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Immunomodulatory Activity and Inhibitory Effects of *Viscum album* on Cancer Cells, Its Safety Profiles and Recent Nanotechnology Development

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Keywords
Viscum album, Santalaceae, immunomodulation, anticancer effects, safety profiles, nanoparticles

received April 4, 2024
accepted after revision August 23, 2024

Bibliography
Planta Med 2024
DOI 10.1055/a-2412-8471
ISSN 0032-0943

Georg Thieme Verlag KG, Rüdigerstraße 14,
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ABSTRACT

Viscum album has been employed traditionally to treat various ailments including as add-on therapy for cancer treatment. *V. album* formulations have been employed as adjuvants in cancer treatment due to their immunomodulatory activities as well as to alleviate the side effects of conventional cancer therapies. The present review provides updated information from the past 10 years on the immunomodulatory activity and inhibitory effects of *V. album* on cancer cells, its safety profile, and recent nanotechnology development. *V. album* extracts and their bioactive phytochemicals, particularly lectins, viscotoxins, and polyphenols, have demonstrated immunomodulatory activity and inhibitory effects against various types of cancer, with low cytotoxicity and side effects, in experimental studies and demonstrated promising anticancer activity in clinical studies in cancer patients. *V. album* extracts have been shown to enhance immune function by promoting cytokine secretion and inducing both innate and adaptive immune responses, which can help improve immune surveillance against cancer cells. The development of *V. album* nanoparticles has boosted their biological activities, including inhibitory activity on cancer cells, and could possibly reduce undesired side effects of the plant. Further prospective studies on the plant as a source of new medicinal agents for use as an adjuvant in the treatment of cancer must be performed to provide sufficient efficacy and safety data.

Introduction

The major immune cells, like polymorph nuclear cells, macrophages, dendritic cells (DCs), mast cytotoxic T cells, natural killer (NK) cells, and mast cells, can detect, combat, and phagocyte tumor cells that are generated early in the body. The detection, combating, and elimination of the tumor cells by the innate immune cells and an induction of an acute immune response play a crucial role in preventing cell propagation [1]. However, some tumor cells are able to develop different mechanisms to elude the strong immune surveillance of the immune system at their early tumor development stage. At this stage, the tumor cells become

less immunogenic. They escape from immune responses by producing immunosuppressive substances, which inhibit the activity of DCs. They are able to evade the immune control by altering molecules on their surface, reducing adhesion molecule expression, downregulating major histocompatibility complex (MHC) molecules, and inhibiting the secretion of immunosuppressive mediators such as transforming growth factor beta (TGF-β), prostaglandin E_2 (PGE_2), and interleukin 10 (IL-10), which suppress T lymphocytes and immune responses [2]. The incidence of cancer generation may be increased during chronic inflammation due to accumulation of proinflammatory cytokines, chemokines, reactive oxygen species (ROS), and growth factors by the immune cells that stimulate mutation of the normal cells into abnormal cells. The tumor cells may undergo phenotypic or genetic changes to enable them to escape immune surveillance. The more immune-resistant cancer cells are able to elude detection and continue to propagate. Understanding these mechanisms is necessary to design and develop targeted therapies to enhance immune surveillance and eliminate cancer cells [3].

Cancer treatments encompass a wide range of approaches that aim to target and control abnormal cell growth. Some commonly used cancer treatments include chemotherapy, radiotherapy, photodynamic therapy, stem cell transplant, hormone therapy, surgery, and active immunotherapy. Cancer immunotherapy is a class of treatments that harness the power of the immune system to recognize and target cancer cells. These therapies work by stimulating or enhancing the body's natural immune response against cancer. Unfortunately, in combating the cancer cells, most of these cancer treatment modalities have serious side effects and immune-related destructive events due to their adverse effects on the surrounding normal cells [4]. An effective cancer treatment must be able to fight the tumor at the first step and arrest any new metastases lesion formation. There has been a growing interest to include complementary and integrative therapy approaches in the management of adverse reactions during anticancer therapy. Several studies have reported that plant extracts and their bioactive phytochemicals demonstrated immunomodulatory and anticancer activities against various types of cancer, with a low risk of side effects and cytotoxicity. Natural immunomodulators have been utilized as adjuvants or supplements together with common therapeutic modalities to treat cancer. As immunostimulants they are employed to inhibit the suppressive effect of chemotherapy and to generally improve the immune response against the tumors, and also to prevent the tumor itself from escaping the immune surveillance. The cell- and humoral-mediated immune responses against the tumor can be stimulated by immunostimulants, which facilitate the recognition and destruction of the already existing tumor. Immunosuppressants can be used as a preventive treatment to inhibit chronic inflammation that leads to the initiation of cancer and also suppress cancer cell proliferation and tumor growth [5].

Viscum album L. (VA), commonly known as mistletoe, has been frequently used traditionally in several countries, especially in Europe, as an integrative oncological drug, particularly as an adjuvant or add-on to standard cancer therapy. VA formulations exert immunomodulatory activities and have been incorporated as supplements in complementary and alternative medicine to treat cancer and reduce side effects of conventional anticancer treatments. The immunomodulatory activity and inhibitory effects and mechanisms of VA on cancer cells have been much investigated, as these properties are characteristics of a good add-on therapy with modern oncological therapies. VA extract (VAE) and its isolated constituents have been greatly studied for their significant inhibitory activities in experimental studies using cancer cells and animal cancer models. VAE has also demonstrated promising anticancer activity in various preclinical and clinical studies in cancer patients [6]. The biological and pharmacological activities of VA were proposed to include many signaling pathways by acting on enzymes, membrane receptors, proteins, transcriptional targets, and ion channels. However, studies on the biological activities of VA have been mainly on its crude extracts. The chemical constituents responsible for most of its individual pharmacological activities have not been identified [7]. VAEs have been shown to enhance immune function by promoting cytokine secretion and inducing both innate and adaptive immune responses such as stimulating NK cell activity. These effects could help improve immune surveillance against cancer cells. Several components of VA, such as lectins, viscotoxins, and polyphenols, have immunomodulatory properties and could increase the production and activity of immune cells, such as T cells, NK cells, and macrophages. They could inhibit tumor growth by inducing apoptosis in cancer cells, inhibiting angiogenesis, and modulating the tumor microenvironment. VAEs have also shown potential in enhancing the efficacy of conventional cancer treatments, such as radiotherapy and chemotherapy, while reducing their side effects [8].

Search Strategy and Objectives

There were previous reviews on the immunomodulatory activity and inhibitory effects and mechanisms of VA on cancer cells and their potential use in cancer therapy [9–15]. The objective of the present paper is to provide updated information from the past 10 years on the immunomodulatory activity and inhibitory effects of VA on cancer cells, including its safety profile and recent nanotechnology development to assess its potential application in nanomedicine, especially for the treatment of cancer. The relevant information on the immunomodulatory activity and inhibitory effects and mechanisms of action of VA on cancer cells over the past 10 years, including its safety profile and its recent nanotechnology development, was collected and compiled by online search engines on updated scientific databases such as Google Scholar, PubMed, Web of Science, Science Direct, SciFinder, ACS Publications Today, and Wiley Online Library. The major topics and data for inclusion are ethnopharmacological importance, phytochemistry, immunomodulation, immunosuppressants, immunostimulants, anticancer activities and mechanisms of action, safety profiles, and VA nanoparticles. The following search terms were used: *Viscum album*, immunomodulation, anticancer effects, safety profiles, nanoparticles, and Boolean operators of 'AND' and 'PLUS' were included between terms. This review also provides a critical assessment of the present knowledge for perspectives and directions for future research and potential applications of VA in cancer therapy.

Distribution, Taxonomy and Ethnopharmacological Uses

VA belongs to the family Santalaceae, order Santalales. Notably, the word "mistletoe" springs from the Anglo-Saxon mistletoe; "mistel" referring to dung and "tan", meaning twig. Hence, it literally means "dung-on-a-twig". The common names of VA are European mistletoe, birdlime mistletoe (Engl.), Sumidad de muerdago, Sumidad de liga (Span.), Sumidad de visco, Mistelkraut, Vogelmistel, Limemistel, Hexenbese (Ger.), Gui, and Blondeau (Fr.). It is a semiparasitic, perennial, epiphytic, angiosperm, and evergreen round bush or shrub that grows mainly on branches and rarely on the trunk of woody deciduous trees. A total of 452 taxa (species, subspecies, varieties, and hybrids) of host woody species belonging to 96 genera of 44 families have been identified [16]. VA is widely distributed around the world, and it is mostly found in Africa, southern Asia, and Madagascar. A few species have been documented as native to temperate Asia, Europe, eastern Australia, and Malaysia. *Viscum* species including VA are distributed on extraordinarily broad host ranges. Distribution on specific hosts rarely occur and only a few plants from a single family or order have been identified as specific hosts to very recently evolved lineages. The host trees are more widely distributed than VA. The local abundance and distribution of VA on the host trees depend on the individual differences among the trees, especially in terms of diameter at breast height. The sex of the host has also been identified to play a major role in influencing VA abundance and distribution [17]. As a semiparasitic plant, it extracts mineral substances in aqueous form from the host tree and in return presents part of its own assimilates to the host that are used to protect the latter from animal pests and fungal diseases. The optimal growth or extension of VA horizontally and vertically depends primarily on temperature. It has been shown that the geographic distribution of the plant might be restricted by both winter and summer temperatures. The limits of the occurrence of VA were shown to be correlated with the mean monthly temperatures of the warmest and coldest months of the year. The stems of the herb are dichotomous yellowish-green, while the leaves are sessile, lanceolate to spatulate. The parts of VA that have medicinal value include the stem, leaves, and pea-sized berries [18].

VA has been utilized for a long time in many countries for various purposes, ranging from medicinal to symbolic and ritualistic. Traditionally, various plant parts are utilized to combat a wide spectrum of diseases. The plant is well recognized as a traditional remedy to manufacture numerous products, among them tinctures, teas, ointments, and nutritional aspects, owing to its practical therapeutic properties [19]. During the Middle Ages, VA preparations were always described for their healing properties since Hippocrates endorsed its use to treat complaints connected with menstruation and ailments of the spleen, and Paracelsus reported its use in the treatment of epilepsy. In addition, Hildegard von Bingen described VA as beneficial in spleen and liver management [20]. In early 1900 s, the plant was used as a complementary oncological remedy as well as to treat epilepsy, spleen ailments, menstruation issues, cardiovascular diseases, infertility, and ulcers [21].

Throughout many nations, including Russia, Armenia, Bulgaria, Ukraine, the Czech Republic, various VA formulae like decoctions, tinctures, and fresh juice of various parts are employed to heal diarrhea, cough, rheumatism, ulcers, broken bones, inflammation of lymphatic glands, gout, and wounds, in addition to being an anti-atherosclerotic, analgesic hypotensive, and sedative remedy [22]. Traditionally, in many European countries, the plant has been utilized for a long time to heal various diseases, including atherosclerosis or internal bleeding, insomnia, hypertension, and anxiety, and as a complement for cancer treatment [23]. VAEs have been utilized in cancer treatment since the 1920 s. Uses of VAE and its preparations as a palliative therapy for malignant tumors and as curative to treat degenerative and inflamed joints have been approved by the German Commission E [24]. In Europe, particularly Germany, young twigs and leaves are used to remedy respiratory and circulatory system issues [25]. The ethnopharmacological use of various parts of VA in curing cardiovascular illness has been documented by the European Medicines Agency [26].

VA has been widely employed in India for healing numerous diseases, such as hypertension, diabetes, degenerative inflammation of the joints, vertigo, asthma, diarrhea, nervousness, epilepsy, and insomnia [27]. The plant is employed to manage problems of stomach, stroke, insomnia, anxiety, chronic cramps, hypertension, heart palpitation, difficulties in breathing, atherosclerosis, and hot flashes during menopause, and to complement cancer healing in Pakistan. In African and Asian traditional medicines, VA have been used to heal numerous ailments as an analgesic, antidiabetic, hypotensive, and anti-inflammatory agent [28]. In early Greece, the tree was used to manage spleen-related diseases, cure ulcers, infertility, and epilepsy, and treat tumors. In France, it was recognized as a golden herb for epilepsy treatment [29]. It is worth noting that the Chinese and Ayurvedic traditional medicines use VA to combat several ailments such as bone fractures, inflammation, arthritis, hypertension, and joint pains [20].

Although VA has a long history of traditional uses, its efficacy for various health conditions in many cases is still inconclusive. Additionally, the plant can be toxic, especially during long-term use. Thus, there has been ongoing strong research interest to provide scientific evidence to support the traditional uses of VA, especially its potential applications in cancer therapy.

Phytochemistry

VA contains diverse biologically active compounds. The most important classes of compounds are its protein compounds, lectins, and viscotoxins, which play a major role in the use of this plant for the treatment of cancer [24]. Other phytochemicals that have been isolated from this plant are flavonoids, phenylpropanoids, phenolic acids triterpenes, phytosterols, and oligo- and polysaccharides, which also contribute significantly to the plant's biological and pharmacological effects. These secondary metabolites may be biosynthesized by the plant, or they are obtained from its host trees. Alkaloids that have been detected in this plant were obtained from the host trees of this hemiparasitic species [30]. Bioactive compounds in VA differ qualitatively and quantitatively according to several factors. Among these factors are host species, organ type, harvesting season, and life cycle of VA [31, 32].

This is consequently reflected on the biological activity of the plant. Therefore, a standardized harvest is highly important for the preparation of herbal products from this plant species. An untargeted LC-MS metabolomic study combined with chemometric analysis indicated that accumulated metabolites depend on the host plant [33]. Moreover, the referred study identified amino acids, lipids, organic acids, and coumarins as the biomarker compounds. The polyphenol profile in VA was analyzed using UHPLC-q-TOF-MS combined with classic molecular networking [34]. It was concluded that these compounds can be used as chemotaxonomical markers of *Viscum* species. In the following section, we discuss the most important compounds produced by this pharmaceutically important plant.

Lectins

Lectins are proteins with a high specificity to interact with various sugar groups that are part of other molecules and form reversible linkages with glyco-conjugate complexes. They are characterized as type II ribosome-inactivating proteins, joined by two polypeptide chains and linked via a disulfide bond. The peptide chains are made up of an A-chain, which is comprised of three distinct individual domains and a B-chain, which contains two domains with similar configurations. The A-chain can inhibit protein synthesis via inactivation of the 60S ribosomal subunit of eukaryotic cells, while the B-chain permits entry into the cells by binding to cell surface glycol conjugates [35]. Different lectins have been isolated from VA and they are known as mistletoe lectins (ML). They are also known as viscumins [36]. They can be classified based on their sugar specificity: ML I (galactose specific), ML II (galactose- and N-acetyl-D-galactosamine specific), and ML III (N-acetyl-D-galactosamine specific). ML I's are glycoproteins having two polypeptide chains linked together by a disulfide bond. The molecular weight of ML I is 115 kDa while both ML II and ML III are 60 kDa [37]. The protein constituents of lectins of European and American varieties of VA exhibit marked similarity [38]. Recently, the high-resolution ML I crystal structure has been refined when it is a complex with the nucleic acid oxidation product 4-N-furfurylcytosine [39]. Lectins are considered the main immunomodulatory and anticancer agents in VAEs. *In vivo* studies have shown that by carefully removing lectins from VAE, the effectiveness of VAE on several cellular immune parameters were markedly reduced [40].

Viscotoxins

Viscotoxins are basic polypeptides of 46 amino acid residues rich in cysteine and containing three or four disulphide bridges. They are a class of lower molecular weight (around 5 kDa) chemicals that belong to the third class of sulfur-containing proteins. VA contains several isoforms of viscotoxins, namely, A1, A2, A3, B, B2, C1, and 1-PS [37]. The composition and content of viscotoxins in VA depends on the subspecies and the host plant. Pressurized water extraction was proposed as an efficient method for isolating lectins and viscotoxins from VA [41]. The conditions used in the referred study were 15 MPa for 15 min at 40 °C. This was found to be more efficient and faster than conventional extraction methods. Homogenizer-assisted and ultrasound-assisted extraction of VA parts (leaf, fruits, and seeds) has been reported [42].

Polyphenols

Phenolic acids (gallic acid, caffeic acid, synaptic acid, protocatechuic acid, and ferulic acid) and flavonoids, including flavonols, flavones, and flavanones, are the main polyphenols identified in VA. The most important flavonoids identified in VA were recently reviewed by [43]. Dai et al. [44] isolated three new flavonoid glycosides from the aerial parts of VA along with rhamnazin-3-*O*-*β*-D-api-(1 → 2)-[6′′-(3-hydroxy-3-methylglutaryl)]-*O*-*β*-D-glucopyranoside, rhamnazin-3-*O*-*β*-D-[6′′-(3-hydroxy-3-methylglutaryl)]-*O*-*β*-D-glucopyranoside, rhamnazine, rhamnazin-3-*O*-*β*-D-glucopyranoside, rhamnazin-4′-*O*-*β*-D-glucopyranoside, rhamnazin-3-*O*-*β*-D-6′′-acetyl-*O*-*β*-D-glucopyranoside, and 3′,4′,3,5-tetrahydroxyl-7-methoxyl flavonoid. A phytochemical study on the stem, leaves, and roots of VA (European variety) resulted in the isolation of albvisosides A–E, dihydroflavonoid glycosides [45]. The phytochemical analysis of the extract of the European variety of VA was performed using ultra-HPLC combined with electrospray ionization tandem-mass spectrometry [7]. A total of 88 compounds, full or in part, mainly flavonoids, hydroxycinnamic acids, and lignans, were identified in the VAE. The effect of host species, place and time of harvest on the phenolic constituents in VA has been reported [46]. It was observed that plant materials harvested in cold weather with less sunshine and the presence of snow showed a correlation between high phenolic content and high antioxidant activity. VA collected from the host species conifers (*Viscum austriacum* and *Viscum abietis*) had the most advantageous antioxidant activity and chemical composition. This result was further confirmed by Kleszken et al. [47], who reported that the host tree contributes significantly to the phenolic profile and antioxidant capacity of VA. Chlorogenic acid and rhamnazin glucosides were the most abundant phenolic acid and flavonoid, respectively. Ligalbumoside A, alangilignoside C, chlorogenic acid, sakuranetin, caffeic acid, isosakuranetin, syringenin 4-O-apiosylglucoside, and syringenin 4-O-glucoside were identified as the phenolic compounds in VAEs by using HPLC with a high-resolution mass spectrometer [48].

Other compounds

The following triterpenes and sterols have been isolated from VA: *β*-sitosterol and its glucosides, stigmasterol, betulinic acid, *β*-amyrin acetate, and oleanolic acid [49, 50]. Oleanolic acid was the major triterpenic acid present in the plant. The contents of ursolic acid, betulinic acid, and oleanolic acid in the stems and leaves of the plant fluctuate in various seasons. The maximum content depends on the host species and the season [51]. Arabinogalactan, pectin, highly methylated homogalacturonan, and 1→*α*4 galacturonic acid methyl ester have also been isolated from the leaves and stems of VA. Rhamnogalacturonanes with arabinogalactan side chains have been identified as major components of the berries of VA [29]. Cao et al. [52] reported the isolation from the leaves of VA of two new acetylene conjugate compounds, dibutyl (2*E*, 6*E*)- octa-2, 6-dien-4-yne dioate and dibutyl (2*Z*, 6*Z*)-octa-2, 6-dien-4-yne dioate.

The chemical constituents of essential oils of VA distilled from three subspecies of the plant growing in Turkey and analyzed by GC-MS have been reported [53]. Recently, GC-MS analysis of VA essential oils in relation to the diverse host trees has been re-

ported [54]. In the referred study, it was concluded that the host tree significantly influenced the chemical composition of essential oils. Proximate and mineral components of the Korean variety of VA from various host trees were measured [55]. Significant differences in the proximate and mineral contents were found depending on the host species. The leaves were reported to contain more crude proteins than the twigs. The twigs were found to be a good source of energy, fiber, and some minerals, especially magnesium. The phytochemical compounds and composition of minerals in the Mexican variety of VA have been determined. The main mineral components found were Ca^{2+} and K^{+}, 56.6 and 25.8%, respectively [19].

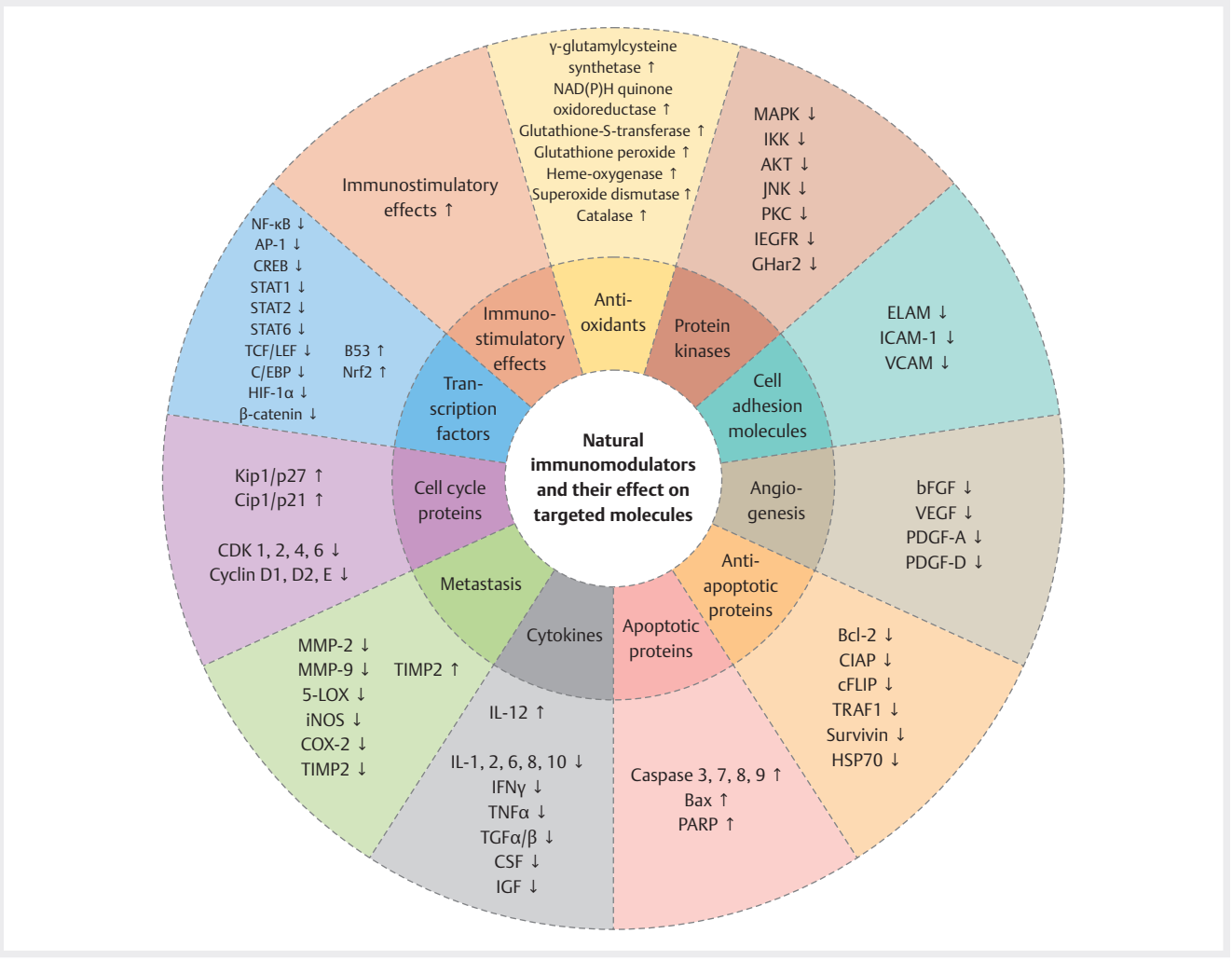
Mechanisms of Immunomodulatory Activity and Inhibitory Effects of Plant Secondary Metabolites on Cancer Cells

Plant secondary metabolites (PSMs) exert immunomodulatory effects by activating both the innate and adaptive immune systems. Within the innate immune system, they generally modulate nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) activation and production of proinflammatory cytokines like TNF-α and interleukins (IL-6, IL-8, IL-1β) [56]. They can also activate NK cells, which directly target and eliminate cancer cells while simultaneously enhancing the phagocytic capabilities of macrophages, the immune cells responsible for engulfing and eliminating foreign invaders, including cancer cells [57]. In the adaptive immune system, these metabolites boost the proliferation and function of T and B cells, the primary white blood cells involved in specific and long-term immune responses. Additionally, they regulate antibody production, aiding in the neutralization of cancer cells and other antigens. The multifaceted immunomodulatory mechanisms of PSMs exhibit substantial potential for enhancing the immune system's capacity to combat cancer and other threats [58].

PSMs such as flavonoids, polyphenolic compounds, alkaloids, and organosulfur compounds possess immunomodulatory properties, which means that they can modulate the immune system to enhance the body's natural ability to fight cancer. PSMs with immunomodulatory activities are widely employed as preventive and therapeutic agents against various types of cancer. They can effectively target a variety of cellular signaling pathways and molecular players, such as mitogen-activated protein kinases (MAPKs), vascular endothelial growth factor (VEGF), Akt/mTOR, and epidermal growth factor receptor (EGFR) to inhibit angiogenesis and tumor growth, which is essential for tumor vascularization [59]. PSMs also use other various tactics to combat cancer while safeguarding healthy cells, which include strong antioxidant and anti-inflammatory properties [60], metastasis suppression [61], epigenetic alterations, and DNA repair [62]. PSMs have the capacity to modulate the tumor microenvironment, comprising cellular and noncellular elements surrounding the tumor. This modulation renders the tumor more vulnerable to immune system responses and treatments like chemotherapy and radiation therapy [63].

The mechanisms of inhibitory activities of PSMs on cancer cells involve inhibiting angiogenesis, triggering apoptosis, and modulating the immune system. Inhibiting angiogenesis refers to preventing the formation of new blood vessels that supply nutrients to cancer cells, thereby limiting their growth. Preventing this process can lead to stopping the development and metastasis of tumors and the formation of new blood vessels. A key regulator of angiogenesis, VEGF, is being downregulated as part of the process. Triggering apoptosis refers to inducing programmed cell death in cancer cells, which helps to eliminate them from the body. The upregulation of proapoptotic proteins like caspase-3 and caspase-9 and the downregulation of antiapoptotic proteins like Bcl-2 are two of the ways by which it occurs. The end outcome is the intrinsic apoptotic mechanism, which is activated, leading to the death of cancer cells. Some PSMs cause cancer cells to undergo apoptosis through a certain number of pathways, including the activation of proapoptotic signals like the mitochondrial and extrinsic pathways, the downregulation of antiapoptotic proteins like Bcl-2 and Bcl-xL [64, 65], and the disruption of the cell cycle, frequently through the inhibition of cyclin-dependent kinases. Another mechanism involves modulating the immune system, which involves enhancing the body's natural defense mechanisms against cancer, such as activating immune cells to recognize and attack cancer cells. PSMs can enhance the activity of NK cells, macrophages, and T cells [66]. This process can play a vital role in strengthening immune response against cancer cells. The above mechanisms work together to target and combat cancer cells, hence aiding in the treatment of cancer. ▶ **Fig. 1** illustrates PSMs as immunomodulators and their suppressive effects against targeted molecules to produce anticancer activity.

Many PSMs such as vincristine, camptothecin, taxol, curcumin, artemisinin, and tymoquinone have shown inhibitory activities toward cancer cells. The mechanism of inhibitory activity of vincristine involves binding to and inhibiting the function of microtubules, which are important for cell division and mitosis. By disrupting microtubule formation and function, vincristine prevents cancer cells from dividing properly, leading to cell cycle arrest and, ultimately, cell death [63]. Camptothecin exhibits inhibitory properties against cancer cells by targeting topoisomerase I, an enzyme involved in DNA replication and transcription. Camptothecin specifically inhibits topoisomerase I activity, leading to the formation of DNA-enzyme complexes. These complexes result in DNA breaks and the accumulation of double-stranded DNA breaks in cancer cells, ultimately causing cell death [67]. Taxol's anticancer activity is attributed to its ability to stabilize microtubules in cancer cells, preventing their dynamic assembly and disassembly. By inhibiting microtubule depolymerization, taxol disrupts the normal cell division process, leading to cell cycle arrest and apoptosis in cancer cells [68]. Curcumin exerts its inhibitory effects through multiple mechanisms, including inhibition of various signaling pathways involved in cancer cell survival, proliferation, and metastasis. Curcumin also exhibits antioxidant and anti-inflammatory activities, which may contribute to its anticancer activities by reducing oxidative stress and dampening chronic inflammation [69]. Artemisinin's primary mechanism of inhibitory action is thought to involve the induction of apoptosis in cancer cells. Artemisinin and its derivatives have been shown to selectively target



▶ **Fig. 1** Plant secondary metabolites as immunomodulators and their targeted molecules to produce anticancer activity.

and kill cancer cells while sparing normal cells. The exact mechanisms of how artemisinin induces cancer cell death are still being investigated, but it is believed to involve ROS generation and damage of DNA in cancer cells [70]. Tymoquinone's mechanism of action involves targeting multiple pathways in cancer cells, including inhibiting cell proliferation, inducing programmed cell death, suppressing inflammation, and inhibiting angiogenesis. Furthermore, tymoquinone has been found to modulate the expression of various genes and proteins involved in cancer development and progression [71].

Immunomodulating Effects and Mechanisms of Viscum album

There are numerous data on the immunostimulant activity and inhibitory effects of VAEs on cancer cells and animal cancer models, and anticancer activity in clinical studies published in the past three decades. Several studies have suggested that the utilization of immunotherapy or chemotherapy in conjunction with VAE has yielded encouraging outcomes in both *in vivo* and *in vitro* experiments [72]. VAE and its chemical constituents have been recognized for their possible immunomodulatory qualities, indicating their ability to influence and regulate the immune system through diverse mechanisms. VA possesses many physiologically active substances, including lectins and viscotoxins, that have been observed to elicit immune response stimulation. These chemicals have the ability to augment the generation and functionality of immune cells, such as T cells, NK cells, and macrophages, which play a crucial role in combating infections and malignancies. The immunomodulatory effects of VA have been evidenced by its capacity to regulate immunological responses, indicating its potential usefulness in the treatment of autoimmune illnesses and immune-related ailments. The influence of the plant on the circulatory system, characterized by vasodilation and potential antihypertensive properties, presents opportunities for the treatment of cardiovascular illnesses. Moreover, the impact of this phenomenon on the growth and programmed cell death of cells presents captivating prospects for the treatment of cancer, potentially

▶ **Table 1** Immunomodulatory activities of *Viscum album* extracts (VAEs) and their mechanism of action.

Plant part/ host tree	Preparation	Type of study	Mechanisms of action	Reference
Viscum album growing on oak trees	VA Qu Spez	*In vitro* study using human lung adenocarcinoma cell line A549	▪ Exhibited potent anti-inflammatory effect by selectively attenuating the COX-2-mediated cytokine-induced secretion of PGE_2. ▪ No changes in the levels of COX-2 mRNA due to destabilization of COX-2 mRNA.	[40]
Viscum album growing on oak trees	Isotonic solution of 5 and 10 mg/mL VAE formulated in 0.9% NaCl	*In vitro* study	▪ Cytokine modulation by suppression of cytokine-induced PGE_2 production through the targeted inhibition of COX-2 and destabilization of COX-2 mRNA.	[74]
Viscum album	Two different VAEs (VAEA + VAEI) VAEA (abnobaviscum fraxini); VAEI (Iscador Qu spez)	*In vitro* cell model	▪ Both VAEs stimulated the maturation of DCs. ▪ VAEI suppressed tumor-induced immunosuppression of DCs. ▪ Coculture experiments with purified $CD4^+$ T cells had no influence on T cell proliferation and activation.	[73]
Viscum album	Methanol extract of VA	*In vitro* and *in vivo* study in LPS-stimulated murine RAW 264.7 macrophages and Wistar albino rats	▪ Exhibited strong anti-inflammatory activity with an IC_{50} value of 57.23 ± 1.922 µg/mL at a concentration of 100 µg by suppressing the protein levels of LPS-induced cells. ▪ Showed strong anti-inflammatory activity at 100 mg/kg.bw on carrageenan-induced paw edema in Wistar albino rats.	[75]
Viscum album growing on oak trees	VA juice extracted with water	Clinical study involving 20 university male rowers after high-intensity exercise	▪ Significant reduction in the levels of TNF-α and IL-6 in the VAE group compared to the control group at baseline, immediately after exercise, and following 30 min of recovery.	[76]
Viscum album	Methanol extract (1 mg/mL) of VA	Peripheral blood monocytes (PBMCs)	▪ Induced an increase in CD69 expression. CD69 plays a crucial role in the activation of many T cell subsets, including CD4+, CD8+, CD25+, and CD25+ T cells.	[72]

functioning as a supplementary therapeutic approach in conjunction with established methods [73].

▶ **Table 1** summarizes some studies on immunomodulatory activities and mechanisms of VAEs in the past 10 years. These studies have demonstrated that the extracts possessed immune stimulating, apoptogenic, and cytotoxic properties, and displayed synergistic effects when combined with chemotherapeutic agents. The inhibitory activity against cancer cells was likely attributed to the presence of lectins and viscotoxins. Lectins were found to hinder the process of protein synthesis and trigger apoptosis and cell cycle arrest [40]. Consequently, they impeded the formation of new blood vessels (angiogenesis) and the growth of cells. VA has the potential to enhance the synthesis of cytokines, specifically IL-2 and IFN-γ. These cytokines play an essential role in upregulating immune cells and facilitating the manifestation of anticancer properties [29]. This phenomenon can confer advantages in the context of autoimmune disorders, as the immune system erroneously targets and damages normal cells and tissues [40]. Intensive research has been carried out on the possible therapeutic effects of VAEs in cancer treatment, specifically in relation to immune regulation. The observed immunomodulatory effects have demonstrated promise, since they encompass the stimulation of immune cells, heightened identification of tumor cells, and improved immune responses against tumors. Several studies have indicated that VA has the potential to increase the efficacy of traditional cancer therapies, including chemotherapy and radiotherapy [4]. The potential involvement of VA in autoimmune illnesses, characterized by the immune system's attack on the body's own tissues, has also been explored in the context of immunological regulation. It is widely believed that this intervention plays a role in regulating the immune response and reinstating immunological homeostasis in medical disorders [15].

In vitro immunomodulatory activity

Research to understand the underlying mechanisms of the modulating effects of VAE and its secondary metabolites is still actively ongoing. However, the impact of VAE as an adjuvant in the treatment of cancer through stimulating the immune response is still unclear. Most of the immunomodulation activities of VA in the past 10 years were investigated involving the use of isolated cells in a controlled laboratory setting. Numerous studies have demonstrated that the administration of VAE could effectively augment the proliferation and activation of immune cells. These studies indicated that it could enhance the synthesis of cytokines, namely those implicated in immunological stimulation and antineoplastic reactions. These studies have demonstrated that VA possessed anti-inflammatory properties via the suppression of proinflammatory cytokine production. Saha et al. [40] reported that VAEs ex-

▶ **Table 2** Immunomodulatory activities of *Viscum album* extracts (VAEs) and their mechanism of action.

VA preparations	Cell lines	Inhibitory effects and mechanisms of action on cancer cells	References
Viscum album ethanol extract	Mouse embryonic fibroblasts (NIH/3 T3)	▪ Demonstrated a less toxic effect to the nontumor cells (3 T3) with an IC_{50} value of 1.60 v/v in comparison to Molt-4 (0.07 v/v) and Yoshida (0.05 v/v).	[26]
Viscum album extracts: tincture A (TA) and tincture B (TB)	Monkey kidney cell (MA-104) and melanoma murine cancer cells (B16F10)	▪ TA and TB enhanced antitumor activity in B16F10 cells in a dose-dependent manner but exhibited a much lower cytotoxicity to MA-104 cells. ▪ The MA-104 cell line was more sensitive to TA, than TB. At 5% v/v, both extracts were equally effective, reducing MA-104 cell viability in approximately 36%.	[48]
Viscum album L. var. *coloratum* agglutinin (VAA)	B16F10 and B16BL6 melanoma cells	▪ Exhibited a significant G0/G1 arrest in both melanoma cells. ▪ Stimulated an increase in both early and late apoptosis in cells. ▪ Dose-dependently increased activated multiple caspases (caspases-1, -3, -4, -5, -6, -7, -8, and − 9) and significantly reduced the expression of pro-caspases-3 and − 8.	[88]
Standardized *Viscus album* preparation and in combination with trastuzumab	Her-2 positive breast cancer (SK-BR-3 human breast carcinoma cells)	▪ VAE and trastuzumab independently and jointly inhibited SK-BR-3 cell proliferation in a dose-dependent manner. ▪ VAE triggered apoptosis at higher concentrations, resulting in G2/M cell cycle arrest, whereas trastuzumab induced G0/G1 arrest. ▪ The combined treatment significantly curtailed VEGF secretion, without diminishing trastuzumab's inhibitory prowess.	[89]
Viscum album extract	Eight human soft-tissue sarcoma (STS) cells and normal primary human fibroblasts	▪ VAE significantly decreased the proliferation of all eight cell lines and reduced viability in seven STS lines. ▪ Proliferation of primary fibroblasts was not affected adversely by VAE compared to DMSO as the negative control. ▪ VAE treatment on the cells resulted in a slightly diminished cell index (CI) in most of the synovial sarcoma and liposarcoma cell lines after 136 h of incubation (CI = 2.94) compared to untreated cells (CI = 3.24).	[90]
Viscum album hot water extract	SK-Hep1 cells, Chang liver cells (CCL-13)	▪ Inhibited the proliferation of SK-Hep1 cells dose-dependently without any cytotoxicity with normal CCL-13. ▪ Inhibited the cell cycle of SK-Hep1 cells via G1 phase arrest. ▪ VA downregulated cyclin-dependent kinase 2 (Cdk2) and cyclin D1 gene expression while p21 was upregulated dose-dependently. These effects of VA on Cdk2, cyclin D1, and p21 collectively may lead to cell death.	[91]
Iscador Qu, aviscumine, and native ML-1	Glioblastoma	▪ VA preparations at high doses induced cell death and inhibited glioma cell proliferation. ▪ Exhibited changes in glioma cell cycle distribution and gene expression but exhibited low toxicity in healthy mouse brain tissue. ▪ Stimulated T cells and NK cells to kill the glioblastoma.	[92]
Ultra-diluted homoeopathic *Viscum album* extract (VAD3)	MCF-7 breast cancer cells and mesenchymal stem cells	▪ Exhibited higher cytotoxic action on MCF-7 cell cultures than on MSCs in the MTT assay. ▪ IC_{50} value for MSC cell lines (62.57 µL/mL) was higher in comparison to MCF-7 breast cancer cells (42 µL/mL).	[93]
Poloxamer 407 hydrogel containing *Viscus album*: 5% of ethanol dry (VA_DEH) and aqueous (VA_AEH) VAE	Human adult keratinocytes (HaCat) cell lines	▪ Cell viability evaluated by the WST-1 colorimetric methodology revealed a dose-dependent toxicity for both formulations with VA_DEH presenting higher activity than VA_AEH.	[94]
Viscus album extract alone and in combination with mebendazole	Malignant gliomas; canine high-grade astrocytoma using the SDT-3 G cell line	▪ VAE exhibited a pronounced dose-dependent cytotoxic response, with an IC_{50} of 5.644 µg/mL, compared to that of mebendazole at 0.03 µM. ▪ Combination of VAE at 5 µg/mL to mebendazole at 0.03 µM led to increased cell death compared to when the VAE and the drug were given separately.	[95]

hibited marked anti-inflammatory activity by selectively attenuating the cyclooxygenase-2 (COX-2)-stimulated cytokine-activated PGE_2 production in A549 cells. However, there were no changes in the levels of COX-2 mRNA, which might be possibly due to COX-2 mRNA destabilization by VA, thereby depleting the functional COX-2 mRNA for protein synthesis and subsequent PGE_2 production. In another study, an isotonic solution of 5 and 10 mg/mL VAE in 0.9% NaCl modulated cytokine production by suppression of cytokine-induced PGE_2 production through the targeted inhibition of COX-2 and the destabilization of COX-2 mRNA [74].

VAE caused an augmentation of both the phagocytosis of *Candida albicans* blastospores and the intracellular killing activity of neutrophils as compared to the negative control. The administration of VAE resulted in the stimulation of Th2-(IL-4)- or Th1-(IFN-γ)-associated cytokines as well as the production of IL-6 and TNF-α [29]. The various immunological events observed could be most effectively elucidated through the process of presenting antigens associated with VA to T and B cells, facilitated by antigen-presenting cells located in the lymph nodes responsible for drainage. Steinborn et al. [73] reported the effect of two different VAEs [abnobaviscum fraxini (VAEA) + Iscador Qu spez (VAEI)] on T cell function and DC maturation in a human *in vitro* cell model. They reported that both VAEs were able to stimulate the maturation of DCs. VAEI was able to suppress tumor-induced immunosuppression of DCs. The T cell function and DC maturation were mediated partially by VAE since VA-specific antibodies and lectin-depleted VAEI almost neutralized the rehabilitative effects of VAEI on DC maturation. It was also observed that coculture experiments with purified $CD4^+$ T cells have an impact on IFN-γ secretion but did not have an influence on T cell activation and proliferation. The findings provided understanding of the mechanism of immunomodulatory effects of VAE as an adjuvant in cancer therapy. Melo et al. [72] demonstrated that the methanol extract (1 mg/mL) of VA could induce an increase in CD69 expression in peripheral blood monocytes. The human CD69 is known to play a crucial role in the activation of many T cell subsets, including CD4+, CD8+, CD25+, and CD25+ T cells. However, further studies need to be carried out to evaluate the impact of VA in *in vivo* situations.

In vivo immunomodulating activity

Although there were several studies to evaluate the *in vivo* immunomodulating effects of VA in animal models in the past 30 years, there is little *in vivo* studies reported in the past 10 years. *In vivo* research conducted on animals has demonstrated that VA could improve the functioning of immune cells, including T cells, NK cells, and macrophages. It elicited the synthesis of cytokines, such as IFN-γ and IL-2. The aforementioned effects result in an enhanced immune response and heightened antitumor activity. Murthuza and Manjunatha [75] investigated the *in vitro* and *in vivo* anti-inflammatory activity of several medicinal plants, including VA, in LPS-activated murine RAW 264.7 macrophages and Wistar albino rats. The methanol extract of VA showed a strong anti-inflammatory effect, with an IC_{50} value of 57.23 µg/mL, by suppressing the protein levels of LPS-induced cells. VAE at 100 mg/kg.bw demonstrated a marked anti-inflammatory effect in the *in vivo* study using carrageenan-induced paw edema in Wistar albino rats. The findings suggested that VA contained bioactive constituents that have promise to be developed into anti-inflammatory agents.

Clinical study for immunomodulating effects

The effect of VAE on inflammatory markers (IL-6, TNF-α, and CRP) in 20 university male rowers after high-intensity exercise was investigated. Blood samples were collected from the rowers who were given 110 mL VAE/dose twice daily for 8 weeks for determination of levels of serum inflammatory markers at baseline, immediately after exercise, and following 30 min of recovery. Administration of VAE resulted in a significant decrease in the levels of TNF-α and IL-6 in the VAE group compared to the control group at baseline, immediately after exercise, and following 30 min of recovery. The findings demonstrated that VAE has anti-inflammatory activity in reducing a high-intensity exercise-stimulated increase in the inflammatory cytokine serum level in active individuals and thus has the potential to mitigate inflammation and support harmonized immune responses [76].

In general, research conducted both *in vivo* and *in vitro* provides evidence in favor of the immunomodulatory characteristics of VA. The extract has demonstrated the capacity to activate immune cells, augment cytokine synthesis, and manifest anti-inflammatory properties. The results indicate that VA exhibits the capacity to regulate the immune system and elicit advantageous outcomes in the context of cancer and autoimmune disorders. Nevertheless, it is crucial to acknowledge that although these studies offer interesting perspectives, additional studies are required to identify the compounds responsible for the bioactivity, comprehensively comprehend the mechanisms of action, and ascertain the most effective dosages and formulations for therapeutic purposes.

Inhibitory effects and mechanisms of *Viscum album* on cancer cells

Generally, natural products are used as a collaborative or supportive therapy in serious diseases and to mask the symptoms of others. The pharmacological effects may vary according to the dosage, route of administration, patient reaction, and the stage of the disease [77]. The birth of the human cancer cell is confusing and unpredictable. Cell division is the normal process by which human cells divide and develop to create new cells as needed by the body. New cells replace old ones when they die because of aging or injury. However, cancer can appear when this well-organized process falters. In many aspects, cancer cells are different from healthy ones. They ignore signals that ordinarily instruct cells to cease dividing or to die through a process called programmed cell death or apoptosis. Cancerous tumors can spread and continue to grow and proliferate even when there are enough cells present. In a process known as metastasis, these aberrant cells can invade neighboring tissues and travel to different areas of the body via the blood and lymphatic systems. There are several types of cancer including stomach, lung, colon and rectum cancers, prostate, skin, and breast cancers. The emergence of cancer is due to many factors, some of them are genetic while others are related to the environment and lifestyle. Drinking alcohol or

smoking, having a bad diet, poor exercise, and being around specific pollutants may be main reasons for cancer [78].

VA has shown several pharmacological properties, including anticancer activity [79]. It has several anticancer effects that are attributed to a combination of different properties, mainly its cytotoxic effect and assistance in killing the cancer cells. It also has immunomodulatory properties, which means it can modulate the immune system to enhance the body's natural ability to fight cancer. Furthermore, it has anti-inflammatory properties that help in reducing inflammation, a common issue in many types of cancer [80]. However, the anticancer mechanism of action for VA is not fully understood, although some articles have provided information about suggested mechanisms of action of some VAEs and their constituents. For almost a century, VA has been utilized as a supplemental anticancer treatment. The preparations made from VA can be separated into two categories: phototherapeutic extracts standardized on a certain lectin level with brand names such as Lektinol, Cefalektin, and Eurixor, and homeopathically manufactured extracts like Isorel, Helixor, Iscador, Abnoba Viscum, and Iscucin products [81].

The main anticancer compounds from VA that have been identified are lectins and viscotoxins. Further studies have demonstrated the anticancer properties of nonpolar molecules, triterpene acids, and phenolic compounds. Lectin B strands have the ability to precisely recognize and attach to markers on cellular boundaries, altering the pathways used for internal cell communication. At the same time, the A strands enter the intracellular fluid and inhibit the synthesis of proteins, which expedites the degradation of a specific ribosomal subunit. Both lectins and viscotoxins are dangerous substances that have characteristics that are detrimental to cancerous cells, causing damage to cells as well as immune system responses [48].

VA has widely been used in combination with available anticancer therapies to minimize the side effects of these products in many cancer types such as ovarian cancer, cervical cancer, neuroblastoma, and lung cancer [48, 82]. The mechanisms of anticancer action of VA and its constituents may involve inhibition of angiogenesis, reduction of tumor growth, promotion of apoptosis, and modulation of the immune system. Prevention of angiogenesis can lead to stopping the development and metastasis of tumors and the formation of new blood vessels. A key regulator of angiogenesis, VEGF, is being downregulated as part of the process [83]. The upregulation of proapoptotic proteins like caspase-3 and caspase-9 and the downregulation of antiapoptotic proteins like Bcl-2 are two of the ways by which apoptosis occurs [84]. The end outcome is the intrinsic apoptotic mechanism, which is activated, leading to the death of cancer cells [85, 86]. VA can enhance the activity of NK cells, macrophages, and T cells. This process can play a vital role in strengthening the immune response against cancer cells [87].

Cytotoxicity and *in vitro* inhibitory effects on cancer cells

Many studies have been carried out to evaluate the *in vitro* cytotoxicity and inhibitory effects of VA on different cancer cell lines. ▶ **Table 2** shows the *in vitro* toxicity and inhibitory effects of VAE on different cancer cell lines. Holandino et al. [26] highlighted the greater sensitivity of tumor cells to VA ethanol extract compared to normal fibroblasts, suggesting the potential of this extract as an antitumor agent. According to the study, the VAE was shown to be less toxic to the non-tumor cells (3 T3), with IC_{50} values of 1.60 ± 0.48 % v/v in comparison to Molt-4 (0.07 ± 0.01 % v/v) and Yoshida (0.05 ± 0.03 % v/v). Melo et. [48] determined the antitumor potential and chemical composition of two types of VAEs, tincture A (TA) and tincture B (TB). Ligalbumoside A, alangilignoside C, chlorogenic acid, caffeic acid, isosakuranetin, sakuranetin, syringenin 4-O-apiosyl-glucoside, and syringenin 4-O-glucoside were identified as the phenolic compounds from the VAEs that enhanced antitumor activity in melanoma murine cancer cells (B16F10) in a dose-dependent manner. Normal kidney cell lines (MA-104) were shown to exhibit much lower cytotoxicity to all hydroalcoholic concentrations used in the study. The MA-104 cell line was more sensitive to TA, which significantly reduced its viability by 50 % in concentrations as low as 1 % v/v, while TB reduced viable cells by 24 % when compared to the control. However, for the concentration of 5 % v/v, TA and TB were equally effective, reducing MA-104 cell viability by approximately 36 %.

The inhibitory effect of *V. album* L. var. *coloratum* agglutinin (VAA), a Korean mistletoe lectin, and its extract on cancer cell growth in melanoma cells has been demonstrated by Han et al. [88]. They reported that VAA-treated cells showed a significant G0/G1 arrest in both B16F10 and B16BL6 melanoma cells. The VAA extract also stimulated an increase in both early and late apoptosis in cells and could increase activated multiple caspases (caspases-1, -3, -4, -5, -6, -7, -8, and − 9) dose-dependently. VAA treatment resulted in a significant reduction in expression of both procaspase-3 and procaspase-8. An *in vitro* study was performed to evaluate the effect of a standardized VA preparation on the effect of trastuzumab on Her-2 positive breast cancer [89]. *In vitro* analysis on SK-BR-3 human breast carcinoma cells indicated that VAE did not negate trastuzumab's antitumor properties. Both agents independently and jointly inhibited SK-BR-3 cell proliferation. The study detailed a dose-dependent antiproliferative effect of VAE, with a significant 60 % growth reduction at a clinically relevant concentration of 1 µg/mL after 7 days, and complete growth cessation at concentrations of 10 and 100 µg/mL. In contrast, trastuzumab reached maximum growth inhibition at 100 µg/mL. VAE triggered apoptosis at higher concentrations, resulting in G2/M cell cycle arrest, whereas trastuzumab induced G0/G1 arrest. Moreover, the combined treatment significantly curtailed VEGF secretion, a key proangiogenic factor, without diminishing trastuzumab's inhibitory prowess. These insights bolster the potential synergy between VAE and standard therapies, highlighting the need for extensive *in vivo* studies to elucidate herb-drug interactions and optimize treatment protocols. This synthesis of traditional and modern medicine suggests a nuanced approach to cancer care, necessitating rigorous clinical trials to corroborate these initial findings [89].

A bromodeoxyuridine (BrdU) assay, real-time cell analysis (RTCA), and MTT assay were performed to assess the cytostatic effects of curcumin and VAE in eight human soft-tissue sarcoma (STS) cells, which include malignant fibrous histiocytoma (U2197), liposarcoma (SW872, T778, MLS-402), synovial sarcoma (SW982, SYO1, 1273), and fibrosarcoma (HT1080). Cell prolifera-

tion, viability, and cell index (CI) on eight cell lines, including normal human fibroblast cell lines as a control, were determined [90]. VAE significantly decreased the proliferation of all eight cell lines and reduced viability in seven STS lines. As indicated by the BrdU assay, VAE had no effect on the proliferation of normal human fibroblast cells compared to DMSO as the negative control. Primary fibroblasts were shown to be not affected adversely by VAE in RTCA. However, treatment with VAE on the cells, as indicated by RTCA, resulted in a slightly diminished CI in most of the synovial sarcoma and liposarcoma cell lines, after 136 h of incubation (CI = 2.94) as compared to untreated cells (CI = 3.24). Interestingly, a hot water extract of VA was found to inhibit the proliferation of SK-Hep1 cells dose-dependently, without any cytotoxicity with normal Chang liver cells (CCL-13) [91]. The extract was shown to inhibit the cell cycle of SK-Hep1 cells via G1 phase arrest based on flow cytometry analysis. Western blot analysis and RT-PCR results indicated that VA downregulated cyclin-dependent kinase 2 (Cdk2) and cyclin D1 gene expression, while p21 was upregulated dose dependently. These effects of VA on Cdk2, cyclin D1, and p21 collectively may lead to cell death.

In another study, the inhibitory effect of VA, particularly its viscumins, recombinant aviscumine, Iscador Qu, and native ML-1, have been investigated *in vitro* and *in vivo* against glioblastoma, a form of brain cancer known for its aggressiveness. The *in vitro* analyses involved comprehensive methodologies, including cell cultures, propidium iodide staining, assays for cell growth, cytotoxicity, clonogenic survival, cell cycle analysis, quantitative RT-qPCR, and immunofluorescence [92]. These rigorous approaches revealed that all VA preparations could induce cell death and inhibit glioma cell proliferation at high doses, accompanied by changes in glioma cell cycle distribution and gene expression. Furthermore, at similar concentrations, these substances were found to exhibit low toxicity in healthy mouse brain tissue, an encouraging indicator for potential therapeutic applications. Notably, the study identified CD75s expression as a potential biomarker, correlating with sensitivity to treatment-induced cell death, providing a pathway for personalized therapeutic strategies. The compounds also exhibited an immunostimulating effect and activated T cells and NK cells to kill the glioblastoma. Viscumins modulated the immune response of proinflammatory related genes in the cells. Moreover, the viscumins were found to possess synergistic effects in the cells when used together with chemotherapy and irradiation. In an *in vivo* study, viscumins as an adjuvant could prolong the survival of mice bearing glioblastoma [92]. These findings suggested that viscumins could be used as an adjuvant in glioblastoma patients; however, further preclinical studies and clinical trials need to be performed to investigate their therapeutic efficacy and safety as an adjuvant in cancer therapy.

Valle et al. [93] examined the concentration-response relationship of ultra-diluted homoeopathic *V. album* 1×10^{-3} (VA3X) in mesenchymal stem cell (MSC) cultures and its cytotoxic effects on MCF-7 cancer cells. The research revealed that higher concentrations of VA3X were required to induce cell death in MSCs compared to MCF-7 cells. The IC_{50} cytotoxicity index, which indicates the concentration required to decrease the cell population by 50%, was determined. It was shown that the IC_{50} for MSC cell lines was 62.57 µL/mL of VA3X, which was higher in comparison to 42 µL/mL of VA3X for MCF-7 breast cancer cells. Furthermore, when treated with 30 µL/mL of VA3X, it was revealed that this concentration led to a 77% mortality rate for MSC cell lines, in contrast to an 86% mortality rate observed in MCF-7 cell lines.

In a recent study conducted by Batista et al. [94], a novel dermic delivery system to harness the therapeutic potential of VAE was studied. Specifically, the study investigated the use of Poloxamer 407 hydrogel formulations that contained 5% of ethanol dry (VA_DEH) and aqueous (VA_AEH) VAE, with concentrations ranging from 2 to 200 mg/mL. The study focused on the application of these formulations in the treatment of human adult keratinocytes (HaCaT) cell lines. Intriguingly, HaCaT cell lines displayed a level of sensitivity to the treatment that was strikingly similar to that observed in T-cell leukemia (Molt-4) cell lines. Both formulations exhibited a dose-dependent toxicity by using the WST-1 *in vitro* assay, with VA_DEH showing stronger activity than VA_AEH. VA_DEH exhibited transdermal potential, as it permeated at 8 h, as shown in the *ex vivo* skin permeation assay with 2.73 ± 0.19 µg/cm^2 chlorogenic acid. These findings indicated that VA_DEH has potential to be developed as a candidate for cancer therapy. Therefore, further studies including *in vivo* and preclinical experiments are necessary to investigate the efficacy and safety of this new dermic delivery system.

VAE is becoming more and more popular as a possible supplemental therapy when used in conjunction with other anticancer medications. Wright et al. [95] expanded this research vista by evaluating VAE 's cytotoxic prowess alone and in combination with mebendazole in an *in vitro* model of canine high-grade astrocytoma using the SDT-3 G cell line. Using the MTT cell viability assay, a 72-h incubation with VAE elicited a pronounced dose-dependent cytotoxic response, with an average IC_{50} of 5.644 ± 0.09 µg/mL, reinforcing the compound's formidable antiproliferative effect. The IC_{50} value of mebendazole was 0.03 µM. The addition of VAE at 5 µg/mL to mebendazole at 0.03 µM led to increased cell death compared to when the VAE and the drug were given separately. The confluence of these agents resulted in a significant decrement in cell viability beyond that achieved by mebendazole alone. This effect was accentuated at higher VAE concentrations, suggesting an additive or potentially synergistic dynamic that could revolutionize therapeutic protocols. These insights not only corroborate VA's therapeutic promise demonstrated in previous studies but also call for intensified research efforts. Such endeavors will be instrumental in demystifying the underlying molecular mechanisms and optimizing treatment modalities, thereby fortifying our armamentarium against formidable foes like malignant gliomas [95].

In vivo inhibitory effects and mechanisms on cancer cells

There was only one recent review on the inhibitory effects of VA treatment in animal cancer models [13]. In this review, a total of 37 studies from 1996 onwards described the VA preparations, study design, treatments and controls, tumor types, animal species used, anticancer effects, mechanisms of action, outcomes, etc. ▶ **Table 3** shows the *in vivo* inhibitory effects and mechanisms of VA on cancer cells based on papers published for the past 10 years. In the realm of alternative cancer therapies, many studies

▶ **Table 3** Inhibitory effects and mechanisms of action of *Viscum album* extracts on cancer cells *in vivo*.

Plant part/ host tree	Preparation	Type of study	Mechanisms of action	Reference
Viscum album that grows on *Malus domestica*	VAE	Intraperitoneal administration of VAE, in combination with doxorubicin in xenograft mouse model	▪ VAE, particularly in combination with doxorubicin, caused a dose-dependent reduction in tumor size and weight compared to the control group. ▪ Immunoblotting analysis of tumor tissues revealed a marked suppression of STAT3 phosphorylation across the groups treated with a high dose of VAE, doxorubicin, and their combination.	[86]
Viscum album var. coloratum (Korean mistletoe)	VAE enteric-coated with a biodegradable polymer (Eudragit) wall	Oral administration of 4% (430 mg/kg/day) to mouse model	▪ A significant decrease in tumor volume on day 14 compared to the negative control group in B16F10 melanoma-inoculated BDF1 mice. ▪ The mice treated with VA had a higher survival rate after day 12.	[88]
Viscum album leaves	Native ML-1, recombinant ML-1 (aviscumine), and Iscador Qu	Intratumoral injection of VAE alone and in combination with irradiation and TMZ in cell cultures, ex vivo murine hippocampal brain slice cultures, human glioblastoma (GBM) cryosections, and a xenograft orthotopic glioblastoma mouse model	▪ A concentration-dependent induction of cell death and reduction in cell growth by inducing cell cycle arrest in the G_2/M phase in GBM cells. ▪ Aviscumine prolonged the survival of GBM-bearing mice when used in combination with irradiation and TMZ for a further 6.5 days compared to radio-chemotherapy. ▪ ML-containing preparations demonstrated synergistic and additive anticancer effects when combined with glioma standard therapy.	[92]
Viscum album	Aqueous *Viscum album* extract with cyclodexdrin Lectins or solubilized triterpene acids containing VAE (VA-TT)	*In vitro* and *ex vivo* studies in human acute myeloid leukemia (AML) cells In vivo study using AML mouse model	▪ VA-TT dose-dependently induced apoptosis and suppressed cell proliferation *in vitro and ex vivo*. VA-TT extracts in combination with lectins showed synergistic effect in enhancing the induction of apoptosis. ▪ All extracts induced apoptosis via caspase-8- and – 9-dependent pathways with downregulation of members of the inhibitor of apoptosis and Bcl-2 families of proteins. ▪ AML mice treated with VA-TT showed a significant reduction in tumor weight, comparable to the effect in cytarabine-treated mice.	[96]

have suggested the potential benefits of VA as an adjuvant in cancer treatment. Park et al. [86] evaluated the *in vivo* effects of VAE on breast cancer cells by employing a xenograft mouse model. VAE was shown to inhibit cell viability and proliferation significantly and induced apoptosis in a dose-dependent manner. The extract also effectively suppressed STAT3 signaling pathway activation through SHP-1 and regulated cell cycle progression. VAE in combination with low-dose doxorubicin produced a synergistic effect. The intraperitoneal administration of VAE, particularly in combination with doxorubicin, resulted in a pronounced reduction in tumor size and weight in a dose-dependent pattern compared to the control group. The effect did not significantly surpass that of doxorubicin used singularly. Immunoblotting analysis of tumor tissues revealed a marked suppression of STAT3 phosphorylation across the groups treated with a high dose of VAE, doxorubicin, and their combination. This finding implicated the STAT3 pathway as a strategic target in VAE-mediated cancer therapy, providing a promising avenue for future research [86]. This outcome nuances our understanding of VAE's role, suggesting its independent action rather than a synergistic effect when used in a combination. A cautionary note was, however, sounded regarding the administration of high VAE concentrations, which were associated with a minor decline in overall body weight, indicating the necessity for meticulous dosing.

Han et al. [88] investigated the *in vivo* effects of VA on the growth of mouse melanoma by orally administrating 430 mg/kg/day of Korean mistletoe enteric coated with a biodegradable polymer. On day 14, there was a significant reduction in tumor volume compared to the negative control group. After 12 days, the mice treated with VA had a higher survival rate. The anticancer effects of native ML-1, recombinant ML-1 (aviscumine), and Iscador Qu were evaluated *in vitro, ex vivo*, and *in vivo* on glioblastoma. ML-containing preparations demonstrated synergistic and additive anticancer effects when combined with glioma standard therapy. The three extracts demonstrated synergistic effects when combined with temozolomide-(TMZ)-based radio-chemotherapy

in cell cultures, human glioblastoma cryosections, and *ex vivo* murine hippocampal brain slice cultures. The cells and glioblastoma expressed ML receptor CD75s, which correlated with the drug-induced cytotoxicity. However, the normal brain did not express the protein. There was a concentration-dependent reduction in cell growth and induction of cell death in the G_2/M phase in glioblastoma cells by inducing cell cycle arrest. In a xenograft orthotopic glioblastoma mouse model, a single intratumoral administration of aviscumine resulted in prolonged and longer median survival of glioblastoma mice compared to those with tumor irradiation. Moreover, when they were given aviscumine in combination with TMZ and irradiation, the survival of glioblastoma-bearing mice was prolonged for a further 6.5 days compared to those receiving radio-chemotherapy [92]. These findings indicate that ML-containing preparations might be beneficial as an adjuvant treatment of glioma patients. This combination therapy not only highlighted the potential of VAEs in enhancing current glioblastoma treatments but also opened new avenues for its application in other cancer treatments.

In vitro, ex vivo, and in vivo investigations were performed on the aqueous extract of VA (cyclodextrins added to solubilize triterpenes, mainly oleanolic acid) to evaluate its effect on human acute myeloid leukemia (AML) cells. Lectins or solubilized triterpene acids containing VAE (VA-TT) induced apoptosis and suppressed cell proliferation dose-dependently in vitro *and* ex vivo. It was found that VA-TT extracts in combination with lectins showed a synergistic effect in enhancing the induction of apoptosis. All the extracts induced apoptosis, with attenuation of members of the Bcl-2 families of proteins and the inhibitor of apoptosis through caspase-8- and −9-dependent pathways. The *in vivo* study on the AML mouse model demonstrated that VA-TT treatment resulted in a significant reduction in tumor weight, comparable to the effect in cytarabine-treated mice. These findings indicate that the combination of VA-TT and lectins might have promise in the treatment of AML [96].

Efficacy and Safety Profiles of Viscum album

In recent years, extracts derived from VA have gained attention for their immunostimulatory properties and dose-dependent cytotoxic effects. These attributes have led to their incorporation into complementary cancer therapies, primarily aimed at improving the overall well-being of cancer patients. Additionally, some treatments use localized and high-dosage injections of VAEs to control tumor growth. However, the use of elevated doses raises concern on whether such treatments may carry adverse effects. In the context of cancer therapy, traditional VA treatments often involve subcutaneous administration of VA preparations, with dosages carefully tailored to individual patient responses. Nevertheless, a pressing need for a comprehensive evaluation of the potential toxicity associated with VAE therapy exists.

Safety profiles in animal models

In comprehensive research, to investigate the safety profiles of VA, animal studies have been pivotal unveiling the plant's potential effect on humans. For instance, studies on rodents to determine the hepatotoxicity of the VA plant have shown intriguing findings. When administered at a certain range of dosages, researchers have observed a paradoxical effect on the liver. In one study, the histopathological analysis of itraconazole-induced hepatocellular injury and acute oxidative stress in female rats showed that intraperitoneal administration of the plant extract (5 mg/kg) for 14 days was able to reduce the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) liver enzymes and improved the total antioxidant status (TAS) level, and no hepatocellular damage was observed in all female rats [97].

The findings from an *in vivo* study of VAE against chlorpyrifos-induced liver damage suggested that the extract had not caused further damage to the hepatic system of the tested Wistar rats [98]. Both low (175 mg/kg) and high (350 mg/kg) dosages of treatment caused a significant reduction in total carbohydrate, total cholesterol, AST and ALT liver enzymes, albumins, and alleviated histopathological changes caused by chlorpyrifos. However, it is important to note that in the aforementioned studies, no high treatment dosages of VA were administered to the subject groups. One study using a high-dosage treatment of the plant extract found that, upon oral administration of VA leaf extract (4000 and 5000 mg/kg) in healthy rats, a significant increase of alkaline phosphatase, AST, and alanine aminotransferase (ALT) liver enzymes was observed [99]. The elevated activities of the liver biomarkers might indicate liver inflammation or liver damage, but no histopathological assessment was carried out in the study. These findings suggest that VA was able to provide protective benefits to the liver within a certain therapeutic window and cause potential harm at excessive doses. The toxicity effects of VAE on the immune response have also been documented by several studies. Most animal studies reported an increase in the immunological effect of the plant, but some cases observed a decrease of immune response towards VA's treatment. To date, no studies have reported any indications of immunosuppression, even at a high dosage of treatment.

In a study to determine the safety of intrauterine fetal exposure to VA, 47 pregnant Wistar rats and 399 fetuses were bred and examined upon the administration of a VA supplement [100]. The therapeutic dose (0.013 mg/kg) and high-dose (12.5 mg/kg) groups resulted in increased weight gain of the rats, placentas, and fetuses compared to the very high-dose (25 mg/kg) treatment group. The histology of the placentas showed a greater inflammatory response in the high-dose and very high-dose groups, but no lesions were found in the fetus' histology analysis. The study also reported that no incidence of abortion, embryotoxicity, and teratogenicity was observed in all treatment groups and VA was considered safe during the pregnancy of the Wistar rats. In one acute toxicity study involving albino rats, the study revealed that the LD_{50} value for the aqueous extract of VA leaves ranged from 1440 to 2440 mg/kg and the stem extract showed LD_{50} values ranging from 600 to 2880 mg/kg [101].

Clinical evaluation of *Viscum album* for efficacy and safety

▶ **Table 4** shows the results of some clinical studies on VAE treatment. A randomized controlled trial was conducted to assess the safety and clinical response of utilizing two VAEs, namely, Helixor A and Iscador M special, as complementary treatments during

▶ **Table 4** Summary of clinical studies on *Viscus album* extract treatment.

Formulation name	Administration	Findings	References
Helixor A, Iscador M Special	Subcutaneous injections	▪ No adverse effects were observed in breast cancer patients at approximately 50 mg VAE. ▪ The tendency of neutropenia was reduced. ▪ The quality of life in terms of pain and loss of appetite increased.	[102]
Abnoba, Helixor, Iscador, Iscucin	Subcutaneous injections, intravenous infusions	▪ No VA-associated adverse events observed in cancer patients with autoimmune conditions. ▪ The overall rates of adverse events were reduced by half.	[4]
Helixor P	Intravenous infusions	▪ MTD was achieved at 2000 mg. ▪ Adverse events observed were fever, fatigue, eosinophilia, and slight temporary elevation of ALT. ▪ Reduction of tumor markers or stable disease observed in three patients.	[106]
Helixor M	Intravenous infusions	▪ MTD was achieved at 600 mg. ▪ Adverse events observed were fever, fatigue, eosinophilia, and slight temporary elevation of ALT. ▪ Five patients had stable disease and three patients experienced tumor reduction.	[108]
Abnova-viscum-F	Injection into chest tubes	▪ Sixty mg of VA extract managed to treat elderly patients with spontaneous pneumothorax. ▪ Adverse events observed were fever, pain, leukocytosis, and dyspnea with desaturation. ▪ Chemical pleurodesis should be proceeded with caution and must be accompanied with adverse event management procedures.	[111]

chemotherapy for breast cancer patients [102]. The patients were subcutaneously injected three times weekly for 18 weeks with a median dosage of 53.8 ± 2.6 injections for Iscador M and 52.3 ± 2.8 injections for Helixor A. The study reported no adverse events associated with VAE treatment. Additionally, compared to the control group, patients receiving VAE treatment showed a trend towards experiencing less neutropenia, as well as improvements in pain and appetite loss scores, as assessed by the EORTC QLQ-C30 questionnaire, designed to evaluate health-related quality of life. Furthermore, this complementary treatment led to a reduction in specific chemotherapy side effects and did not affect the frequency of relapse and metastasis over a 5-year period in breast cancer patients. The non-toxicity and effectiveness of VAE as second-line therapy has been demonstrated in other randomized controlled trials involving patients with metastatic pancreatic cancer [103] and osteosarcoma [104]. Next, the safety of adding VAE therapy to the treatment protocols of cancer patients with preexisting autoimmune conditions, predominantly those with Hashimoto's thyroiditis, psoriasis, and ulcerative colitis, was investigated in an observational cohort study [4]. The study was based on documented data of 106 patients from the Network Ontology Registry in Germany. Patients received VAEs via subcutaneous injections or intravenous infusions, sourced from manufacturers like Abnoba, Helixor, Iscador, and Iscucin. The study observed that the therapy did not result in increased rates of VA-associated adverse events. Additionally, the overall rates of adverse events (unrelated to VA) were significantly reduced by half during periods of VA therapy. This corroborates earlier findings from Bock et al. [105], indicating that combining conventional cancer treatments with VA therapy led to significantly fewer side effects and treatment-related symptoms observed in patients.

In a phase I Good Clinical Practice trial, individuals with advanced or metastasized cancer received a VA formulation, Helixor P, treatment in escalating dosage groups of 200, 400, 700, 1200, and 2000 mg, according to a 3 + 3 dose escalation protocol [106]. While no dose-dependent trend in adverse events was observed, a subset of predominantly mild events was possibly linked to the study medication, particularly in the 2000 mg dose group. These adverse events include grade 1 of fever, fatigue, and eosinophilia, and slight temporary elevation of ALT. Despite the occurrence of a dose-limiting toxicity in one patient at the highest dose level due to an allergic reaction, the study highlighted the manageable safety profile of Helixor P up to 2000 mg, with no evidence of direct hepatotoxicity or significant alterations in liver enzyme levels over the course of treatment. The finding of the absence of hepatotoxicity effects of Helixor P was supported by the study Steele et al. [107]. Therefore, weekly infusions of the mistletoe product Helixor P were well tolerated up to an initial dose of 2000 mg, although an elevated risk of side effects such as allergic reactions and fever has to be expected.

A recent similar study was conducted on heavily pretreated patients with advanced cancer, wherein Helixor M was administered intravenously three times a week, with escalating dosage groups of 150, 300, 600, and 900 mg [108]. This study observed treatment-related adverse events similar to those reported by Huber et al. [106]. However, the study concluded that the maximum tolerated dose (MTD) was 600 mg due to the higher frequency of administration. This highlights the significance of considering dose frequency and disease severity when determining MTD for intravenous infusions of VAE. Both studies reported temporary improvements in tumor markers and instances of stable disease among patients who received doses within the determined MTD [106, 108]. Paller et al. [108] suggested that potentially more fa-

vorable responses might have been achieved if the treatment were extended to less severely ill patients. Various researchers have studied the administration of high-dose intravenous VA treatments across different tumor types to prevent recurrence, mitigate adverse drug reactions linked to chemotherapy, and enhance overall quality of life [107, 109, 110]. The collective findings suggest that this treatment approach is safe, with few and manageable adverse events.

For patients who cannot undergo surgery, chemical pleurodesis with sclerosants like tetracycline, doxycycline, minocycline, and bleomycin is considered an alternative treatment option for spontaneous recurrent pneumothorax. Kim et al. [111] examined the effectiveness and safety of using VAE, specifically 60 mg abnovaviscum-F, to manage spontaneous pneumothorax in elderly patients aged over 65 years. The treatment-related adverse events such as fever and pain were managed by propacetamol and morphine sulphate IV injections, and leukocytosis returned to normal levels after 3 days without any specific intervention. Serious adverse events, particularly dyspnea with desaturation due to severe pleural inflammation, were effectively managed using a fentanyl regimen. While all patients were successfully treated without enduring any long-term complications and were subsequently discharged, caution should be exercised when performing chemical pleurodesis with VAE, especially in elderly patients, given the elevated likelihood of experiencing dyspnea with desaturation [111].

In a pilot observational cohort study conducted by Thronicke et al. [112], the safety of combining immune checkpoint inhibitors (ICMs) with VA therapy was evaluated in patients with advanced or metastatic cancer. The study involved a total of 16 cancer patients, with 56% receiving combined ICM/VA therapy and 44% receiving ICM alone. The results indicated no statistically significant difference in the rate of adverse events between the two groups. Furthermore, 85% of the adverse events recorded were typical reactions to ICM, with no severe (grade 3 or higher) events reported for the entire cohort. The study concluded that the overall adverse event rate was consistent with those found in other ICM treatments, suggesting that concurrent VA application might not alter ICM-induced adverse event rates. However, the study emphasized the need for larger, more comprehensive trials to confirm these findings and further explore the potential interactions between ICM and VA, especially concerning safety, clinical efficacy, and impacts on quality of life [112]. Generally, VA can be used as a complementary and cotreatment with other available anticancer drugs to generate an anticancer effect, potentiate the synergistic effect, and minimize the adverse effects.

Viscum album Nanoparticles and Their Potential Application in Nanomedicine

Nanotechnology is the engineering of atoms and materials/molecules at a nanoscale level, spanning from 1 to 100 nm [113]. In this contemporary epoch, natural products like plants, marines, and bee products are used to biogenesis nanoparticles and nanocomposites [114]. When compared to their counterparts that are generated via physical and chemical processes, natural product-based or green nanoparticles are distinguished by being more ecofriendly, affordable, nontoxic, and less expensive [115]. Generally, green nanostructures play a pioneer role in diverse arenas, i.e., cancer therapy, drug delivery, diseases diagnosis, biosensors, pharmaceutical formulations, DNA technology, cosmetics and industrial applications, food industry, agriculture, and solar energy [116]. Recently, natural product-based nanoparticles have been applied for the treatment of various human ailments, including cancer. Application of nanoparticles as drug carriers has overcome the limitations of conventional drug delivery systems, such as side effects, non-specificity, low bioavailability, aqueous solubility, and multidrug resistance. Although the advent of nanotechnology in cancer therapy is recent, it has progressed rapidly to overcome a large number of drawbacks associated with the conventional cancer therapeutic modalities (chemotherapy, radiotherapy, immunotherapy, and surgery) such as systemic toxicity, structural deformities, damage to proliferating healthy tissues, tumor cells becoming resistant to drugs, and long-term side effects. The nanoparticles can enhance the specificity and efficacy of cancer therapeutic modalities by specifically targeting tumor cells, which in turn improves patient response and survival [117].

Since VA contains crucial bioactive complexes (viscotoxins, lectins, flavonoids, alkaloids, terpenoids, and polyphenols), it has proven to be a good candidate for ecofriendly fabrication of a wide range of nanoparticle structures [24]. In this context, Mushtaq et al. [118] employed VA methanol extract of the leaves to manufacture zinc oxide nanoparticles. Transmission electron microscopy and X-ray diffraction (XRD) images revealed that the nanoparticles created by VA have a quasi-spherical shape at a nanoscale range (8.14–39.82 nm). Importantly, VA aqueous and methanol-mediated zinc nanoparticles at a concentration of 100 µg/mL showed high antioxidant potential toward DPPH radicals by 94 and 98%, respectively. The antibacterial activity of the same nanoparticles was also evaluated against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Gold nanoparticles (AuNPs) could be used as a carrier assembly for VA particles. FT-IR, scanning electron microscopy, XRD, and UV techniques were employed for full morpho-structural representation of the *Viscum*-AuNPs carrier assembly. Segneanu et al. [28] expected that the *Viscum*-AuNPs would boost antitumor activity and reduce undesired side effects.

In another study, VA was used as a reducing agent and *Chenopodium album* (CA) as an active carbon source in the preparation of VA-Ag/CA NPs. The morphology and chemical properties of VAA/CA NPs were determined by transmission electron microscopy and XRD analysis. The green synthesized silver nanoparticles were evaluated for their effectiveness and induction of oxidative stress in teratogenic changes and neurotoxic pathways in zebrafish. The VAA/CA NPs were approximately 1600 times less toxic than nanoparticles prepared by different methods based on the assessment of histopathological alterations, apoptosis, and oxidative DNA damage of the aquatic organism. However, the green silver nanoparticles at high doses could cause morphological, histopathological, and neurological changes. The outcomes of this study indicated that VA prepared as green silver nanoparticles could provide safety and stability of such synthesis products towards aquatic organisms [119].

▶ **Table 5** Nanoparticles biosynthesized using *Viscum album* L. and their applications.

Type of extract	Mode of synthesis/reducing and capping agent	Nanoparticles	Size (nm)	Morphology/nature	Application	References
Aqueous	Extracellular/lectines, viscotoxins, polyphenols, flavonoids, terpenoid, phytosterols, and carbohydrates	CeO	5.59	Spherical	Antioxidant ▪ DPPH radical scavenging	[123]
Aqueous	Extracellular/terpenoids, phenols	Zn	39.82	Quasi-spherical/crystals	Antioxidant ▪ DPPH radical scavenging. At 100 g/mL, scavenged 94 %	[118]
Antibacterial ▪ *Escherichia coli* – At 25 g/mL, gave an inhibition zone of 44 ± 0.2 mm ▪ *Staphylococcus aureus* – At 25 g/mL, gave an inhibition zone of 40 ± 83 mm ▪ *Pseudomonas aeruginosa* – At 25 g/mL, gave an inhibition zone of 36 ± 0.9 mm						
Methanol	Extracellular/terpenoids, phenols	Zn	8.14	Quasi-spherical/crystals	Antioxidant ▪ DPPH radical scavenging. At 100 g/mL, scavenged 98 %	[118]
Antibacterial ▪ *E. coli* – At 25 g/mL, gave an inhibition zone of 43 ± 0.2 mm ▪ *S. aureus* – At 25 g/mL, gave an inhibition zone of 39 ± 0.3 mm ▪ *P. aeruginosa* – At 25 g/mL, gave an inhibition zone of 33 ± 0.6 mm						
Powder	Extracellular/flavonoids, amino acids and peptides, terpenoids, phenolic acids, fatty acids, organic acids, nucleosides, alcohols and esters, amines, coumarins, alkaloids, lignans, steroids, aldehydes, and miscellaneous	Au	≥ 20	Spherical	*Viscum*–AuNPs carrier assembly	[28]
Ethanol	Phenolic compounds and flavonoids	ZnO/SnO_2	≥ 100	Uniform and spherical	Photocatalytic ▪ Congo red (CR). At 40 mg/L, showed a reaction rate (K) of 0.0067 min^{-1} ▪ Bisphenol A (BPA). At 40 mg/L, showed a reaction rate (K) of 0.0071 min^{-1} ▪ Tetracycline (TC). At 40 mg/L, showed a reaction rate (K) of 0.0006 min^{-1}	[122]

continued next page

▲ **Table 5** *Continued*

Type of extract	Mode of synthesis/reducing and capping agent	Nanoparticles	Size (nm)	Morphology/nature	Application	References
Aqueous	Extracellular	Ag	75–100	Spherical	Antibacterial Wastewater treatment ▪ Gram-negative (*E. coli*) exhibited ▪ Inhibition zone of 16.2 ± 0.1 mm ▪ Gram-positive bacteria (*S. aureus*) exhibited an inhibition zone of 17.8 ± 0.12 mm ▪ Removal of sulfamethazine; removal efficiency reached up to ~ 91 %	[121]
Aqueous	Extracellular	Ag	34.56	Spherical	▪ Reduction of oxidative DNA damage, apoptosis, and histopathological alterations on the aquatic organism ▪ At 100 mg/L, severe 8-OHdG and Bax expression	[119]
Methanol	Extracellular	Ag	99.56–119.68	Irregular with slightly rounded edges	Antioxidant ▪ Silver nanoparticle antioxidant capacity (SNPAC). At 50 uL, showed 0.5645–2.2665 uM	[120]

The methanol extract of VA was used to synthesize AuNPs. Scanning electron microscopy indicated that the shape of the nanoparticles was irregular with slightly rounded edges, and dynamic light scattering (DLS) determined the nanoparticles size ranged from 99.56 to 119.68 nm. Notably, the antioxidant activity of VAA/NPs was also determined using a DPPH method [120]. Similarly, Khalatbary et al. [121] used VAE of leaves to prepare a nanocomposite of multi-walled carbon nanotubes decorated with Ag nanoparticles (γ-Fe_2O_3/MWCNT/Ag). In this procedure, the leaves of VA were employed to ecofriendly reduced and cap silver nanoparticles, which were further used to generate the carbon nanotubes. Importantly, this nanocomposite was verified to be a promising antibacterial and nano-adsorbent for wastewater treatment. In another study, ZnO/SnO_2 nanocomposite was ecofriendly generated by an ethanol extract of VA vegetative tissues via mixing 0.1 mL of zinc nitrate [$Zn(NO_3)_2$:$6H_2O$] with 50 mL of the VA ethanol extract. Then, 100 mL of 0.02 M tin chloride [$SnCl_2$·$2H_2O$] were added to the solution and stirred for 2 h at 80 °C. Basically, XRD, FTIR, EDX, UV, FESEM, and DLS techniques were used to illustrate the crystal skeleton, functional group, elemental composition, absorbance, morphology, and size of ZnO/SnO_2 nanocomposite, respectively [122].

Recently, Fifere et al. [123] prepared cerium oxide nanoparticles using an aqueous extract of fresh VA. It was supposed that chemical constituents of the plant (lectins, viscotoxins, polyphenols, and flavonoids) have an efficient role in synthesis, capping, and stabilizing of cerium oxide nanoparticles. XRD depicted the nanoparticles size at 5.59 nm using the Scherrer equation. Overall, since the plant has a plethora of compounds within a variety of functional groups such as hydroxyl, carboxyl, polysaccharides, lectins and proteins, it showed a potential talent in the biosynthesis of different nanoparticles, among them silver, gold, cerium oxide, zinc oxide, ten oxide, and selenium nanoparticles/nanocomposites. Chemically and physically, these compounds have a good efficiency in reducing, stabilizing, and capping nanoparticles/nanocomposites of various shapes, nano-sizes, and structures, with numerous jobs, for example, antioxidant, antibacterial, apoptosis, photocatalytic, and wastewater treatment. ▶ **Table 5** shows some nanoparticles biosynthesized using VA and their potential applications. With the beneficial physicochemical properties of the nanoparticles as effective carriers, we expect that VA nanoparticles could boost antitumor activity and reduce undesired side effects when used as adjuvants in conjunction with anticancer agents.

Conclusion

This review presents an updated mechanistic insight into the immunomodulatory and anticancer effects, safety profiles, and recent nanotechnology development of VA. The current status of understanding VA as a modulator of the immune system and potential anticancer candidate as a potential new adjuvant for add-on therapy with modern oncological therapies are highlighted. The impact of VAEs and its bioactive metabolites on different cellular pathways associated with their immunological responses in the battle against cancerous cells has not been fully understood. The possible mechanisms involved in the immunomodulatory

and anticancer effects of bioactive components of VA, such as lectins, viscotoxins, and polyphenols, are depicted in ▶ **Fig. 1**. Moreover, the immunomodulating and anticancer studies of VA were mainly carried out using its crude extracts and the chemical constituents responsible for its individual immunomodulatory and anticancer activities have not been fully identified. Standardized VAEs must be used in future studies to ensure consistent and reproducible concentrations of active ingredients, then the intended pharmacological effects can be achieved consistently. Several experimental studies using cellular and animal models have proven VAE possessed strong antitumor and immunomodulatory properties; however, their clinical efficacy in cancer and associated survival benefits require further investigation. It is important to note that while some studies have shown promising results, more research is needed to fully understand the mechanisms and effectiveness of VA therapy in cancer treatment.

Contributors' Statement

All the authors were involved in collection and interpretation of data, drafting and editing of the manuscript. I. Jantan, S. Abouzid and N. Yosri participated in the concept and design of the manuscript. I. Jantan edited the final version to be submitted for publication.

Funding Information

This work received no specific grant from any funding agency.

Conflict of Interest

The authors declare that they have no conflict of interest.

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