THE BIOLOGICAL ACTIVITY OF EUROPEAN MISTLETOE
*(Viscum album)* EXTRACTS AND THEIR PHARMACEUTICAL
IMPACT

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Key words:

Abstract: Extracts of *Viscum album* (mistletoe) are widely used as complementary cancer therapies in Europe. The mistletoe lectins and viscotoxins have been identified as the main principle of mistletoe extracts that participating in biological activity of *V. album*. These compounds were isolated and studied *in vitro* and *in vivo* for their biological activity and mechanism of action. A comparison of the results to those using whole extracts indicated that lectins and viscotoxins are not the only bioactive compounds present in the mistletoe. In this paper, we review the recent studies regarding with cytotoxic activity on tumor cells of mistletoe extracts, as well as, the role of this semiparasitic plant in diabetics and hypertension illness.

INTRODUCTION

In recent year, antioxidants derived from natural resources, mainly from plants, have been intensively used to prevent oxidative damages. Natural antioxidants have also some advantages over synthetic ones, being obtained easily and economically and have slight or negligible side effects.

Aqueous extracts of the European mistletoe have been widely used for decades as alternative treatment and adjuvant cancer therapy, particularly in Germany, Austria and Switzerland.

European mistletoe (*Viscum album L.*, family Loranthaceae) is an evergreen, semiparasitic plant (it depends on the host tree for nutrients and water) normally found growing on a variety of trees, especially pine and apple. Although there are many varieties of mistletoe, including the American (*Phorandendron serotinum* or *Phorandendron flavescens*), the European (*Viscum album L.*), and the Korean (*Viscum album L. coloratum*), most investigative work has been done on European mistletoe. A number of biological effects, such as anticancer, antimycobacterial, apoptosis – inducing, antiviral, and immunomodulatory activities have been reported.

The main ingredients of the *Viscum album* extract are it’s three ribosome inactivating proteins or lectins (mistletoe lectins, ML) ML-1, ML-2, and ML-3, the glycoprotein binding with D-galactose and N-acetyl-D-galactosamine, viscotoxins (VT), as well as, oligo- and polysaccharides, alkaloids. The flavonoid patterns of *V. album* from various hosts were investigate by Becker and Exner (1980). They identified quercetin and a series of quercetin methyl ethers, which may be assumed to be accumulated on the plant surface. The epicuticular material of the *V. album* contains preferably the flavonol quercetin and its methyl derivatives, occasionally also the flavonol kaempferol and some of its methyl derivatives, and rarely the flavanone naringenin (Haas et al., 2003).
It has been suggested that pharmacologically active compounds may pass from the host trees to the parasitic plants (Büssing and Schietzel., 1999).

One of the most important problems for researchers working on parasitic plants concerns the nature of the biological connections established between the host and the parasite. The influence of the host tree may play a very significant part in the assessment of the mistletoe as a plant raw material. In spite of intensive investigations carried out by many authors, there are only single data sources available in this respect. Although it is know that there are morphological and, especially, phytochemical differences in mistletoes occupying different species of host trees, the aspect of mistletoe’s taxonomy is not quite clear (Ochocka and Piotrowski, 2002).

**IN VITRO EXPERIMENTAL EVIDENCE FOR CYTOTOXIC AND APOPTOTIC EFFECTS OF VISCUM ALBUM**

The European mistletoe extracts are used in an adjuvant cancer therapy because of their immunostimulatory and simultaneously cytotoxic properties. These effects are usually more evident for the whole extracts than for purified mistletoe lectins and viscotoxins alone.

Different mistletoe preparations are available for the treatment of cancer (Table 1). Abnobaviscum®, Helixor, Iscador®, Isucin® and Isorel® are produced according to anthroposophical methods; other mistletoe preparations include Cefalektin®, Eurixor® and Lektinol®. Iscador was first proposed for the treatment of cancer in 1920, by Rudolf Steiner, PhD, (1861-1925), founder of the Society for Cancer Research, in Arlesheim, Switzerland and introduced in the treatment of human cancer as early as 1921. The anthroposophical preparations are available from different host trees such as oak, apple, pine and others. Harvesting procedure is standardized, and the juices from summer and winter harvests are mixed together. The total extract of VAE (mistletoe extracts) is considered essential for full effectiveness and concentration of its compounds is ensured through process standardization. The non-anthroposophical preparations are harvested in winter from poplars; they are standardized and dosed according to mistletoe lectin content (ranging from 1 ng/kg up to 15 ng/kg bodyweight) on the premise that mistletoe lectin is the main active ingredient (http://wissenschaft.misteherapie.de/- Mistletoe in cancer treatment).

**Table 1. Mistletoe preparations in cancer treatment**

<table>
<thead>
<tr>
<th>Proprietary names</th>
<th>Host tree*</th>
<th>Harvest season</th>
<th>Extraction</th>
<th>Parts used</th>
<th>Dosage according to lectin content</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthroposophical preparations</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Abnobaviscum</td>
<td>A, Ac, Am, B,C, F, M, P, Qu</td>
<td>Winter and summer juices mixed</td>
<td>Pressing</td>
<td>Fresh leafy shoots, fruits</td>
<td>Seldom</td>
</tr>
<tr>
<td>Helixor</td>
<td>A, M, P</td>
<td></td>
<td>Aqueous</td>
<td>Fresh leafy shoots, fruits</td>
<td></td>
</tr>
<tr>
<td>Iscador (Iscar)</td>
<td>M, P, Qu, U</td>
<td></td>
<td>Aqueous fermentation</td>
<td>Fresh leafy shoots, fruits</td>
<td></td>
</tr>
<tr>
<td>Isucin</td>
<td>A, C, M, P, Po, Qu, S, T</td>
<td></td>
<td>Aqueous</td>
<td>Dried leafy shoots, fruits, sinker</td>
<td></td>
</tr>
<tr>
<td>Isorel</td>
<td>A, M, P</td>
<td></td>
<td>Aqueous</td>
<td>Fresh leafy shoots, fruits, sinker</td>
<td></td>
</tr>
<tr>
<td><strong>Other preparations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefalektin</td>
<td>Po</td>
<td>Winter</td>
<td>Aqueous</td>
<td>Dried leafy shoots</td>
<td>Yes</td>
</tr>
<tr>
<td>Eurixor</td>
<td>Po</td>
<td>Winter</td>
<td>Aqueous</td>
<td>Dried leafy shoots</td>
<td></td>
</tr>
<tr>
<td>Lektinol</td>
<td>Po</td>
<td></td>
<td>Aqueous</td>
<td>Dried leafy shoots</td>
<td></td>
</tr>
</tbody>
</table>

*A: Abies = fur; Ac: Acer = maple; Am: Amygdalus = almond tree; B: Betula = birch; C: Crataegus = whitethorn; F: Fraxinus = ash tree; M: Malus = apple tree; P: Pinus = pine; Po: Populus = poplar; Qu: Quercus = oak; S: Salix = willow; T: Tilia = lime; U: Ulmus = elm
Commercial mistletoe preparations differ significantly in their lectin and viscotoxin content, e.g. Iscador™ is a rich viscotoxin preparation with a relatively small amount of lectins possible forming complexes with viscotoxins (Jung et al., 1990), while Helixor™ contains significantly more lectins in comparison with viscotoxins (Büssing and Schietzel, 1999). Additionally, there is presumably synergism in the biological activity of the constituents of mistletoe preparations, indicating that there are complex interactions among them, which makes a prediction of the overall activity difficult. The mechanism of synergism between the lectin ML-1 and viscotoxins in the complete extract had been proposed by Hajto et al., (1989). It was suspected that NK cells activated by ML-1 in vivo may be more effective in killing tumor targets that have already been acted on by viscotoxins. Inhibition of the suppressor-cell activity by viscotoxins could be an additional important factor in the entire process. However, Büßing et al, 1996, suggested another explanation for the action of whole extracts since it was hard to find any close correlation with the content of lectins or viscotoxins, although the activity was specific to the host tree and exceeded that of purified lectins. It was proposed that mistletoe lectins form complexes with other substance and other lectin-like proteins. Nevertheless, in the experiments where isolated lectins, viscotoxin and oligosaccharides (oligosaccharides are also suspected to play a role in the biological activity of crude V. album extracts) were tested separately, the strongest apoptosis-inducing effect was obtained in the experiments with lectins. Apoptosis occurred together with direct or indirect killing of the cells by damage to the cell membrane with the subsequent influx of Ca^{2+}. It had also been suggested that the loss of membrane integrity and, as the result, the leakage of calcium within the cells led to the activation of DNA-cleaving endonucleases.

Kelter and Fiebig, (2006) investigated the direct effect of the three mistletoe extracts Iscador M Spezial, Iscador Qu Spezial and Iscador P on tumor growth in a panel of 26 human tumor cell lines in vitro using cellular proliferation assays. Antitumor activity of the three preparations at high concentrations was investigated in a panel of 12 cell lines. The results showed no evidence of stimulation of tumor growth. On the contrary, the lectin containing preparations Iscador M Spezial and Iscador Qu Spezial expressed a pronounced antitumor activity exhibiting a nearly identical antitumor profile compared to isolated mistletoe lectin 1.

Cytotoxicity assays in vitro (MTT test) showed that the different breast cancer cell lines Kpl-1, MCF-7 and Mfm-223 respond differently to the mistletoe preparations: Iscador. Quercus (Qu), Abies (A), Malus (M) and Pinus (P) (Eggenschwiler et al., 2006). In order to determine the differences in the responsiveness of the cells more exactly, the gene expression profiles were determined by cells, which were treated with Mistletoe extracts, compared with untreated control cells. Such differences can be analysed in more detail by looking at the gene expression using Human Whole Genome microarray chips (41,000 genes). The results of the transcriptome analyses suggested that Iscador preparations influenced the overregulation of genes regarding immune defense, stress response, apoptosis and cell-cell adhesion pathways. The authors conclude that Iscador Qu and M have a greater influence on the immune defense and stress response genes whereas Iscador A tends to affect the cell-cell adhesion and cytoskeleton pathways.

The molecular and cellular mechanisms by which mistletoe (Viscum album L.) extracts exert cytotoxic and immunomodulatory anti-tumoral effects are largely unknown.

Hülsen et al., 1986, tested the extracts from mistletoe growing on different species of host trees. The extract from mistletoe growing on Malus sp. appeared to be most toxic against Molt4 cells in vitro: 500 µg/ml of extract caused 91.19% decreased of living cells after 72-h incubation. This was followed by the extract from mistletoe growing on Abies
sp., which was weaker (72.08% mortality of living cells). The extract from mistletoe growing on *Pinus* sp. Caused a very weak effect (19.93% mortality of living cells), which could result from a different qualitative and quantitative lectin and viscotoxins composition. The authors proposed two general explanations for those differences in biological activity:

i) relevance with genetic differences among mistletoes occupying different species of the host tree or

ii) nutritional influence of the host tree.

Table 2 summarizes the recent papers regarding with the anti-carcinogenic effects of mistletoe extracts on tumor cell lines.

**MISTLETOE AND DIABETES**

*Diabetes mellitus*, characterized by a lack of the pancreatic hormone insulin (important in regulating the blood sugar level) have also been documented as being treatable by mistletoe. A tea prepared from the leaves of the plant has traditionally been used in the West Indies for diabetics. It has been shown that extracts of mistletoe evoked a 1.1 to 12.2 fold stimulation of insulin secretion from clonal pancreatic B-cells in the laboratory (Gray et al, 1999), providing evidence for the presence of insulin-releasing natural products in *Viscum album* that may contribute to the reported anti-diabetic property of the plant.

In recently paper Nwanjo, (2007), investigated the role of aqueous V. album extract on hypoglycaemic and antioxidant potentials in streptozotocin (STZ) induced diabetic Wistar rats. This investigation shows that the aqueous extract of *V. album* leaves in addition to being hypoglycaemic seems to be effective for reducing oxidative stress and free radical-related diseases including diabetes.

**MISTLETOE AND HYPERTENSION**

A decoction of the leaves of mistletoe is traditionally used in the treatment of hypertension. There exists little scientific literature on the effect of *V. album* on blood pressure. Ofem et al., 2007, investigated the effect of the crude aqueous extract from *V. album* leaves on arterial blood pressure and heart rate in albino Wistar rats. The results of this study shown that the crude mistletoe extract significantly lowered the blood pressure but had no effect on the heart rate in normotensive rats.

**CONCLUSIONS**

Mistletoe shows promise in adjuvant cancer therapies, and research is currently being undertaken into other components of mistletoe extracts, what effects they may have, as well as their interactions with other components. The World Health Organisation has also recommended that traditional plant treatments for diabetes warrant further investigation (World Health Organisation, 1980), and this could lead to another important use for mistletoe.

There are other potential applications of *V. album* components, not necessarily related to anticancer studies: the utilization of mistletoe lectins for the construction of immunotoxins could be prospective, and their role in the protection of plant cells against frost requires further studies. As for viscotoxins, they seem to have a potential, among other thionins, as plant protection agents against pathogens, especially in transgenic plants, which would help to reduce the general tendency for pesticides application.
References

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7. Hajto T., Hostanska K., Gabius, 1989, Modulatory potency of the β-galactoside-specific lectin from mistletoe extract (Iscador) on the host defense system in vivo in rabbits and patients, Cancer Res., 49, 4803-4808
Table 2. *In vitro* experimental evidence for cytotoxic and apoptotic effects of *Viscum album*

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cell line</th>
<th>Mistletoe</th>
<th>Anti-cancer mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>COLO 320 HSR</td>
<td>Korean mistletoe lectin&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Activated caspase-2,-3 and -8, which are mediated by death receptors, and caspase -2 and -9, which are mediated by the mitochondrial pathways</td>
<td>Khil et al, 2007</td>
</tr>
<tr>
<td>Mammary</td>
<td>MFM-223, KPL-I, MCF-7, HCC-1937</td>
<td>VAP-Qu, VAP-M, VAP-P, VAP-A&lt;sup&gt;2&lt;/sup&gt;</td>
<td>The four bladder carcinoma cell lines were more sensitive to mistletoe preparations than the four breast carcinoma cell line. The IC&lt;sub&gt;50&lt;/sub&gt; values of VAP-Qu, VAP-M and VAP-A for the various carcinoma cell lines were lower than those obtained with VAP-P, indicating that this latter preparation has the weakest effect on viability/proliferation of carcinoma cells. VAP-A had even slightly less mistletoe lectin than VAP-P, but the strongest cytotoxic effect of all four mistletoe preparations. This result suggest that other components might play a role in the cytotoxic effect of mistletoe preparations.</td>
<td>Eggenschwiler et al., 2007</td>
</tr>
<tr>
<td>Bladder</td>
<td>T-24, TCC-SUP, UM-UC-3, J-82</td>
<td>Iscador Qu&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Iscador Qu has a differential effect on different human tumors cell lines. Iscador caused early cell cycle inhibition followed by apoptosis in a dose-dependent manner. Apoptosis was induced by activating the mitochondrial but not the death receptor-dependent pathway.</td>
<td>Harmsma et al., 2004</td>
</tr>
<tr>
<td>Lung</td>
<td>MR 65, NCI-H125, NCI-H82</td>
<td>Iscador M, Iscador Qu, Iscador P&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Iscador preparations containing a high lectin concentration showed antitumor activity in the mammary cancer line at the 15µg/ml dose level with a more than 70% growth inhibition compared to untreated control cells.</td>
<td>Maier and Fiebig, 2002</td>
</tr>
<tr>
<td>Colon</td>
<td>Caco-2, HT-29</td>
<td>Iscador Qu&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary</td>
<td>MCF-7</td>
<td>Iscador M, Iscador Qu, Iscador P&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
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</tr>
<tr>
<td>Mammary</td>
<td>MAXF 401NL</td>
<td>Iscador M, Iscador Qu, Iscador P&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
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</tbody>
</table>

<sup>1</sup>*Viscum album coloratum* agglutinin

<sup>2</sup>*Viscum album* preparations obtained from mistletoe growing on oak VAP-Qu, apple tree VAP-M, pine VAP-P or white fir VAP-A

<sup>3</sup>Iscador Qu is a fermented aqueous extract of mistletoe plants growing on oak trees

<sup>4</sup>The ampoules contain 1 ml of an aqueous extract from 5 mg total plant of *V. album* with 250 ng total lectins/ml (Iscador M), 5 mg total plant of *V. album* with 375 ng total lectins/ml (Iscador P) or 10 mg total plant of *V. album* (Iscador P).