



Mistletoe (*Viscum album* L.) for human papillomavirus: A seven-case series Mistletoe for HPV

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ABSTRACT

Background: Human papillomavirus (HPV) is a significant public health concern, as 50 % of the population are at risk of being infected by this virus at least once in their lifetime. Although the host immune system clears most infections, a small percentage of patients do not fully clear genital HPV. They have an increased risk of developing HPV-associated cancers and can transmit HPV. Currently, no conventional treatments are available to eliminate the virus in HPV carriers.

Cases presentation: This study describes the clinical evolution of seven consecutive outpatients with HPV treated with *Viscum album* extract (VAE). In four patients, the persistence of HPV infection was six months or longer (in two of these patients, it had persisted for 22 months); in three patients, the diagnosis was recent. The patients were treated with topical (vaginal) VAE. For those with high-risk HPV or cervical intraepithelial neoplasia, subcutaneous injections of VAE were administered. *Calendula officinalis* (vaginal ovules) was prescribed for genital inflammation, and *Thuja occidentalis* ointment for verrucous lesions. Clinical and laboratory parameters were monitored throughout the treatment. All medications were well tolerated, with only mild skin reactions reported at the VAE injection site. Following treatment, six out of seven patients had negative hybrid capture tests for HPV. One of these six patients required a repeat course of treatment, after which the HPV hybrid capture test turned negative. In one patient, treatment failed to eradicate HPV.

Conclusions: Although this case series has a small sample size, there is a therapeutic potential to be further investigated regarding VAE for genital HPV. The treatment needs to be validated by future studies, which should be followed by a clinical trial to rigorously assess the efficacy of mistletoe for genital HPV infection.

Introduction

Human papillomavirus (HPV) is the etiological agent of the most common sexually transmitted infection. It is estimated that men and women have a 50 % risk of being infected at least once in their lifetime (Handler et al., 2015). HPV infects humans exclusively, targeting the cutaneous and mucosal epithelia of the genitals (penis, perianal region, vulva, perineum, vagina, and cervix), as well as the oral cavity and pharynx (Egawa et al., 2015). Transmission occurs through skin-to-skin or mucosa-to-mucosa contact, typically following cutaneous or mucosal trauma.

Clinically, lesions can be single or multiple, localized or diffuse, and vary in size. Morphologically, they present as circumscribed, hyperkeratotic, rough, and painless papules. The vast majority of low-risk

HPV (LR-HPV) infections are resolved by the host immune system. Similarly, most high-risk HPV (HR-HPV) infections are also cleared by the immune system. It is estimated that 85 % of HR-HPV infections are subclinical and transient (Stanley, 2010; Scarth et al., 2021).

Clinical infections generally resolve within several months due to the host immune response. These infections do not lead to cancer. However, if the viral genome and/or infected host cells undergo modifications, what was initially a transient infection can become persistent and not be cleared by the immune system, leading to a possible progression to cancer. Consequently, HPV infection can result in cervical disease that precedes cervical cancer. Although carcinogenesis is not a regular outcome of the HPV life cycle, it can occur through a non-productive infection, a process that does not produce virions, eventually leading to the virus's demise (Graham, 2017). In over 99 % of cervical cancer

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cases and a significant percentage of oropharyngeal and anogenital cancers, the cause is persistent HR-HPV infection (Scarth et al., 2021; Graham, 2017).

Cervical carcinoma is the third most common cancer in terms of incidence and mortality among the female population, after breast cancer and colorectal cancer. Effective strategies for preventing HPV infection include the use of barrier contraception methods, HPV vaccination during adolescence for both sexes and abstention from smoking. Diagnostic techniques, including cervical cytology, HPV testing, and colposcopy with targeted biopsy, when necessary, facilitate the early diagnosis of cervical dysplasia and early-stage cervical carcinoma, thereby enabling appropriate treatment and follow-up (D'Augè et al., 2024a).

Advancements in early diagnosis, surgical techniques, and adjuvant therapies have led to improvements in the treatment of early-stage cervical cancer in recent decades, consequently enhancing prognosis and enabling tailored management, as reviewed by D'Augè et al. (2024b).

The diagnosis of HPV infection is relatively simple and is based on tests that detect HPV DNA, one of which is the hybrid capture assay. The Papanicolaou test (Pap smear), which examines cells from the cervical surface, is a less specific but essential screening tool for cervical cancer.

To date, 229 HPV genotypes have been identified according to the International Human Papillomavirus Reference Center (Karolinska Institutet) (2025), with around 40 of these genotypes known to infect the anogenital region (Pennycook and McCready, 2022). Based on their oncogenic potential, HPV types are classified as either HR-HPV (oncogenic types, which are potentially carcinogenic, including subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) or LR-HPV (non-oncogenic types, mainly found in warts, such as subtypes 6 and 11) (Cheng et al., 2020). The most common HPV types worldwide are HPV 16 and 18, which are preventable through vaccination. These types are most strongly linked to carcinogenesis (Egawa et al., 2015).

Although they represent a relatively low percentage, patients who do not spontaneously clear HPV infection deserve appropriate treatment because they face an increased risk of developing HPV-associated cancers and can transmit the virus.

Mistletoe

White-berry European mistletoe (*Viscum album* L.) extracts exert various antitumor, pro-apoptotic, anti-proliferative, and immunomodulatory effects (Kienle and Kienle, 2007, 2010, 2017; Kienle et al., 2009). Since 1917, *Viscum album* extract (VAE) has been used in cancer treatment, administered parenterally, usually subcutaneously, in accordance with anthroposophic principles (Leroi, 1987; Gardin, 2007). In recent decades, its immunomodulatory effects have been extensively demonstrated in numerous studies involving cancer patients and healthy volunteers. In cellular immunity, VAE has been shown to increase the number and activity of lymphocytes, natural killer cells, monocytes, macrophages, and dendritic cells, as well as to promote the maturation and differentiation of CD4+ T cells (Fischer et al., 1997; Stein et al., 1998). VAE also directly stimulates the migration and tissue distribution of T lymphocytes, exerting a greater effect on CD4+ T cells than on CD8+ T cells (Nikolai et al., 1997). Additionally, it promotes the proliferation and phagocytic activity of granulocytes (Stein et al., 1999; Hajto, 1986). Subcutaneous (SC) and intravenous administration of VAE have been shown to increase neutrophil counts in patients (Büssing, 2006; Gorter et al., 1998).

VAE induces functional alterations in the microcirculation of immunologically active tissues, enhancing leukocyte adhesion. These changes are crucial for the initiation of immune responses (Koop et al., 2001).

In terms of humoral immunity, VAE stimulates the release of proinflammatory cytokines, such as tumour necrosis factor- α , IL-1, IL-2, and IL-6 (Hajto et al., 1990).

A study published by Gorter (1998) involved 100 patients with cervical dysplasia who received two SC doses of VAE per week (Iscador Spezial, Iscador AG, Arlesheim, Switzerland) for six months. Initially, all patients had Papanicolaou class III-D (low-grade and/or moderate dysplasia). After six months of therapy, 77 % showed improvement: 19 % had class I (normal cytology), 58 % had class II (inflammation and/or infection), and only 23 % remained in class III-D.

These studies provide the foundation for the indication of VAE in HPV treatment.

Medicines and doses

These seven patients, described below, were treated at a general practitioner's clinic following the principles of anthroposophic medicine. For HR-HPV or cervical intraepithelial neoplasia (CIN), SC injections of VAE were prescribed: Helixor M (Helixor Heilmittel GmbH, Rosenfeld, Germany) or Iscador M (Iscador AG, Arlesheim, Switzerland). Dosages were individualised according to anthroposophic principles.

Topical (vaginal) VAE was prescribed in all cases. Viscum Mali 10 % ointment (25 g tube) is a magistral preparation by Weleda AG Switzerland, imported by patients from Victoria Apotheke Zürich (Switzerland). It was applied vaginally at a dose of 1 mL at bedtime, three times per week, tube was finished (approximately 8 weeks). When it was not possible for the patient to import Viscum Mali 10 %, a VAE vaginal ovule was used, although a more diluted preparation: VAE Mali D5 (magistral, Farmácia Weleda, São Paulo, Brazil). "D5" indicates that the substance (VAE) was diluted and agitated 1:10 five times.

If inflammation was present (cervicitis, colpitis or leucorrhoea), *Calendula officinalis* 10 % vaginal ovules (magistral, Farmácia Equilíbrio, São Paulo, Brazil) were prescribed, to be used two to three times per week (a total of 10 ovules), based on its recognised effects in treating inflammation and promoting healthy tissue regeneration (Pelikan, 1997; Gardin and Schleier, 2009).

Thuja occidentalis 10 % ointment (magistral, Farmácia Equilíbrio, São Paulo, Brazil) was prescribed topically for external warty lesions, to be applied once per day for two weeks. This is a renowned medicinal plant, traditionally known for its effect on verrucous lesions (Pelikan, 1997; Gardin and Schleier, 2009).

Cases presentation

All seven consecutive patients were seen in a private clinic in São Paulo, Brazil. Each patient provided written and signed consent for the publication of their data. This case series was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Patient 1: A 27-year-old female presented with a 22-month history of colpitis and vulvar verrucous lesions. Hybrid capture testing showed HR-HPV (types 51 and 58) in the vagina, cervix, and anus. One year after the initial diagnosis, the patient still tested positive for HR-HPV infection. Biopsy results revealed chronic cervicitis and vulvar intraepithelial neoplasia (VIN) grade 1. She received SC injections of VAE Helixor M 1 mg three times per week for 11 weeks (32 ampoules in total), Calendula 10 % vaginal ovules three times per week (10 ovules in total), and Viscum Mali 10 % ointment, 1 mL vaginally three times per week for 8 weeks. External lesions were cauterized, and Thuja 10 % ointment was applied daily for two weeks. Five months after completing treatment, a new HPV test using hybrid capture was performed and returned negative (undetectable). Upon examination, no external lesions or colpitis were observed.

Patient 2: A 36-year-old female was diagnosed with atypical squamous cells of undetermined significance (ASC-US) by Pap smear six months prior. Her colposcopy results were normal, but hybrid capture testing showed HR-HPV (type 33) in the cervix. Her treatment included SC injections of VAE Helixor M, starting with 0.1 mg twice

per week for 4 weeks (8 ampoules), followed by 1 mg twice per week for an additional 4 weeks (8 ampoules). Topical treatments consisted of Calendula 10 % vaginal ovules used three times per week (10 ovules in total) and Viscum Mali 10 % ointment, 1 mL applied vaginally three times per week for 8 weeks. One month after completing treatment, her HPV test using hybrid capture was negative, and colposcopy results remained normal.

Patient 3: A 47-year-old female was diagnosed with CIN 2, despite her hybrid capture test showing LR-HPV 19 months before the initial appointment. Cervicitis was observed during colposcopy. Two months after diagnosis, the patient underwent conization. However, two months later, a new lesion appeared in the cervix. She received two cycles of topical imiquimod prescribed by another physician. A subsequent biopsy revealed CIN 1, and LR-HPV persisted. A Pap smear showed ASC-US findings. Calendula 10 % vaginal ovules were prescribed three times per week (10 ovules), along with SC injections of VAE Helixor M 1 mg twice per week for 8 weeks (16 ampoules). Following the Calendula treatment, she used Viscum Mali 10 % ointment, 1 mL vaginally three times per week for 8 weeks. Two weeks after completing the treatment, hybrid capture testing showed persistent LR-HPV, and a biopsy of a vaginal lesion revealed vaginal intraepithelial neoplasia (VaIN) grade 1. The relative light units to positive control ratio (RLU/CO) was 628 prior to treatment and 79.1 post-treatment, indicating a partial response. The treatment was repeated, consisting of SC injections of VAE Helixor M 1 mg twice per week (16 ampoules) and Viscum Mali 10 % ointment, 1 mL vaginally, three times per week for 8 weeks. Three months after completing the second treatment, hybrid capture testing for HPV was negative, the Pap smear showed ASC-US, and colposcopy results were normal.

Patient 4: A 35-year-old female presented with chronic colpit on colposcopy and a Pap smear showing CIN 1. Two months later, a cervical biopsy revealed CIN 2. Her hybrid capture test detected HR-HPV (type 16), the same result she had reported five years earlier. She was treated with Calendula 10 % vaginal ovules, administered three times per week (10 ovules), and SC injections of VAE Helixor M, starting with 0.1 mg twice per week for 4 weeks (8 ampoules), followed by 1 mg twice per week for an additional 4 weeks (8 ampoules). Following the Calendula treatment, she used Viscum Mali 10 % ointment, 1 mL vaginally three times per week for 8 weeks. Two months after completing treatment, hybrid capture testing revealed persistence of HPV 16, indicating treatment failure. The patient was then referred for conventional treatment (conization).

Patient 5: A 26-year-old female visited the office with a recent diagnosis of HR-HPV (type 18) in vulvar and perianal verrucous lesions. A Pap smear showed inflammation, but colposcopy was normal. The external lesions were cauterized, and Thuja 10 % ointment was applied daily for two weeks. Subsequently, she used Viscum Mali 10 % ointment, 1 mL vaginally three times per week for 8 weeks, along with subcutaneous (SC) injections of VAE Iscadur M, 1 mg twice per week (7 ampoules). Five months after completing the treatment, her hybrid capture test for HPV was negative, and vulvoscopy results were normal.

Patient 6: A 40-year-old female was recently diagnosed with HR-HPV (type 39) affecting the vulva and cervix. A Pap smear showed inflammation, and colposcopy revealed chronic cervicitis. A biopsy indicated squamous metaplasia. She received SC injections of VAE Helixor M, 1 mg twice per week for 16 weeks (32 ampoules). She was also prescribed Calendula 10 % vaginal ovules, three times per week (10 ovules), followed by VAE Mali D5 vaginal ovules, three times per week (10 ovules). One month after completing treatment, her hybrid capture test for HPV was negative, and follow-up colposcopy results were normal.

Patient 7: A 22-year-old female visited our office with a recent diagnosis of LR-HPV infection affecting the vagina and cervix. A Pap smear showed inflammation, colposcopy revealed cervicitis, and a

biopsy indicated squamous metaplasia. She was treated with VAE Mali D5 vaginal ovules, three times per week (10 ovules), followed by Calendula 10 % vaginal ovules, twice per week (10 ovules). One month after completing treatment, her hybrid capture test for HPV was negative, and follow-up colposcopy results were normal.

All treatments were well tolerated. Patients reported only a mild local inflammatory reaction (redness) at the VAE injection site, which lasted one day or less. According to anthroposophic principles, this reaction is considered a sign of a desired immunological response. Topical treatments (Calendula, Thuja, and VAE ointments and ovules) did not cause any adverse effects reported by the patients. [Table 1](#) summarizes the key data for all seven patients.

Discussion

HPV infection poses a significant public health challenge due to its high prevalence and carcinogenic potential. While most individuals successfully clear the virus, persistent infections require ongoing surveillance, leading to anxiety and financial burdens associated with medical procedures and testing. Additionally, HPV carriers have a high risk of transmitting the virus during unprotected sexual intercourse.

HPV vaccination is a crucial public health intervention designed to prevent HPV-related diseases, including cervical cancer, anogenital warts, and other malignancies such as oropharyngeal, vulvar, vaginal, penile, and anal cancers. Women who have undergone hysterectomy for CIN 2 or early-stage cervical cancer may still benefit from HPV vaccination, as it provides protection against lower genital tract dysplasia, with an estimated 67 % efficacy, according to a multicentre retrospective study by [Bogani et al. \(2024\)](#). Additionally, males are encouraged to receive the vaccine to prevent HPV-related diseases and reduce viral transmission.

The conventional treatment for CIN 3 typically involves ablation or excision. However, no conventional treatment has been proven to eliminate HPV infection in chronic carriers.

Currently, limited data exist in the medical literature on VAE for HPV eradication; only one study involving 100 patients has been published ([Gorter, 1998](#)). Nevertheless, some studies have reported beneficial outcomes with VAE for treating HPV-related CIN and cervical cancer. [Portalupi \(1995\)](#) conducted a prospective, non-controlled study using SC VAE as a complementary treatment for HPV-related CIN (grades 1 to 3), achieving complete remission in 41 % of cases and partial remission in 27 %. [Reynel et al. \(2018\)](#) successfully treated cervical carcinoma in situ using VAE both intralesionally and subcutaneously. Additionally, [Jach and Basta \(1995\)](#) reported positive outcomes with VAE combined with recombinant interferon alpha in treating CIN 1 and CIN 2. [Vögler \(2003\)](#) described the successful complementary treatment of a child with laryngeal papillomatosis using subcutaneous VAE. Histological testing confirmed the presence of HPV subtype 6. It was later discovered that the mother had condyloma acuminata at the time of birth.

Limitations

As a case series, this study cannot establish causal relationships. The primary limitation is the small sample size. Nevertheless, we hope that these findings will encourage further research into the potential use of this medicinal plant in the treatment of genital HPV. According to [Kienle and Kiene \(2009\)](#), case reports and case series are valuable tools for physicians to scientifically document their experiences, thereby advancing medical knowledge. Such reports play a crucial role in discovering new diseases, treatments, and unexpected or adverse effects. Additionally, they serve as important educational resources for medical professionals. Many therapeutic innovations have their origins in case reports or case series.

Table 1
Summary of clinical and laboratorial features.

| Patient | Age - years | HPV type | HPV Site | Persistence of HPV | Pap smear | Colposcopy and vulvoscopy | Biopsy | Treatment | Result – hybrid capture |
|-------------------|-------------|-------------|----------------------|--------------------|--------------|----------------------------------|---------------------------|--|---|
| 1 | 27 | HR (51, 58) | Vagina, cervix, anus | 22 months | Not done | Colpitis, external warty lesions | Chronic cervicitis, VIN 1 | SC VAE, Calendula, VAE ointment, Thuja | Negative 5 months after completing treatment |
| 2 | 36 | HR (33) | Cervix | 6 months | ASC-US | Normal | Not done | SC VAE, Calendula, VAE ointment | Negative 1 month after completing treatment |
| 3 | 47 | LR | Cervix | 19 months | ASC-US | Cervicitis | CIN 2 | SC VAE, Calendula, VAE ointment | Persistence of LR-HPV 2 weeks after completing treatment |
| 3 (2nd treatment) | 47 | LR | Cervix | 22 months | ASC-US | Not done | VaIN 1 | SC VAE, VAE ointment | Negative 3 months after completing treatment |
| 4 | 35 | HR (16) | Cervix | 5 years | CIN 1 | Chronic colpitis | CIN 2 | SC VAE, Calendula, VAE ointment | Persistence of HR-HPV 2 months after completing treatment |
| 5 | 26 | HR (18) | Vulva, anus | Recent | Inflammation | External warty lesion | Not done | SC VAE, Thuja, VAE ointment | Negative 5 months after end the of treatment |
| 6 | 40 | HR (39) | Vulva, cervix | Recent | Inflammation | Chronic cervicitis | Squamous metaplasia | SC VAE, Calendula, VAE ovules | Negative 1 month after completing treatment |
| 7 | 22 | LR | Vagina, cervix | Recent | Inflammation | Cervicitis | Squamous metaplasia | VAE ovules, Calendula | Negative 1 month after completing treatment |

ASC-US = atypical squamous cells of undetermined significance, CIN = cervical intraepithelial neoplasia, HR = high risk, HPV = human papillomavirus, LR = low risk, Pap = Papanicolaou, SC = subcutaneous, VAE = *Viscum album* extract, VaIN = vaginal intraepithelial neoplasia, VIN = vulvar intraepithelial neoplasia.

Conclusion

This small case series highlights the potential of VAE as a treatment for HPV. To obtain more robust conclusions, the authors recommend conducting randomized, placebo-controlled clinical trials to rigorously evaluate its efficacy in treating genital HPV.

What is remarkable in this case series is that it demonstrates that HPV can be eliminated, even in long-term carriers, using VAE. The treatment was successful in six out of seven patients. Although Patient 3 did not initially achieve viral clearance, the first course of treatment resulted in a significant reduction in RLU/CO values. This prompted a repeat treatment. Subsequent testing revealed that HPV had become undetectable by hybrid capture.

The authors hope to inspire other researchers to continue exploring the therapeutic potential of this medication.

Clinical trial number

Not applicable.

Written informed consent for publication

Written consent was obtained from all patients for the publication of the data. Copies of written consent are available for review.

Statement of ethics

This case series was conducted according to the ethical principles set and internationally accepted standards for research practice and reporting. A case series article doesn't require ethics committee approval because it involves retrospective data use and no identifiable information.

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Author agreement

All authors have seen and approved the final version of the manuscript being submitted.

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CRedit authorship contribution statement

Nilo E. Gardin: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beatriz Gardin-Machado:** Writing – review & editing, Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest and no funding sources.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.phyplu.2025.100805](https://doi.org/10.1016/j.phyplu.2025.100805).

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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