the reagent per well. The luminescence and fluorescence were measured in a BioTek, Synergy LX plate reader after incubating the plates for 8 hours at 37°C and 5% CO₂.

Results & Discussion

We demonstrate here that the mother tincture of our proprietary mistletoe (*Viscum album* L.) of the sub species mali, robinia, populus, and tilia significantly inhibits the proliferation of the prostate cancer cell line LNCAp while no such significant effect was seen caused by *V. salix*. The same results were seen at the D23 dilution level, and no significant inhibitory effects were seen at dilutions from D24 to D30 (Table 1). On the other hand, the same five sub-species of mistletoe mali, robinia, populus, tilia and salix all had an opposite, stimulating, effect on the normal cell line RWPE (Table 2). The cell viability and stimulation studies were done using the CellTiter Glo® assay which, according to the manufacturer, provides a homogeneous method to determine the number of viable cells in culture by quantitating the amount of ATP present, which indicates the presence of metabolically active cells. The RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay kit was also used to measure the real-time exposure of phosphatidylserine (PS) on the outer leaflet of cell membranes during the apoptotic process. Annexin V luciferase fusion proteins supplied in the assay reagent bind to PS during early apoptosis and are detected with a simple luminescence signal. The assay reagent also includes a DNA-binding dye, which enters the cell and generates a fluorescent signal upon loss of membrane integrity. The combination and timing of luminescent (annexin V binding) and fluorescent (DNA release) signals were used to differentiate secondary necrosis occurring during late apoptosis from necrosis caused by other cytotoxic events.

The mother tinctures of all five mistletoe sub-species caused necrosis of the LNCAp line at the 8-hour time point. The D23 dilution of three sub-species, mali, tilia, and salix but not robinia or populus also caused necrosis (Table 3, Figure 1). Significant apoptosis was observed only by *V. populus* and *V. salix* (Table 4, Figure 2). The weakness of this preliminary study consists in not providing more data to elucidate the mechanisms of action. However, the current results encourage further work. This is the first time that the effect of mistletoe on the prostate cancer line was compared with the effect of mistletoe on the normal human

prostate cell line. The results encourage exploration of other mistletoe effects on similar experimental set ups with other cancer and normal cell lines. The finding of the “sparing effect” of the mistletoe on the normal cell line tentatively confirms the intuitive sense gained over decades of clinical use of pharmaceutically processed mistletoe extracts that their therapeutic use is safe as anti- tumoral agents. The availability of androgen receptors positive LNCaP prostate cancer cells has greatly helped the

study of prostate cancer at pre-clinical stages. The cell line was obtained initially from a metastatic lymph node lesion of a patient with prostate cancer and are unique in their ability to model key stages of prostate cancer progression. Analyses of LNCaP cells and their derivatives have been invaluable for elucidating important translational aspects of prostate tumorigenesis, metastasis, and drug response, particularly in the context of androgen receptor signaling [35,36].

Table 1: Inhibition of LNCaP cell line proliferation. Both mother tincture and the D23 high dilution of mistletoe (*Viscum album*) from various host trees: *V. mali*, *V. robinia*, *V. populus*, *V. tilia* inhibit the LNCaP cells at a statistically significant level of $p < 0.05$. No statistically significant inhibition was seen with *V. salix*. *The media was subtracted from the relative luminescence unit (RLU) to remove any background. All $n=4$.

<i>V. mali</i>				<i>V. robinia</i>			<i>V. populus</i>		
	Control	Ø	D23	Control	Ø	D23	Control	Ø	D23
AVER*	2238789.75	195612.13	2128099.50	2246543.94	204988.28	2094070.42	2276093.13	14140.54	2054683.50
STDEV	162551.00	115961.34	163089.76	185513.91	87340.59	167971.64	247005.97	4672.32	217133.52
%Growth	0.00	-148.01	-16.60	0.00	-138.71	-7.48	0.00	-157.00	-18.45
P VALUE	1	6.34E-26	0.0013083	1	1.007E-42	0.0390773	1	6.57E-24	0.0002747
<i>V. salix</i>				<i>V. tilia</i>					
	Control	Ø	D23	Control	Ø	D23			
AVER	2187572.28	35467.78	2102675.87	2313414.58	47379.40	2162106.83			
STDEV	1165814.37	3441.52	1146600.70	300116.30	13679.24	335584.93			
%Growth	0.00	-167.18	-12.80	0.00	-174.85	-11.20			
P VALUE	1	2.672E-14	0.5070325	1	2.603E-36	0.0451435			

Table 2: Stimulation of RWPE cell line proliferation. Both mother tincture and the D23 high dilution of mistletoe (*Viscum album*) from various host trees: *V. mali*, *V. robinia*, *V. populus*, *V. salix* stimulate the RWPE cells at a statistically significant level of $p < 0.05$. No statistically significant stimulation was seen with *Viscum tilia*. *The media was subtracted from the relative luminescence unit (RLU) to remove any background. All $n=4$.

<i>V. mali</i>				<i>V. robinia</i>			<i>V. populus</i>		
	Control	Ø	D23	Control	Ø	D23	Control	Ø	D23
AVER*	153561.50	228276.63	162725.88	113786.75	233588.25	154712.88	129718.25	65092.75	149829.63
STDEV	8646.52	10835.39	7470.89	28664.1	23055.31	11844.22	2659.13	4575.09	3261.61
%Growth	0.00	427.74	493.96	0.00	282.55	91.45	0.00	-129.35	40.61
P VALUE	1	7.96E-10	1.48E-05	1	1.29E-06	1.69E-05	1	9.03E-13	7.41E-08
<i>V. salix</i>				<i>V. tilia</i>					
	Control	Ø	D23	Control	Ø	D23			
AVER	140140.50	216396.00	197964.88	199559.38	349027.88	187142.63			
STDEV	2939.02	6021.43	12431.02	11130.16	29735.57	9389.37			
%Growth	0.00	274.24	225.77	0.00	166.30	-10.58			
P VALUE	1	1.47E-11	129E-06	1	3.43E-07	0.11			

Table 3: Necrosis of LNCaP cells when exposed to different mistletoe (*Viscum album*) grown on various host trees. The mother tincture (Ø) and D23 of the *Viscum tilia*, *mali*, *salix*, *robinia*, and *populus* potency significantly promote necrosis in the prostate carcinoma cell line LNCaP. *The media was subtracted from the relative fluorescence unit (RFU) to remove any background.

LNCaP Necrosis When Exposed to Mistletoe												
	<i>V. tilia</i>		<i>V. mali</i>		<i>V. salix</i>		<i>V. robinia</i>		<i>V. populus</i>		Control	
	Ø	D23	Ø	D23	Ø	D23	Ø	D23	Ø	D23	Positive	Negative
RFU-Media*	2855.88	1750.75	4144.00	1373.75	2082.83	1556.92	5730.79	722.29	1832.25	848.25	912.50	1127.58
Std. Dev.	250.44	89.88	367.71	108.21	212.68	112.69	185.16	72.36	320.50	65.85	214.57	112.44
P Value	6.99E-09	1.11E-05	1.64E-08	7.84E-05	1.55E-10	1.06E-03	7.7E-15	1.2E-08	3.60E-04	6.91E-05	<0.05	1

Table 4: Apoptosis of LNCaP cells when exposed to different mistletoe (*Viscum album*) grown on various host trees. The mother tincture ($\emptyset$) and D23 of the *Viscum tilia*, *mali*, *robinia*, and *populus* potency significantly promote apoptosis in the prostate carcinoma cell line LNCaP. *The media was subtracted from the relative luminescence unit (RLU) to remove any background.

LNCaP Apoptosis When Exposed to Mistletoe												
	<i>V. tilia</i>		<i>V. mali</i>		<i>V. salix</i>		<i>V. robinia</i>		<i>V. populus</i>		Control	
	$\emptyset$	D23	$\emptyset$	D23	$\emptyset$	D23	$\emptyset$	D23	$\emptyset$	D23	Positive	Negative
RFU-Media*	879.38	5207.25	5053.88	1988.13	761.83	12237.42	10084.75	4923.00	3732.50	29383.63	6381.58	8170.83
Std. Dev.	104.02	204.25	123.11	50.10	50.01	348.03	900.12	231.76	256.37	769.68	4039.73	1265.43
P Value	9.48E-06	2.40E-02	1.30E-03	2.59E-10	8.47E-09	8.74E-10	1.40E-04	1.10E-02	6.40E-04	1.10E-11	<0.05	1

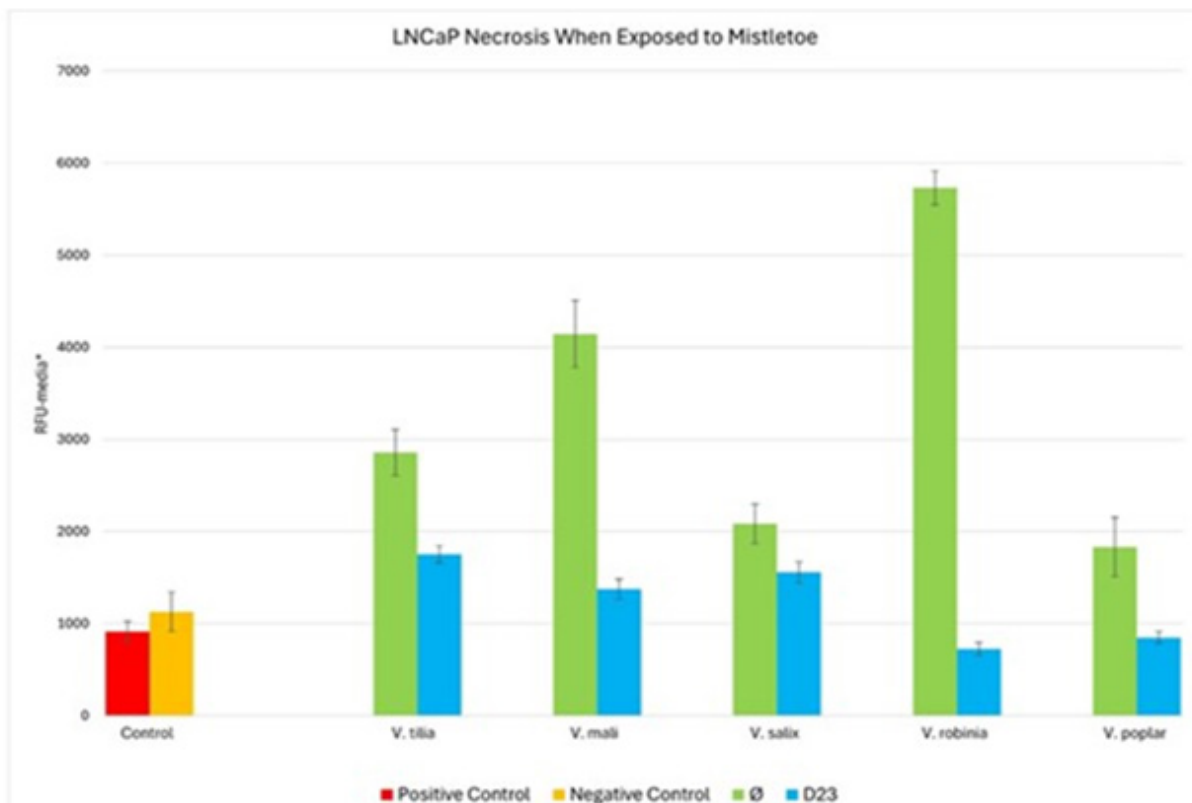


Figure 1: Necrosis of LNCaP cells when exposed to different mistletoe (*Viscum album*) grown on various host trees. The mother tincture ($\emptyset$) and D23 of the *Viscum tilia*, *mali*, *salix* and the of *robinia*, and *populus* potencies significantly promote necrosis in the prostate carcinoma cell line LNCaP when compared to controls. Standard deviation is indicated by the error bars. *The media was subtracted from the relative fluorescence unit (RFU) to remove any background.

The study of prostate cancer in vitro was facilitated, as well, by the development of the normal human prostate cell line RWPE-1. It is a well characterized cell line that can display development and differentiation of features of normal prostatic epithelium. The RWPE line was created by immortalization with HP virus, and the injection of RWPE-1 cells into naked mice does not cause the cells to proliferate and create tumors. In essence RWPE-1 cells are considered a cell line of choice to study the control of prostate

growth and carcinogenesis [35]. It needs to be emphasized that to be useful in the treatment of cancer the mistletoe needs to be processed in specific ways. While unprepared wild mistletoe extracts were reputed in folk medicine for centuries to be beneficial in a multitude of conditions of the cardiovascular, digestive, immune system, etc. [37]. Rudolf Steiner was the first, in the 1920s, to discover that specific processing of the mistletoe juices would have anti-tumor effects [38]. In presentations to

doctors and pharmacists he then specified the novel processing details that consisted of mixing the summer and winter extracts through a complicated centrifugation process [39]. Since then,

several European companies have worked on perfecting this method [40-43].

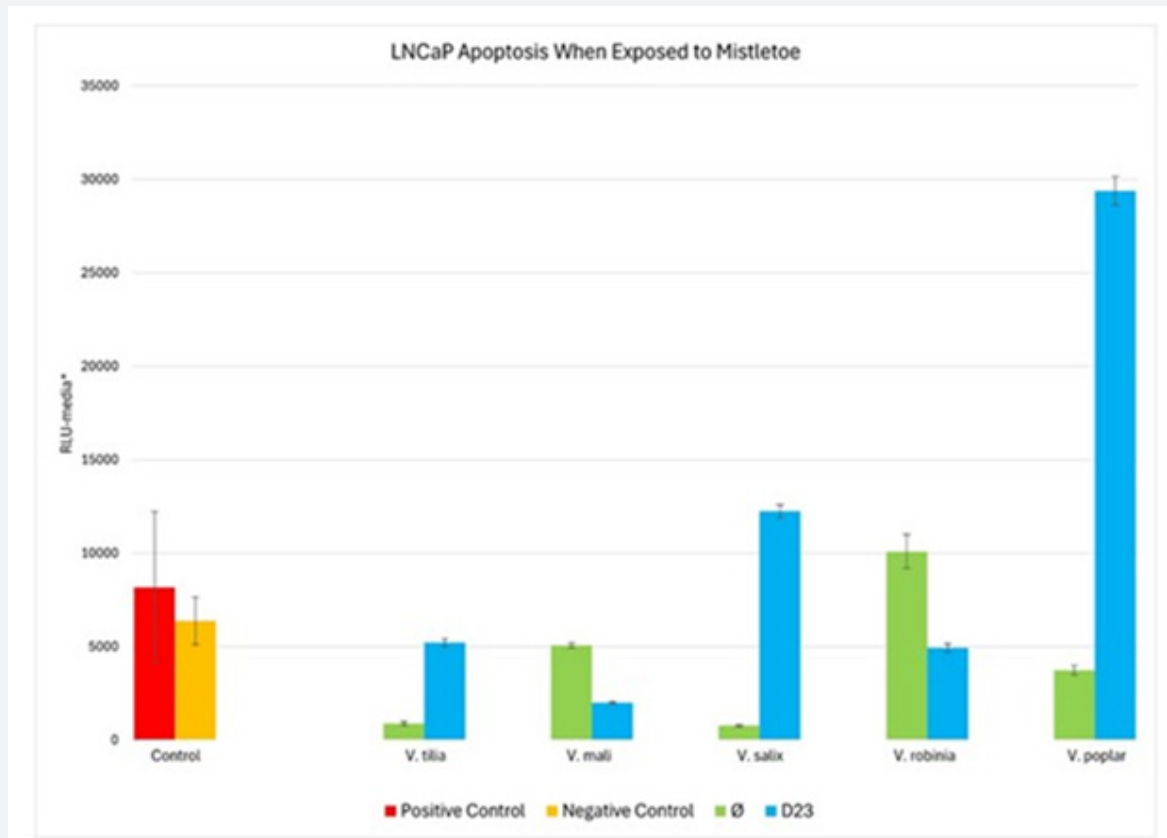


Figure 2: Apoptosis of LNCaP cells when exposed to different mistletoe (*Viscum album*) grown on various host trees. Standard deviation is indicated by the error bars. The Media was subtracted from the relative fluorescence unit (RFU) to remove any background.

At the Kolisko Institute we have developed a similar pharmaceutical process that consists of the same essential elements that R. Steiner suggested, mixing mistletoe extracts from opposite times of the year and uniting them through a centrifugation process. Our finding that highly diluted mistletoe (D23) has the same anti tumoral effect (albeit not as pronounced) as the mother tincture is of high potential importance and aligns with other similar findings in the literature. Even the high dilution of the mistletoe (D23) demonstrating a potential anti-tumoral effect is thus of significance.

A few of the future advantages of using high and ultra-high dilutions of mistletoe merit mentioning here:

1. High and ultra-high dilution of substances have well known strong safety profiles.
2. In addition to anti-tumoral effects high and ultra-high dilutions have also been found to convey a mental health advantage over the concentrated or low dilution tinctures extracts

[44-46]

3. The commercial high and ultra-high dilutions are less expensive to produce.

4. Use of high and ultra-high dilutions will lead to environmental preservation of the viscum species since more usable material can be produced from significantly lesser amounts of harvested wild growing mistletoe. Especially in the case of this semi parasitic plant growing on host trees, this is important since the life cycle of the mistletoe from seed implantation on the branch to maturity is spread over many years. The propagation of the mistletoe is made complicated by the fact that for new individual plants to “take” on a branch the berries of the old plant must pass through the digestive tract of specific birds who then eliminate the berries from their digestive tract. The berries then stick to a tree branch, and it takes several years for the seeds to germinate. The mistletoe bushes thus need to be carefully and only partially harvested since complete removal may not have the guarantee of a new plant being naturally implanted [47-49].

Adding to the problem is the fact that since different mistletoe sub species have a particular affinity to specific host trees (growing in relative abundance on the poplar tree, much less so on oak trees and extremely rarely on f.ex. elm tree), plants harvested one year may not be found again on that tree for many years after [50,51].

This interestingly has led the large manufacturers of pharmaceutically prepared European mistletoe to start to artificially seed the plant on their host trees to assure supply.

5. Finally, due to the presence of sensitive proteins, which mainly account for the anti-tumoral effects of the mother tinctures, the recommendation has always been that the application of the “viscum drug” in cancer therapy had to have been by the injectable route. No such constraints would exist for the dilutions which then could be given orally. In conclusion, continuing these pilot experiments toward development of mistletoe in high and ultra-high dilutions for ultimate clinical use will be of value.

Authors Participation

i. R Rentea MD - designed the experimental project, wrote the article; and participated in the extraction of the mistletoe material.

ii. M Mueller M. Sc – executed, calculated the experimental data, and wrote the Materials & Methods section.

iii. M Kamsler MD - participated in the extraction of mistletoe material.

Conflict of Interest

The authors report no conflict of interest.

References

1. Miller KD, Nogueira L, Devasia T, Mariotto AB, Yabroff KR, et al. (2022) Cancer treatment and survivorship statistics CA: A Cancer Journal for Clinicians 72(5).
2. Prostate Cancer Foundation (2017) What Are the Side Effects of Prostate Cancer Treatment? Prostate Cancer Foundation.
3. Abrams DI (2018) An Integrative Approach to Prostate Cancer. Journal of alternative and complementary medicine (New York, NY) 24(9-10): 872-880.
4. Hann DM, Baker F, Roberts CS, Witt C, McDonald J, et al. (2005) Use of Complementary Therapies Among Breast and Prostate Cancer Patients During Treatment: A Multisite Study. Integrative Cancer Therapies 4(4): 294-300.
5. Auerbach L (2006) Complementary and alternative medicine in the treatment of prostate cancer. Journal of Men's Health and Gender 3(4): 397-403.
6. Wilkinson S, Chodak G (2003) Critical Review of Complementary Therapies for Prostate Cancer. Journal of Clinical Oncology 21(11): 2199-210.
7. Nakayama A, Ide H, Lu Y, Takei A, Fukuda K, et al. (2021) Effects of Curcumin Combined With the 5-alpha Reductase Inhibitor Dutasteride on LNCaP Prostate Cancer Cells. In Vivo 35(3): 1443-1450.
8. Moga KD (2018) Antioxidant and Antiproliferative Activities of Plant Derived Extracts Against Cervical and Prostate Cancer Cell Lines. Jomo Kenyatta University of Agriculture and Technology.
9. Stanisławska IJ, Figat R, Kiss AK, Bobrowska-Korczak B (2022) Essential Elements and Isoflavonoids in the Prevention of Prostate Cancer. Nutrients 14(6): 1225.
10. Abouhamra H (2013) Effect of Eurycoma longifolia (Tongkat Ali) on the prostate cancer cell line LNCaP. Western Cape, South Afrika: UWCSolar.
11. Tembugade D, Tardalkar K, Pawal DA, Kashte S, Joshi MG (2021) Repurposing homeopathic drug Lachesis 200C as an anticancer activity: In vitro and in ovo study. Journal of the Indian Chemical Society 98(10): 100176.
12. MacLaughlin BW, Gutsmuths B, Pretner E, Jonas WB, Ives J, et al. (2006) Effects of homeopathic preparations on human prostate cancer growth in cellular and animal models. Integrative Cancer Therapies 5(4): 362-372.
13. Dos Santos AP, Cardoso TN, Waisse S, Bonamin LV (2020) Homeopathy in Experimental Cancer Models: A Systematic Review. Homeopathy 110(2): 76-85.
14. Witt CM, Bluth M, Albrecht H, Weißhuhn TER, Baumgartner S, et al. (2007) The in vitro evidence for an effect of high homeopathic potencies-A systematic review of the literature. Complementary Therapies in Medicine 15(2):128-138.
15. Khuda-Bukhsh AR, Saha SK, Roy S (2021) Evidence in support of gene regulatory hypothesis: Gene expression profiling manifests homeopathy effect as more than placebo. International Journal of High Dilution Research 12(45): 162-167.
16. Jonas WB, Gaddipati JP, Rajeshkumar NV, Sharma A, Thangapazham RL, et al. (2006) Can Homeopathic Treatment Slow Prostate Cancer Growth? Integrative Cancer Therapies 5(4): 343-349.
17. Thangapazham RL, Gaddipati JP, Rajeshkumar NV, Sharma A, Singh AK, et al. (2006) Homeopathic Medicines Do Not Alter Growth and Gene Expression in Prostate and Breast Cancer Cells In Vitro. Integrative Cancer Therapies 5(4): 356-361.
18. Melzer J, Iten F, Hostanska K, Saller R (2009) Efficacy and Safety of Mistletoe Preparations (Viscum album) for Patients with Cancer Diseases. Complementary Medicine Research 16(4): 217-226.
19. Lange lindberg AM, Garrido MV, Busse R (2006) Mistletoe treatments for minimising side effects of anticancer chemotherapy. GMS Health Technology Assessment 19: 2.
20. Pelzer F, Loef M, Martin DC, Baumgartner S (2022) Cancer-related fatigue in patients treated with mistletoe extracts: a systematic review and meta-analysis. Supportive Care in Cancer. 30(8): 6405-6418.
21. Kienle GS, Kiene H (2010) Review Article: Influence of Viscum album L (European Mistletoe) Extracts on Quality of Life in Cancer Patients: A Systematic Review of Controlled Clinical Studies. Integrative Cancer Therapies 9(2): 142-157.
22. Tröger W, Galun D, Reif M, Schumann A, Stanković N, Miličević M (2014) Quality of Life of Patients with Advanced Pancreatic Cancer During Treatment With Mistletoe. Deutsches Aerteblatt Online 111(29-30): 493-502.
23. Wilkens J (2015) Misteltherapie. Georg Thieme Verlag.
24. Twardziok M, Kleinsimon S, Rolff J, Jäger S, Eggert A, et al. (2016) Multiple Active Compounds from Viscum album L. Synergistically Converge to Promote Apoptosis in Ewing Sarcoma. Loeb DM, editor. PLOS ONE 11(9): e0159749.

25. Kempenich R, Wileke M, Meyer U (2009) Das Prostatakarzinom und seine Behandlung mit Iscucin® Populi - Einführung und klinische Fälle aus der Praxis. Der Merkurstab 62(3): 255-261.
26. Association of Anthroposophic Physicians in Germany (2019) Best Practices for Mistletoe Use in Cancer Care. Der Merkurstab 72(1).
27. Devi S, Carsten Gründemann, Huber R, Kowarschik S (2023) Characterization of Viscum album L. Effect on Immune Escape Proteins PD-L1, PD-L2, and MHC-I in the Prostate, Colon, Lung, and Breast Cancer Cells. Complementary Medicine Research 30(5): 386-392.
28. Szurpnicka A, Kowalczyk A, Sztark A (2020) Biological activity of mistletoe: in vitro and in vivo studies and mechanisms of action. Archives of Pharmacal Research 43(6):593-629.
29. Park YK, Do YR, Jang BC (2012) Apoptosis of K562 leukemia cells by Abnobaviscum F®, a European mistletoe extract. Oncology Reports 28(6): 2227-2232.
30. Rentea R (2024) Pilot Study: Evidence that High and Ultra-high Diluted Extracts of the anti-tumor active Mistletoe (Viscum Album L.) Plant Inhibit but may also Stimulate in Vitro Cell Proliferation of K562 Leukemia Cells. Cancer Therapy & Oncology International Journal 27(1).
31. Viana Valle AC, Matard PF, Brunel H dos SS, Andrade RV (2022) Homeopathic Viscum Album at Potencies D3 and 200CH Presents Cytokine Modulatory Effect Produced by in vitro Culture of Mesenchymal Stem Cells. Current Research in Complementary & Alternative Medicine 6(2).
32. Valle ACV, Aguiar LR, Brunel H dos SS, Malard PF, Andrade RV (2020) Homoeopathic Viscum album extract inhibits the growth of osteosarcoma cells. Journal of Integrated Standardized Homoeopathy 3(3): 59-63.
33. Juengel E, Meiborg M, Felenda J, turek C, Stinzing F, Blaheta R (2020) Preclinical Studies on the significance of Mistletoe preparations in the therapy of urological tumors. In: Die Mistel in der Tumor Therapie, 5. Essen, Germany pp: 85-97.
34. Boyapati K, Kumar R, Wilson L, Yang Y, Topiwala D, et al. (2021) Abstract LB102: Effect of mistletoe extract on growth and proliferation of prostate cancer cells. Cancer Research 81(13_Supplement):LB102-LB12.
35. Naeem A, Abdulsamad S, Bayati A (2022) Prostate cell lines. Journal of Oncology and Medicine 5(2): 534-538.
36. Abate-Shen C, Nunes de Almeida F (2022) Establishment of the LNCaP Cell Line – The Dawn of an Era for Prostate Cancer Research. Cancer Research 82(9): 1689-1691.
37. Rippe (Hrsg.) (2017) O. Die Mistel. Muenchen: Pflaum verlag.
38. Steiner R (1999) Introducing Anthroposophical Medicine. Steiner Books.
39. Rudolf Steiner Archive (2021) First Discussion-GA 314. Meetings with Practicing Physicians - Rudolf Steiner Archive Rsarchive.org.
40. Hirschberger S, Giesder M (2021) Beschreibung und Hintergründe der Iscucin-Herstellung bei der WALA Heilmittel GmbH. Der Merkurstab 74(3): 227-232.
41. Debus M (2014) Die Mistel in der Krebs Therapie. Der Merkurstab 67(1): 54-61.
42. Leroi R (1987) Die Mischung der Mistelsäfte; Angaben Rudolf Steiners. Der Merkurstab 40(5).
43. D Schlodder (2021) Hintergründe des Helixor-Herstellungsprozesses. Der Merkurstab 74(3): 215-218.
44. Shannon S (2001) Handbook of Complementary and Alternative Therapies in Mental Health. Burlington: Elsevier.
45. Rentea R, Kamsler M, Mueller M (2024) Therapeutic applications of Viscum Robinia RK 30X. Der Merkurstab 77(1): 97-103.
46. Krauss E, rispens JA (2025) Anthromedics - Der Merkurstab : Mensch, Mistel und Wirtsbaum. Gesichtspunkte zur rationellen Misteltherapie. Neue Methodologie, Anthromedics.org.
47. Kunz C (2008) Wirtsbäume der Mistel - Die Grundpolarität. Der Merkurstab 61(2): 123-128.
48. Goedings P (1993) Die Bildprozesse der Mistel (Viscum album L.), Teil I. Der Merkurstab 46(6).
49. Goedings P (1994) Die Bildprozesse der Mistel (Viscum album L.), Teil II. Der Merkurstab 47(1).
50. Sommer M, Soldner G (2000) Die Mistel und ihre Wirtsbäume. Differenzierungen zur Optimierung der Therapie. Der Merkurstab 53(1): 29-53.
51. Melo MN de O, Ochioni AC, Zancan P, Oliveira AP, Grazi M, et al. (2022) Viscum album mother tinctures: Harvest conditions and host trees influence the plant metabolome and the glycolytic pathway of breast cancer cells. Frontiers in Pharmacology 31: 13.

Appendix

Appendix A: Designation of the varieties of Mistletoes depending on the Host Tree they grew on

Mistletoe Grown On	
Apple	<i>Viscum mali</i>
Poplar	<i>Viscum populus</i>
Robinia	<i>Viscum robinia</i>
Willow	<i>Viscum salix</i>
Linden	<i>Viscum tilia</i>



This work is licensed under Creative Commons Attribution 4.0 License
DOI: [10.19080/CTOIJ.2025.28.556240](https://doi.org/10.19080/CTOIJ.2025.28.556240)

**Your next submission with Juniper Publishers
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats

(Pdf, E-pub, Full Text, Audio)

- Unceasing customer service

Track the below URL for one-step submission

<https://juniperpublishers.com/online-submission.php>