European Viscum album: a potent phytotherapeutic agent with multifarious phytochemicals, pharmacological properties and clinical evidence

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Abstract

Viscum album L. or European mistletoe (Loranthaceae), a semi-parasitic shrub has been used as a traditional medicine in Europe for centuries to treat various diseases like cancer, cardiovascular disorder, epilepsy, infertility, hypertension and arthritis. *V*. *album* contains diverse phytochemicals, which exert a large number of biological and pharmacological activities. The aim of this review is to compile the developments in the domain of *V*. *album* and research trends, with a focus on ethnopharmacology, phytochemistry and pharmacological properties to illustrate the potential of this phytotherapeutic as an attractive commercial herbal medicine. Crude extracts and isolated chemical constituents from *V*. *album* exhibited significant medicinal effects in experimental models and in patients with cancer, autoimmune and inflammatory conditions. Importantly, recent randomized clinical trials have suggested an improved overall survival and quality of life in cancer patients treated with different mistletoe preparations. The current phytochemical studies have shown that lectins, hetero-dimeric glycoproteins, polysaccharides, viscotoxins, alkaloids, lipids, triterpenes, peptides, vesicles, flavonoids, cyclitols and amines are principal bioactive phytochemicals of *V. album*. Clinical studies and experimental models have revealed that *V*. *album* exhibits several pharmacological activities, such as immunomodulatory, anti-hypertensive, anti-oxidant, cytotoxicity, anti-tumor, anti-inflammation, anti-diabetic, anti-microbial and sedative activities. It is conceivable that the heterogenous profile of biochemical compounds provides the basis for the broad diversity of pharmacological activities of mistletoe as each single component contributes diverse modes of actions in addition to imparting to a synergistic beneficial action in conjunction with other molecules.

Abbreviations

2AA, 2-aminoanthracene; ADRs, adverse drug reactions; Aps, arabinogalactan proteins; Bcl-2, B-cell lymphoma 2; cb, chitin-binding; COX-2, cyclooxygenase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FME, fermented mistletoe extract; HPIV-2, human parainfluenza virus type 2; HUVEC, human umbilical vein endothelial cells; IFN-γ, interferon gamma; IL, interleukin; JNK, c-jun N-terminal kinase; KME, Korean mistletoe extract; KML, Korean mistletoe lectin; KML-C, lectins from Korean *V*. *album* spp. *coloratum*; KVA, Korean *V*. *album*; LPS, lipopolysaccharide; ML, mistletoe lectin; NK, natural killer; NO, nitric oxide; QOL, quality of life; rVAA, recombinant *V*. *album* agglutinin, TNF-α, tumor necrosis factor-alpha; VAA, *V*. *album* ssp. *album;* VAA-I, *V*. *album* agglutinin-I; VAC, *V*. *album* var. *coloratum;* VAE, *Viscum album* extract; VCA, *V*. *album* var. *coloratum* agglutinin; VF-2, viscum fraxini-2

Contents

1. Introduction

Viscum album L., (Loranthaceae) commonly known as mistletoe or European mistletoe, is recognised by various names: European white-berry mistletoe, bird lime, birdlime, all-heal, and masslin. In German, *V*. *album* is called by Mistel, Vogelmistel, Leimmistel, Affolter, and Bocksfutter; gui, gui commun, and gui de druides in French; vischio, visco, vescovaggine, guatrice, pania, and scoaggine in Italian; muerdago in Spanish, and common mistletoe in Asia and Africa. [1-4] It is native to Europe and western and southern Asia. [5, 6] *V*. *album* has been commonly used in local medicine in Europe and Asia for thousands of years. [2] In Europe, folk medicinal uses of *V*. *album* are recorded for curing various ailments such as cancer, hypertension, anxiety, insomnia, headache, and internal bleeding or atherosclerosis. The compounds isolated from *V*. *album* so far mainly include hetero-dimeric glycoproteins, polysaccharides, lectins, amines, triterpenes, viscotoxins, alkaloids, lipids, peptides, cyclitols, vesicles, and flavonoids. [7-10] European *V*. *album* with its bioactive phytochemicals is possessed of wide-reaching biological activities, including immunomodulatory, anti-oxidant, cytotoxicity, anti-tumor, anti-hypertensive, sedative, anti-diabetic, and hepato-protective. Meanwhile, *V*. *album* has also displayed significant inhibitory bio-activity against human cancer cell lines. [11, 12] In addition, extract and phytochemicals can inhibit inflammation and prevent the development of cancer. [4, 6] A number of phyto-pharmacological providers like WELEDA, ABNOBA HEILMITTEL, HELIXOR HEILMITTEL, NOVIPHARM and MADAUS market a range of different mistletoe preparations. Brand names of various preparations of mistletoe extract are Iscador®, ABNOBAViscum, Cephalektin, Eurixor®, Helixor®, Isorel and Lektinol™.

Owing to its extensive use as a potent phytotherapeutic agent in European countries, it has been in the spotlight of researchers for a long time. The present review provides an update on the developments in the domain of *V*. *album* and research trends, with a focus on ethnopharmacology, phytochemistry, pharmacological properties and clinical use.

2. Botany and distribution

V. *album* L., a dioecious, insect-pollinated and hemi-parasitic evergreen shrub mostly grows on a number of host trees**.** It is commonly found in the crowns of broad-leaved deciduous trees including apple, ash, hawthorn, lime, cedar of lebanon, larch, and other trees. On the Oak and pear, it grows very rarely. *V*. *album* receives additional nutrients via a haustorial attachment to a host, but is also able to photosynthesize. It has stems 30-100 cm long, yellowish, and smooth with dichotomous branching. The leaves are tongue-shaped, in apposite pairs, broader towards the end, 2-8 cm long, 1-2.5 cm wide, leather textured and of a dull yellow-green colour. Small flowers are inconspicuous, clusters in the forks of the branches, yellowish-green and 2-3 cm diameter. Neither male nor female flowers have a corolla. The fruit is a white or yellow berry, smooth, ripening in December, glutinous pulp containing one (very seldom) seed covered in the very sticky pulp. [13] Based on fruit colour, leaf shape and size, and most obviously in the host trees utilized, numerous subspecies of *V*. *album* have been classified. These include *V*. *album* subsp. *abietis* (Wiesb.) having white fruit and leaves up to 8 cm; *V*. *album* subsp. *album* having white fruit and leaves 3–5 cm; *V*. *album* subsp. *austriacum* (Wiesb.) having yellow fruit and leaves 2–4 cm; *V. album* subsp. *meridianum* (Danser) having yellow fruit and leaves 3–5 cm; *V. album* subsp. *creticum* having white fruit and short leaves; and *Viscum album* subsp. *coloratum* Kom, which is now considered as a separate species *Viscum coloratum* (Kom) Nakai by the Flora of China.

Geographically, Mistletoe is distributed from North Africa to southern England and southern Scandinavian regions, across Central Europe to southwest and east Asia to Japan. In Europe, three subspecies have been identified depdneing on their growth on different species of host trees. *V. album* subsp. *album* grows on hardwoods*, V. album* subsp. *abietis* uses fir as host trees and *V. album* subsp. *laxum* is dependent on pines and spruce. *V. album* with coloured fruits are also recorded in further east.[13] In United Kingdom, *V. album* is distributed from east

Devon to Yorkshire, and is exceptionally common across London and regions of central and southern England.

3. Traditional uses and ethnopharmacology

The European *V*. *album* is a pharmaceutical plant and a symbol in mythology. It is the first plant, which was termed as “mistletoe”. According to G. P. Secundus (23-79 AC) this plant was considered to be an antidote for poisons and the plant became a miracle because of its ability to cure each illness.

The traditional curative use of mistletoe infusion has been for high blood pressure, dizziness, and hives. The Greek author and physician (15-85 AC) reported that during 460-377 BC, spleen-related diseases were treated using Oak tree mistletoe. During 23-79 AC, Plinius explained the beneficial role of mistletoe in the treatment of infertility, ulcers, epilepsy. Platonist around 150 AC, described the utilization of mistletoe to treat tumors. In a French work on domestic remedies, in the year 1682, it was considered as a golden herb for treating epilepsy. During the year 1731, mistletoe was used for various purposes including labour pain and deworming in children. Later, it had been used for curing convulsions delirium, hysteria, neuralgia, nervous debility, urinary disorders, heart disease, and many other complaints arising from a weakened and disordered state of the nervous system. Mistletoe extracts contain several toxic components, several of which are lectins, or proteins capable of binding to specific sugars. In 1921, the Austrian anthroposophical spiritual leader Rudolf Steiner recommended that mistletoe could be used to treat cancer, based on the observation that mistletoe, like cancer, is a parasitic and lethal to its host. Swiss and German clinics were founded to implement this idea and still actively use a mistletoe preparation fermented with a strain of *Lactobacillus* for 3 days.

Despite having a strong historical background of mistletoe, in the 19th Century scientific community rejected mistletoe remedy and the interest was re-awakened in the 20th century

when Gaultier demonstrated oral/subcutaneous administration of fresh mistletoe extract to cure blood pressure-related issues both in animals and humans. Traditionally, the European mistletoe has been widely used for many years with remarkable therapeutic effects for the treatment of hypertension, anxiety, insomnia, internal bleeding or atherosclerosis and in complementary cancer therapies. In the year 1920, the founder of anthroposophy, Rudolf Steiner, introduced *V. album* as an anti-cancer remedy [14]. Although local medicine at the end of the 19th century still regarded mistletoe as a crucial part of the medicine box, academic medicine in the growing scientific age lost considerable attention in mistletoe as a remedy.

4. Bioactive constituents of European *V. album*

European mistletoe is characterized by a number of phytochemicals including lectins, polysaccharides, alkaloids, terpenoids, proteins, amines, peptides, polyphenols, flavonoids, phytosterols, and amino acids (Table 1). Interestingly, some therapeutic phytochemicals such as certain alkaloids are not produced by the mistletoe rather absorbed from the host tree.

4.1. Viscotoxins

Viscotoxins, a mixture of low-molecular weight cysteine rich and basic proteins belong to plant thionins (α and β) and are synthesized in the leaves and stems. [15, 16] They are amphipathic in nature consisting of 46 amino acids with a molecular mass of 5 kDa. The polypeptide chains are attached through three or four disulphide bonds at highly conserved positions (Cys3/Cys40, Cys3/Cys32, and Cys16/Cys26), giving them a compact structure and high stability against denaturing conditions such as heat and proteases. To date, seven different isoforms have been characterized (A1, A2, A3, B, B2, C1 and 1-PS) and are differed mainly in their sequence of amino acids. [17] The content of viscotoxins varies from 0.05 to 0.1%, while composition depends on the host tree. For example, the presence of viscotoxins A2 and A3 were observed in *V.*

album ssp. a*lbum* (VAA), however the predominance of PS-V was detected in *V. album* ssp. *austriacum*. All viscotoxins, with the exception of A2, were detected but A3 was predominant in *V*. *album* ssp. *abietis*. [18]

Investigation on the 3D-structures of viscotoxins also provided information on a specific phosphate-binding site.[19] It has also been assumed that the phosphate-binding site and amphipathic structures of the viscotoxins help in the inducing cytotoxicity in eukaryotic cells by interfering with cell membrane and altering its integrity. In addition to their high structural homology, biological effect of viscotoxins can vary according to their different isoforms. [20] The viscotoxins reveal a high structural and pharmacological association with snake (cobra) cardiotoxins. [21]

4.2. Mistletoe lectins (MLs)

The main compounds isolated from *V*. *album* are MLs (a mixture of high-molecular-weight polypeptides) and the total content is in the range of 340-1000 µg/g dried plant material or their content is not less than 2% of total polypeptides and proteins. [22, 23] The lectin content is highest in the winter. Sprouts and shoots contain the highest concentrations. Three different MLs (ML-I, ML-II, and ML-III) with differential sugar-binding specificities have been isolated from European mistletoe by affinity chromatography on partially hydrolyzed Sepharose and human immunoglobulin-Sepharose. [18, 24, 25] These include galactose-specific ML-I (115 kDa, dimer), galactose- and *N*-acetyl-D-galactosamine-specific ML-II (60 kDa) and N-acetyl-D-galactosamine-specific ML-III (60 kDa). All three MLs have high reactivity with human erythrocytes without specificity for the A, B, and O blood groups.

Peumans and colleagues reported that deciduous trees contain mostly ML-I and Eurupean mistletoe growing on fir and pine trees found to be rich in ML-III. [22] The subdomains of ML-I and ML-III were identified to be responsible for sugar binding. These authors also first

219 described chitin-binding (cb) MLs with a molecular weight of 10.8 kD. The three cbMLs including
220 cbML1, cbML2, and cbML3 found very closely related primary structures with hevein. MLs,
221 categorized as type-2 ribosome-inactivating proteins which consist of two peptide chains such
222 as chain A comprising three distinct individual domains and chain B having two domains with
223 similar configurations. [18, 26, 27] The chains are linked by a disulfide bridge. The chain A inhibits
224 protein synthesis by degrading the 28S rRNA in ribosomes of eukaryotic cells and also
225 accelerates apoptosis. While, the chain B is capable of binding to glycoconjugates of cell
226 surface and thereby permitting into the cell of the toxic subunit. [22] Based on glycosylation
227 patterns of MLs, more than 20 different isoforms have been separated by isoelectric focusing.
228
229 *4.3. Carbohydrates*

230 Further constituents of European *V*. *album* include oligo- and polysaccharides. Structurally
231 different types of mistletoe polysaccharides have been identified in the berries and leaves. A
232 highly methylated homogalacturonan, pectin (42 kD), 1→α4 galacturonic acid methyl ester, and
233 arabinogalactan (110 kD) were characterized in leaves and stem, while berries were specially
234 rich in other polysaccharides such as rhamnogalacturonans with arabinogalactan side chains
235 (1,340 kD), arabinogalactans and small amounts of xyloglucans. [28-30] Monosaccharides and
236 polyols were also identified, but after hydrolysis of mistletoe extract. The content of
237 polysaccharides is varied depending on the host plants. For example, inositol (58%) was
238 recorded at early stage of lime tree, however in the latter stage, galactose (44%) was dominant.
239
240 *4.4. Polyphenols and phenylpropanoids*
241 A range of flavonoids, phenylpropanoids, and phenolic acids were isolated from European *V*.
242 *album* and the host tree has an influence on their contents. For example, high contents of
243 salicylic acid and rosmarinic acid were detected in *Sorbus aucuparia* and *Malus domestica*,

respectively. It has also been noticed that mistletoe grown on *Fraxinus excelsior* had the highest quantity of total phenolic acids and total flavonoids. The diversified qualitative and quantitative amount of phenolic acids including caffeic, phydroxybenzoic, salicylic, protocatechuic, ferulic, and sinapic acids were observed in a free state and as glycosides.[31, 32] Recently, two new phenolic acids have been isolated from European white-berry *V*. *album*, including gallic acid and 3-(3'-carbomethoxypropyl)-7→3''-protocatechoyl galloate. [33]

Two classes of flavonoids such as chalcones and flavanones were isolated from alcoholic extract of *V*. *album* in their glycosidic form and with methoxyl groups in the molecules [18]. They were 5,7-dimethoxyflavanone-4'-*O*-[2''-*O*-(5'''-*O*-trans-cinnamoyl)-apiosyl]-glucoside), 2'-hydroxy-4',6'-dimethoxychalcone-4-*O*-[2''-*O*-(5'''-*O*-trans-cinnamoyl)-apiosyl]-glucoside, 5,7-dimethoxy-flavanone-4'-*O*-glucoside, 2'-hydroxy-4',6'-dimethoxychalcone-4-*O*-glucoside, 2'-hydroxy-3,4',6'-trimethoxychalcone-4-*O*-glucoside, 5,7-dimethoxyflavanone-4'-*O*-[apiosyl-(1→2)]-glucoside and (2S)-3',5,7-trimethoxyflavanone-4'-*O*-glucoside, and 2'-hydroxy-4',6'-dimethoxychalcone-4-*O*-[apiosyl(1→2)] glucoside.[34, 35] A promising antioxidant flavonoid quercetin has been detected only after acid hydrolysis of *V*. *album* extract. [36] Other flavonoids including quercetin, kaempferol and their mono-, di and trimethylethers were also characterized in epicuticular waxes of different subspecies of the European mistletoe. [37] Chaudhary and colleagues reported that the 80% methanolic extract of mistletoe contains many polyphenolic constituents viz. 5,7-dimethoxy-4'-hydroxy flavanone, 3-(4-acetoxy-3,5-dimethoxy)-phenyl-2E-propenyl-β-glucoside, 5,7-dimethoxyflavanone-4'-*O*-b-glucoside, 4'-*O*-[bapiosyl(1→2)]-β-glucosyl]-5-hydroxy-7-*O*-sinapylflavanone, 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2E-propenyl-β-glucoside, and 4',5-dimethoxy-7-hydroxy flavanone. [38]

Recently, four new flavonoid glycosides were isolated and identified from the leaves and twigs of *V*. *album* such as 3,7,3'-tri-O-methylquercetin-4'-O-β-d-apiofuranosyl-(1→2)-O-β-d-glucopyranoside, 7,3'-di-O-methylquercetin-4'-O-β-d-glucopyranosyl-3-O-[6‴-(3-hydroxy-3-

methylglutaroyl)]-α-d-glucopyranoside, 7,3'-di-O-methylquercetin-4'-O-β-d-glucopyranosyl-3-O-[(6''''→5'''')-O-1''''-(sinap-4-yl)-β-d-glucopyranosyl-6-(3-hydroxy-3-methylglutaroyl)]-α-d-glucopyranoside, and (2S)-5-hydroxy-7,3'-dimethoxyflavanone-4'-O-β-d-apiofuranosyl-(1→5)-O-β-d-apiofuranosyl-(1→2)-O-β-d-glucopyranoside. [39]

Phenylpropanoids are the important bioactive molecules of the European mistletoe, with extremely diverse structures and wide-spectrum medicinal effects. The leaves and stem were found to contain several phenylpropanoids including coniferyl alcohol 4-*O*-β-D-glucoside (coniferin), syringenin 4-*O*-β-D-glucoside (syringin), syringenin 4-*O*-β-D-apiosyl(1→2)-β-D-glucoside, and lignans such as syringaresinol 4',4''-di-*O*-glucoside (eleutheroside E) and syringaresinol-*O*-glucoside. [40, 41] Lignan skeleton also contained trihydroxy-tetramethoxy-epoxy glucosides such as ligalbumosides A to E and alangilignoside C. [42] The quantity of phenylpropanoids also varied according to the mistletoe subspecies. Maximum levels of syringin and coniferin were noticed in the extract of VAA by HPLC, having isocratic mobile phase (methanol : water : 0.1 N sodium acetate; 20 : 73.5 : 6.5). Apart from these phenylpropanoids, kalopanaxin D (4-[2-O-(apiosyl)-b-D-glucosyloxy]-3-methoxycinnamyl alcohol) was detected. *V*. *album* ssp. *austriacum* contains trace amount of coniferin, and both syringin and coniferin were characterized in *V*. *album* ssp. *abietis*. However, both these subspecies of mistletoe were unable to synthesize kalopanaxin D. [43]

4.5. Lipid soluble compounds

Terpenoids, liposoluble compounds are the main components of European *V*. *album*. The lipid soluble extract of *V*. *album* showed the presence of oleanolic acid, β-amyrin acetate, β-amyrinacetate, lupeol, lupeol acetate, betulinic acid, and ursolic acid.[44, 45] A mixture of phytosterols including β-sitosterol and stigmasterol and their esters were also identified.[46, 47] Moreover, other lipophilic compounds specially saturated fatty acids palmitic, arachidic,

lignoceric, behenic, and cerotic acids and the unsaturated oleic, linoleic, and linolenic acids were presented in the extract of Turkey *V*. *album*. [48, 49] Long-chain fatty acids and hydrocarbons including loliolide, vomifoliol trans-α-bergamotene, and trans-β-farnesene were identified in an extract obtained from supercritical fluid extraction method. [50] Due to poor solubility of triterpene in water, it is difficult to extract from the mistletoe. However, 2-hydroxypropyl-β-cyclodextrin and sodium phosphate (pH 7.3), as solubilizers have been developed and represent excellent tools for the extraction of triterpenes. [47, 51]

4.6. Trace minerals

In addition to organic components, mistletoe contains trace mineral elements including potassium, calcium, manganese, sodium, nickel, phosphate, selenium, silica, magnesium, and zinc. Importantly, calcium has detected mainly in its non-soluble oxalate form. [52, 53] Mistletoe grown on oak and fir have high levels of manganese. [53]

4.7. Other chemical constituents

Cyclic peptides, amino acids, proteins (9.3%), alkaloids, amines (histamine and cetylcholine), jasmonic acid, cysteine, glutathione, vitamin C, and xanthophyll are other. [54, 55] Three new diarylheptanoids including (3S,5R)-3-hydroxy-5-methoxy-1,7-bis(4-hydroxyphenyl)-6E-heptene (1), (3S,5S)-3-hydroxy-5-methoxy-1,7-bis(4-hydroxyphenyl)-6E-heptene (2), and (3S)-3-hydroxy-1,7-bis(4-hydroxyphenyl)-6E-hepten-5-one have been isolated and determined from the leaves and twigs of *V*. *album*. [39] Arabinogalactan proteins (APs) were isolated from berries and aerial part of European mistletoe. The rations of arabinose and galactose in APs were 1:0.7 and 1:1.18 in barriers and aerial part, respectively.

5. Multifarious pharmacological properties

V. *album* has been used as a folk herbal remedy in Europe and was a mythical shrub that had a strong influence on people in ancient times. Owing to the presence of a range of therapeutic and bioactive chemical constituents, European mistletoe exhibits multifarious pharmacological activities by altering molecular events in the cells (Fig 1; Table 2).

5.1 Anti-inflammatory

Our group has found that VAQu Spez impedes cytokine-induced prostaglandin E2 (PGE_2), by selectively inhibiting cyclooxygenase-2 (COX-2) which is transcriptionally activated in response to various pro-inflammatory cytokines. [6] Further dissection of the molecular events revealed that *V*. *album* significantly reduces the COX-2 mRNA half-life without influencing its protein stability, implicating that *V*. *album* induces destabilization of COX-2 mRNA. [4] Excessive amount of PGE_2 is also associated pro-tumoral condition and enhances dendritic cell-mediated regulatory T cell expansion. [56-59] Thus, inhibition of PGE_2 by *V. album* was also important for anti-tumoral functions. To assess the anti-nociceptive and anti-inflammatory activities of isolated flavonoids of *V*. *album*, the p-benzoquinone-induced writhing test and carrageenan-induced hind paw edema model were used, respectively. The ethyl acetate fraction (250 mg/kg) as well as 2'-hydroxy-4',6'-dimethoxy-chalcone-4-O-beta-D-glucopyranoside and 5,7-dimethoxy-flavanone-4'-O-[β-D-apiofuranosyl-(1-->2)]-β-D-glucopyranoside at the concentration of 30 mg/kg dose were shown to possess remarkable anti-nociceptive and anti-inflammatory activities, without inducing any apparent acute toxicity as well as gastric damage. [60]

A single intraperitoneal (IP) injection of the mistletoe preparation Isorel (100 mg/kg) decreased the size of the tumour and triggered abundant tumour necrosis with inflammatory response, oedema and destruction of the malignant tissue. [61] Moreover, the Isorel-treated

342 melanoma cells were found to be more sensitive to the cytotoxic activity of the lymphocytes in
343 the presence of Isorel-treated mice plasma than the control tumour cells.

344

345 *5.2. Immunomodulatory*

346 *V*. *album* and their chemical constituents have well-known immunomodulatory properties. *V.*
347 *album* significantly enhanced interferon gamma (IFN-γ) responses. [62] Our group has reported
348 that QU FrF mistletoe preparation significantly inhibits tumor growth *in vivo* in an IL-12-
349 dependent mechanism. [63] As dendritic cells are key players in regulating the immune
350 responses, [64-68] we examined the effect of *V*. *album* on these innate cells. *V*. *album* Qu Spez
351 enhanced the expression of several antigen presenting and co-stimulatory molecules on human
352 dendritic cells and additionally induced secretion of pro-inflammatory cytokines such as IL-6 and
353 IL-8 and stimulated the proliferation of $CD4^+$ T cells. [69] A transcriptome analysis of the gene
354 expression profile induced by *N*-acetyl-*D*-galactosamine-specific lectin of *V*. *album* var.
355 *coloratum* agglutinin (VCA, Korean mistletoe lectin) following incubation in human T cells
356 revealed activation and inhibition of 3000 genes involved in a wide range of immune functions.
357 These genes were related to cytokines, cell adhesion, cell motility, cell growth and maintenance,
358 cell death, and the response to stress and to external stimulus. [70]

359 A diet enriched by 1% and 2% of Korean mistletoe extract positively enhanced innate
360 immunity responses such as respiratory burst and phagocytic activity in kelp grouper
361 *Epinephelus bruneus* against *Philasterides dicentrarchi*. [71] A recombinant form of *Escherichia*
362 *coli*, producing ML (aviscumine) was developed. Immunomodulatory and cytotoxic activities
363 have been observed in *in vivo* and clinical phase I studies. [72] The natural killer (NK) cells have
364 been anticipated as one of the candidates for direct tumour cell destruction. [73] Under *in-vitro* and
365 *in-vivo* systems, Korean mistletoe lectin was found to enhance the immune system through
366 modulation of lymphocytes, natural killer cells, and macrophages. [74] Subcutaneous (SC)

administration of mistletoe causes increase in relative number of lymphocytes with activated phenotype, NK cells and specific subsets of lymphocytes including B cells, CD4+ T cells and cytotoxic T cells.[75] These results were also confirmed in other studies and found that *V. album* treatment can result in normalization of initial immune indices.[25, 76-78] Further, mistletoe extracts obtained from apple (mali) or pine (pini) induced *in vitro* oligoclonal activation of $CD4^+$ T cells from mistletoe-treated cancer patients. [77] A placebo-controlled study in healthy individuals found that *Iscador Quercus* causes eosinophilia due to stimulation of IL-5 and GM-CSF by ML. [78] Nontoxic doses of ML-1 or its carbohydrate-binding subunit prompted significant increase in components of the cellular host defense system including natural killer cytotoxicity or release of various cytokines including IL-1, TNF-α and IL-6.[79]

The effect of VCA on murine splenocytes was investigated to examine whether VCA acts as an immunomodulator. VCA in a dose-dependent manner (4-64 ng/mL) decreased IFN-γ secretion in concanavalin A (ConA)-stimulated murine splenocytes without changing IL-4 levels. [80] Treatment of VCA also resulted in an anti-proliferative effect at 2-8 ng/mL and 1-8 ng/mL in human peripheral blood mononuclear cells (hPBMC) and T lymphocytes, respectively. However, at lower doses (4-16 pg/mL and 4-32 pg/mL respectively), a proliferative effect was noticed in hPBMC and T lymphocytes. [62] The RT-PCR result confirmed the release of pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8, and IFN-γ, when cells were treated with low doses of VCA (4-32 pg/mL). Another report also confirmed enhanced expression of aforementioned cytokine genes upon stimulation of hPBMC with *V. album* agglutinin-I. [81] These data might suggest new perspective of VCA to regulate the balance between cell proliferation, cytokine production and apoptotic cell death. Induction of these cytokine genes and protein production in the cultures of hPBMC was also observed upon treatment with ML-I. [82]

VAA extract increased phagocytic activity and candidacidal activity of neutrophils, and decreased adhesion function of epithelial cells. Furthermore, extract stimulated the levels of $CD4^+CD25^+$ and $CD8^+CD25^+$ T cells and $CD3^-CD16^+CD56^+$ natural killer cells. [83] A study

393 reported that the cell killing capacities of mistletoe extracts are host tree-specific and not
394 correlated with ML or viscotoxin content. [84] The newly isolated mistletoe viscotoxins such as
395 VTA1 (85 nm), VTA2 (18 nm) and VTA3 were found to increase natural killer cell-mediated
396 cytotoxicity. [85] Impact of the viscotoxins on human granulocytes was studied by flow cytometry
397 and it was found that viscotoxins at 25 and 250 µg/mL concentrations enhanced phagocytosis
398 and burst activity against *E. coli* infection in respiratory track. [86] The SC treatment of aqueous
399 mistletoe extract in 8 volunteers was reported to induce the secretion of Th1 (IFN-γ) or Th2 (IL-
400 4) cytokines and also the release of TNF-α and IL-6. [87]

401 Numerous studies have reported a strong stimulatory response of hPBMC by Iscador
402 Pini (a fermented extract of mistletoe) in normal and allergic individuals. A study was conducted
403 to examine the cell subtypes involved in this *in vitro* reactivity. Flow cytometry results clearly
404 showed that Iscador activates T cells ($CD3^+$), especially $CD4^+$ T helper cells, as well as
405 monocytes at concentrations of 0.1 to 1.0 mg/mL. No evidence for a key involvement of B cells
406 ($CD19^+$), NK cells ($CD56^+$), and T suppressor cells/cytotoxic T lymphocytes ($CD8^+$) was
407 detected. [88] A recombinant *V. album* agglutinin (rVAA) enhanced the secretion of an active form
408 of IL-12 and potentiated the cytokine-induced NK cell activation in cultured rat splenocytes.
409 These Authors also stated that the effects of rVAA could be associated with its enhancing
410 effects on MHC-unrestricted cytotoxicity *in vivo*. [89] However, the contradictory report on
411 phagocytic activity of lectin is also reported and investigators found that various concentrations
412 of lectin ranging from 0.025 to 20 ng/mL had only marginal effect on phagocyte activity.

413

414 *5.3. Cytotoxicity*

415 ML can induce apoptosis depending on the apoptosis-associated factor-1 (Apaf-1) pathway by
416 stimulating mitochondrial membrane potential (MMP) breakdown and stimulating caspase-3. [90,
417 91] The c-Jun N-terminal kinase (JNK) stimulation by ML-I led to translocation of the pro-

apoptotic proteins Bax and Bad. ML-I down regulates B-cell lymphoma 2 (Bcl-2) and up regulates TNF-α and hence provoke apoptosis. We have demonstrated that VA Qu FrF, induces significant cell toxicity *in vitro* in the human T cell lines CEM and in monocyte cell lines HL-60 and MM-6. [92] The viscotoxin-free *V*. *album* extract significantly enhanced granulocyte activity, and this effect was correlated with the content of the ML. [93]

Treatment of *V*. *album* preparations from eight dissimilar host trees (Iscucin Abietis, Pini, Populi, Mali, Salicis, Crataegi, Quercus and Tiliae) showed a significant cytotoxic effect on the medulloblastoma cell lines including Daoy, D342, D425, and UW-288-2, yet the cell susceptibility was unrelated against the different extracts. The reduction in mitochondrial activity and enhancement in apoptotic cell death correlated with the lectin content of the used preparation in a dose-dependent manner. [94] ML-I, ML-II, and ML-III were found to be toxic for Molt 4 cells at pg concentrations, ML-III being the most cytotoxic. Interestingly, the digalactosides Gal beta 1,2Gal beta-allyl and Gal beta 1,3Gal beta-allyl were able to bind to the B-chain of these lectins and inhibit their toxic activity. *N*-acetyl-D-galactosamine and rho-nitrophenyl *N*-acetylgalactosamine prevented the toxic effects of ML-II and III. [95]

Major cytotoxic components were fractionated from Korean mistletoe and the changes of their cytotoxic effects due to heat treatment were studied. ML-I showed maximum toxicity, but was disappeared by heating for 30 min. The study suggested that the ML is not responsible for inducing apoptosis, but the involvement of other components might be possible. [96] Viscotoxins and alkaloids were found to retain their effects even after heating for 60 and 180 min, respectively. Moreover, the alkaloid fraction was more effective to tumor MSV cells than to non-tumor A31 cells. [97] The isolated KML-C showed strong cytotoxicity against various human and murine tumor cells by inducing apoptosis mediated by Ca^{2+}/Mg^{2+} -dependent endonucleases. However, the cytotoxic activity of KML-C was higher than that of a lectin from European mistletoe *V*. *album* spp. *loranthaceae*. [98]

443

444 *5.4. Anti-angiogenic*

445 Treating B16L6 melanoma cells with *V*. *album* suppressed tumor growth and resulted in DNA
446 fragmentation and nuclear morphological changes, suggesting that *V*. *album* inhibits tumor
447 growth and metastasis by elevating apoptosis and blocking angiogenesis. [99] Our group has
448 shown that VAQU FrF induces apoptosis of endothelial cells in human umbilical vein endothelial
449 cells and in immortalized human venous endothelial cell line. [12] Fermented mistletoe extract
450 (FME) treatment of glioblastoma cells down-regulated cytokine TGF-β and matrix-metallo-
451 proteinase genes expression, which involve in glioblastoma progression and malignancy. In
452 addition, FME reduced the migratory and invasive potential of glioblastoma cells. [100] VAA-I is a
453 plant lectin, which possesses anti-tumoral properties. VAA-I was reported to induce apoptosis in
454 PLB-985 cells and cells from chronic granulomatous disease via caspase-mediated pathway. [101]

455 The role of VAA-I on activated neutrophils and pro-inflammatory properties have not
456 much explored so far. Lavastre et al. demonstrated that VAA-I at 1000 ng/mL activate apoptotic
457 cell death in lipopolysaccharide (LPS)-treated human neutrophils *in vitro* as well as in murine
458 neutrophils isolated from LPS-induced neutrophil influx.[102] They concluded that VAA-I can inhibit
459 LPS-induced pro-inflammatory response *in vivo*. [102] VAA-I induces apoptosis in human
460 neutrophils by accelerating the loss of anti-apoptotic Mcl-1 expression and the degradation of
461 cytoskeletal paxillin and vimentin proteins via caspases. [103] IscadorQu, an aqueous fermented
462 extract from the European mistletoe grown on oaks caused early cell cycle inhibition followed by
463 apoptosis in a dose-dependent manner in endothelial cell cultures. Apoptosis was induced by
464 activating the mitochondrial activity. [104]

465 Epi-oleanolic acid, a triterpene was isolated from the dichloromethane extract of Korean
466 *V*. *album* (KVA) by repeated silica gel chromatography and recrystallization. Treatment of
467 triterpene showed a typical pattern of apoptotic cell death, including morphological changes and

DNA fragmentation in human and marine cancer cells. [105] A study on the anti-cancer mechanisms of action of VCA from Korean mistletoe suggested that VCA induces apoptosis in hepatocarcinoma Hep3B cells by inducing ROS production and a loss of DeltaPsim, in which JNK phosphorylation plays a key role in these events. [106] The β-galactoside- and *N*-acetyl-D-galactosamine-specific lectin II, polysaccharides, and viscotoxin of mistletoe were found to induce apoptosis in U937 cells through the activation of phosphotransferase activity in JNK1/stress-activated protein kinase and was characterized by DNA ladder pattern fragmentation. [107] However, protein kinase A or C protected the apoptosis induced by MLII of KVA in the human leukemic HL-60 cells. [108] The viscotoxins induced cell death by producing mitochondrial Apo2.7 molecules and by generating ROS-intermediates in lymphocytes. [109]

5.5 Anti-oxidant

It is well known that the antioxidant activity effects of *V*. *album* extracts are varied depending on the host tree and the harvesting time. It was observed that the extract from lime tree or white locust tree completely inhibits mitochondrial DNA damage induced by H_2O_2 in HeLa cells, while extract from hedge maple tree inhibits mitochondrial DNA damage only by 50%. [110]

Organic extracts of *V*. *album*, which contains polyphenolic compounds were reported to exert anti-glycation and anti-oxidant properties. [38] Oxidative stress protective activity of Korean mistletoe lectin was examined under in vitro system using LLC-PK1 renal epithelial cells. Korean mistletoe lectin exhibited strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potential with an IC_{50} value of 42.6 μg/mL. [111] In addition, it exerted free radical quenching potential against nitric oxide (NO), superoxide anion, and hydroxyl radical in a concentration-dependent manner. Further, inhibition of COX-2, inducible NO synthase, SIN-1-induced nuclear factor kappa B, and the phosphorylation of inhibitor kappa B alpha was also seen in lectin-treated LLC-PK1 cells. [111]

Methanol extracts of mistletoe grown on different host trees were studied for their potential anti-oxidant activity. The extract from mistletoe grown on lime tree in summer showed the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ant-lipid peroxidation activities. [112]

5.6. Anti-tumoral

A pre-clinical study suggested that aqueous mistletoe extract exhibits potent anti-tumoral activity by depleting hypoxanthine and activating xanthine oxidase in the cancer cells, which lead to lowered salvage pathway activity required for the cancer cells to proliferate in the cancerous colon tissue. [113] Lipophilic extract of *V. album* and its predominant triterpene oleanolic acid significantly decreased monocyte chemotactic protein-1-induced monocyte transmigration. [114] Ethanol extract of *V. album* containing viscotoxin enhanced the anti-tumor effect of doxorubicin. [115] Pretreatment of C6 glioma cells with 100 μg/mL of VAE before heat shock significantly decreased expression levels of Hsp27 (73%), 14-3-3β (124%), 14-3-3γ (23%), and 14-3-3ζ (84%) proteins. Increased apoptosis was also observed through caspase-3 activation (60%). [116] VAA-I enhanced anti-proliferating potential of cycloheximide in the human lung carcinoma cell line A549 by inducing G1-phase accumulation. [117] The KML-II was also found to induce apoptosis in U937 cells via activation of caspase cascades. [118]

A large number of studies on synergistic effect of mistletoe extract and its components are available. IFN-γ enhanced the apoptotic response to ML-II through augmentation of Fas/Fas L expression and caspase activation in human myeloid U937 cells. [119] The MLs activated apoptotic pathway in various tumour cell lines and human lymphocytes. The ML-III was also found to reduce the expression of nuclear p53 and Bcl-2. [120]

Oleanolic acid, a component of the leaves and roots of *V. album* induced apoptosis by altering cellular morphology as well as DNA integrity in HaCaT cells in a dose-dependent

manner, with comparatively low cytotoxicity. [121] Either solubilized triterpene acids or lectins and combinations thereof were found to induce dose-dependent apoptosis in the acute lymphoblastic leukaemia cell line NALM-6 via caspase-8 and -9 dependent pathways *in vitro* and *in vivo*. [122] *V*. *album* from apple and pine increased anti-tumoral activity of activated human macrophages by inducing the production of NO. [123] Anti-tumor activity of Iscador M Spezial, Iscador Qu Spezial and Iscador P preparations of *V*. *album* at high concentrations were investigated in a panel of 12 cell lines. [124, 125] The lectin-containing Iscador M Spezial and Iscador Qu Spezial showed a noticeable anti-tumor activity in the mammary cancer MAXF 401NL cells at 15 µg/mL concentration with more than 70% growth inhibition compared to untreated control cells. [124, 125]

Anti-proliferative effect of *V*. *album* extracts was characterized in the bladder carcinoma T24, TCCSUP, J82 and UM-UC3 cell lines. Necrosis and apoptotic cell death were the fundamental mechanisms of anti-tumoral effect of *V*. *album* extracts. [126] The primary structure and anti-tumor activity of a novel peptide from stem and leaves of mistletoe (*V*. *coloratum* (Kom.) Nakai) was examined on the rat osteoblast-like sarcoma 17/2.8 cells. [127] The primary structure of the peptide named as viscotoxin B2 was KSCCKNTTGRNIYNTCRFAGGSRERCAKLSGCKIISASTCPSDYPK and its IC_{50} value was 1.6 mg/L^{-1}. Viscin, betulinic acid, oleanolic acid and ursolic acid are lipophilic compounds of *V*. *album* and found to inhibit growth and induce apoptotic cell death in Molt4, K562 and U937 leukaemia cells. However, the growth inhibitory effect of viscin was more prominent in Molt4 and U937 cells with IC_{50} values 118±24 and 138±24 µg/mL respectively. [48]

The VCA was shown to induce apoptosis by decreasing Bcl-2 level and telomerase activity and by inducing of Bax through p53- and p21-independent pathway in hepatoma cells. Later on, the induction of apoptosis via activation of caspase-3 and the inhibition of telomerase activity through transcriptional suppression of hTERT in the VCA-treated A253 cells was

reported. [128] Treatment of VCA also induced apoptosis in both SK-Hep-1 (+p53) and Hep 3B (-p53) cells through p53- and p21-independent pathways. Apoptosis induction was related to down-regulation of Bcl-2 and up-regulation of Bax functioning upstream of caspase-3. Moreover, VCA caused down-regulation of telomerase activity in both cells. [91] Signaling through lectins could involve modulation of protein kinase activities. However, the alpha/beta-galactoside-binding lectins, isolated from mistletoe leaves, did not inhibit the epidermal growth factor receptor tyrosine kinase activity of rat liver. [129]

Administration of lectin (KML-C) from Korean mistletoe (20-50 ng/mouse) for 2 days by intravenous route before tumor implantation significantly reduced lung metastases of B16-BL6 and colon 26-M3.1 cells. Importantly, KML-C treatment one-day post-tumor implantation not only significantly suppressed lung metastasis of B16-BL6 and 26-M3.1 cells, but also reduced liver and spleen metastasis of L5178Y-ML25 lymphoma cells. Mechanistically, it was found that KML-C treatment (50 ng/mouse) for 2 days significantly increased NK cell-mediated cytotoxicity against tumor cells and tumoricidal activity of peritoneal macrophages. [130] Similar results were also observed with Korean mistletoe extract KM-110, wherein administration of KM-110 (100 μg/mouse) for 2 days by intravenous route before tumor implantation significantly blocked lung metastases of B16-BL6 and 26-M3.1 cells, and liver and spleen metastasis of L5178Y-ML25 cells. This effect on tumor metastasis was also mediated by NK cell activation. [131] Additionally, multiple administration of KM-110 into tumour-bearing mice resulted in significant inhibition of primary tumour growth.[131] Administration of the Iscador (1 mg Iscador/dose, IP) inhibited lung metastasis of B16F10 melanoma cells in mice by reducing nodule formation (92%) and enhanced the life-span (71%) of animals. [132] The IC_{50} was found to be 0.0166 mg Iscador/dose. However, galactoside-specific mistletoe lectin failed to inhibit N-methyl-N-nitrosourea-induced tumor development in the urinary bladder of rats and to mediate a local cellular immune

567 response following long-term administration. [133] Therefore, anti-tumoral functions are not
568 mediated by all lectin preparations of mistletoe.

569 Intravenous treatment with a standardized mistletoe extract at 3, 30 or 150 ng/kg doses
570 once daily for 3 weeks exerted inhibitory effects (58 to 95%) on the lung metastasis of B16. A
571 significant reduction in the percentage of bronchoalveolar lavage pigmented cells was also
572 noticed. [134] The study conducted by Srdic-Rajic and co-workers investigated synergistic anti-
573 tumor effects of *V. album* and doxorubicin chemotherapeutic agent on chemo-resistant chronic
574 myelogenic leukemia K562 cells. Authors found that *V. album* enhanced the anti-leukemic
575 efficiency of doxorubicin against chemo-resistant K562 cells by checking the G2/M arrest and by
576 stimulating apoptosis. [135]

577

578 *5.7. Anti-diabetic*

579 *V*. *album* has been well-documented as a traditional treatment for diabetes. The Korean
580 mistletoe *V*. *album* var. *coloratum* enhanced the insulin secretion from pancreatic β-cells without
581 any cytotoxicity effects. Moreover, upregulated pattern of insulin genes such as PDX-1 and
582 β2/neuroD was also observed in *V*. *album* var. *coloratum*-treated mice. Thus, VAC could be
583 considered as a useful source for the development of antidiabetic drug to reduce blood glucose
584 level of type I diabetic patients. [136] Structural analysis of ML-1 complexed with galactose and
585 lactose revealed unique sugar binding abilities. [137] Among the medicinal plants, *V*. *album*
586 showed potent alpha-glucosidase inhibitory activity. [138] The aqueous extract of mistletoe (1-10
587 mg/mL) was reported to stimulate secretion of insulin (1.1- to 12.2-fold) from clonal pancreatic
588 β-cells. The ability of extract to enhance insulin secretion was not mediated by lectins. [139] The
589 results indicated the presence of insulin-releasing natural product(s), which might contribute to
590 the reported anti-diabetic property of the mistletoe.

591

592 *5.8. Anti-hypertensive*

593 Acute effect of different extracts of mistletoe stem on arterial blood pressure was studied in
594 Wistar rats. [140] The ethanol extract showed the superlative effect even at the lowest applied
595 concentration (3.33×10^{-5} mg kg^{-1}) and significantly reduced the blood pressure after applied
596 concentration 1.00×10^{-3} mg kg^{-1}). However, other extracts such as ether and ethyl acetate
597 showed the activity only at higher concentrations.

598

599 *5.9. Anti-microbial*

600 Methanol extract of *V*. *album* showed anti-microbial activity against 9 out of 32 pathogenic
601 microorganisms. [141] Different extracts from the leaves of *V*. *album* L. ssp. *album* were prepared
602 and analyzed for their effect on human parainfluenza virus type 2 (HPIV-2) growth in Vero cells.
603 [142] The aqueous extract (1 µg/mL) was observed to prevent HPIV-2 replication and that virus
604 production was inhibited >99% without any toxic effect on host cells. This activity could neither
605 be credited to the direct HPIV-2 inactivation nor to the inhibition of adsorption to Vero cells. Five
606 patients with chronic hepatitis C showed 6-20% reduction in the viral load and normalization of
607 liver inflammation (two patients) without side effects following treatment with Iscador for one
608 year.[143] Two other patients were also in complete remission of their elevated aspartate
609 transaminase and alanine transaminase. However, IFN-γ increase in the serum of HIV-positive
610 and healthy subjects was not noticed following subcutaneous injection of a non-fermented *V*.
611 *album* extracts. [144]

612

613 *5.10. Anti-mutagenic*

614 *V*. *album* var. *coloratum* was evaluated for its anti-mutagenic activity against the mutagens such
615 as 2-aminoanthracene (2AA) and furylfuramide-2 for *Salmonella typhimurium* strain TA98, and

sodium azide (NaN_3) and 2AA for *S. typhimurium* strain TA100 using Ames test. *V*. *album* var. *coloratum* was more effective in preventing the mutagenicity of the indirect-acting mutagen 2-AA, when tested with both the strains. [145]

5.11. Miscellaneous properties

European mistletoe is known for its anti-cancer and immune enhancing activities but few data exist on anti-convulsant activity. Treatment of *V*. *album* managed refractory childhood absence epilepsy of a 4.5-year old girl. [146] *V*. *album* lipophilic extract (10 µg/mL) and its oleanolic acid (1 µg/mL) have shown excellent wound healing activity. It was associated with the stimulation of migration of NIH/3T3 fibroblasts. [147] Administration of *V*. *album* var. *coloratum* (50 µg/mL) increased the mean survival time by 9.61 and 19.86 % in *Caenorhabditis elegans* and *Drosophila melanogaster*, respectively. [148] Treatment with *V*. *album* var. *coloratum* extract (3 g/kg/day) had an anti-obesity effect and protected against hepatic steatosis in mice with high-fat diet-induced obesity. The effects appear to be mediated through an increased mitochondrial activity. [149] The KME induced mitochondrial activity possibly by activating PGC-1α and SIRT1, and improved the endurance of mice. Authors also strongly suggested that KME could be used as a novel mitochondria-activating agent. [150] A pre-clinical study suggested that aqueous extract of *V*. *album* leaves exhibits sedative, anti-epileptic and anti-psychotic activities in mice and rats.[2]

To find out the promising pancreatic lipase (triacylglycerol acylhydrolase) suppressors from natural products, 61 medicinal plants from Korea were tested for their anti-lipase activity for prevention of obesity. The *V*. *album* extracts showed anti-lipase activity with IC_{50} values of 33.3 µg/mL and 35.15 µg/mL for anti- phosphodiesterase. [151] Aqueous extract of *V*. *album* decreased the serum cholesterol and HDL-cholesterol, triglyceride concentrations in the mice fed with high-cholesterol diet without inducing any gastric damage, suggesting potent hypocholesterolaemic activity. [152] The aqueous extract of *V*. *album* leaves exhibited a significant coronary vasodilator

activity on the Langendorff's isolated and perfused heart model. Authors also suggested that extract contains some bioactivity constituents that may act as inducers of the nitric oxide/soluble guanylate cyclase pathway. [153] Formation of lactose-resistant aggregates of human platelets induced and differential signaling responses to cell contact formation by the ML was also detected. [154]

6. Clinical trials

Although mistletoe preparations are currently being used in different clinical settings, the most important clinical use has been in the field of cancer as a complementary therapy to reduce the adverse reactions of conventional chemotherapies. In fact, mistletoe extract therapy is among the most thoroughly studied complementary treatments in Europe. Several systematic reviews and meta-analysis have found a benefit from mistletoe treatment in cancer patients and in minimizing the side effects of anticancer chemotherapy.[155-159] However, these reviews have also found that nearly all studies suffered from methodological shortcomings to some degree, and many of the studies were not conclusive. Earlier review had found that even statistical pooling is not possible because of the heterogeneity of the primary studies, [160] therefore only a narrative systematic review was conducted.

Furthermore, a Cochrane review was done with the objective to determine the effectiveness, tolerability and safety of mistletoe extracts either as a monotherapy or administered as an adjunct to conventional cancer treatment.[161] Cochrane reviewers have found that from 80 mistletoe studies examined for the purpose of assessing mistletoe therapy in oncology 58 had no prospective trial design with randomized treatment allocation and were excluded from the analysis. Although 6 trials among 13 that investigated survival upon mistletoe therapy showed certain evidence of therapeutic benefit, none of them met with high methodological quality.

Among 16 trials that explored the efficacy of mistletoe extracts for either improved quality of life (QOL), psychological parameters, performance index, symptom scales or the reduction of adverse effects of chemotherapy, only 2 of them were of a superior methodological quality.[161] Thus, the overall conclusion was that independent clinical research of superior quality is required to accurately assess the safety and therapeutic effects of mistletoe extracts.

A study published by Gerhard et al. in 2004 illustrated the difficulties in enrolment and randomization of cancer patients for the therapy with mistletoe. [162] Among 1,922 patients who were operated for breast tumor, 154 patients who met the inclusion criteria agreed to participate in the study. However, 80 patients were subsequently excluded from the study following evaluation of the final results on tumor staging and conventional treatment plan. This study suggested that only 29 (39%) of the remaining 74 patients would have agreed to participate in a randomized trial on mistletoe therapy for breast cancer. However, several randomized clinical trials assessing safety and effectiveness of mistletoe preparations have been published in recent years providing clear evidence for improved survival and QOL of cancer patients treated with mistletoe preparations.

6. 1 Pancreatic cancer

Two hundred and twenty patients with inoperable or metastatic pancreatic cancer were included in a prospective randomized clinical trial. Hundred and ten patients received Iscador® and remaining 110 patients received no anti-neoplastic therapy. Patients treated with Iscador® survived better (4.8 months) than the control group (2.7 months) (HR=0.55; p=0.0031). Importantly, no therapy related adverse events reported in the Iscador group. *V. album* therapy thus exhibited a significant and clinically relevant prolongation of overall survival. [163] In the same single-center, group-sequential, randomized phase III trial (ISRCTN70760582), [164] data on QOL

691 and body weight were obtained from 96 patients treated with mistletoe and 72 control patients.
692 Patients treated with mistletoe performed better on all the 6 functional scales and on 7 of the 9
693 symptom scales (EORTC QLQ-C30), including pain (95% confidence interval [CI] -29 to -17),
694 fatigue (95% CI -36.1 to -25.0), appetite loss (95% CI -51 to -36.7), and insomnia (95% CI -45.8
695 to -28.6). This was reflected by the body weight trend of the patients during the study period.
696 The results indicated that mistletoe treatment significantly improves the QOL in comparison to
697 best supportive care alone. [164] The study suggested that *V. album* is non-toxic and effective
698 second-line therapy for patients with locally advanced or metastatic pancreatic cancer.

699

700 *6.2. Breast cancer*

701 A prospective randomized open label pilot study on 95 breast cancer patients showed an
702 improvement of QOL when treated with a combination of chemotherapeutic agents
703 cyclophosphamide, adriamycin and 5-fluoro-uracil (CAF) and Iscador® M special (IMS). The
704 control group received only CAF. [165] A descriptive analysis of all 15 scores of the EORTC-QLQ-
705 C30 displayed better QOL in the IMS group compared to the control group. Significant
706 differences were observed among 12 scores ($p < 0.02$) and clinically pertinent and significant
707 difference of minimum 5 points were noticed in nine scores. IMS group showed a trend of lower
708 frequency of CAF-induced neutropenia. This pilot study thus showed the importance of IMS to
709 improve the QOL of the patients treated with CAF. A five-year follow-up study suggested that
710 adding *V. album* during chemotherapy of early stage breast cancer patients does not influence
711 the frequency of relapse or metastasis within 5 years. [166]

712

713 *6.3. Bone cancer*

714 A recent randomized study investigated post second metastatic relapse (12 month)
715 disease-free survival rate in osteosarcoma patients following treatment with *Viscum sc* or oral

716 Etoposide (a topoisomerase inhibitor anticancer drug). Twenty patients with a median age of 34
717 years (ranging 11-65 years) were enrolled and were treated randomly with *Viscum* sc or oral
718 Etoposide. Patients were monitored for a median follow-up time of 38.5 months (3-73). The
719 median PRDSF in the oral Etoposide was 4 months (1-47) and it was 39 months (2-73) in the
720 *Viscum* group. Also, because of lower toxicity, *Viscum*-treated patients reported a higher QOL.
721 [167] However, authors have also suggested that a larger study is obligatory for the firm
722 determination of the efficacy and immunomodulatory mechanisms of *Viscum* therapy in
723 osteosarcoma.
724
725 *6.4. Lung Cancer*
726 A randomized phase II study was conducted in chemotherapy-naïve advanced non-
727 small-cell lung cancer (NSCLC) patients to evaluate the influence of Iscador therapy on
728 carboplatin-containing treatments-related side-effects and QOL. Seventy-two patients were
729 registered for this study with 39 patients for control and 33 for Iscador. Majority of the patients
730 (65%) were in stage IV and had squamous histology (62%). Iscador therapy did not modify the
731 overall survival of the patients and median overall survival was 11 months in both the groups.
732 Although not significant, Iscador group showed a tendency of higher TTP. Median TTP was 4.8
733 months for the controls and 6 months in the Iscador. Grade 3-4 hematological toxicities were
734 similar between both the groups. However, patients in the control group had significantly higher
735 chemotherapy dose reductions (44% vs 13%, p=0.005), grade 3-4 non-hematological toxicities
736 (41% vs 16%, p=0.043) and hospitalizations (54% vs 24%, p=0.016), suggesting that Iscador
737 reduces the chemotherapy-related toxicity. Additional clinical trials are required to cinfirm and
738 validate these results. [168]
739 A multi-center, randomized, open, prospective clinical trial was conducted to assess the
740 impact of standardized mistletoe extract (sME) therapy on QOL in various types of cancers. [169]
741 The study enrolled 233 patients with NSCL (n=94), breast (n=68) and ovarian (n=71). The 224

742 patients who fulfilled all the criteria were grouped into two. One hundred and fifteen patients
743 were treated with sME HELIXOR A and 109 control group patients were treated with the
744 approved immunomodulating phytopharmacon Lentinan. All the patients with treated with sME
745 or Lentinan complimentary therapy during chemotherapy regimen. QOL was determined by the
746 Functional Living Index-Cance, Traditional Chinese Medicine Index and the Karnofsky
747 Performance Index. Authors found that patients complementarily treated with sME had
748 significantly improved QOL ($p<0.05$) as compared to control group. Adverse effects were also
749 less frequent and self-limiting in sME-treated patients.[169] This trial suggested that
750 complementary sME therapy can improve the QOL in cancer patients by reducing the side-
751 effects of chemotherapy.
752
753 *6.5. Advanced solid tumors*
754 The phase I study of gemcitabine (GEM) and *V. album* in patients with advanced solid
755 cancers (ASC) was conducted for the evaluation of safety, toxicity, and maximum tolerated dose
756 (MTD); absolute neutrophil count (ANC) recovery; formation of mistletoe lectin antibodies (ML
757 ab); plasma cytokine concentrations; clinical response; and pharmacokinetics of GEM. [170] Forty
758 four patients with advanced pancreatic, non-small cell lung cancer (NSCLC), recurrent
759 metastatic colorectal or breast cancer was included. In the first stage, increasing does of *V.*
760 *album* and fixed dose GEM dose was used. In the second stage, increasing does of *GEM* and
761 fixed dose *V. album* dose was used. This study found that all the patients showed immune
762 response to mistletoe injections as determined by ML3 IgG Abs. Compliance with mistletoe
763 therapy was high and the median survival was 200 days with % of partial response in 6%
764 patients and stable disease in 42%. Dose-limiting toxicities attributed to *V. album* were G4
765 neutropenia, G4 thrombocytopenia, G4 acute renal failure, and G3 cellulitis. MTD was GEM
766 1300 mg/m^2 and mistletoe 250 mg combined and *V. album* did not affect pharmacokinetics of
767 GEM. This study indicated that combined GEM and *V.* album is well tolerated by patients with

advanced sold tumors. Clinical response in the group received combination therapy was similar to GEM alone treated patients.

6.6. Cancer-related fatigue

Although not examined in a randomized clinical trial, the use of mistletoe preparations has shown an improvement of cancer-related fatigue. [171] The fatigue levels among 324 patients with non-metastasized colorectal cancer (UICC stage I-III) during the first-line chemo- or radio-chemotherapy protocols were assessed. Iscador(®) Qu was given to 181 patients compared to control group of 143 patients without this supportive care treatment. At the end of the median treatment period, CRF was diagnosed in 16 patients (8.8%) treated with Iscador(®) Qu and was 60.1% in chemo- or radio-chemotherapy group without Iscador(®) Qu. Multivariable-adjusted OR = 10.651 (95% CI 5.09-22.28; $p < 0.001$) at the first visit was dropped to OR = 0.054 (95 CI 0.02-0.13; $p < 0.001$) at the end of therapy.

7. Toxicological studies

For the development of remedies in general, knowledge of toxicology is crucial to confirm drug safety. Cancer patients who received subcutaneous injections of mistletoe extracts were examined for adverse drug reactions (ADRs). Out of 1923 patients, 14.7% patients reported local reactions less than 5 cm and raised body temperature less than 38°C. Among 162 patients who reported ADRs, these reactions were mild (50.8%) to moderate (45.1%) in majority of the patients. Only 4.2% patients reported severe ADR. There were no recognizable risk factors for ADRs. The ADR rate augmented as the dose of mistletoe increased. However, patients receiving concurrent conventional therapies reported less ADRs during mistletoe therapy. The study indicated that mistletoe therapy is safe. [172] In another report, SC injections of ML-1 (1mg/kg, weekly twice) for a month period resulted in significant

enhancement of several acute phase reactants including C-reactive protein, haptoglobin and complement component C3. [173] Viscotoxins were also shown to stimulate the generation of reactive oxygen species in human lymphocytes, as well as cell death.[174] Altogether, mistletoe preparations at high doses were reported to cause hypotension, pupil contraction, vomiting, intestinal cramps, diarrhea and seizures. In addition to pain and irritation at the site of injection, SC route of administration of mistletoe can also trigger mild to severe headaches, chills, angina, fever and allergic reactions.

Experimental studies have shown that mistletoe viscotoxins and phoratoxin effects on the circulation were responsible for the reflex bradycardia, negative inotropic effects and vasoconstriction observed in the cardiac muscles of cats.[21] In addition, viscotoxins reduced isometric twitch and caused contracture and progressive depolarization in rabbit heart preparations.[21, 175] It was also proposed that phenylpropanoids might mediate cardiovascular effects by suppressing cAMP phosphodiesterase.[176] Therefore, use mistletoe in patients with cardiovascular diseases requires caution. A systemic review on the safety of mistletoe in animals and humans revealed that this therapy is not associated with immunosuppression. The side effects were mostly dose-dependent flu-like symptoms, local reactions at the site of injection of mistletoe and miscellaneous mild effects. Some reports of allergic reactions and reversible hepatotoxicity with high doses of recombinant ML were also recorded. [177]

Toxicity of oral mistletoe exposure is controversially discussed. In 1952, Winterfeld stated that oral application of powdered *V. album* extracts or drops were well tolerated and did never induce toxic reactions. [178] Further, it was also observed that consumption of berries up to three or one to two leaves of American mistletoe Phoradendron serotinum (Loranthaceae) seems unlikely to cause severe toxicity. [179] However, consumption of a herbal product

containing mistletoe as one of the ingredients caused hepatitis in a women. [180] However, the role of mistletoe was not proved in this case. Weeks and Proper also reported a case of chronic active hepatitis after ingestion of a herbal remedy containing mistletoe, skullcap, valerian and other plants. Again, this study could not prove mistletoe as an underlying cause. [181]

Toxicity of Iscador and a purified protein fraction of *V. album* by parenteral route were examined *in vivo* in mice. [182] Authors could observe long-term toxicity only with the purified but not well-characterized proteins. Mortality accompanying with liver atrophy and other organs involved in metabolism, and thymus disintegration was recorded 3-4 days post *V. album* injection. However, 5-10% of the LD_{50} concentrations of these proteins caused enlarged spleen and thymus. However, precise concentration of the ingredients in the injected preparation was not known in this study. Subsequent study by Rentea and colleagues tried to determine the LD_{50} of a Iscador by using precise concentration of the product. [183] They found LD_{50} dose following IP injection varies among different strains and species of animals. Thus, LD_{50} was 700 mg/kg for CD-1 outbred albino mice; 348 mg/kg for C57/BL6 mice and 378 mg/kg for Sprague-Dwaley rats. These animals at lethal doses showed hemorrhagic peritonitis and died with tonic and clonic seizures. On the other hand, LD_{50} of VA-E (*Iscador* Mali) in mice was lower (168 mg/kg). [184] However, LD_{50} of VA-E (*Iscador* Quercus) in mice was at higher range: 500 mg/kg by i.v. route and 1200 mg/kg by SC route. [185] Studies in the animals did not give any indications on the adverse effects of mistletoe on the reproduction and genotoxic effects[177]

Administration of high dose VA-E (*Lektinol*) at 100 mg/kg caused mortality of all the rats within 5 min.[186] These animals experienced dyspnea, ataxia, sedation, exophthalmos and spasms. However at 25 mg/kg, animals showed dyspnea and sedation with no death. The sub-chronic toxic doses of 0.2, 1.5 and 5 mg/kg for 4 weeks did not reveal any organ toxicity. [186]

Toxic effects of purified components of mistletoe were also been explored. In mice, the LD_{50} of ML-1 was found to be 80 µg/kg. [187] Another report suggested that LD_{50} of ML-1 and ML-

3 were 28 and 49 mg/kg, respectively. [185] But the lectin activity and route of application were not clear in these reports. Subsequent study however reported lower LD_{50} values when different lectins were injected IP route: ML-1: 28 µg/kg, ML-2: 1.5 µg/kg and ML-3: 55 µg/kg. [24] ML-1 in rat was lethal within 24 hours at 100 µg/kg by IP route. However, 10 µg/kg caused mortality in 3-4 days. [188] These animals experienced pancreatic hemorrhages, ascites, and congested intestine and these symptoms were similar to those observed with ricin. Thus, toxicological values for ML preparations showed large variations probably due to differences in the methods that calculate lectin activity. High production of TNFα and hemagglutinating activity of the lectins were proposed as underlying mechanisms of ML toxicity. [189]

8. Conclusions and perspectives

V. *album*, a plant that has been described from mythological times as a potent remedy for several pathologies continues to evoke interest and scientific curiosity among researchers. Even after a century after its introduction as a treatment for cancer, the clinical use as a component of supportive care, and knowledge on the mechanisms of action of mistletoe continue to expand. Over hundred clinical studies have provided evidence in support of the beneficial effects of mistletoe in cancer patients and mistletoe thus remains as one of the remedies most often used. The results from several randomized clinical trials suggested that mistletoe preparations are safe and improved overall survival and QOL of cancer patients.

Our essay is focused on the active components of mistletoe extracts and pluripotent biological activities. A wide spectrum of pharmacologically active metabolites that belong to a variety of chemical entities of proteins, polysaccharides, liposoluble compounds, and secondary metabolites have been identified in V. *album* extracts. It is conceivable that the heterogenous profile of biochemical compounds provides the basis to the broad diversity of pharmacological activities of mistletoe as each single component contributes diverse modes of actions in addition

to imparting to a synergistic beneficial action in conjunction with other molecules. Although a large number of the anti-tumoral properties of mistletoe preparations have been attributed to the lectins, it is possible that enlarging the scope of research to other components especially polyphenols would open new perspectives.

Although a number of elegant pre-clinical studies and numerous powerful clinical trials have provided ample lines of evidence in favor of potent anti-cancer activity of mistletoe if used as concomitant therapeutics in parallel to standard therapy, the field is plagued by a certain degree of skepticism. This may be due to homeopathic origin of the therapy, or inconclusive beneficial effects in Cochrane review or highly scattered technically sound scientific reports of exploring the molecular and cellular mechanisms underlying the beneficial effects of mistletoe in cancer patients. Thus, further studies examining the results of *V*. *album* extracts in the adjunct therapy of cancer should aim at evidence-based clinical data, superior quality, transparent study-design and clear end-points to deliver higher perceptiveness into a supportive therapy that is frequently disapproved as ineffective. Such careful analysis of the effects of *V*. *album* should help in clarifying certain skepticism clouding over its use, and provide more effective pointers to the clinicians in adopting appropriate treatment regimes.

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Figure caption

Figure 1. Molecular targets of *V. album* for different biological properties.

Table 1

Chemical constituents of *V. album*

S.No.	Chemical constituents	Content	Source	References
1	**Viscotoxins**	0.05-0.1%		18
	Isoforms: A1, A2, A3, B, B2, C1 & 1-PS		Leaves Stem	17
2	**Lectins**	0.34-1.0 mg/g dried material		22, 24-26
	Isoforms: ML-I, ML-II, & ML-III		Older stems	
3.	**Carbohydrates**	44-58%/dry wt.	Leaves Stem Berries	28
	Methylated homogalacturonan		Stem	30
	Pectin		Berries Leaves	18
	1→α4 galacturonic acid methyl ester		Berries	29
	Arabinogalactan		Berries	28
5.	Polyphenols and phenylpropanoids	50-85 mg/100 g dry wt.	Leaves Stem Berries	33
	Flavonoids			35, 39
	Phenolic acids			33
	Lignans			40
	Kalopanaxin D			43

4.	**Vitamin C**	750 mg/100g fresh wt.	Leaves Berries	18
5.	**Proteins**	9.3%	Leaves Stem Berries	136
6.	**Lipophilic compounds**	-		
	Terpenoids		Leaves Stem Berries	47
	Phytosterols		Berries	46
	Saturated fatty acids		Leaves Stem	48, 49 50
7.	**Inorganic elements**			
	Potassium, calcium, manganese, sodium, nickel, phosphate, selenium, silica, magnesium, and zinc	-	Leaves Stem	18
8.	**Others**			
	Cyclic peptides, alkaloids, amines (histamine and cetylcholine), jasmonic acid, cysteine, glutathione, and xanthophyll	-	Leaves Stem Berries	54

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Table 2

Pharmacological properties of *V*. *album* and its bioactive compounds

Bioactivity	Extract/constituent	Model system	Mechanism	Dose	References
Antioxidant activity					
	Extracts of V. album grown on lime tree or white locust tree	HeLa cells	Inhibits mitochondrial DNA damage induced by H_2O_2	10 µg/mL for 48 h	110
	Organic extracts	*In-vitro*	Show anti-glycation and antioxidant properties		38
	Lectin	LLC-PK(1) cells	Exhibits free radical scavenging, expression of cyclooxygenase-2, inducible NO synthase, SIN-1-induced nuclear factor kappa B and the phosphorylation of inhibitor kappa B alpha	IC_{50} 42.6 µg/mL	111
	Methanolic extract	Rats	Shows DPPH radical scavenging and anti-lipid peroxidation activities	500 mg/kg	112
Anti-inflammatory	Preparation (VA Qu Spez)	A549 cells	Inhibits prostaglandin E2, by selectively inhibiting COX-2	100 µg/mL	6
	Preparation (VA Qu Spez)	A549 cells	Reduces COX-2 mRNA half-life	50 µg/mL	4
	flavonoids	Rats	Inhibit carrageenan-induced hind paw edema without any toxic effects	30 mg/kg	60
	Preparation (Isorel)	Mice	Triggers abundant tumour necrosis with inflammatory response, oedema and destruction of the malignant tissue	100 mg/kg	61
Immunomodulatory					
	Preparation (QU FrF)	B16 mouse melanoma	Abrogates IL-12 expression	20 µg/mouse/day	190

VA Qu Spez	Dendritic cells	Stimulates proliferation of CD^{4+} T cells	5-15 µg/mL	69
N-acetyl-*D*-galactosamine-specific lectin	T cells	inhibits 3000 immune functions-regulating genes	600 ng/mL	70
KME	*Epinephelus bruneus*	Enhances phagocytic activity	1% and 2%	71
Aviscumine	*In-vivo* and clinical phase I studies	Activates immune system	1.5 mg/kg/day	72
KML	Tumoral implantation	Modulates lymphocytes, natural killer cells, and macrophages		74
VCA	Murine splenocytes	Decreases interferon (IFN)-gamma secretion	4-64 ng/mL	80
VCA	hPBMC cells T-lymphocytes	Releases IL-1α, IL-1β, IL-6, IL-8, and IFN-γ	4-16 pg/mL 4-32 pg/mL	62
Lectin	T-lymphocytes	Enhances expression of IL-1 alpha, IL-1 beta, IL-6, IL-10, TNF-α, interferon-gamma, and granulocyte-monocyte colony stimulating factor genes	1-8 ng/mL	62
ML-I	Peripheral blood mononuclear cells	Induces cytokines gene expression and protein production	1 ng/mL	82
ML-I	Peripheral blood mononuclear cells	Induces IL-6 and TNF-alpha production	10 ng/mL	82
VAA extract	Epithelial cells	Stimulates the levels of CD4(+)CD25(+) and CD8(+)CD25(+) T cells and CD3(-)CD16(+)CD56(+) natural killer cells	10% ethanolic extract	83
VTA1 (85 nm), VTA2 (18 nm) and VTA3	K562 and NK effector cells	Increase natural killer cell-mediated cytotoxicity	6-25 nM	85

	Viscotoxins	Rats	Enhance phagocytosis and burst activity against *E. coli* infection	25 and 250 µg/mL	86
	Aqueous extract	Human trail	Induces the secretion of Th1- (IFN-γ) or Th2- (IL-4)		87
	rVAA	Rat splenocytes	Enhances the secretion of an active form of IL-12	100 pg/mL	89
	Iscador Pini	Peripheral blood mononuclear cells	Activates T-helper cells (CD4+)	0.1-1.0 mg/mL	88
Cytotoxicity	Mistletoe lectin	SK-Hep-1 (p53-positive) and Hep 3B (p53-negative) cells	Induces apoptosis by stimulating mitochondrial membrane potential (MMP) breakdown and stimulating caspase-3	10-50 ng/mL	91
	VA Qu FrF	CEM, HL-60 and MM-6 cells	Reveals cell cytotoxicity	100-200 µg/mL	92
	Viscotoxin-free *V. album* extract		Enhances granulocyte activity		93
	Preparations	Daoy, D342, D425 and UW-288-2 cells	Induces cytotoxicity by reducing mitochondrial activity	50 mg/mL	94
	MLI, MLII, and MLIII	Molt 4 cells	Exhibits cytotoxic activity		95
	Viscotoxins and alkaloids	Tumor MSV cells	Show cytotoxicity	10 µg/mL	97
	MLI		Exhibits cytotoxicity		96
	KML-C	Human and murine tumor cells	Shows strong cytotoxicity by inducing apoptotic cell death	0.4-307 mg/mL	98
Anti-angiogenic					
	ME	B16L6 melanoma cells	Suppresses tumor growth and metastasis by elevating fragmentation and nuclear morphological changes	100 ng/mL	191

VA QU FrF	Human umbilical vein endothelial and immortalized human venous endothelial cells	Induces apoptosis	12.5-50 µg/mL	12
FME	Glioblastoma cells	Regulates cytokine TGF-β and matrix-metallo-proteinases central genes expression	100 µl/mL	100
VAA-I	PLB-985 and chronic granulomatous disease cells	Induces apoptosis via caspase activation	1 mg/mL	101
VAA-I	LPS-treated human neutrophils and murine neutrophils	Activates apoptosis	1-100 ng/mL	102
VAA-I	Human neutrophils	Induces apoptosis via acceleration the loss of antiapoptotic Mcl-1 expression and the degradation of cytoskeletal paxillin and vimentin proteins	1-10 mg/mL	103
IscadorQu	Endothelial cell cultures	Causes early cell cycle inhibition followed by apoptosis		104
Epi-oleanolic acid	Human and marine cancer cells	Activates apoptotic cell death, characterized by morphological changes and DNA fragmentation	4, 20, and 100 µg/mL	105
VCA	Hepatocarcinoma Hep3B cells	Induces apoptosis by increasing ROS production and a loss of DeltaPsim	20 ng/mL	106
β-galactoside, N-acetyl-D-galactosamine-specific lectin II, polysaccharides, viscotoxin	U937 cells	Induce apoptosis through activation of the phosphotransferase activity of c-Jun N-terminal kinase 1 (JNK1)/stress-activated protein kinase (SAPK)	100 ng/mL	107

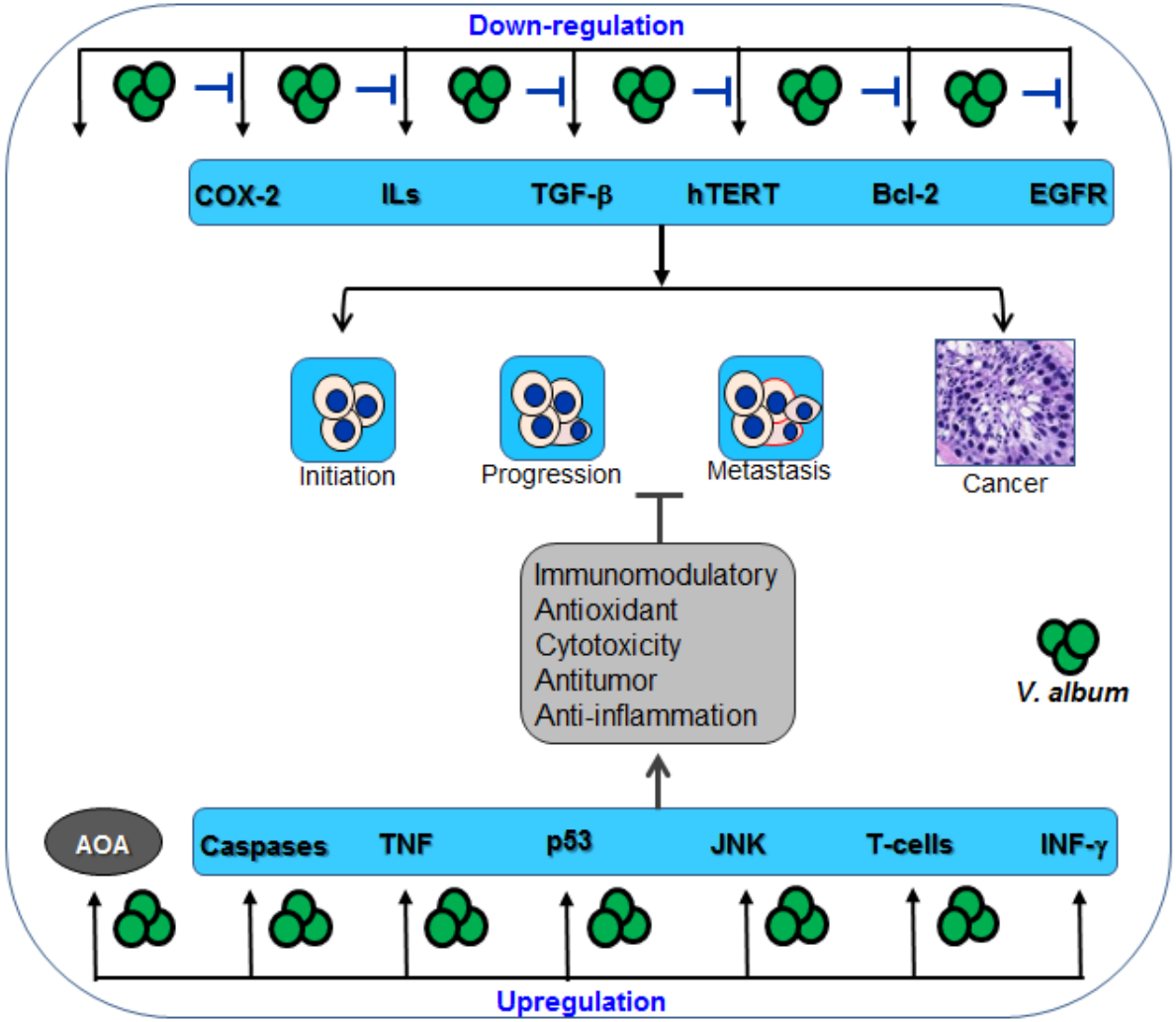
	Viscotoxins	Lymphocytes	Induce cell death by producing mitochondrial Apo2.7 molecules and by generating ROS-intermediates	100 ng/mL	174
Anti-tumoral					
	Aqueous extract	Cancer cells	Depletion of hypoxanthine concentration and xanthine oxidase activation		113
	Lipophilic extract and its predominant triterpene oleanolic acid	Tumour cells	Decreases MCP-1 induced monocyte transmigration	25 µg/mL	114
	Ethanolic extract containing viscotoxin	Swiss female mice	Enhances the anti-tumor effect of doxorubicin	50 mg/kg	115
	VAE	C6 glioma cells	Induces apoptosis by activating caspase-mediated pathway	100 µg/mL	116
	VAA-1	Lung carcinoma A549	Enhances anti-proliferating potential of cycloheximide by inducing G1-phase accumulation		117
	Synergistic effect of mistletoe extract and its components	U937 cells	Induce apoptosis via activation of caspase cascades	100 ng/mL	118
	ML-II	U937 cells	Enhances apoptotic response through augmentation of Fas/Fas L expression and caspase activation	100 ng/mL	119
	ML-III	Tumour cell lines and human lymphocytes	Reduce the expression of nuclear p53 and Bcl-2		120
	Oleanolic acid	HaCaT cells	Induces apoptosis by altering cellular morphology as well as DNA integrity	12.5-200 µM	121

Either solubilized triterpene acids or lectins and combinations thereof	Acute lymphoblastic leukaemia cell line NALM-6	Induce dose-dependent apoptosis via caspase-dependent pathways	8 ng/mL	122
VE from apple and pine	Human macrophages	Increases anti-tumoral activity of activated human macrophages by inducing the production of NO		123
Lectin containing Iscador M Spezial and Iscador Qu Spezial	Mammary cancer MAXF 401NL cells	70% growth inhibition	15 µg/mL	124, 125
VAE	Bladder carcinoma T24, TCCSUP, J82 and UM-UC3 cell lines	Induces necrosis and apoptotic cell death	10-1000 µg/mL	126
Viscotoxin B2	Rat Osteoblast-like Sarcoma 17/2.8 cells	Exhibits antitumor activity	IC_{50} 1.6 mg/L	127
Viscin	Molt4 U937 leukaemia cells	Inhibits growth and induce apoptotic cell death	IC_{50} 118 $\pm$ 24 &138 $\pm$ 24 µg/mL	48
VAC	Hepatoma cells	Induces apoptosis by decreasing Bcl-2 level and telomerase activity and by inducing of Bax	10 ng/mL	128
Lectins	Rat liver	Modulate protein kinase activities	100 µg/mL	129
Lectin	Yac-1 tumor cells	Increases natural killer-mediated cytotoxicity	50 ng/mouse	130
KM-110	B16-BL6, 26-M3.1, L5178Y-ML25 cells	Inhibits lung metastasis	100 µg/mouse	131

	Iscador	Melanoma cells in mice	Inhibits lung metastasis by reducing nodule formation (92%) and by enhancing a life span (71%)	IC_{50} 0.0166 mg/dose	132
	ME	Mice	Inhibits pulmonary metastatic colonization	3, 30 or 150 ng/kg	133
	KM-110	L5178Y-ML25 lymphoma cells	inhibit liver and spleen metastasis	100 µg/mL	131
Anti-diabetic					
	VAC	Mice	Enhances the insulin secretion from the pancreatic β-cell without any effects of cytotoxicity	2 mg/mL	55
	VAC	Mice	Upregulates pattern of insulin genes such as PDX-1 and β2/neuroD	2 mg/mL	55
	ML-I		Mimics the sugar compound		137
	VE		Shows potent alpha-glucosidase inhibitory activity	IC_{50} 10.1 mg/mL	138
	Aqueous extract		Stimulate secretion of insulin (1.1 to 12.2-fold) from clonal pancreatic B-cells	1-10 mg/mL	139
Anti-hypertensive					
	ethanol extract	Wistar rats	Reduces the blood pressure	$3.33x10^{-5}$ mg kg^{-1}	140
Anti-microbial					
	Methanolic extract	Pathogenic microorganisms	Shows antimicrobial activity		141
	Aqueous extract	Vero cells	Prevent HPIV-2 replication and the virus production	1 µg/mL	142
Anti-mutagenic					
	VAC	*Salmonella typhimurium* strains TA98 and TA100	Prevents the mutagenicity of the indirect-acting mutagen 2-aminoanthracene	100-400 µg/mL	145

Anticonvulsant					
	VE	4.5-year old girl	Manages refractory childhood absence epilepsy		146
Wound healing activity					
	Lipophilic extract	Rats	Stimulates migration of NIH/3T3 fibroblasts	10 µg/mL	147
Anti-ageing					
	VAC	*Caenorhabditis elegans* and *Drosophila melanogaster*	Promotes the mean survival time	50 µg/mL	148
Anti-obesity					
	VAC	Mice	Protects against hepatic steatosis	3 g/kg/day	149
Endurance promoting					
	KME	Cell lines	Activates PGC-1α and SIRT1	400 & 1000 mg/mL	150
Others					
	Aqueous extract of leaves	Mice and rats	Exhibits sedative, antiepileptic and antipsychotic activities	50 & 150 mg/mL, p.o.	2

Figure . 1



Molecular targets of *V. album*

Graphical Abstract