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

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21 **Abstract**

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

43

44

## 45 **Abbreviations**

46 2AA, 2-aminoanthracene; ADRs, adverse drug reactions; Aps, arabinogalactan proteins; Bcl-2,  
47 B-cell lymphoma 2; cb, chitin-binding; COX-2, cyclooxygenase; DPPH, 2,2-diphenyl-1-  
48 picrylhydrazyl; FME, fermented mistletoe extract; HPIV-2, human parainfluenza virus type 2;  
49 HUVEC, human umbilical vein endothelial cells; IFN- $\gamma$ , interferon gamma; IL, interleukin; JNK, c-  
50 jun N-terminal kinase; KME, Korean mistletoe extract; KML, Korean mistletoe lectin; KML-C,  
51 lectins from Korean *V. album* spp. *coloratum*; KVA, Korean *V. album*; LPS, lipopolysaccharide;  
52 ML, mistletoe lectin; NK, natural killer; NO, nitric oxide; QOL, quality of life; rVAA, recombinant  
53 *V. album* agglutinin, TNF- $\alpha$ , tumor necrosis factor-alpha; VAA, *V. album* ssp. *album*; VAA-I, *V.*  
54 *album* agglutinin-I; VAC, *V. album* var. *coloratum*; VAE, *Viscum album* extract; VCA, *V. album*  
55 var. *coloratum* agglutinin; VF-2, *viscum fraxini*-2

56

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## 93 1. Introduction

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

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

118 **2. Botany and distribution**

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

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

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

### 145 **3. Traditional uses and ethnopharmacology**

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

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

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



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

176

#### 177 **4. Bioactive constituents of European *V. album***

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

182

##### 183 *4.1. Viscotoxins*

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

193 *album* ssp. *album* (VAA), however the predominance of PS-V was detected in *V. album* ssp.  
194 *austriacum*. All viscotoxins, with the exception of A2, were detected but A3 was predominant in  
195 *V. album* ssp. *abietis*.<sup>18</sup>

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

203

204

#### 205 4.2. Mistletoe lectins (MLs)

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

216 Peumans and colleagues reported that deciduous trees contain mostly ML-I and  
217 European mistletoe growing on fir and pine trees found to be rich in ML-III.<sup>22</sup> The subdomains  
218 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.<sup>18, 26, 27</sup> 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.<sup>22</sup> 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→ $\alpha$ 4 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.<sup>28-30</sup> 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*,

244 respectively. It has also been noticed that mistletoe grown on *Fraxinus excelsior* had the highest  
245 quantity of total phenolic acids and total flavonoids. The diversified qualitative and quantitative  
246 amount of phenolic acids including caffeic, phydroxybenzoic, salicylic, protocatechuic, ferulic,  
247 and sinapic acids were observed in a free state and as glycosides.<sup>31, 32</sup> Recently, two new  
248 phenolic acids have been isolated from European white-berry *V. album*, including gallic acid and  
249 3-(3'-carbomethoxypropyl)-7→3''-protocatechoyl galloate.<sup>33</sup>

250 Two classes of flavonoids such as chalcones and flavanones were isolated from  
251 alcoholic extract of *V. album* in their glycosidic form and with methoxyl groups in the molecules  
252 <sup>18</sup>. They were 5,7-dimethoxyflavanone-4'-O-[2''-O-(5'''-O-trans-cinnamoyl)-apiosyl]-glucoside),  
253 2'-hydroxy-4',6'-dimethoxychalcone-4-O-[2''-O-(5'''-O-trans-cinnamoyl)-apiosyl]-glucoside, 5,7-  
254 dimethoxy-flavanone-4'-O-glucoside, 2'-hydroxy-4',6'-dimethoxychalcone-4-O-glucoside, 2'-  
255 hydroxy-3,4',6'-trimethoxychalcone-4-O-glucoside, 5,7-dimethoxyflavanone-4'-O-[apiosyl-  
256 (1→2)]-glucoside and (2S)-3',5,7-trimethoxyflavanone-4'-O-glucoside, and 2'-hydroxy-4',6'-  
257 dimethoxychalcone-4-O-[apiosyl(1→2)] glucoside.<sup>34, 35</sup> A promising antioxidant flavonoid  
258 quercetin has been detected only after acid hydrolysis of *V. album* extract.<sup>36</sup> Other flavonoids  
259 including quercetin, kaempferol and their mono-, di and trimethylethers were also characterized  
260 in epicuticular waxes of different subspecies of the European mistletoe.<sup>37</sup> Chaudhary and  
261 colleagues reported that the 80% methanolic extract of mistletoe contains many polyphenolic  
262 constituents viz. 5,7-dimethoxy-4'-hydroxy flavanone, 3-(4-acetoxy-3,5-dimethoxy)-phenyl-2E-  
263 propenyl-β-glucoside, 5,7-dimethoxyflavanone-4'-O-β-glucoside, 4'-O-[bapiosyl(1→2)]-β-  
264 glucosyl]-5-hydroxy-7-O-sinapylflavanone, 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2E-propenyl-β-  
265 glucoside, and 4',5-dimethoxy-7-hydroxy flavanone.<sup>38</sup>

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

269 methylglutaroyl)]- $\alpha$ -d-glucopyranoside, 7,3'-di-O-methylquercetin-4'-O- $\beta$ -d-glucopyranosyl-3-O-  
270 [(6'''' $\rightarrow$ 5''')-O-1''''-(sinap-4-yl)- $\beta$ -d-glucopyranosyl-6-(3-hydroxy-3-methylglutaroyl)]- $\alpha$ -d-  
271 glucopyranoside, and (2S)-5-hydroxy-7,3'-dimethoxyflavanone-4'-O- $\beta$ -d-apiofuranosyl-(1 $\rightarrow$ 5)-O-  
272  $\beta$ -d-apiofuranosyl-(1 $\rightarrow$ 2)-O- $\beta$ -d-glucopyranoside.<sup>39</sup>

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

287

#### 288 4.5. Lipid soluble compounds

289 Terpenoids, liposoluble compounds are the main components of European *V. album*. The lipid  
290 soluble extract of *V. album* showed the presence of oleanolic acid,  $\beta$ -amyrin acetate,  $\beta$ -  
291 amyrinacetate, lupeol, lupeol acetate, betulinic acid, and ursolic acid.<sup>44, 45</sup> A mixture of  
292 phytosterols including  $\beta$ -sitosterol and stigmasterol and their esters were also identified.<sup>46, 47</sup>  
293 Moreover, other lipophilic compounds specially saturated fatty acids palmitic, arachidic,

294 lignoceric, behenic, and cerotic acids and the unsaturated oleic, linoleic, and linolenic acids  
295 were presented in the extract of Turkey *V. album*.<sup>48, 49</sup> Long-chain fatty acids and hydrocarbons  
296 including loliolide, vomifoliol trans- $\alpha$ -bergamotene, and trans- $\beta$ -farnesene were identified in an  
297 extract obtained from supercritical fluid extraction method.<sup>50</sup> Due to poor solubility of triterpene  
298 in water, it is difficult to extract from the mistletoe. However, 2-hydroxypropyl- $\beta$ -cyclodextrin and  
299 sodium phosphate (pH 7.3), as solubilizers have been developed and represent excellent tools  
300 for the extraction of triterpenes.<sup>47, 51</sup>

301

#### 302 4.6. Trace minerals

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

307

#### 308 4.7. Other chemical constituents

309 Cyclic peptides, amino acids, proteins (9.3%), alkaloids, amines (histamine and cetylcholine),  
310 jasmonic acid, cysteine, glutathione, vitamin C, and xanthophyll are other.<sup>54, 55</sup> Three new  
311 diarylheptanoids including (3S,5R)-3-hydroxy-5-methoxy-1,7-bis(4-hydroxyphenyl)-6E-heptene  
312 (1), (3S,5S)-3-hydroxy-5-methoxy-1,7-bis(4-hydroxyphenyl)-6E-heptene (2), and (3S)-3-  
313 hydroxy-1,7-bis(4-hydroxyphenyl)-6E-hepten-5-one have been isolated and determined from the  
314 leaves and twigs of *V. album*.<sup>39</sup> Arabinogalactan proteins (APs) were isolated from berries and  
315 aerial part of European mistletoe. The ratios of arabinose and galactose in APs were 1:0.7 and  
316 1:1.18 in barriers and aerial part, respectively.

317

## 318 **5. Multifarious pharmacological properties**

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

323

### 324 *5.1 Anti-inflammatory*

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

339 A single intraperitoneal (IP) injection of the mistletoe preparation Isorel (100 mg/kg)  
340 decreased the size of the tumour and triggered abundant tumour necrosis with inflammatory  
341 response, oedema and destruction of the malignant tissue.<sup>61</sup> 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- $\gamma$ ) responses.<sup>62</sup> Our group has reported  
348 that QU FrF mistletoe preparation significantly inhibits tumor growth *in vivo* in an IL-12-  
349 dependent mechanism.<sup>63</sup> As dendritic cells are key players in regulating the immune  
350 responses,<sup>64-68</sup> 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<sup>+</sup> T cells.<sup>69</sup> 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.<sup>70</sup>

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*.<sup>71</sup> 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.<sup>72</sup> The natural killer (NK) cells have  
364 been anticipated as one of the candidates for direct tumour cell destruction.<sup>73</sup> 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.<sup>74</sup> Subcutaneous (SC)



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

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

390 VAA extract increased phagocytic activity and candidacidal activity of neutrophils, and  
391 decreased adhesion function of epithelial cells. Furthermore, extract stimulated the levels of  
392 CD4<sup>+</sup>CD25<sup>+</sup> and CD8<sup>+</sup>CD25<sup>+</sup> T cells and CD3<sup>+</sup>CD16<sup>+</sup>CD56<sup>+</sup> natural killer cells.<sup>83</sup> 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.<sup>84</sup> 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.<sup>85</sup> 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.<sup>86</sup> 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.<sup>87</sup>

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<sup>+</sup>), especially CD4<sup>+</sup> 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<sup>+</sup>), NK cells (CD56<sup>+</sup>), and T suppressor cells/cytotoxic T lymphocytes (CD8<sup>+</sup>) was  
407 detected.<sup>88</sup> 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*.<sup>89</sup> 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.<sup>90</sup>  
417 <sup>91</sup> The c-Jun N-terminal kinase (JNK) stimulation by ML-I led to translocation of the pro-

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

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

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

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.<sup>99</sup> 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.<sup>12</sup> Fermented mistletoe extract  
450 (FME) treatment of glioblastoma cells down-regulated cytokine TGF- $\beta$  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.<sup>100</sup> 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.<sup>101</sup>

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.<sup>102</sup> They concluded that VAA-I can inhibit  
459 LPS-induced pro-inflammatory response *in vivo*.<sup>102</sup> 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.<sup>103</sup> 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.<sup>104</sup>

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

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

478

#### 479 *5.5 Anti-oxidant*

480 It is well known that the antioxidant activity effects of *V. album* extracts are varied depending on  
481 the host tree and the harvesting time. It was observed that the extract from lime tree or white  
482 locust tree completely inhibits mitochondrial DNA damage induced by H<sub>2</sub>O<sub>2</sub> in HeLa cells, while  
483 extract from hedge maple tree inhibits mitochondrial DNA damage only by 50%. <sup>110</sup>

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

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

497

#### 498 5.6. Anti-tumoral

499 A pre-clinical study suggested that aqueous mistletoe extract exhibits potent anti-tumoral activity  
500 by depleting hypoxanthine and activating xanthine oxidase in the cancer cells, which lead to  
501 lowered salvage pathway activity required for the cancer cells to proliferate in the cancerous  
502 colon tissue.<sup>113</sup> Lipophilic extract of *V. album* and its predominant triterpene oleanolic acid  
503 significantly decreased monocyte chemotactic protein-1-induced monocyte transmigration.<sup>114</sup>  
504 Ethanol extract of *V. album* containing viscotoxin enhanced the anti-tumor effect of doxorubicin.  
505<sup>115</sup> Pretreatment of C6 glioma cells with 100 µg/mL of VAE before heat shock significantly  
506 decreased expression levels of Hsp27 (73%), 14-3-3β (124%), 14-3-3γ (23%), and 14-3-3ζ  
507 (84%) proteins. Increased apoptosis was also observed through caspase-3 activation (60%).<sup>116</sup>  
508 VAA-I enhanced anti-proliferating potential of cycloheximide in the human lung carcinoma cell  
509 line A549 by inducing G1-phase accumulation.<sup>117</sup> The KML-II was also found to induce  
510 apoptosis in U937 cells via activation of caspase cascades.<sup>118</sup>

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

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

518 manner, with comparatively low cytotoxicity.<sup>121</sup> Either solubilized triterpene acids or lectins and  
519 combinations thereof were found to induce dose-dependent apoptosis in the acute  
520 lymphoblastic leukaemia cell line NALM-6 via caspase-8 and -9 dependent pathways *in vitro*  
521 and *in vivo*.<sup>122</sup> *V. album* from apple and pine increased anti-tumoral activity of activated human  
522 macrophages by inducing the production of NO.<sup>123</sup> Anti-tumor activity of Iscador M Spezial,  
523 Iscador Qu Spezial and Iscador P preparations of *V. album* at high concentrations were  
524 investigated in a panel of 12 cell lines.<sup>124, 125</sup> The lectin-containing Iscador M Spezial and  
525 Iscador Qu Spezial showed a noticeable anti-tumor activity in the mammary cancer MAXF  
526 401NL cells at 15 µg/mL concentration with more than 70% growth inhibition compared to  
527 untreated control cells.<sup>124, 125</sup>

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

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

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

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



567 response following long-term administration.<sup>133</sup> 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.<sup>134</sup> 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.<sup>135</sup>

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  $\beta$ -cells without  
581 any cytotoxicity effects. Moreover, upregulated pattern of insulin genes such as PDX-1 and  
582  $\beta$ 2/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.<sup>136</sup> Structural analysis of ML-1 complexed with galactose and  
585 lactose revealed unique sugar binding abilities.<sup>137</sup> Among the medicinal plants, *V. album*  
586 showed potent alpha-glucosidase inhibitory activity.<sup>138</sup> 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  $\beta$ -cells. The ability of extract to enhance insulin secretion was not mediated by lectins.<sup>139</sup> 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. <sup>140</sup> The ethanol extract showed the superlative effect even at the lowest applied  
595 concentration ( $3.33 \times 10^{-5}$  mg kg<sup>-1</sup>) and significantly reduced the blood pressure after applied  
596 concentration  $1.00 \times 10^{-3}$  mg kg<sup>-1</sup>). 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. <sup>141</sup> 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 <sup>142</sup> 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. <sup>143</sup> 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. <sup>144</sup>

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

616 sodium azide (NaN<sub>3</sub>) and 2AA for *S. typhimurium* strain TA100 using Ames test. *V. album* var.  
617 *coloratum* was more effective in preventing the mutagenicity of the indirect-acting mutagen 2-  
618 AA, when tested with both the strains.<sup>145</sup>

619

#### 620 5.11. Miscellaneous properties

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

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

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

646

## 647 **6. Clinical trials**

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

658

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

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

671

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

682

### 683 *6. 1 Pancreatic cancer*

684 Two hundred and twenty patients with inoperable or metastatic pancreatic cancer were  
685 included in a prospective randomized clinical trial. Hundred and ten patients received Iscador®  
686 and remaining 110 patients received no anti-neoplastic therapy. Patients treated with Iscador®  
687 survived better (4.8 months) than the control group (2.7 months) (HR=0.55; p=0.0031).  
688 Importantly, no therapy related adverse events reported in the Iscador group. *V. album* therapy  
689 thus exhibited a significant and clinically relevant prolongation of overall survival.<sup>163</sup> In the same  
690 single-center, group-sequential, randomized phase III trial (ISRCTN70760582),<sup>164</sup> 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.<sup>164</sup> 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.<sup>165</sup> 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.<sup>166</sup>

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 <sup>167</sup> 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 confirm and  
738 validate these results. <sup>168</sup>

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. <sup>169</sup>  
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 phytopharmakon 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.<sup>169</sup> 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.<sup>170</sup> 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<sup>2</sup> 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



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

770

#### 771 *6.6. Cancer-related fatigue*

772 Although not examined in a randomized clinical trial, the use of mistletoe preparations  
773 has shown an improvement of cancer-related fatigue.<sup>171</sup> The fatigue levels among 324 patients  
774 with non-metastasized colorectal cancer (UICC stage I-III) during the first-line chemo- or radio-  
775 chemotherapy protocols were assessed. Iscador(®) Qu was given to 181 patients compared to  
776 control group of 143 patients without this supportive care treatment. At the end of the median  
777 treatment period, CRF was diagnosed in 16 patients (8.8%) treated with Iscador(®) Qu and was  
778 60.1% in chemo- or radio-chemotherapy group without Iscador(®) Qu. Multivariable-adjusted  
779 OR = 10.651 (95% CI 5.09-22.28; p < 0.001) at the first visit was dropped to OR = 0.054 (95 CI  
780 0.02-0.13; p < 0.001) at the end of therapy.

781

#### 782 **7. Toxicological studies**

783 For the development of remedies in general, knowledge of toxicology is crucial to  
784 confirm drug safety. Cancer patients who received subcutaneous injections of mistletoe extracts  
785 were examined for adverse drug reactions (ADRs). Out of 1923 patients, 14.7% patients  
786 reported local reactions less than 5 cm and raised body temperature less than 38°C. Among  
787 162 patients who reported ADRs, these reactions were mild (50.8%) to moderate (45.1%) in  
788 majority of the patients. Only 4.2% patients reported severe ADR. There were no recognizable  
789 risk factors for ADRs. The ADR rate augmented as the dose of mistletoe increased. However,  
790 patients receiving concurrent conventional therapies reported less ADRs  
791 during mistletoe therapy. The study indicated that mistletoe therapy is safe.<sup>172</sup> In another report,  
792 SC injections of ML-1 (1mg/kg, weekly twice) for a month period resulted in significant

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

800

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

812

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

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

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

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

841 Toxic effects of purified components of mistletoe were also been explored. In mice, the  
842 LD<sub>50</sub> of ML-1 was found to be 80 µg/kg. <sup>187</sup> Another report suggested that LD<sub>50</sub> of ML-1 and ML-

843 3 were 28 and 49 mg/kg, respectively.<sup>185</sup> But the lectin activity and route of application were  
844 not clear in these reports. Subsequent study however reported lower LD<sub>50</sub> values when different  
845 lectins were injected IP route: ML-1: 28 µg/kg, ML-2: 1.5 µg/kg and ML-3: 55 µg/kg.<sup>24</sup> ML-1 in  
846 rat was lethal within 24 hours at 100 µg/kg by IP route. However, 10 µg/kg caused mortality in 3-  
847 4 days.<sup>188</sup> These animals experienced pancreatic hemorrhages, ascites, and congested  
848 intestine and these symptoms were similar to those observed with ricin. Thus, toxicological  
849 values for ML preparations showed large variations probably due to differences in the methods  
850 that calculate lectin activity. High production of TNFα and hemagglutinating activity of the lectins  
851 were proposed as underlying mechanisms of ML toxicity.<sup>189</sup>

852

## 853 **8. Conclusions and perspectives**

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

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

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

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

884

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886

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1189 **Figure caption**

1190 Figure 1. Molecular targets of *V. album* for different biological properties.

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1210 **Table 1**1211 Chemical constituents of *V. album*

S.No.	Chemical constituents	Content	Source	References
1	<b>Viscotoxins</b>	0.05-0.1%		18
	Isoforms: A1, A2, A3, B, B2, C1 & 1-PS		Leaves Stem	17
2	<b>Lectins</b>	0.34-1.0 mg/g dried material		22, 24-26
	Isoforms: ML-I, ML-II, & ML-III		Older stems	
3.	<b>Carbohydrates</b>	44-58%/dry wt.	Leaves Stem	28
	Methylated homogalacturonan		Berries Stem	30
	Pectin		Berries Leaves	18
	1→ $\alpha$ 4 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.	<b>Vitamin C</b>	750 mg/100g fresh wt.	Leaves Berries	18
5.	<b>Proteins</b>	9.3%	Leaves Stem Berries	136
6.	<b>Lipophilic compounds</b>	-		
	Terpenoids		Leaves Stem Berries	47
	Phytosterols		Berries	46
	Saturated fatty acids		Leaves Stem	48, 49 50
7.	<b>Inorganic elements</b>			
	Potassium, calcium, manganese, sodium, nickel, phosphate, selenium, silica, magnesium, and zinc	-	Leaves Stem	18
8.	<b>Others</b>			
	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
<b>Antioxidant activity</b>	Extracts of <i>V. album</i> grown on lime tree or white locust tree	HeLa cells	Inhibits mitochondrial DNA damage induced by H <sub>2</sub> O <sub>2</sub>	10 µg/mL for 48 h	110
	Organic extracts	<i>In-vitro</i>	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 <sub>50</sub> 42.6 µg/mL	111
	Methanolic extract	Rats	Shows DPPH radical scavenging and anti-lipid peroxidation activities	500 mg/kg	112
<b>Anti-inflammatory</b>	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
<b>Immunomodulatory</b>	Preparation (QU FrF)	B16 mouse melanoma	Abrogates IL-12 expression	20 µg/mouse/day	190



VA Qu Spez	Dendritic cells	Stimulates proliferation of CD <sup>4+</sup> T cells	5-15 µg/mL	69
<i>N</i> -acetyl- <i>D</i> -galactosamine-specific lectin	T cells	inhibits 3000 immune functions-regulating genes	600 ng/mL	70
KME	<i>Epinephelus bruneus</i>	Enhances phagocytic activity	1% and 2%	71
Aviscumine	<i>In-vivo</i> 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-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and IFN- $\gamma$	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- $\alpha$ , 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 <i>E. coli</i> 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
<b>Cytotoxicity</b>	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 <i>V. album</i> 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
<b>Anti-angiogenic</b>	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

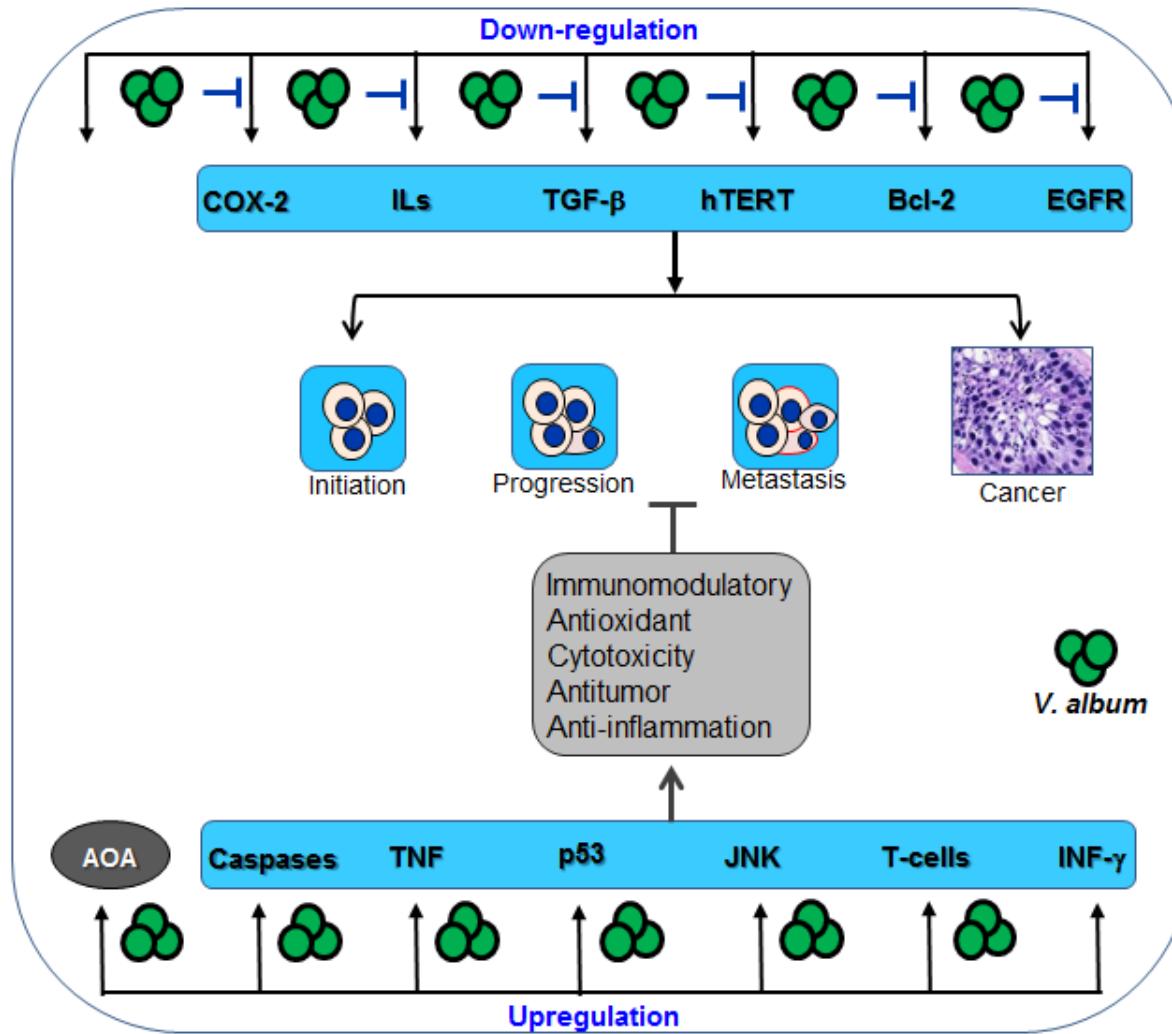
<b>Anti-tumoral</b>	Viscotoxins	Lymphocytes	Induce cell death by producing mitochondrial Apo2.7 molecules and by generating ROS-intermediates	100 ng/mL	174
	Aqueous extract	Cancer cells	Depletion of hypoxanthine concentration and xanthine oxidase activation		113
	Lipophilic extract and its predominant triterpene oleanolic acid	Tumour cells	Decreases MCP-1 induced monocyte transmigration	25 µg/mL	114
	Ethanollic 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 <sub>50</sub> 1.6 mg/L	127
Viscin	Molt4 U937 leukaemia cells	Inhibits growth and induce apoptotic cell death	IC <sub>50</sub> 118 ± 24 & 138 ± 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 <sub>50</sub> 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
<b>Anti-diabetic</b>	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 <sub>50</sub> 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
<b>Anti-hypertensive</b>	ethanol extract	Wistar rats	Reduces the blood pressure	3.33x10 <sup>-5</sup> mg kg <sup>-1</sup>	140
<b>Anti-microbial</b>	Methanolic extract	Pathogenic microorganisms	Shows antimicrobial activity		141
	Aqueous extract	Vero cells	Prevent HPIV-2 replication and the virus production	1 µg/mL	142
<b>Anti-mutagenic</b>	VAC	<i>Salmonella typhimurium</i> strains TA98 and TA100	Prevents the mutagenicity of the indirect-acting mutagen 2-aminoanthracene	100-400 µg/mL	145

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



## Graphical Abstract

