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Chapter IV

THE ROLE OF PLANT LECTINS IN CANCER TREATMENT

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ABSTRACT

Social behavior of cells depends on membrane glycosylation, including cell communication, adhesion and migration. Malignant transformation is associated with alterations in cell surface carbohydrates expression, which suggests that such molecules play an important role in malignant transformation. Plant lectins are oligomeric proteins lacking enzymatic activity and are distinct from immunoglobulins. They can have several carbohydrate-binding sites per molecule that allow them to specifically interact with other carbohydrate moieties, hence the name lectin (from the Latin *legere*, to select or choose). Lectins are commonly used in biochemistry, cell biology and immunology, as well as for diagnostic and therapeutic purposes in cancer investigation. They are important tools for investigating structural and functional complex carbohydrates, for the evaluation of changes that occur in the cell surface during physiological and pathological processes and for the identification of cancer cells. Because of the ability of lectins to recognize cancer cells as well as for their cytotoxic activity against them, the role of plant lectins as anticancer agents is discussed.

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INTRODUCTION

Lectins are proteins or glycoproteins from non-immune origin that specifically recognize cell surface molecules with at least two binding sites to carbohydrates (hence their ability to agglutinate cells), precipitating the corresponding glycoconjugates. They are found in all kinds of organisms, including animals, plants, fungi, bacteria and viruses [1,2]. Lectins have no single action and a wide spectrum of functions has been related to them (Figure 1). Their relative abundance is not necessarily related to the importance of their function [3].

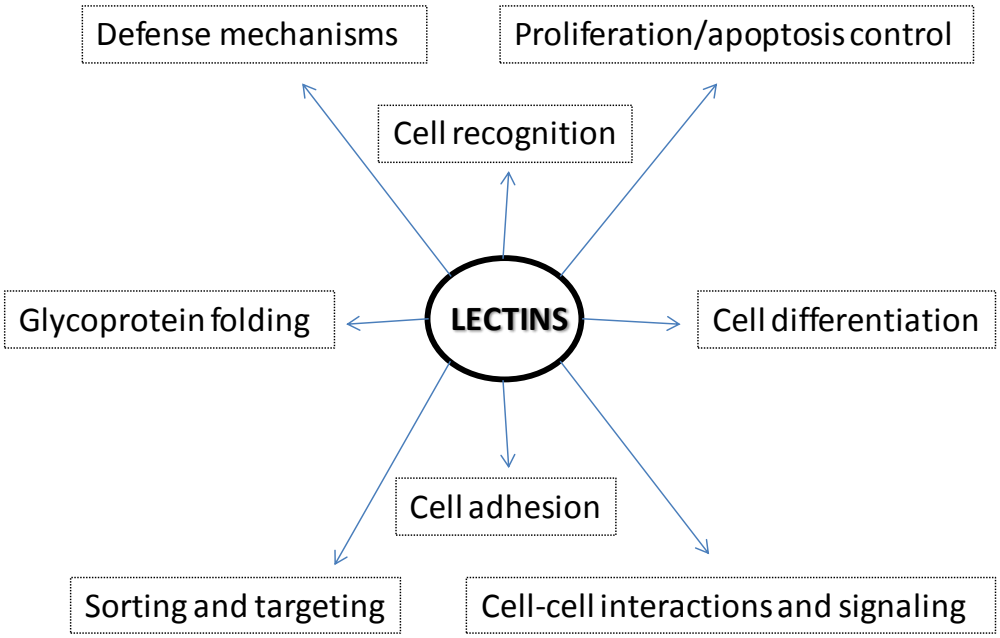

Figure 1. Some biological functions of lectins in live organisms [3,4].

Plant lectins can be defined as all plant proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide [5]. Classification of plant lectins is based on different criteria. On the basis of the overall structure of the mature lectins they can be divided in four groups (Table 1) but analysis of the available sequences distinguishes seven families of evolutionary related proteins (Table 2). Some lectins, however, do not fit the classification system or cannot be classified because there is no sequence information available [6].

The main source of lectins in the human diet is found in plants. Lectins are mainly present in seeds cotyledons and kernels where they represent 2 to 10% of the total protein. It is suggested that, within the plant, these proteins may have different functions such as: physiological regulation, defense against microorganisms attack, storage protein, carbohydrate transport, mitogenic stimulation, recognition of the nitrogen-fixing bacteria of *Rhizobium* genus, and some more. Plant lectins represent a unique group of proteins with potent biological activity. They occur in foods like wheat, corn, tomato, peanut, kidney bean, banana, pea, lentil, soybean, mushroom, rice, and potato. Many lectins resist digestion, survive gut passage, and bind to gastrointestinal cells and/or enter the circulation intact,

maintaining full biological activity including specific agglutination of lymphocytes, erythrocytes, spermatozoa, platelets, bacteria and tumor cells, induction of mitosis or cytotoxic effects on lymphocytes. Once they are consumed, different biological properties are observed at biochemical and molecular level. Binding between lectins and surface cell molecules or internalization into cells involve a wide variety of signals that are important for cell regulation, including [7,8]:

1) Cell agglutination and/or aggregation.
2) Induction of apoptosis or cell cycle arrest.
3) Down regulation of telomerase activity and inhibition of angiogenesis.
4) Increase of drug sensitivity of tumor cells, hence their utility in the design of immunotoxins for cancer treatment.
5) Direct effects on the immune system by altering the production of various interleukins, or by activating certain protein kinases.
6) Ingestion of lectins also sequesters the available body pool of polyamines, thereby thwarting cancer cell growth.
7) Some lectins can bind to ribosomes, inhibiting protein synthesis.

Due to their ability to bind reversibly with specific carbohydrate structures, lectins have commonly been used as molecular tools in several disciplines of biology and medicine. Lectin affinity chromatography (LAC) and various histochemical methods, provide practical applications for the observation of changes occurring at the cell membrane in different stages of physiological and pathological development of human or animal organisms [9].

Table 1. Plant lectins classification based on mature lectin structure

Lectin type	Definition
Merolectins	Single carbohydrate-binding domain, they are monovalent and hence cannot precipitate in glycoconjugates or agglutinate cells.
Hololectins	Contain at least two carbohydrate-binding domains that are either identical or very homologous and bind either the same or structurally similar sugars. They are di- or multivalent and hence agglutinate cells and/or precipitate glyco-conjugates.
Chimerolectins	They are fusion proteins consisting of one or more carbohydrate-binding domains and a well-defined enzymatic domain or another biological activity that act independently from the carbohydrate-binding domain. Depending on the number of carbohydrate-binding sites, chimerolectins behave as merolectins or as hololectins.
Superlectins	Consist of at least two carbohydrate-binding domains that recognize structurally unrelated sugars. They can also be considered a special group of chimerolectins.

Adapted from [6].

Table 2. Plant lectins classification based on molecular structure

Lectin group	Definition
Legume lectins	Plant lectins that are found exclusively in the *Leguminoseae,* but not all lectins found in legume species belong to the legume lectins. All legume lectins are built up of protomers of approximately 30 kDa that give rise to the so-called 'one-chain' legume lectins. In some instances the protomers are cleaved into two smaller polypeptides. The legume lectins composed of such cleaved protomers are usually referred to as 'two chain' legume lectins. Legume lectins contain divalent cations (Mn^{2+} or Ca^{2+}) at specific metal-binding sites which are essential for the carbohydrate-binding activity. Many, but not all, legume lectins are glycosylated and possess one or two glycan chains of high-mannose or complex type that may be present on a single lectin protomer. Differences in glycosylation result in the formation of glycoforms, which can mistakenly be considered as isolectins. Native legume lectins are composed of two or four protomers held together by noncovalent interactions. The possible combinations imply that legume lectins can occur in eight different molecular forms.
Chitin-binding lectins	This family comprises all proteins containing at least one hevein domain (small 43 amino acid protein from the latex of the rubber tree, *Hevea brasiliensis*) but there are also chitin-binding lectins without hevein domains. The family of chitin-binding lectins comprises merolectins, hololectins, as well as different types of chimerolectins.
Type 2 RIP and related lectins	Ribosome-inactivating proteins (RIP) are commonly known as proteins that catalytically inactivate eukaryotic ribosomes, as a result, protein synthesis is arrested and the cell dies. All type 2 RIP are built up of similar protomers consisting of disulfide bridge linked A and B chains. The A chain (25 to 30 kDa) possesses *N*-glycosidase activity, whereas the B chain (30 to 35 kDa) has one or more carbohydrate-binding sites. Because the A chain of type 2 RIP shares a high sequence similarity with type 1 RIP, type 2 RIP are considered as chimerolectins composed of a RIP subunit and a lectin subunit. Native type 2 RIP consist of one or two, and, in a few exceptional cases, four identical [A-s-s-B]-pairs. Because the [A-s-s-B]-pair is a single structural unit, type 2 RIP consisting of one, two, and four [A-s-s-B]-pairs are considered as monomeric, dimeric, and tetrameric proteins, respectively.
Monocot mannose-binding lectins	Super families of strictly mannose-specific lectins, which have been found exclusively in a subgroup of the monocotyledonous plants, consist of subunits with a similar sequence and overall three-dimensional structure. According to the size of the protomers, these lectins can be divided into one-domain protomers of 11 to 14 kDa and two-domain protomers of about 30 kDa.
Jacalin-related lectins	Jacalin is the trivial name for the lectin from the seeds of jack fruit (*Artocarpus integrifolia*). All lectins that are structurally and evolutionary related to the jack fruit lectin belong to this group that comprises two subgroups of lectins. A first subgroup is the GalNAcspecific *Moraceae* seed lectins, which are very similar to the jack fruit lectin. The second subgroup is the *Convolvulaceae* lectins, which share sequence similarity with the *Moraceae* lectins but exhibit specificity toward mannose/maltose.
Amatanthin lectin family	The term amaranthin, from the seed lectin of *Amaranthus caudatus,* is now used as a collective name for the closely related GalNAc-specific seed lectins from various *Amaranthus* species. The amaranthins are not related to any other lectin family. Detailed specificity studies have been performed only with the *Amaranthus caudatus* lectin. The lectin is inhibited by GalNAc but has a much higher affinity for the disaccharide Galb(1,3)GalNAc.
***Cucurbitaceae* phloem lectins**	Small family of chitin-binding agglutinins found in the phloem exudates of *Cucurbitaceae* species. They are not related to other *Cucurbitaceae* lectins and do not contain the vein domains. The *Cucurbitaceae* phloem lectins exhibit specificity toward oligomers of GlcNAc.
No classified lectins	Plant families in which lectins occur that, in the absence of clear criteria, have not been classified: *Apiaceae, Araucariaceae, Celastraceae, Cucurbitaceae, Euphorbiaceae, Gramineae, Labiatae*

Adapted from [6].

CELL MEMBRANE GLYCOSYLATION AND LECTINS: THE KEY OF SELECTIVITY

Tumor cells display aberrant patterns of glycosylation in carbohydrates linked to ceramides and cell surface proteins [10,11,12]. Alterations on membrane glycosylation are present in all cancer cells and some of them are well known as progression markers. Each type of cancer presents differential alteration patterns even during the different stages of the disease [13]. Two major glycosylation changes have been described in cancer cells: blockage of carbohydrate synthesis or neo synthesis [11]. Glycosylation alterations that occur in cancer cells may involve loss or changes in function of certain structures, presence of truncated structures or their precursors and, to a lesser extent, the appearance of new structures. Carbohydrates expressed in tumor cells are either adhesion molecules *per se* or modulate adhesion receptor functions. Among the more common changes are the increase of N-glycans and sialic acid content in the cell surface, the abnormal production of mucin, expression of Lewis X/A structures in glycosphingolipids (identified at first as a tumor antigen), and the increased expression of galectins (Figure 2). All these changes correlate with the ability of metastatic cancer cells and/or the increase in migration and their ability to evade the immune system [10,14]. In some cases membrane glycoproteins are also modified, so that they act as oncogenic antigens. Several lines of evidence accumulated in recent years implicate tumor cell lectins in cellular interactions such as adhesion, cell growth, tumor cell differentiation, and metastasis. The involvement of lectins in processes such as cell–cell and host–pathogen interactions, serum-glycoprotein turnover and innate immune responses are of particular relevance to tumor growth and metastatic spread [13].

Changes in glycosylation involve not only interactions with endogenous, but also with exogenous lectins, that can alter the response of cancer cells. The knowledge of the interaction of lectins with cancer cells and how they can affect the biology of the tumor will explain the role of carbohydrates in the acquisition of malignant status and therefore its inhibition [13]. The study of lectins as biological tools has led to the conclusion that their main significance lies in their properties in cell recognition (i.e. red blood cells, lymphocytes, platelets, sperm, bacteria, viruses and tumor cells) [7]. Several studies have focused on their ability to show preferential agglutination on cancer cells [16] therefore, one important area where lectins are used is in the detection of malignant changes in transformed cells due to the changes on cancer cells surface [7,14,17]. Higher affinity has been observed between human cancer cells and lectins, than between healthy cells and the same lectins [18]. Evidence of this is shown in the selective binding of plant lectins, such as Concanavalin A (ConA) and the wheat germ agglutinin (WGA) to tumor cells [13]. The link between membrane glycoproteins and lectins is weak, but a stronger one is formed by multiple binding sites of a lot weak joints. Through this mechanism, lectins can induce apoptosis, cytotoxicity, and inhibition of tumor growth [8, 17]. Selective binding of lectins to specific carbohydrates allows them to be used as diagnostic tools, some examples of differential recognition are:

- Mistletoe lectins, (MLs, ML-I, ML-II, and ML-III) in which the binding of the B-chain to carbohydrates inhibit their toxic activities. Digalactosides Gal-β-1,2Gal-β-allyl and Gal-β-1,3Gal-β-allyl were 60 and 30 times, respectively, more potent than D-galactose, protecting the cells from ML-I cytotoxicity. GalNAc and nitrophenyl

galNAc protected mostly from the effects of ML-II and ML-III. The serum glycoproteins haptoglobin, 1-acid glycoprotein, and transferrin notably inhibited the toxicity of the lectins but deglycosylated haptoglobin had no protective activity on the Molt 4 cells [19].

- The potential and the applicability of different plant lectins using 5637 bladder cancer cells as a model for human urinary carcinoma were studied. As a result, wheat germ agglutinin (WGA) and *Ulex europaeus* agglutinin (UEA) revealed strongest interaction with single cells demonstrating a high presence of N-acetyl-d-glucosamine, sialic acid and α-l-fucose residues on the membrane surface [20].
- *Griffonia simplicifolia* lectin-I (GS-I) and *Vicia vilosa* agglutinin (VVA) showed significant associations with nuclear grade of ductal carcinoma in situ (DCIS). DCIS specimens with nuclear grades II and III showed significantly more intense reactivity than DCIS cases with nuclear grade I to GS-1 and VVA. Those results suggest that the expression of VVA- and GS-I-reactive carbohydrate antigens may contribute to forming higher grade DCIS and increase the recurrence risk [21].
- Different fluorescence labeled lectins: DBA (*Dolichos biflorus*) PNA, LCA (*Lens culinaris*), STL (*Solanum tuberosum*), UEA-I (*Ulex europaeus* I), and WGA showed binding specificity on three cell lines of human colorectal carcinoma (CaCo-2, HT-29 and HCT-8) [22].
- Bioadhesive properties of fluorescein-labeled plant lectins with different carbohydrate specificities, investigated by flow cytometry at 4 and 37°C using Du-145 prostate cancer cells. At both temperatures lectin association rate increased following the order: *Dolichos biflorus* agglutinin (DBA) peanut agglutinin, Ulex *europaeus* isoagglutinin I, *Lens culinaris* agglutinin, *Solanum tuberosum* lectin, wheat germ agglutinin (WGA), reflecting the glycosylation pattern of Du-145 cells [23]
- ABL (*Agaricus bisporus*) lectin specifically binds to a galactosilated disaccharide expressed in keratinocytes and this lectin reversibly inhibits proliferation of cancer cell lines without cytotoxicity [24].
- Comparative analysis of glycoproteins patterns from human melanoma cells using different lectins (SNA: *Sambucus nigra*, MAA: *Maackia amurensis* and PHA: *Phaseolus vulgaris*) suggest an increased expression of branching N-oligosaccharides in human melanoma from metastatic sites. It suggests that carbohydrates are associated with the acquisition of the metastatic potential of tumor cells [25].
- Peanut agglutinin lectin (PNA) binds the Thomsen–Friedenreich (TF) oncofetal carbohydrate antigen that is increased in colon cancer, adenomas, and inflammatory bowel disease. However, PNA has a mitogenic effect, both *in vitro* and *in vivo*, for colon epithelial cancer cells mediated by phosphorylation of c-Met and MAPK [26].
- Lymphatic invasion, lymph node metastasis, and peritoneal metastasis correlated with staining with lectins that bind galactose/N-acetylgalactosamine residues (Gal/GalNAc) such as Maclurapomifera (MPA), Arachishypogaea (PNA), Helixpomatia (HPA), and Viciavillosa (VVA). In contrast, hepatic metastasis correlated with staining with *Anguilla anguilla* lectin (AAA), anti-LewisX (LEX-2), anti-sialyl Lewisa (NS19-9), andanti-sialyl-dimeric LewisX (FH-6) MAbs, all of which bind preferentially to fucosylated carbohydrate chains. The five-year survival

rate of patients was related to the staining of cancers with MPA, HPA, FH-6 or NS19-9, and MPA and FH-6 staining were in dependent prognostic factors. Carbohydrate expression profiles of cancer cells are relevant to the route of tumor cell dissemination, metastatic pattern as well as prognosis of colorectal cancer [27].

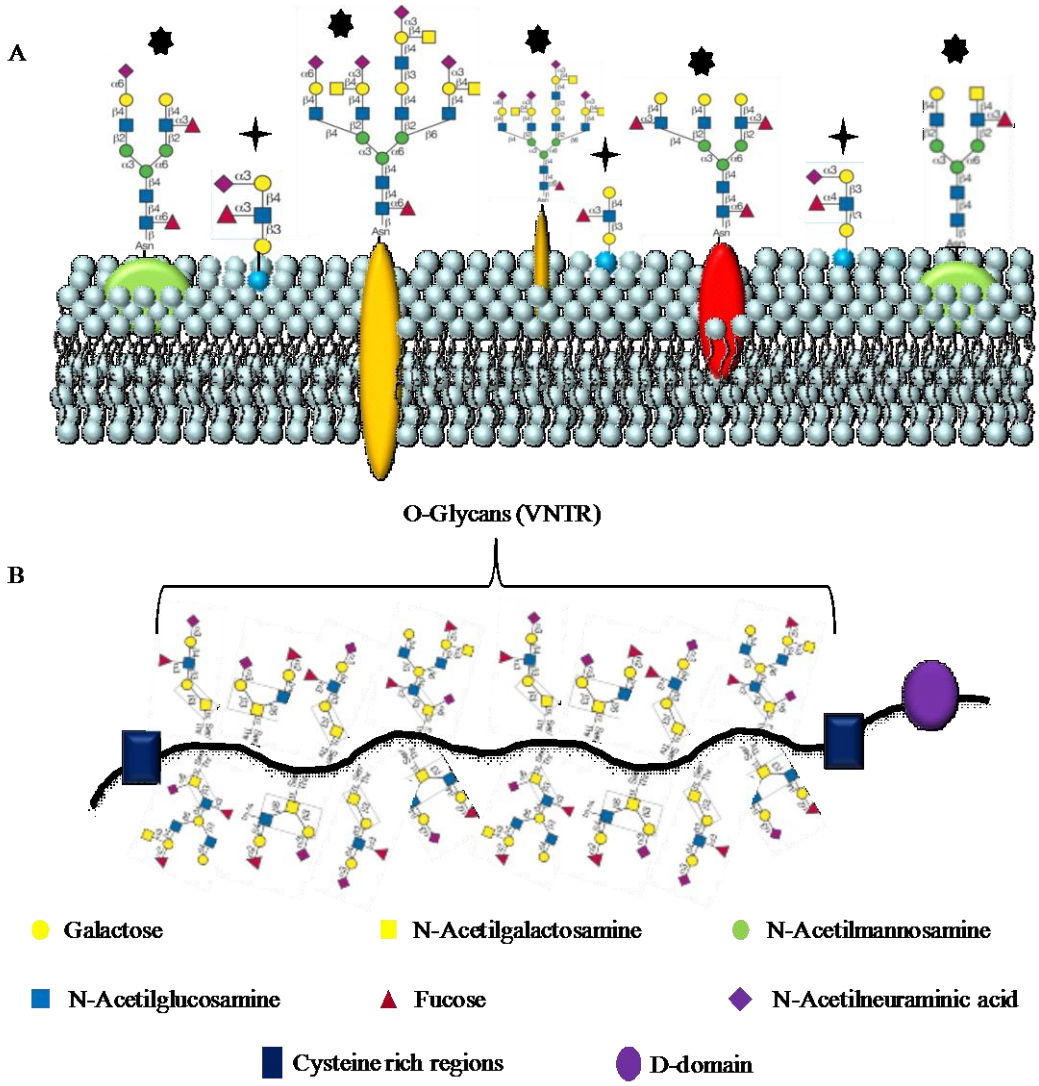

Figure 2. Simplified structure of cell oligosaccharides. (A) Glycoproteins and glycolipids of cell membrane with (✹)N-glycan structures and (✦)Type-1 and -2 Lewis determinants. (B) O-GalNAc glycans on a simplified model of mucin. The VNTR (variable number of tandem repeat) region rich in serine, threonine, and proline is highly O-glycosylated and the peptide assumes an extended "bottle brush" conformation. Hundreds of O-GalNAc glycans with many different structures may be attached to serine or threonine residues in the VNTR domains. The cysteine-rich regions at the ends of the molecules are involved in disulfide bond formation to form large polymers of several million daltons. D domains are also involved in polymerization [15].

LECTINS AS ANTICANCER AGENTS

Lectins have anticancer properties *in vitro* and *in vivo*, preferentially binding to cancer cell membranes or their receptors, causing cytotoxicity, apoptosis, and inhibition of tumor growth [8]. Antitumor effect and anticarcinogenic activity of lectins are due through different mechanisms as the induction of remission in certain tumors, having a direct anti-tumor cytotoxic effect, by improving the antineoplastic effect of radiation and chemotherapy, by promoting restoration of normal growth in cancer cells, by amplifying the immunogenicity of tumor cells and because of their differential cytotoxic effect on malignant cells with respect to normal cells, they exhibit minimal risk of anti-tumor cytotoxic activity [28].

It has been observed that lectins from different sources inhibit cancer cells growth depending on their concentration and in a differential manner [29]. They are able to induce apoptosis and activate the immune system by stimulating the proliferation of T lymphocytes [30] and also food lectins can stimulate differentiation of colon cancer cells [31]. The ability of lectins to modulate growth, differentiation, proliferation and apoptosis are mainly mediated by surface receptors [32].

First studies had focused on cytotoxic properties of lectins like ricin (RCA) and abrin (APA) as potential therapies for human cancer treatment [33,34]. Later on, studies performed using Con A (*Canavalia ensiformis*) showed tumor growth inhibition in hamsters [35]. Some studies using either pure or semi-pure plant lectins against different cancer cell lines or tumors are shown in Table 3.

Comparative studies using several lectins as antitumoral or cytotoxic agents have shown differential effect depending on lectin source and cell line or cancer type. For example, lectins from common bean, soybean, and wheat were tested on lymphoma cells. After *in vitro* treatment, cells were inoculated into normal animals. All lectins were found to possess therapeutic effects, as revealed by inhibition of tumor growth and delayed tumor progression. Wheat lectin was most effective in controlling tumor growth and improving the life expectancy of the host, probably by activating the host immune response (macrophages increased three-fold). Although cell viability was retained, the ability of the cells to multiply was possibly affected. Tumor cells become more susceptible to attack by macrophage-mediated cytolysis, which induces the binding of effector cells that trigger the non-specific lysis of target cells [93]. On the other hand, a study with five different lectins: PHA (*Phaseolus vulgaris*) GSA (*Griffonia simplicifolia*) Con-A (*Concavalina A*), WGA (*Triticum vulgare*) and PNA (*Arachis hypogea*) on three colon cancer cell lines (Lovo, HCT-15 and SW837) showed that growth was affected in different ways depending on the concentration and type of lectin tested. It was concluded that these lectins have a potential to affect the growth of cancerous colonies *in vitro* [94]. Lectin from common bean (*Phaseolus vulgaris*) has mitogenic action on immune system cells and has the ability to specifically agglutinate malignant cells. This has developed a strong interest in research to use it as a treatment for tumor growth control [95].

Table 3. Cytotoxic and anticancer effects of some plant and mushroom lectins

Lectins or lectins extracts	Effects	Ref
AAL ***Agrocybe aegerita***	Antitumoral effect via apoptosis with DNAase activity. Growth inhibition on HeLa, SW480, SCG-7901, MGC80-3, BGC-823, HL-60 cells and murine S-180 sarcoma.	[36]
ABL ***Algaricus bisporus***	Cell proliferation inhibition on HT29 cells. Internalization and blockage NLS dependent nuclear channels.	[37,38]
	Internalization, proliferation inhibition and blockage of nuclear proteins importation.	[39]
Abrin-a	Cytoagglutination against human cultured cell lines derived from acute lymphoblastic leukemia and adult T-cell leukemia, weak agglutination against normal lymphocytes.	[40]
Abrin	Antitumoral effects on transplanted mice.	[41]
AHL ***Arisaema helleborifolium***	Inhibits proliferation of human cancer cell lines HOP-62 (95%), HCT-15 (92%), HEP-2 (66%), HT-29 (68%), PC-3 (39.4%), and A-549 (20.7%).	[42]
Alocasia cucullata	Inhibition of SiHa (human cervix) cancer cell line.	[43]
ATL (*Arisaema tortuosum*)	*In vitro* proliferation inhibition of human cancer cell lines HT29, SiHa and OVCAR-5.	[44]
DSA (*Datura stramonium*)	Irreversible differentiation induction on glioma C6 cells, dose-dependent proliferation inhibition and DNA synthesis suppression. Recognition between astrocitic and neuronal glycoreceptors.	[45]
GS-1 (*Griffonia simplicifolia*)	Tumoral growth inhibition in mice, cytotoxic effect.	[46,47]
Iscador M (*Viscum album*)	Increase in life span, reduction in tumor growth, and hyperplasia of mice and rats with lymphoma and lung cancer.	[48]
Iscador M special, Iscador Qu special, and Iscador P. Aqueous mistletoe extracts (*Viscum album*)	Preparations containing high lectin concentration showed antitumor activity in the mammary cancer cell line MAXF 401NL. Apoptosis and cytotoxicity were positively correlated at low and intermediate concentrations, and the effects observed in long intervals and high concentrations of the lectin were mostly necrotic. Proliferation inhibition on 16 tumor cell lines.	[49]
Isorel Mistletoe extract (*Viscum album*)	Stimulation of immune system, protein synthesis inhibition in various malignant cell lines. Almost complete inhibition of tumor growth, increase of apoptosis and necrosis, and reduction in mitosis was apparent only for tumors in the vicinity of the tumor exposed to mistletoe. Reduction of lung metastases. Local and systemic effects.	[50]
	Prolonged survival time and a reduction in the number of tumor colonies. Histology revealed an increase of apoptosis and necrosis in the tumors, while a reduction in mitosis was noticed only for the tumors in the vicinity of the tumor exposed to Isorel. Immunomodulation combined with tumor growth inhibition and a reduction in metastasis was observed.	[51]
KM-110 Extract from korean mistletoe (*Viscum album* var. coloratum)	Inhibition of lung metastasis of melanoma and colon cells. Liver and spleen metastasis of lymphoma cells by various administration routes (subcutaneous, oral, intranasal and intravenous) was dose-dependent. Stimulation of host defense system and NK cell activation.	[52]
KML-C Korean mistletoe lectin (*Viscum album* var. coloratum)	Stimulation of immune system, NK cells and macrophages activation.	[53]
Kurokawa mushroom (*Boletopsis leucomelas*)	Inhibition of proliferation of human monoblastic leukemia U937 dose-dependently due to apoptosis induction.	[54]
Mesquite seed lectin (*Prosopis*)	Antiproliferative effect on cervical human tumor cells (HeLa) but no effect on normal cells.	[55]

Table 3. (Continued)

Lectins or lectins extracts	Effects	Ref
ML Mistletoe lectin (*Viscum album*)	Antitumor activity by cytotoxicity. Bladder carcinoma was reduced, and survival times were prolonged in mice as a function of concentration.	[56]
	Growth-inhibition on HeLa-S3, Molt-4, MFM-223, COR-L51, KPL-1 and VM-CUB1 tumor cell lines	[57]
	Blocks the growth of bladder carcinoma cells.	[58]
ML-I Mistletoe lectin (*Viscum album*)	Apoptosis induction on leukemic T and B cell lines. Ribosomal inactivation.	[59]
	Reduction of tumor growth of a murine non-Hodgkin lymphoma.	[60]
	Reduced mitotic activity of murine non-Hodgkin lymphoma tumors, lower degree of mitotic activity, CD3 cells infiltration in tumors, apoptotic bodies, poorly developed blood supply, and reduction in tumor weight.	[61]
ML II Mistletoe lectin (*Viscum album*)	Strong inducer of pro-oxidants that mediate the activation of caspase-9 and caspase 3-like proteases, apoptotic death of human myeloleukemic U937 cells.	[62]
ML-I and ML-III Mistletoe lectins (*Viscum album*)	Differential induction of apoptosis on leukemic B-cells from patients with B chronic lymphocytic leukemia and on the leukemic T-cell line Molt-4.	[63]
PCL *Polygonatum cyrtonema* Lectin	Induced HeLa cell apoptosis.	[64]
PHA Common bean agglutinin (*Phaseolus vulgaris*)	Reduction in number of Krebs II tumor cells in the ascitic fluid of mice and tumor-cell growth.	[65]
	Increase in the activity of polyamine oxidase.	[66]
	After including the lectin in the diet of mice, reduction of intraperitoneal tumors and subcutaneous no-Hodgkin lymphomas in mice.	[67,68]
	Reduction of tumorogenesis in animals.	[69]
***Pleurotus ostreatus* lectin**	Differential effect on human hepatoma (H3B), human choriocarcinoma, mouse melanoma, and rat osteosarcoma cell lines. Lectin was more efficient on sarcoma S-180 than on hepatoma H-22 tumor inhibition, improvement of the host immune system.	[70]
Ricin	Protein synthesis inhibition by binding to ribosomes. Internalization and trigger cell proliferation.	[71]
rML Recombinant mistletoe lectin (*Viscum album*)	Immunomodulation can influence tumor growth in breast cancer patients	[72]
	The inhibitory effect not related with interferon gamma (IFN-γ) and/or interleukin-10-dependent mechanisms in rat urothelial carcinogenesis	[73]
	Antitumor activity if administered locally into the peritoneum of a human ovarian cancer harboring SCID mouse.	[74]
	Alone or in combination with ionizing radiation showed down regulation of the proliferative activity and cell killing of transformed murine tumor cells in a dose response manner.	[75]
SBA Soybean agglutinin	Inhibition of ascitic lymphoma cells and immune system stimulation.	[76]
	Inhibition of proliferation of breast cancer MCF7 cells and hepatoma HepG2 cells.	[77]
SVL (*Sauromatum venosum*)	Proliferation inhibition of murine cancer cell-lines (WEHI-279, J774, P388D1 and A-20). *In vitro* anti-proliferative activity on T-47D (breast), SiHa (cervix), SW-620 (colon), HT-29 (colon), HEP-2 (liver), OVCAR-5 (ovary) and PC-3 (prostate) cells except on SK-N-MC (CNS), SK-N-SH (CNS) cells.	[78]
Tepary bean lectin extracts (*Phaseolus acutifolius*)	Differential cytotoxic effect on breast, cervix and colon human cancer cell lines.	[79]
TMA I and TMA II (*Tricholoma mongolicum*)	Inhibition of sarcoma 180 cells and increment of life span.	[80]
VAA Mistletoe agglutinin (*Viscum album*)	VAA therapy alone stimulated tumor growth as well as lung metastasis.	[81]

Table 3. (Continued)

Lectins or lectins extracts	Effects	Ref
VAA-1 Mistletoe agglutinin-*1* (*Viscum album*)	Synergistic antineoplastic activity alone and in combination with other chemotherapeutic drugs on A549human lung carcinoma cell line. Induction of nonapoptotic G1-phase accumulation mechanisms.	[82]
VCA Korean mistletoe agglutinin (*Viscum album* var. corolatum)	Dose-dependent effect on promieloid leukemia HL-60 cells viability and apoptosis induction via caspase 3.	[83]
	Dose-dependent effect on melanoma B16-BL6 cells growth, apoptosis induction, antimetastasic effect, increased life span observed in inoculated mice, dose-dependent angiogenesis inhibition.	[84]
	Apoptosis induction on human hepatocarcinoma SK-Hep-1 and Hep3B cells via Bax activation and Bcl-2 inhibition, caspase 3 activation and telomerase inhibition.	[85]
VFA (*Vicia faba*)	Colorectal adenocarcinoma cell lines (LS174T, SW1222 and HT29) showed cell aggregation, morphologic differentiation and dose-dependent proliferation inhibition. Morphological differentiation and reduction of malignant phenotype of colon cancer cells. Aggregation of cancer cells binding directly to EpCAM.	[86, 87]
WGA	Inhibitory effect on the rat pancreatic tumor cell line AR42J, accompanied by a small decrease in α-amylase secretion.	[88]
	Highly toxic to human pancreatic carcinoma cells *in vitro*, with high membrane binding to sialic acid residues, with lectin internalization and apoptosis induction.	[89]
	Restriction of tumor growth of lymphoma cells.	[90]
	Isolectins showed differential interaction with leukemic cells and different cytoagglutinating and cytotoxic activities.	[91]
	Differential effects on cell growth of several human breast cancer cell lines *in vitro* (MCF-7, T47D, HBL 100, BT 20).	[92]

European mistletoe (*Viscum album*), used as complementary cancer therapies in Europe, has been used parenterally for more than 80 years as an anticancer agent with strong immunomodulating action. The quality of life of patients with pancreatic cancer stages III and IV improved as a result of exposure to Eurixor in a phase I and II study [96], on the opposite, patients with head and neck squamous cell carcinoma did not experience an improvement in their quality of life [97]. Mistletoe lectins or extracts from *Viscum album* (European variety, VAA) and *Viscum album* var. *coloratum* (Korean variety, VCA) have been widely studied against cancer. These varieties presented similar cytotoxic activity (IC50 of 1.2 ng/mL) against Molt-4 cells (T cell lymphoblasts, leukemia) [98]. Lectin from Chinese mistletoe showed important effects on human T cells cytotoxicity, apoptosis and cytokine production. ML increased tumor necrosis factor (TNF)-α release and inhibiting the release of anti-inflammatory interleukin (IL)-10 [99].

Specificity of lectins has triggered numerous applications in experimental medical sciences. Although the antitumor activity of lectins has been described, it is important to consider that their use may present, in some cases, adverse effects [7,16]. While not all lectins are toxic, many of them may cause different degrees of toxicity with severe negative effects, even death [100,101]. Toxicity of lectins depends on the administration route and some of them had been reported to be highly allergenic under certain conditions [8,16,101,102].

Due to the properties of some lectins as RIP, some studies have focused on using them for production of immunotoxins against cancer cells, where the lectin is attached to a monoclonal antibody, which has a specific receptor site for tumor cells. However, there have been reports after clinical trials that one of the major adverse effects, that limit the therapeutic

dose in patients treated with immunotoxins formed by ricin A chain, is the vascular infiltration syndrome. This effect is even more frequent and severe in patients previously treated with radiotherapy [7]. Therefore, due to the toxicity of certain lectins, it is necessary to evaluate its systemic toxicity before testing their therapeutic effectiveness. On top of that, it will be recomended to take in consideration whether the patient has been receiving a special treatment which could pose an additional effect in the use of lectins.

CONCLUSION

Plant lectins have shown unique characteristics against different types of cancer cells and, in some cases, they present differences in the recognition between normal and transformed cells; their effects involve death and growth inhibition of cancer cells. The two main properties of lectins; selectivity and cytotoxicity, have become the focus of attention in research against cancer. Considering the extensive number of different lectins present in living organisms, and taking into account their different structures as well as differences in their mechanism of action, these compounds represent the opening of new avenues in the search for different cancer treatments. There is still the need to prove the inocuity of those lectins proposed for their possible use for the cancer treatment. Nonetheless, even those lectins that could be found to be toxic to humans or to animals, they still have the potential to be used as diagnostic tools, particularly oriented to the early recognition of different types of cancer cells.

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