Phytomedicine Plus 1 (2021) 100039

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.elsevier.com/locate/phyplu

Mistletoe lectins: From interconnecting proteins to potential tumour inhibiting agents

Mahak Majeed[a], Khalid Rehman Hakeem[b,*], Reiaz Ul Rehman[a,*]

[a] Department of Bioresources, University of Kashmir, Hazratbal, Srinagar, Jammu and Kashmir, India
[b] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Keywords:
Apoptosis
Cytotoxic
Lectins
Mistletoe
Oncotargets

ABSTRACT

Background: Mistletoe lectins (MLs) are obtained from the diverse species of *Viscum album* L., sharing an extensive history of their use as potent anticancer agents in the prevention and treatment of various cancers. Mistletoe lectin-I (ML-I) is highly investigated among all MLs for the anti-proliferative activity that arises from its cytotoxic and immunomodulatory functions.

Methods: Various databases, such as PubMed, Scopus, Web of Science, and Google Scholar, were searched up to 2020 using relevant keywords.

Results: *In vitro* and *in vivo* studies have signified apoptosis and cytokine production by immune cells as main mechanisms responsible for antitumor activities. MLs elicit the apoptotic pathway in tumour cell by regulating the expression of genes resulting in activation of caspases causing cell death. During apoptosis the disruption of the mitochondrial membrane leads to the release of cytochrome C and pro apoptotic factor Apaf-1 causing activation of Caspases. In addition, studies conducted on animal models have suggested that c-Myc, JNK and immune signalling systems as main oncotargets that mediate the antitumor functions. MLs are known to regulate the expression of genes involved in tumour survival, spread and growth. This includes down-regulation of anti-apoptotic proteins (Bcl-2), metalloproteinases, c-Myc protein, growth factor β genes while as up-regulation of pro-apoptotic proteins (Bax, Bad) and tumour necrosis factor (TNF).Transcriptomic and proteomic data also reported that cancer cells treated with whole plant Mistletoe extract constituting liphophillic Mistletoe compounds (triterpenes) and water soluble (lectins and viscotoxins) showed better anticancer efficacy than individual components while significantly affecting the genes involved in cell survival and death.

Conclusion: This review aims at exploring the antitumor mechanisms of MLs against various cancer cell lines and mice animal models which encourages its application as effective bio-therapeutic tools to address range of human cancers.

Introduction

Lectins are sugar-binding proteins of non-immunogenic origin that show specific binding properties to carbohydrate moiety of glycoconjugates (Pervin et al., 2015). The term “lectin” denotes a group of plant agglutinins that agglutinate erythrocytes and are known for their marked sugar-binding, biotechnological and pharmaceutical properties (Fohona et al., 2017; Varrot et al., 2013).The specific molecular sites on lectin bind with mono- or oligosaccharides through non-covalent forces that involve van der Walls, hydrophobic interactions and hydrogen bonds with high affinity and specificity but without any catalytic and immune responses. Although ubiquitously found in diverse living forms such as animals, fungi, bacteria but plant derived lectins mediate diverse biological functions such as anti-inflammatory, antitumor, antiviral, antibacterial, immunomodulatory, and antifungal effects. They also have potential application as histochemical markers, drug delivery agents and biosensors of diseases. (Mishra et al., 2019; Coelho et al., 2017).Plant lectins can cause cell death in cancer cells expressing aberrant glycan structures on their cellular surfaces. They specifically sense and target such changes in cancer cells, which demonstrate their effective use in cancer diagnostics and treatment.The ability of lectins to selectively target diverse glycosylated biological molecules on the cell surface have revolutionised their application in the area of pharmaceutical sciences especially in cure of challenging diseases (Vojdani, 2015).

Abbreviations: MLs, Mistletoe lectins; TNF, tumour necrosis factor; VAA-I, Viscum album agglutinin-I; APC, adenomatous polyposis coli; ARMS, alveolar Rhabdomyosarcoma; ROS, reactive oxygen species; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; mAbs, monoclonal antibodies.

* Corresponding authors.
E-mail addresses: kur.hakeem@gmail.com (K.R. Hakeem), rreiazbiores@gmail.com (R.U. Rehman).

https://doi.org/10.1016/j.phyplu.2021.100039
Received 2 December 2020; Received in revised form 17 January 2021; Accepted 8 February 2021

Table 1
Sources of some plant lectins with specific carbohydrate binding and anticancer potential.

Plant source	Lectin group	Carbohydrate binding specificity	Anticancer activity	References
European Mistletoe (*Viscum album* L.)	ML-I	Galactose (Gal)	Breast cancer, hepatocarcinoma	Krauspenhaar et al. (1999) Wacker et al. (2004) Lyu et al. (2002) Schumacher et al. (1995)
	ML-II	Galactose & N-acetylgalactosamine (GalNAc)		
	ML-III	N-acetylgalactosamine (GalNAc)		
Korean Mistletoe (*Viscum album* Coloratum)	KML–C	Galactose and GalNAc	lung cancer, hepatoma	Kang et al. (2007) (Panda et al., 2014)
Arachis hypogea	Peanut agglutinin	Gal, Galb3Gal NAc	Breast cancer	Reviewed in (Lavanya et al., 2016)
Glycine max	Soybean agglutinin	Gal/GalNAc	Breast cancer, hepatoma	
Prosopis juliflora	Mesquite seed lectin	Fruc	Cervical cancer	
Musa accuminate	Banlec	Fruc	Leukemia, hepatoma	
Triticum aestivum	Wheat germ agglutinin	(Glc NAc)1,3 Neu5 Ac	Pancreatic cancer	
Vicia faba	Vicia faba agglutinin	D-mannose & D-glucose	Colon cancer	
Artocarpus integrifolia	Jacalin	Gal, Galb3GalNAc	Colon cancer	
Agaricus bisporos	Agaricus bisporos lectin	Gal, Galb3GalNAc	Breast cancer	
Phaseolus vulgaris	Phaseolus agglutinin	Nonspecific sugar binding	Breast cancer, colon cancer	

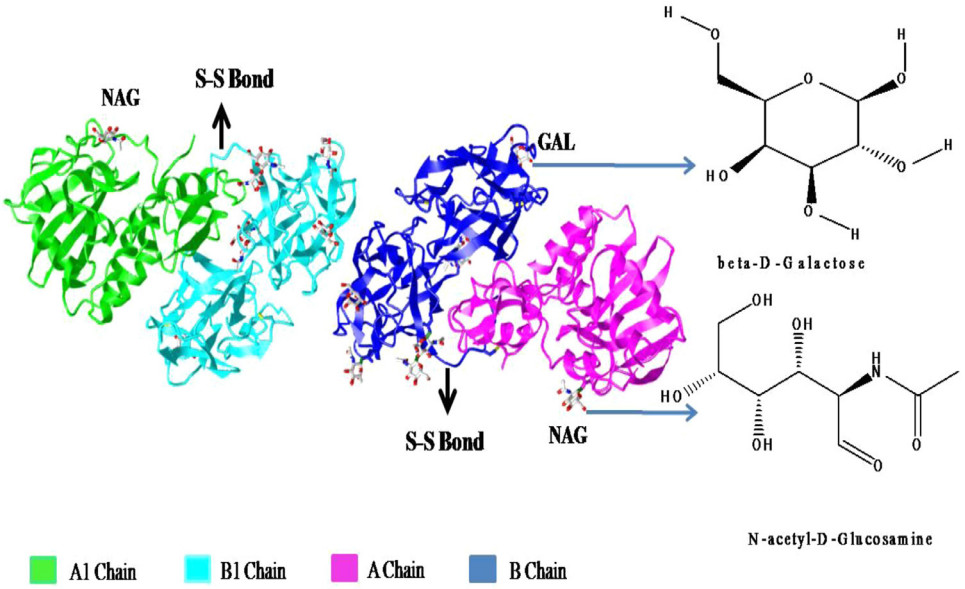


Fig. 1. X-ray crystallographic structure of Mistletoe lectin-I belonging from type II ribosome inactivating protein (RIP) complex with galactose. MLI-I forms homodimer $(A\text{-}B)_2$ between A and B chains involving weak polar and non-polar interactions between N-terminal positions of two B chains. Galactose binding occur by common hydrogen bonds and hydrophobic contact with aromatic ring (Niwa H. et al., (2003).

Plant-derived lectins have become significant therapeutic tools in treating cancers due to their ease in preparation and hence abundant availability on a commercial scale (Liu et al., 2013; Fohona et al., 2017) **(Table 1).**

Based on carbohydrate binding specificities, lectins are classified as poly- and monospecific either binding with one or more sugars like glucose, mannose, galactose or mannose containing glycans (Barre et al., 2001; Liu et al., 2010). In other nomenclature, MLs are also known as *Viscum album* agglutinin (VAA) due to their monosaccharides that inhibits the lectin-induced agglutination of erythrocytes. In terms of chemical structure and antigenicity, Mistletoe plants contain three similar lectins (Hajto et al., 2005), ML-I, II and III which differ from each other only in the specificity of carbohydrate-binding. These lectin types are formed of chain A and chain B bound together by a disulphide bond (Kang et al., 2007) **(Fig 1).** A-Chain of MLs is composed of three distinct individual domains and a B-Chain is formed of two domains with the same configurations (Liu et al., 2010). Mistletoe lectin I (ML-I or VAA-I) has been observed as most important galactoside specific lectin. It is constituted of cytotoxic A-Chain (29 kDa) and a specific carbohydrate-binding B-chain (34 kDa) that is immunomodulatory in function.VAA-II (ML-II) specifically binds with galactoside as well as N-acetylgalactosamine glycans and VAA-III (ML-III) shows N-acetylgalactosamine specificity (Holtskog et al., 1988). The differential specificities of B-chain in binding with sugars play the determining role in explaining the selective cytotoxicity of lectins towards cancer cells on interacting with their putative receptors. This further showed that A- and B-Chains are both involved in the cytotoxic action of lectins (Liu et al., 2010). Chain A mainly cleaves the glycosidic bond in 28S RNA of 60S ribosome causing thereby interruption in the elongation step during protein synthesis **(Fig 2)** (Fohona et al., al.,2017; Lee et al., 2007).

1. Extraction and purification of mistletoe lectins

Leaves and stem harvested from Mistletoe plant were crushed in liquid nitrogen to attain a fine powder with mortar and pestle. In 10 ml of Tris-EDTA buffer, soluble proteins were extracted out at alkaline pH around 8.5 (Barberaki et al., 2015). This extract was then purified from

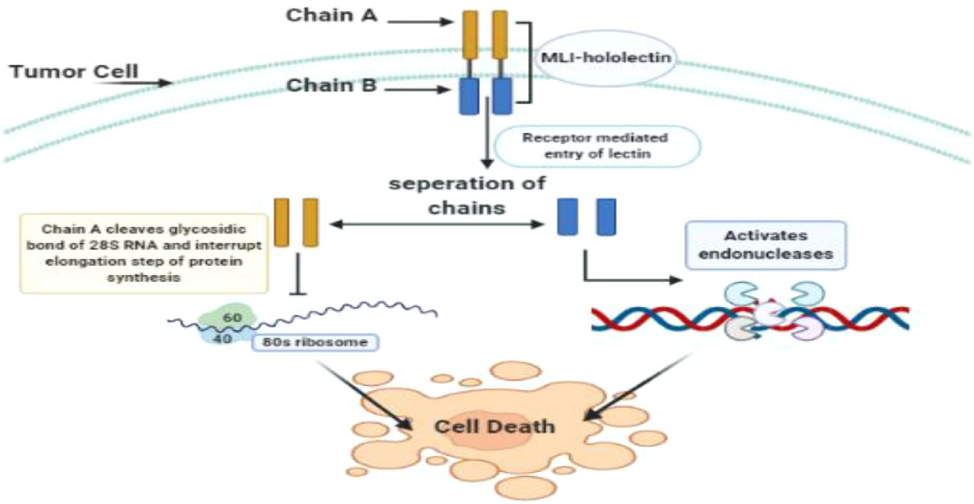


Fig. 2. The cytotoxic action of chain A & B of Mistletoe lectin ML-I.

debris by two times centrifugation at 5000 × *g* and 4 °C for 15 min. To attain maximum saturation of proteins, supernatant was treated with solid ammonium sulfate to precipitate proteins. Using affinity chromatography, the extract was purified with lactosyl sepharose affinity resin packed columns. 4 ml plant extracts at flow rate of 300μl/min were loaded onto these columns for retention time of 2 min and the fractions were further elluted. The bound and unbound fractions were further spectrophotometrically detected for presence of proteins at $O.D_{(280\ nm)}$. Elution was performed at pH 6 with base buffer used was constituted of 1 mM ethylenediaminetetraacetic acid and 300 mM 20 mM sodium dihydrogen phosphate.The proteins obtained from Mistletoe extracts were quantified using altered Bradford method (Buyel and Fischer., 2014). The purity, relative abundance and molecular weight of target proteins in different eluted fractions of crude protein extract were then analysed with 10% sodium dodecyl sulphate polyacrylamide gel (SDS-Page) electrophoresis The protein bands derived from SDS-Page electrophoresis were visualised using silver nitrate dye (Chevallet et al., 2006). **Fig 4** shows a brief outline of the extraction and purification of MLs.

Anticancer activity of mistletoe lectins

Since very remote times there has been an extensive use of Mistletoe extracts in treatment of cancer in European countries. Recent study conducted on antiproliferative activities of an aqueous extracts of *Viscum album* L. namely Iscucin® has revealed more anticancer efficacy than isolated ML-I. This further suggested that increased antioxidative property of phenolic compounds and cytotoxicity of viscotoxin A (VT-A) enhance the antiproliferative potential of whole extract than an equivalent concentration of isolated ML-I (Felenda et al., 2019). Recent studies concluded that Mistletoe extract contain hydrophilic lecins and viscotoxins and hydrophobic triterpens such as betulinic acid and oleanolic as fundamental tumor inducing phytochemicals.The *in vitro* application of aqueous Mistletoe extract and Triterpenes dissolved in cyclodextrins showed better tumor inhibiting results against Ewing sarcoma.It was also aberved that both hydophillic and hydrophobic components in the extract influence the proteomic profile of treated cells (TC-71) resulting in downregulation of protein synthesis associated genes and upregulating the protein genes involved in protein degeneration. (Twardziok et al., 2017). In other study, the anticancer efficacy of aqueous extract of *viscum album* was enhanced with liphophillic triterpenes against rhabdomyosarcoma. the study showed that aqueous exytact along with triterpenes induced intrinsic and extrinsic apoptosis by dysregulation of mitochondrial membrane potential and stimulation of Caspases- 8 and 9 (Stammer et al., 2017).

Various *in vitro* and *in vivo* studies conducted using Mistletoe extracts against various tumors have indicated their selective toxicity to cancer cells and also the immune-stimulating effects that results from their cytotoxic action on lymphocytes (Kienle et al., 2009; Sunjic et al., 2015).The results from Clinical studies have suggested that *Viscum album* extracts can positively influence the survival and longevity of cancer patients (Ostermann et al., 2009) Research has revealed an inverse relationship between the progression of tumors and binding of Mistletoe lectins (ML-I) to cancer cells. In about one-third of breast cancers, Mistletoe lectins (ML-I) is reported to show strong binding with breast cancer tissues (Fritz et al., 2004). In leukemic PLB-985D cells, VAA-I has been reported to stimulate the breakdown of cytoskeletal associated proteins such as lamin B, paxillin and vimentin (Lavastre et al., 2007) **(Fig: 5).**Studies also showed that ML-I decreased the motility of glioma cells by changing the expression of genes involved in TGF- signaling.(Schotterl et al., 2019). Lectins obtained from the leaves of *Viscum album* Coloratum revealed inhibitory effect in lung, liver and spleen metastasis of mice on treatment with 20–50 ng of lectin (Panda et al., 2014).The inhibitory role of MLs (CM1) was seen on Wnt signaling, a key pathway in progression of colorectal cancers by decreasing expression of miR-135 a&b and stimulating the expression of the adenomatous polyposis coli (APC) gene. (Li et al., 2011) **(Table 2).**

In Europe, Iscador®, Helixor®, Eurixor®, Lektinol® and Isorel® are some popular trade names of anticancer drugs prepared from Mistletoe extracts containing lectins as an important components.These drugs have noticeably increased the longevity and quality of life in cancer patients with minimum side effects as reported in radiation-chemotherapy (Patel and Suryakanta, 2014; Marvibaigi et al., 2014). The application of lectin-containing mistletoe preparation Iscador® inhibited the tumor growth of glioblastoma xenograft in mice and caused inhibition of genes associated with tumor progression including downregulation of matrix-metalloproteinases and growth factor-β (Podlech et al., 2012). In other study taken on tumor mouse model E.G7, It was observed that KML- B chain treated dendritic cells stimulated anticancer activity by enhancing the activities of Th 1 lymphocytic cells. Thus KML-B treated DCs can act as novel immunotherapeutic approach to inhibit tumors under *in vivo* conditions (Kim et al., 2017)

Mistletoe Lectins induced tumor cell death by targeting apoptosis: Apoptosis is the main pathway to induce tumor cell inhibition shown by MLs. During this pathway, several *caspases* such as *caspase 3, caspase 8* and *caspase 9* are activated along the deactivation of telomerase resulting in reduced expression of protein Bcl-2 (Yau et al., 2015; Fohona et al., 2017). Further studies revealed that antitumor activities of MLs are dependent on disruption of mitochondrial membrane potential that elicits the release of cytochrome C and apoptosis-associated factor-1 (Apaf-1)

Table 2
Mechanism of anticancer activity of Mistletoe lectins (MLs) against different cancer cell lines.

Type of Mistletoe lectin	Cancer cell line/s	Mechanism of Action	References
Mistletoe lectins MLs	Cervical cancer cell lines Hela cells	stimulates oxidative stress mediated cell death by Caspase-3 activation leading to cell death and higher ROS levels	Mavrikou et al. (2020)
ML-I and oleanolic acid	Human acute myeloid leukemia cell lines	Induces apoptosis by releasing s cytochrome C	Delebinski et al. (2015)
	Human osteosarcoma cell lines	Induces apoptosis by inhibiting antiapoptotic proteins (BIRC5, XIAP, BCL2)	Kleinsimon et al. (2017)
Mistletoe lectin extact (ML-I, II, III)	Ewing sarcoma cells	Activates intrinsic and extrinsic apoptotic pathways with activation of caspase –8 and –9	Twardziok et al. (2016)
	Ewing sarcoma cells	Promotes gene and protein expression by activation of MAPK signalling pathway	Twardziok et al. (2017)
	Human ARMS cell lines RH-30 cells	Induced apoptosis by inhibiting antiapoptotic proteins and disruption of mitochondrial membrane potential	Stammer et al. (2017)
Mistletoe (growing on *Fraxinus*)	Hepatocarcinoma cells Hep3B	Inhibited proliferation and expression of c-Myc protein through activation of c-Myc signalling pathway	Yang et al. (2019)
	CT26 cells	Induces cell inhibition by stimulating apoptosis, ROS generation and activates SEK/JNK signalling pathways	Beztsinna et al., 2018
	Mice glioma cells	Stimulates Caspase dependant apoptosis pathway and activation of NK cells	Schotterl et al. (2018)
Korean Mistletoe lectin	B16BLB and B16F10	Stimulates the activation of caspases-1,3,4,5,6,7,8, 9. Also inhibits the activity of caspase-3 and –8	Han et al. (2015)
	Human lung cancer cell lines	Stimulates the activation of caspase-3 and caspase-9	Fan et al. (2019)
Recombinant ML-II aviscumine	Lung cancer cells H460 & A549	Induces apoptosis leading to cell toxicity	Mazalovska and Kouokam (2020)

causing activation of *caspase-3*. ML-I are also associated with transport of pro-apoptotic proteins Bax and Bad in tumor cell through the activation of JNK pathway. ML-I also triggers the repression in anti-apoptotic molecule such as Bcl-2 and up-regulation of TNF-α that stimulates the apoptosis in leukemia cells, murine lymphocytes and peripheral blood lymphocytes (Liu et al., 2013; Kim et al., 2000) **(Fig 5).**VAA-I was reported to induce apoptotic death in chronic granulomatous disease (X-CGD) cells and PLB-985 cells by inducing rapid release of cytochrome c in these cells while in other studies cancer cells treated with VAA-I showed both extrinsic and intrinsic pathway leading to cell apoptosis (Lavastre et al., 2005) **(Fig 6).** MLs induced apoptosis in Hep3B cells that showed reduced expression of c-Myc protein in a dose dependant manner. In mice bearing human pancreatic cancer xenograft, intratumoral injection of Fraxini showed the appreciable results of tumor inhibition (Rostock et al., 2005). Lectins have been suggested as key components in *Viscum album* L. extract inducing apoptosis through the activation of mitochondrial pathway (Troger et al., 2013).Furthermore, research showed *Viscum album* hosted by ash trees (Fraxinus) indicated highest content (>10 μg/ml) of bioactive MLs (Mabed et al., 2004). The aqueous extracts of Viscum (Fraxinus) containing lectins inhibited proliferation of Hep3B cells and protein expression of c-Myc. MLs induced apoptosis in Hep3B cells that showed reduced expression of c-Myc protein in a dose dependant manner. In mice bearing human pancreatic cancer xenograft, intratumoral injection of Fraxini showed the appreciable results of tumor inhibition (Rostock et al., 2005). It was further reported that the c-Myc signalling pathway is the main oncotarget of MLs in preventing the proliferation of hepatocarcinoma cells and mice xenograft models (Yang et al., 2019).Studies documented that Mistletoe lectin stimulates downregulation of Bcl-2 and telomerase and up-regulation of Bax in SK-Hep-1 (p53-positive) and Hep 3B (p53-negative) human hepatocarcinoma cells (Lyu et al., 2002).

Recently, aviscumine, a recombinant form of naturally occurring ML-I has been investigated for its anticancer activities. Aviscumine has been evaluated for dose limited toxicity and maximum tolerated dose before its administration in clinical trials of cancer patients (Schoffski et al., 2004). The recombinant VAA (rVAA) performed similar biological functions as naturally occurring (VAA-I) including induction of apoptosis, selective binding, the release of cytokines and stimulation of natural killer (NK) functions (Hostanska et al., 1999). Studies have demonstrated that treatment of Recombinant ML to immunodeficient (SCID) mice bearing human ovarian cancer cells elongates their survival time (Schumacher et al., 2000). Studies carried on mouse models of lymphosarcoma, melanoma and pre-BALL have also documented the strong anticancer effects of MLs (Seifert et al., 2008). Moreover, the treatment of hepatocellular carcinoma cell line (SMMC7721) with rVAA-I results in influx of cyt C in cellular cytoplasm leading to apoptosis (Yang et al., 2012) **(Table 2).**

Mistletoe lectins stimulated tumor cell death by its immunostimulatory effects

The *in vitro* and *in vivo* studies conducted on MLs purified from European mistletoe (*Viscum album*) and Korean mistletoe (*Viscum album* coloratum) have documented them as potential anticancer agents due to their immunomodulatory activities (Lavanya et al., 2016).The chain B in *Viscum album* L. lectins is specificaly associated with immunomodulatory function to stimulate the natural killer cells for releasing the cytokines leading to the production of nitric oxide that further kill the target cancer cells by interrupting the signal cascade mediated from membrane lipids. (Fohona et al., al.,2017; Lee et al., 2007) Mistletoe lectins stimulate the human Neutrophils to release superoxide anion that leads a generation of H_2O_2 causing cellular toxicity **(Fig 3)** (Timoshenko et al., 1999). ML-I was shown to inhibit melanomas in the mouse at low dose mainly by immune-signaling (Troger et al., 2013).ML-I induced apoptosis and cytokine production in human peripheral mononuclear cells (Hajtò et al., 2005). Studies showed anti-proliferative and pro-apoptotic effects of *Viscum album* agglutinin-I (VAA-I) enable its modulating function between cellular proliferation and programmed cell death. At higher concentrations in clinical trials,VAA-I showed anti-inflammatory properties (Liu et al., 2010). Recently, it has been described that mistletoe extract drug AbnobaViscum mediated anticancer effects through mitogenic stimulation of specific subtype of T cells (Vγ9Vδ2) and also promoted their funtions such as production of cytotoxic granules and release of cytokines including IFN and TNF(Sebestyen et al., 2020 and

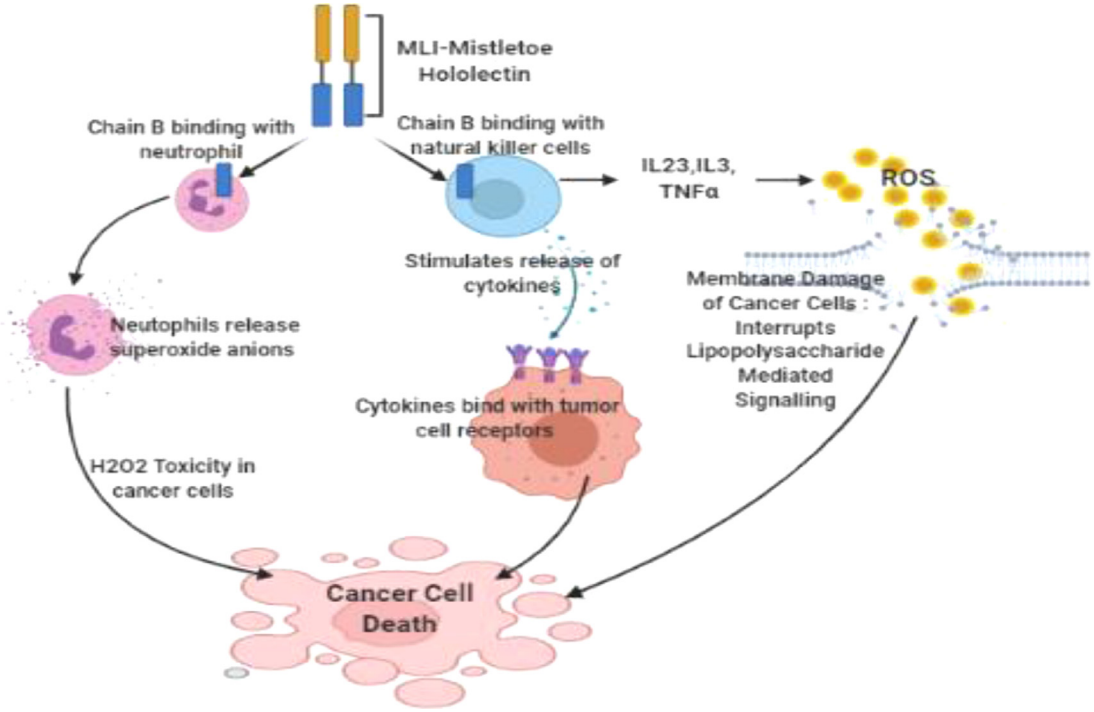


Fig. 3. Immunomodulatory effects of chain-B of ML-I on cancer cells. Binding of chain-B with neutrophil causes release of superoxide radicals (O^{2-}) which transforms into peroxide radicals (H_2O_2) in tumor cells leading to death. Binding of chain-B with natural killer cell stimulate it to produce cytokines (interleukin-23,3) & TNF α (Tumor necrosis factor alpha) resulting in tumor cell lysis. Cytokines also produce reactive oxygen species (ROS) such as nitric oxide (NO) which binds with signalling lipid on cancer cell phospholipid membrane causing interruption in signal cascade pathway for the survival of cancer cell.

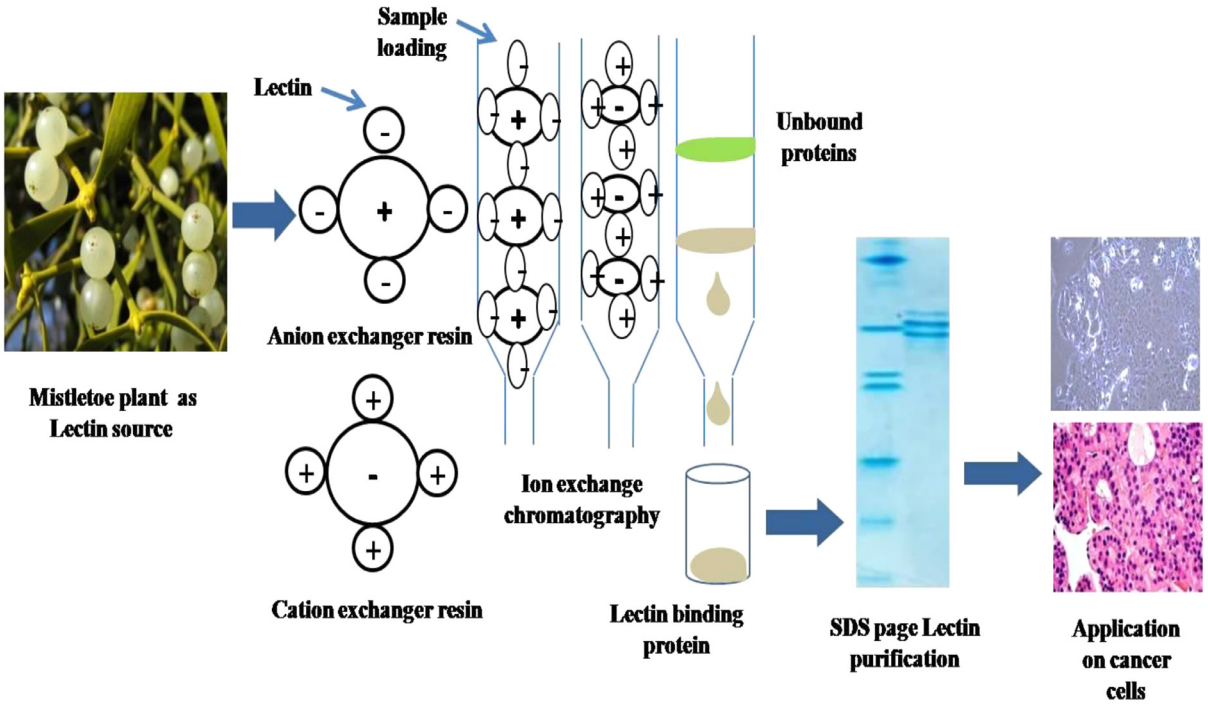


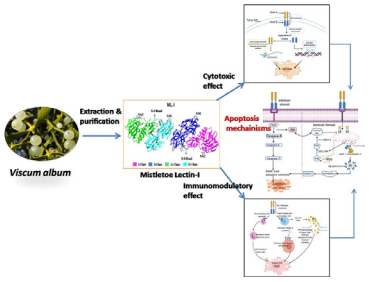
Fig. 4. Schematic presentation of, extraction, purification and *in vitro* antiproliferative application of Mistletoe lectins against cancer cell lines.

Silva-Santos et al., 2019). Results obtained in advanced oral carcinoma showed applications of Mistletoe preparations strongly impacted the dentritic cells and macrophages resulted in cytoxicity in target tumor (Metelmann et al., 2020).

The *in vitro* application of Korean Mistletoe extract on cultured tumor cells showed immunomodulatory effect by inducing the maturation of dendritic cells (Kim et al., 2014). Korean Mistletoe lectin (KML) was further reported to promote the immunomodulatory functions by stimulating the expression of cytokines like IL-23, IL-3, TNF α and generation of ROS (intracellular reactive oxygen species) and inhibiting the signalling events caused by lipopolysaccharides leading to the production of NO and IL-10 (Lee et al., 2007).

Fig. 5. Main mechanisms of Apoptosis induced by Mistletoe lectins.

Synergistic anticancer effect of Mistletoe lectins (combinatorial Mistletoe therapy): Many documented reports suggested MLs enhanced the cytotoxic action of conventional anticancer drugs used in chemotherapy (Sunjic et al., 2015) (Fig. 5). Studies undertaken on combinatorial therapy of VAA-1 in combination with chemotherapeutic drugs such as doxorubicin, cisplatin, and taxol showed potent synergism in comparison to the effect of every single drug in human lung carcinoma cell line A549. Similar results were also derived when cancer cell lines were treated alone with VAA-1 (Siegle et al., 2001). Also, the treatment of transforming murine tumor cells either with recombinant ML (aviscumine) or its combination with ionizing radiation resulted in cell death and inhibition of proliferation (Hostanska et al., 2003). Further studies carried on anticancer effects of the lectins such as recombinant ML-I (aviscumine), purified ML-1 and *Viscum album* extract, Iscador Q in glioma cells have shown them to induce the differential expression of genes involved in the regulation of cell invasion, migration, and adhesion.These drugs mainly regulated the expression of genes responsible for motility resulting in a decrease of glioma cell motility in a ML-I dose-dependant manner (Schotterl et al., 2019). Another combinatorial approach of anticancer therapy, that uses *Viscum album* prepration (Helixor) along with different kinds of mAbs (monoclonal antibodies) such as reduced the



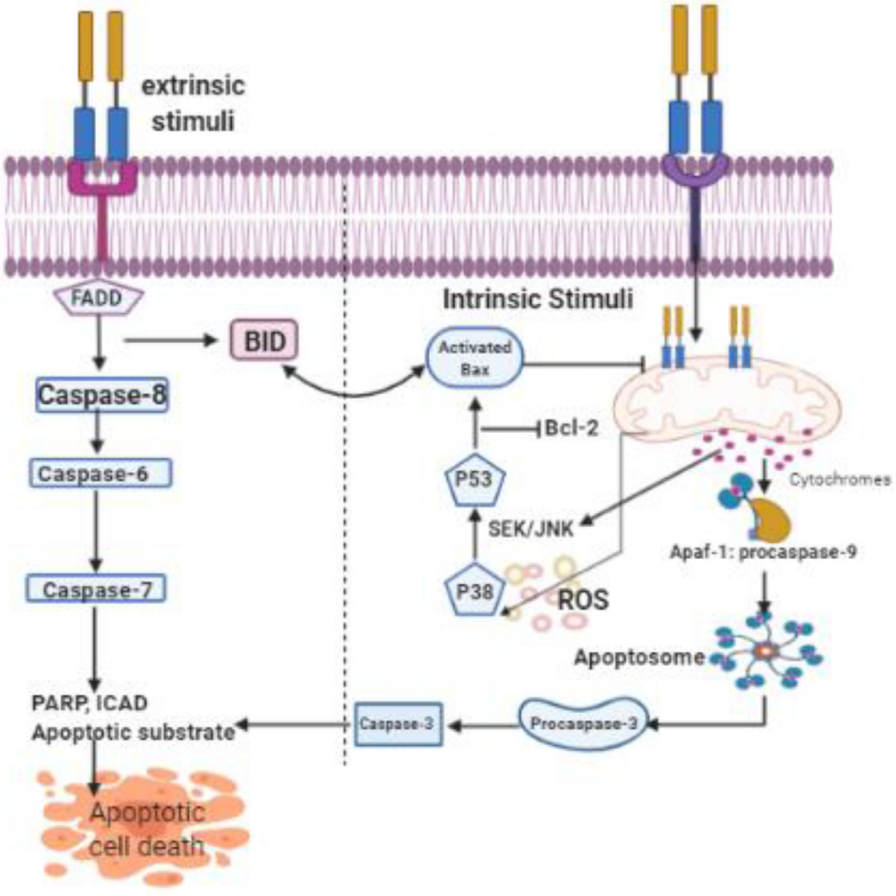


Fig. 6. MLs binding with tumor cell acting as extracellular and intracellular stimuli for mediating extrinsic and intrinsic apoptotic pathways. In extrinsic apoptotic pathway, binding of ML with death receptor: FADD (Fas associated protein with death domain) on tumor cell membrane leads to activation of caspases-8,6,7 causing apoptotic cell death along with activation of apoptotic substrates PARP (Poly ADP-ribose polymerase) and ICAD (inhibitor of caspase-3 activated DNase). Moreover, there is activation of BID (BH3 interacting domain death agonist) that further facilitates intrinsic apoptotic pathway. Binding of ML with mitochondrial membrane results in disruption of mitochondrial membrane potential and release of cytochrome C, Apaf-1(apoptotic protease activating factor) leading to formation of apoptosome and activation of caspase-3. Intrinsic pathway also up regulates pro-apoptotic protein TNF-α (tumor necrosis factor), down regulates anti-apoptoic protein Bcl-2 (B-cell lymphoma 2) and activates JNK (c-Jun N-terminal kinase, signalling cassette of Mitogen activated protein kinase) and Sek (dual specificity mitogen activated protein kinase) pathaway that causes ROS generation and activation of p38, p53, BAX (Bcl-2 like protein, pro apoptotic protein) and BAD (Bcl-2 associated agonist of cell death is a pro apoptotic member of Bcl-2 family).

adverse reactions caused by mAbs which futher ensures its safer application in human trials (Schad et al., 2018).

Novel drug strategies used in mistletoe therapy

Various limitations have been recognised in lectin based cancer research that arises from their difficulty in production, stability, nonspecific binding and low stability. To address these problems in lectin anticancer therapy, the liposomal formulation of *Cratylia mollis* lectin (Cra) was devised. Cra-Lectin a mannose and glucose binding lectin was evaluated for its anticancer potential in mice sarcoma 180 cell line. Unlike free solution form, the nanoparticle formulation of Cra-lectin showed enhanced tumor inhibition, protein stability and minimum tissue toxicity (Andrade et al., 2004). ML was also rendered into alginate chitosan microparticle for efficient drug release and to withstand the acidic conditions in the gut to be used in oral delivery (Lyu et al., 2004). Similarly, many approaches were adopted for lectin based cancer therapy which included use of *Haliotis discus discus* sialic acid-binding lectin (HddSBL) gene against hepatocellular carcinoma cell line Hep3B and lung cancer cell lines A549 and H1299 (Yang et al., 2014). In lung and colon cancers, WGA-conjugated isopropyl myristate (IPM)-incorporated PLGA showed an improved cellular uptake and intracellular retention anticancer drug paclitaxel with reduced toxicity and enhanced antiproliferative effects (Mo and Lim 2004;Mo and Lim 2005; Wang et al., 2010). Meanwhile, a nanogold particle formulation was observed to target cervical cancer cells with high target precision (Wang et al., 2009). Recently, theranostic application of lectin conjugated nanoparticles as drug delivery systems has received great attention due to ease in delivery of drugs and examining the therapeutic response. The theranostic application of lectin was seen in leukaemia that involves the use of lectin conjugated paclitaxel loaded nanoparticle. Unlike the native application of paclitaxel, a nanoparticle formulation of paclitaxel showed higher efficacy against myelogenous leukaemia cells (Singh et al., 2011).

Conclusion and future prospects

Mistletoe lectin-containing extracts have gained huge significance as alternative and adjuvant therapy to treat various kinds of malignancies. Although the data derived till date from clinical and preclinical studies have clearly stated that lectin containig Mistletoe extracts under the names Iscador®, Helixor®, Eurixor®, Lektinol® and Isorel® noticeably inhibited tumors, improved the quality of life and stimulated the host immune system with much-reduced side effects as reported in conventional cancer therapies.However, a thorough investigation needs to be undertaken to understand biological mechanisms elicited by various components in Mistletoe extracts such as hydrophobic (triterpenes) and hydrophilic (lectins) entities causing synergistic anticancer action. Besides, many studies are being carried out regarding fatigue- specific assessment of cancer patients, safety parameters and drug cointerventions and dosage standardization used in clinical trials.

MLs are known as valuable probes for histochemical detection of altered glycans or glycomarkers expressed on the surface of tumor cells. The specified interaction of lectins with the changed receptors on the surface of cancer cell causes agglutination of cancer cells and apoptosis. In addition to this, MLs promote the activity of the immune system by elevating production of interleukins that further impedes the tumor. Various *in vivo* studies conducted to evaluate the therapeutic implications of MLs have revealed that mistletoe lectins used in combination with other chemotherapeutic agents like Taxol or different types of mistletoe lectins applied together can exert more synergism in cytotoxic, immunomodulatory, anti-inflammatory and apoptotic effects during cancer therapies. Meanwhile, new prospectives on lectin research focuses on the surge for novel drug delivering strategies that can address the shortcomings about stability, undesired toxicity and unfavourable binding interactions of lectins with gut epithelium arising from oral administration and skin allergies and inflamation associted with subcutaneous application. (Marvibaigi et al., 2014). There is also a need to understand the fundamental pathways or molecular mechanisms involved in biological functions of lectins for the development of safer and effective lectin based drugs with reduced toxicity. The novel lectin microarray technologies have broadened the understanding of specificity in lectin bindings and the recognition of even the slight alterations on cells, tissue surfaces and serum samples. Thus amplifying the therapeutic effects of lectins to be used as an effective relevant marker to assess and curb the spread of tumor cells.

Declaration of Competing Interest

The authors declare that there is no conflict of interest among the authors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2021.100039.

References

Andrade, C.A., Correia, M.T., Coelho, L.C., Nascimento, S.C., Santos- Magalhaes, N.S., 2004. Antitumor activity of Cratylia mollis lectin encapsulated into liposomes. Int. J. Pharm. 278, 435–445.

Barre, A., Bourne, Y., Van Damme, E.J.M., Peumans, W.J., Rouge, P., 2001. Mannose binding plant lectins: different structural scaffolds for a common sugar-recognition process. Biochimie 83, 645–651.

Barberaki, M., Dermitzaki, E., Margioris, N., Theodosaki, A., Grafakos, M., Kintzios, S., 2015. Protein extracts from somaclonal mistletoe (*Viscum album* L.) callus with increased tumor cytotoxicactivity *in vitro*. Curr. Bioact. Compd. 11, 1.

Buyel, J.F., Fischer, R., 2014. Flocculation increases the efficacy ofdepth filtration during the downstream processing of recombinantpharmaceutical proteins produced in tobacco. Plant Biotechnol. J. 12, 240–252.

Beztsinna, N., de Matos, M.B.C., Walther, J., Heyder, C., Hildebrandt, E., Leneweit, G., Mastrobattista, E., Kok, R.J., 2018. Quantitative analysis of receptor-mediated uptake and pro-apoptotic activity of mistletoe lectin-1 by high content imaging. Sci.Rep. 8, 1–10.

Chevallet, M., Luche, S., Rabilloud, T., 2006. Silver staining of proteinsin polyacrylamide gels. Nat. Protoc. 1, 1852–1858.

Coelho Barroso, L., Silva Santos, P., Lima Menezes, V., De Lúcia, V., PontualViana., E., PaivaGuedes, P., Napoleão Henrique, T., Correia1 Santos,, M., 2017. Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic applications. Evi-Based Complement Altern. Med. 22, 1–22.

Delebinski, C.I., Twardziok, M., Kleinsimon, S., Hoff, F., Mulsow, K., Rolff, J., Jager, S., Eggert, A., Seifert, G., 2015. A natural combination extract of *Viscum album* L. containing both triterpene acids and lectins is highly effective against AML *in vivo*. PLoS One 10, e0133892.

Fan, J., Wu, M., Wang, J., Ren, D., Zhao, J., Yang, G, 2019. 1,7-Bis(4-hydroxyphenyl)-1,4-heptadien-3-one induces lung cancer cell apoptosis via the PI3K/Akt and ERK1/2 pathways. J. Cell. Physiol. 234, 6336–6349.

Felenda., J.E., Turek, C., Stintzing, F.C., 2019. Antiproliferative potential from aqueous Viscum album L. preparations and their main constituents in comparison with ricin and purothionin on human cancer cells. J. Ethnpharm. 236, 100–107.

Fohona, S., Coulibaly., Bi-Botti, C., Youan., 2017. Current status of lectin-based cancer diagnosis and therapy. AIMS Mol. Sci. 4, 1–27.

Fritz, P., Dippon, J., Kierschke, T., Siegle, I., Möhring, A., Moisa, A., Mürdter, T.E., 2004. Impact of mistletoe lectin binding in breast cancer. Anticancer Res. 24 1187–119.

Hajtó, T., Hostanska, K., Berki, T., Pálinkás, L., Boldizsár, F., Németh, P., 2005. Oncopharmacological perspectives of a plant Lectin (Viscum album Agglutinin-I): overview of recent results from *in vitro* experiments and *in vivo* animal models, andtheir possible relevance for clinical applications. eCAM 1, 59–67.

Han, S.Y., Hong, C.E., Kim, H.G., Lyu, S.Y., 2015. Anti-cancer effects of enteric-coated polymers containing mistletoe lectin in murine melanoma cells *in vitro* and *in vivo*. Mol. Cell Biochem. 408, 73–87.

Holtskog, R., Sandvig, K., Olsnes, S., 1988. Characterization of a toxic lectin in Iscador, a mistletoe preparation with alleged cancerostatic properties. Oncology 45, 172–179.

Hostanska, K., Hajto, T., Fischer, J., Mengs, U., Weber, K., Lentzen, H., Saller, R., 1999. Selective modulation of phosphatitylserine expression on various subpopulations of human peripheral blood lymphocytes by a plant lectin, *Viscum album* agglutinin VAA-I and its recombinant form (rVAA) *in vitro*. Cancer Detect Prev 23, 511–523.

Hostanska, K., Vuong, V., Rocha, S., Soengas, M.S., Glanzmann, C., Saller, R., Bodis, S., Pruschy, M., 2003. Recombinant mistletoe lectin induces p53- independent apoptosis in tumour cells and cooperates with ionising radiation. Br. J. Cancer 88, 1785–1792.

Kang, T.B., Song, S.K., Yoon, T.J., Yoo, Y.C., Lee, K.H., Her, E., Kim, J.B., 2007. Isolation and characterization of two Korean Mistletoe. Lectins J. Biochem. Mol. Bio. 40, 959–965.

Kienle, G.S., Glockmann, A., Chink, M., Kiene, H., 2009. *Viscum album* L. extracts in breast and gynaecological cancers: a systematic review of clinical and preclinical research. J. Exp. Clin Cancer Res. 28, 79.

Kim, M.S., So, H.S., Lee, K.M., Park, J.S., Lee, J.H., Moon, S.K., Ryu, D.G., Chung, S.Y., Jung, B.H., Kim, Y.K., Moon, G., Park, R., 2000. Activation of caspase cascades in Korean mistletoe (*Viscum album* var. coloratum) lectin-II-induced apoptosis of human myeloleukemic U937 cells. Gen. Pharmacol. 34, 349–355.

C. Kim, J.J., Hwang., Y.H., Kang, K.Y., Kim, I., Kim, J.B., Park, J.H., Yoo, Y., Yee, S.T., 2014. Enhanced dendritic Cell maturation by the B-chain of Korean mistletoe lectin (KMLB), a novel TLR4 agonist. Inter. Immuno. pharmacol. 21, 309–319.

C. Kim, J.J., Hwang., Y.H., Kang, K.Y., Kim, I., Kim, J.B., Park, J.H., Yoo, Y., Yee, S.T., 2017. Antitumor effect of KML-B treated dedritic cells via induction of lymphocyte activation. J Immnol. Res., 2471627 2017.

Kleinsimon, S., Kauczor, G., Jaeger, S., Eggert, A., Seifert, G., Delebinski, C., 2017. ViscumTT induces apoptosis and alters IAP expression in osteosarcoma *in vitro* and has synergistic action when combined with different chemotherapeutic drugs. BMC Complem. Altern Med. 17, 26.

Krauspenhaar, R., Eschenburg, S., Perbandt, M., Kornilov, V., Konareva, N., Mikailova, S., Stoeva, R., Wacker, T., Maier, T., Singh, A., Mikhailov, W., Voelter., Betzel, C., 1999. Crystal structure of mistletoe lectin I from *Viscum album*. Biochem. Biophys. Res. Commun. 257, 418–424.

Lavastre, V., Chiasson, S., Cavalli, H., Girard, D., 2005. *Viscum album* agglutinin-I (VAA-I) induces apoptosis and degradation of cytoskeletal proteins in human leukemia PLB-985 and X-CGD cells via caspases: lamin B1 is a novel target of VAA-I. Leuk. Res. 29, 1443–1453.

Lavastre, V., Binet, F., Moisan, E., Chiasson, S., Girard, D., 2007. *Viscum album* agglutinin-I induces degradation of cytoskeletal proteins in leukaemia PLB-985 cells differentiated toward neutrophils: cleavage of non-muscle myosin heavy chain-IIA by caspases. Br. J. Haematol. 138, 545–554.

Lavanya, V., Adil Mohamed, A.A., Ahmed, N., Jamal, S., 2016. Lectins-the promising cancer therapeutics. Oncobio. Targets 1, 12.

Lee, J.Y., Kim, Y.J., Lee, Y.G., Byeon, S.E., Kim, B.H., Rhee, M.H., Lee, A., Kwon, M., Hong, S., Cho, J.Y., 2007. *In vitro* immunoregulatory effects of Korean mistletoe lectin on functional activation of monocytic and macrophage-like cells. Bio.Phar. Bull. 30, 2043–2051.

Li, L.N., Zhang, H.D., Zhi, R., Yuan, S.J., 2011. Down-regulation of some miRNA by degrading their precursors contributes to anti-cancer effect of mistletoe lectin-I. Br. J. Pharmacol. 162, 349–364.

Liu, B., Bian, H., Bao, J., 2010. Plant lectins: potential antineoplastic drug from bench to clinic. Cancer Lett. 287, 1–12.

Liu, Z., Luo, Y., Zhou, T.T., Zhang, W.Z., 2013. Could plant lectins become promising anti-tumour drugs for causing autophagic cell death? Cell Prolif. 46, 509–515.

Lyu, S.Y., Choi, S.H., Park, W.B., 2002. Korean mistletoe lectin-induced apoptosis in hepatocarcinoma cells is associated with inhibition of telomerase via mitochondrial controlled pathway independent of p53. Arch. Pharm. Res. 25, 93–101.

Lyu, S.Y., Kwon, Y.J., Joo, H.J., Park, W.B., 2004. Preparation of alginate/chitosan microcapsules and enteric coated granules of mistletoe lectin. Arch. Pharm. Res. 27, 118–126.

Mabed, M., El-Helw, L., Shamaa, S., 2004. Phase II study of viscum fraxini-2 in patients with advanced hepatocellular carcinoma. Br. J. Cancer. 90, 65–69.

Marvibaigi, M., Supriyanto, E., Amini, N., Abdul Majid, F.A., Jaganathan, S.K., 2014. Preclinical and clinical effects of mistletoe against breast cancer. Biomed. Res. Int. 2014, 785479. doi:10.1155/2014/785479, 15 pages.

Mavrikou, S., Tsekouras, V., Karageorgou, M.A., Moschopoulou, G., Kintzios, S., 2020. Anticancer and biochemical effects of *Viscum album* L. protein extracts on HeLa cells. PCTOC 140, 369–378.

Mazalovska, M., Kouokam, C.J., 2020. Transiently expressed Mistletoe Lectin II in *Nicotianabenthamiana* demonstrates anticancer activity *In Vitro*. Molecules 25, 2562. doi:10.3390/molecules25112562.

Metelmann, H.R., Woedtke, T.V., Masur, K., Hyckel, P., Bekeschus, S., Podmelle, F., Seebauer, C., 2020. Immunotherapy and Immunosurveillance of Oral Cancers: Perspectives of Plasma Medicine and Mistletoe. In: Rezaei, N. (Ed.), Cancer Immunology. Springer, Cham doi:10.1007/978-3-030-57949-4_14.

Mishra, A., Behura, A., Mawatwal, S., kumar, A., Naik, L., Mohanty, S., Manna, D., Dokania, P., Mishra, A., Patra, S., Dhiman, R., 2019. Structure-function and application of plant lectins in disease biology and immunity. Food Chem.Toxicol. 134, 110827.

M.o, Y., Lim, L.Y., 2004. Mechanistic study of the uptake of wheat germ agglutinin-conjugated PLGA nanoparticles by A549 cells. J. Pharm. Sci. 93, 20–28.

M.o, Y., Lim, L.Y., 2005. Preparation and *in vitro* anticancer activity of wheat germ agglutinin (WGA)-conjugated PLGA nanoparticles loaded with paclitaxel and isopropyl myristate. J. Control. Rel. 107, 30–42.

Ostermann, T., Raak, C., Büssing, A., 2009. Survival of cancer patients treated with mistletoe extract (Iscador): a systematic literature review. BMC Cancer 451, 125–134.

N. Panda, P.K., Mukhopadhyay, S., Behera, B., Bhol, C.S., Dey, S., Das, D., Sinha, N., Bissoyi, A., Pramanik, K., Maiti, T.K., Bhutia, S.K., 2014. Antitumor effect of soybean lectin mediated through reactive oxygen species-dependent pathway. Life Sci. 111, 27–35.

Patel, S., Suryakanta, P., 2014. Emerging roles of mistletoes in malignancy management. Biotech 4, 13–20.

Pervin, M., Koyama, Yu., Isemura, M., Nakamura, Y., 2015. Plant lectins in therapeutic and diagnostic cancer research. Int. J. Plant Biol. Res. 3, 1030–1035.

Podlech, O., Harter, P.N., Mittelbronn, M., Poschel, S., Naumann, U., 2012. Fermented mistletoe extract as a multimodal antitumoral agent in gliomas. Evid. Based Complement. Altern. Med. 50 2012.

Rostock, M., Huber, R., Greiner, T., Fritz, P., Scheer, R., Schueler, J., Fiebig, H.H., 2005. Anticancer activity of a lectin-rich mistletoe extract injected intratumorally into human pancreatic cancer xenografts. Anticancer Res. 25, 1197–1969.

Sebestyen, Z., Prinz, I., Déchanet-Merville, J., Silva-Santos, B., Kuball, J., 2020. Translating gammadelta (gamma delta) T cells and their receptors into cancer cell therapies. Nat. Rev. Drug Discov. 19, 169–184.

Schoffski, P., Riggert, S., Fumoleau, P., Campone, M., Bolte, O., Marreud, S., Lacombe, D., Baron, B., Herold, M., Zweirzina, H., Ogunbiyi, K.W., Lentzen, H., Twelves, C., 2004. Phas I trial of intravenous aviscumine (r-viscumine) in patients with solid tumors: a study of the European organization for research and development group. Ann. Oncol. 15, 1816–1824.

Schötterl, S., Miemietz, J.T., Ilina, E.I., Wirsik, N.M., Ehrlich, I., Gall, A., Huber, S.M., Lentzen, H., Mittelbronn, M., Naumann, U., 2019. Mistletoe based drugs work in synergy with radio-chemotherapy in the treatment of glima *in vitro* and *in vivo* in glioblastoma bearing mice. Evi. Compl. Alter. Med. 2019, 1–17.

Schumacher, U., Stamouli, A., Adam, E., Peddie, M., Pfüller, U., 1995. Biochemical, histochemical and cell biological investigations on the action of mistletoe lectins I, II and III with human breast cancer cell lines. Glycoconj. J. 12, 250–257.

Schumacher, U., Feldhaus, S., Mengs, U., 2000. Recombinant mistletoe lectin (rML) is successful in treating human ovarian cancer cells transplanted into severe combined immunodeficient (SCID) mice. Cancer Lett. 150, 171–175.

Schad, F., Axtner, J., Kröz, M., Matthes, H., Steele, M.L., 2018. Safety of Combined Treatment With Monoclonal Antibodies and *Viscum album* L PreparationsIntegrative. Cancer Therap. 17, 41–51.

Schotterl, S., Huber, S.M., Lentzen, H., Mittelbronn, M., Naumann, U., 2018. Adjuvant therapy using mistletoe containing drugs boosts the T-cell-mediated killing of glioma cells and prolongs the survival of glioma bearing mice. Evid. Based Compliment. Altern. Med., 3928572. doi:10.1155/2018/3928572, PMID: 30224928; PMCID: PMC6129785.

Seifert, G., Jesse, P., Laengler, A., Reindl, T., Luth, M., Lobitz, S., Henze, G., Prokop, A., Lode, H.N., 2008. Molecular Mechanism of Mistletoe plant extracts- induced apoptosis in acute lymphoblastic leukemia*in vivo* and *in vitro*. Cancer Lett. 264, 218–228.

Siegle, I., Fritz, P., McClellan, M., Gutzeit, S., Murdter, T.E., 2001. Combined cytotoxic action of *Viscum album* agglutinin-1 and anticancer agents against human A549 lung cancer cells. Anticancer Res. 21, 2687–2691.

Silva-Santos, B., Mensurado, S., Co_elt, S.B., 2019. T cells: pleiotropic immune effectors with therapeutic potential in cancer. Nat. Rev. Cancer 19, 392–404.

Singh, A., Dilnawaz, F., Sahoo, S.K., 2011. Long circulating lectin conjugated paclitaxel loaded magnetic nanoparticles: a new theranostic avenue for leukemia therapy. PLoS One 6, e26803.

Stammer, M.R., Kleinsimon, S., Rolff, J., Jager, S., Eggert, A., Seifert, G., Delebinski, C.I., 2017. Synergistic antitumour properties of viscumTT in alveolar rhabdomyosarcoma. J Immnol. Res., 4874280 2017.

Sunjic, S.B., Gasparovic, A.C., Vukovic, T., Weiss, T., Weiss, E.S., Soldo, I., Djakovic, N., Zarkovic, T., Zarkovic, N., 2015. Adjuvant cancer biotherapy by *Viscum Album* extract isorel: overview of evidence based medicine findings. Coll. Antropol. 39, 701–708.

Timoshenko, A.V., Dubovskaya, L.V., Karvatskaya, O.D., Zharkov, V.V., Gabius, H.J., 1999. NO-dependent regulation of lectin- and menadione-induced H2O2 production by cells from pleural effusions of lung cancer patients and by immune cells. Int. J. Oncol. 14, 793–798.

Troger, W., Galun, D., Reif, M., Schumann, A., Stankovic, N., Milic´evic, M., 2013. *Viscumalbum* [L.] extract therapy in patients with locally advanced or metastatic pancreatic cancer: a randomised clinical trial on overall survival. Eur. J. Cancer 49, 3788–37978.

Twardziok, M., Kleinsimon, S., Rolff, J., Jäger, S., Eggert, A., Seifert, G., Delebinski, C.I., 2016. Multiple active compounds from *Viscum album* L. synergistically converge to promote apoptosis in Ewing sarcoma. PLoS One 11, e0159749.

Twardziok, M., Meierhofer, D., Börno, B., Timmermann, B., Jäger, S., Boral, S., Eggert, A., Delebinski, C.I., Seifert, G., 2017. Transcriptomic and proteomic insight into the effects of a defined European mistletoe extract in Ewing sarcoma cells reveals cellular stress responses. BMC Complement. Altern. Med. 17, 237.

Varrot, A., Basheer, S.M., Imberty, A., 2013. Fungal lectins: structure, function and potential applications. Curr. Opin. Struct. Biol. 23, 678–685.

Vojdani, A., 2015. Lectins, agglutinins, and their roles in autoimmune reactivities. Altern.Ther. Health Med. 21, 46–51.

Wacker, R., Stoeva, S., Pfuller, K., Pfuller, U., Voelter, W., 2004. Complete structure determination of the A chain of mistletoe lectin III from *Viscum album* L. ssp. album. J. Pept. Sci. 10, 138–148.

Wang, J., Duan, T., Sun, L., Liu, D., Wang, Z., 2009. Functional gold nanoparticles for studying the interaction of lectin with glycosyl complex on living cellular surfaces. Anal. Biochem. 392, 77–82.

Wang, C., Ho, P.C., Lim, L.Y., 2010. Wheat germ agglutinin-conjugated PLGA nanoparticles for enhanced intracellular delivery of paclitaxel to colon cancer cells. Int. J. Pharm. 400, 201–210.

Yang, X., Jiang, S., Liu, Y., Zhang, P., Xie, S., Wang, G., 2012. Recombinant VAA-I from Viscum album induces apoptotic cell death of hepatocellular carcinoma SMMC7721 cells. Molecules 17, 11435–11446.

Yang, X., Wu, L., Duan, X., Cui, L., Luo, J., Li, G., 2014. Adenovirus carrying gene encoding Haliotis discus discussialic acid binding lectin induces cancer cell apoptosis. Mar Drugs 12, 3994–4004.

Yang, P., Jiang, Y., Pan, Y., Ding, X., Rhea, P., Ding, J., Hawke, D., Felsher, D., Narla, G., Lu, Z., Le, R., 2019. Mistletoe extract Fraxini inhibits the proliferation of liver cancer by down-regulating c-Myc expression. Sci. Rep. 9, 6428.

Yau, T., Dan, X., Ng, C.C., Ng, T.B., 2015. Lectins with potential for anti-cancer therapy. Molecules 20, 3791–3810.