2. LITERATURE OVERVIEW

2.1 INTRODUCTION

Lectins have been purified from varied sources and their properties, characteristics and biological functions have been extensively studied. Though they are ubiquitously present in animals, microorganisms such as bacteria, viruses, fungi and in marine sources such as fishes, marine cyanobacteria, algae, mollusks, sea urchin, etc., plants are the major source of lectins. Based on the plant species, lectins have been shown to be abundantly present in the seeds, leaves, fruit pulp, barks, bulbs and tubers [91,92]. Lectins isolated from varied sources exhibit specificity towards distinct carbohydrates such as sialic acid, mannose, galactose/N-acetylgalactosamine, fucose, N-acetylglucosamine and glycans [93]. Research on lectins received considerable attention mainly owing to the significance of protein-carbohydrate interactions in various recognition events. Since many decades, numerous lectins have been purified and the structures of most of them have been elucidated. The ability of lectins to specifically interact with glycoconjugates in cells and biological fluids are being exploited in various diagnostic and therapeutic applications [94].

2.2 CONFORMATIONAL ANALYSIS OF LECTINS

The functions of lectins depend on their CRD, which is a predominant part of their three-dimensional structure. Proteins are held together by hydrophobic interactions, van der Waals forces, ionic interactions, hydrogen and disulfide bonds [95]. When these forces are perturbed due to surrounding environmental factors, the protein loses its biological activity. Hence, it is important to measure the conformational stability of lectins so as to define the physical interactions that stabilize them and to further optimize their stabilities. The stability is usually determined as a function of temperature, pH or chemical denaturant such as urea or Guanidine Hydrochloride (GdnHCl). Various techniques such as fluorescence spectroscopy, UV-Vis spectroscopy,

differential scanning colorimetry and circular dichroism are often used to follow the unfolding of lectins.

Thermal and chemical denaturation studies of a number of lectins, belonging to different families have been extensively studied so as to gain an insight into their unfolding behavior [96-98]. While most lectins follow the two-state unfolding mechanism, in some lectins, intermediate states exist between the native and the denatured states [99]. It is important to study such partially folded conformations, so as to gain more knowledge regarding the folding/unfolding pathway. It has been possible to identify and characterize such intermediates by using techniques like the nuclear magnetic resonance (NMR) spectroscopy [100].

2.3 ANTIPROLIFERATIVE EFFECTS OF DIFFERENT CLASSES OF LECTINS

Tumor cells are known to exhibit differential patterns of glycosylation on their cell surface which are considered the hallmark of cancer progression and metastasis [101,102]. Lectins possess the ability to specifically recognize cancer cells by binding to the aberrant glycosylation on the tumor cell surface [103]. There are interesting discrepancies that were observed in the ensuing effects of lectins on cancer cell proliferation. While a majority of lectins were found to act as cytotoxic or non-cytotoxic inhibitors of cancer cell growth, some lectins were shown to stimulate the cells to undergo mitotic division. There are various reports of *in vitro* and *in vivo* antiproliferative effects of lectins from varied sources on cancer cells belonging to different lineages [104]. Also, certain lectins such as Con A, mistletoe lectins (MLs) and *Phaseolus vulgaris* lectin have shown promise as effective anti-cancer agents in preclinical research phase [105].

2.3.1 Legume lectins

Among the lectin families, the antitumor properties of legume lectins are the most comprehensively studied and the underlying mechanism has been

elucidated in most cases. There are various reports of antiproliferative effects of Con A, the first and the most extensively studied legume lectin [106,107]. Con A, for instance, was shown to instigate apoptosis of murine macrophage PU5-1.8 cells by release of cytochrome c [108]. Furthermore, Con A induced caspase-mediated apoptosis thereby resulting in cytochrome c release and mitochondrial transmembrane potential (MMP) collapse in human melanoma A375 cells [109]. The legume lectins, Con A and *Sophora flavescens* lectin (SFL) selectively resulted in apoptosis of MCF-7 breast cancer cells but did not affect the proliferation of the normal human mammary MCF-10 A cells [110].

SFL, a mannose-specific legume lectin and Astragalus membranaceus lectin (AML), a galactose-binding legume lectin were found to induce apoptosis of HeLa cervical cancer cells and K562 cells, respectively [111,112]. A galactose-binding lectin obtained from the seeds of *Lotus corniculatus* effectively hindered cell migration and further caused cell cycle arrest and apoptosis of human leukemic THP-1 cells at G_0-G_1 phase [113], while another galactose-binding lectin isolated from tubers of *Dioscorea opposita* cv.Nagaimo inhibited cell proliferation and induced mitochondrial depolarization of MCF-7 cells resulting in cell death [114].

Lectins or PHA are the major storage proteins of most of the cultivars of common bean (*Phaseolus vulgaris*) and other *Phaseolus* species. Different cultivars of *Phaseolus vulgaris* have differential carbohydrate binding specificities and exhibit varying degrees of antiproliferative effects; for instance, the lectin isolated from French bean cultivar no.35 induced apoptosis of MCF-7 cells through the death receptor-mediated pathway [115], while the lectin isolated from the seeds of *Phaseolus vulgaris* cv. extralong autumn purple bean was reported to inhibit growth of nasopharyngeal carcinoma cells and breast cancer cells and induced production of apoptotic bodies in HepG2 liver cancer cells [116]. Another lectin from Blue tiger king bean was shown to be selectively toxic to the human hepatoma HepG2 cells but had no effects on the normal liver cells [117]. A specific lectin that binds glucosamine that was obtained from seeds of brown kidney bean inhibited proliferation of HepG2,

CNE1 and CNE2 cells and induced apoptosis of MCF-7 cells [118]. Lectin from tepery bean (*Phaseolus acutifolius*) was shown to inhibit proliferation as well as colony formation of colon cancer Sw480 cells and the cervical carcinoma C33-A cells [119]. A sialic acid-binding lectin isolated from *Phaseolus coccineus* was found to be highly toxic and induced apoptosis of murine L929 cells [120]. Interestingly, inhibition of sialic acid-specific activity was shown to reduce the cytotoxic effects of the lectin. More recently, Lunatin, a glycosylated lectin isolated from seeds of *Phaseolus lunatus* billb was reported to be as a strong inhibitor of K562 cell proliferation [121].

2.3.2 GNA-related lectins

There are various reports of anti-cancer effects of GNA-related lectins wherein they are shown to instigate apoptosis and/or autophagy. Previously, a typical GNA-related lectin from garlic bulbs induced apoptosis of myeloleukemic U937 and HL60 cells by inhibiting DNA synthesis [122]. Another mannose-binding, GNA-related lectin *Polygonatum crytonema* lectin (PCL), isolated from the rhizomes of PCL Hua has been recognized as a potential antineoplastic drug due to its ability to target multiple apoptotic and autophagic pathways [123]. PCL instigated apoptosis and autophagy in A375 cells by activating the ROS-p38-p53 pathway [124] and in murine fibrosarcoma L929 cells by inhibiting PI3K-AKT and Ras-Raf signaling pathways [125]. More recently, PCL was shown to instigate autophagy and apoptosis in A549 cells by generation of reactive oxygen species and by activating signaling proteins such as ERK, JNK and p38 [126].

Polygonatum odoratum lectin (POL), another mannose-specific, GNA-related lectin was found to induce apoptosis of various cancer cells. POL was found to induce apoptosis of L929 cells through the death receptor as well as the mitochondrial pathway [127]. In another study, POL concurrently induced autophagy and apoptosis in non-small lung cancer A549 cells. Interestingly, the lectin was shown to trigger apoptosis by inhibiting AKT-NFκB pathway and induce autophagy by inhibiting AKT-mTOR pathway [128]. Likewise, POL instigated apoptosis and autophagy of MCF-7 cells by activating the ERK

signaling pathway by targeting the epidermal growth factor receptor (EGFR) [129].

Pinellia ternata agglutinin, a GNA-related lectin isolated from tubers of *Pinellia ternate,* induced apoptosis of Bel-7404 human hepatoma cells [130]. In a recent study, *Remusatia vivipara* lectin altered the mitochondrial membrane potential which will in turn increase the reactive oxygen species, thereby inducing apoptosis in MDA-MB-468 and MCF-7 breast cancer cells [131].

2.3.3 Type II ribosome inactivating proteins

The type 2 RIPs consists of two polypeptide chains, namely, the A-chain that is enzymatically active and the B-chain that consists of the sugar-binding domain. While the toxic A-chain of galactose/N-acetylgalactosamine specific type 2 RIPs acts as a potent inhibitor of protein biosynthesis, the toxic effects of the B-chain involve induction of apoptotic or autophagic pathway [132].

In spite of their sequence similarity, different type 2 RIPs exhibit different modes of cytotoxicity; for instance, while the lectin from *Sambucus nigra* was known to strongly inhibit protein synthesis *in vitro,* they were found to be less toxic as compared to ricin, when administered *in vivo*. This discrepancy occurs probably due to differences in their cellular uptake and intracellular transport of the lectins.

The lectin from Korean mistletoe (*Viscum album* var. *coloratum* agglutinin or VCA) induced apoptosis of hepatocarcinoma cells, Hep3B and A253 through p21 and p53 independent pathways, by inhibition of telomerase [133,134]. Ricin, a toxic R-type lectin isolated from *Ricinus communis* induced apoptosis of L540 human Hodgkin's lymphoma-derived cells by activating the extrinsic caspase pathway [135].

Abrus agglutinin (AGG), a heterodimeric RIPII isolated from *Abrus precatorius* induced caspase-dependent apoptosis of HepG2 cells both *in vitro* and *in vivo* [136]. Further, AGG was found to exert anti-proliferative as well as

anti-angiogenic effects in human breast cancer cells [137]. Also, the lectin simultaneously induced apoptotic and autophagic-dependent cell death in cervical cancer cells [138]. In a recent study, AGG was found to induce cell cycle arrest and mitochondrial-dependent apoptosis in oral squamous cell carcinoma (FaDu cells). The growth of FaDu xenografts was also found to be inhibited in AGG administered athymic nude mice [139].

2.3.4 Jacalin related lectins (JRLs)

JRLs that exhibit similar tertiary and quaternary structures are known to exert different cytotoxic effects. This discrepancy probably arises due to differences in their oligosaccharide binding specificities. Moringa M and Artocarpin are mJRLs that exhibit similarity in their tertiary and quaternary structure. However, while, Moringa M was found to induce apoptosis of Jurkat T cell leukemia cells, no such effects were observed with Artocarpin [140]. Artin M, another mJRL isolated from *Artocarpus heterophyllus,* mediated apoptosis of human myeloid leukemia cells (NB4) and Jurkat T cells [141,142]. Fruitalin, a gJRL isolated from *Artocarpus incisa* was shown to exert irreversible, time and dose-dependent inhibitory effect on proliferation of HeLa cells [143].

2.3.5 Chitin-binding lectins

The anti-cancer studies involving chitin-binding lectin family were limited as compared to other families that include a plethora of lectins. WGA, a typical chitin-binding lectin-induced profound toxicity in various pancreatic carcinoma cells. Upon binding to the sialic acid residues that are abundantly expressed on the pancreatic cell surface, WGA was found to localize into the nucleus, resulting in apoptosis [144]. Further, WGA was shown to induce remarkable dose-dependent inhibition of breast cancer cells including BT 20, HBL 100 and T47D cells [145]. Another chitin-binding lectin isolated from the rhizome of *Setcreasea purpurea* induced dose-dependent apoptosis of CNE-1 cells [146].

2.3.6 Anticancer effects of fungal lectins

Lectins isolated from mushrooms belonging to different genus and species exert remarkable anti-cancer effects both *in vitro* and *in vivo* [147,148]. The anti-cancer effects of ABL and *Agrocybe aegerita* lectin (AAL), the TFD-binding lectins isolated from edible mushrooms have been extensively studied. While ABL inhibits the growth of HT29 cells and MCF-7 cells, AAL exerts its inhibitory effect on the growth of human tumor cell lines including BGC-823, HL-60, HeLa, MGC80-3, SGC-7901 and SW480 [149,150]. Further, AAL has been reported to induce apoptosis of H22 hepatoma cells, *in vitro* [151].

Sclerotium rolfsii lectin (SRL), another TFD-binding mushroom lectin exerts remarkable *in vitro* and *in vivo* antitumor effects. SRL was shown to incite caspase-8 and -9 mediated apoptosis of HT29 and DLD-1, *in vitro* and also lead to tumor regression *in vivo*, in mice bearing HT29 xenografts [152]. Likewise, SRL retarded PA-1 human ovarian cancer cell growth by activation of intrinsic and extrinsic apoptotic pathways [153]. Also, SRL had a marked anti-proliferative activity on MCF-7 and ZR-75, which are breast cancer cells but induced only a slight inhibition in proliferation of normal breast epithelial cells, MCF 10A and human mammary epithelial cells (HMECs) [154].

Unlike other fungal lectins that induced apoptosis, few others were found to be cytostatic and thus act as non-cytotoxic inhibitors. For instance, the *Volvariella volvacea* lectin was shown to inhibit proliferation of S 180 mouse sarcoma cells by arresting the cell cycle arrest at G2/M phase [155]. Likewise, the N-acetyl-D-glucosamine (GlcNAc) specific lectin isolated from the fruiting bodies of *Psathyrella asperospora* inhibited HT29 cell growth by hindering the progression of cell cycle beyond the G2/M phase [156]. The *in vivo* antiproliferative effects of certain mushroom lectins have also been investigated. The lectins isolated from the fruiting bodies of *Pleurotus ostreatus* and *Pleurotus citrinopileatus* were found to significantly inhibit tumor growth in mice bearing sarcoma S-180 [157,158], while AAL inhibited tumor growth in H22 hepatoma tumor-bearing mice and further increased the life span of the mice bearing the specific tumor [151].

2.4 LECTIN-BASED CANCER DIAGNOSIS/PROGNOSIS

The ability of lectins to specifically recognize profound alterations on the cancer cell membrane molecules has been exploited in cancer diagnosis and prognosis. Lectins have been used as molecular probes for cancer screening in virtue of their potential to distinguish subtle changes in cell glycosylation. Various techniques have been developed that makes use of lectins as probes for diagnosis and prognosis of cancer [5,159]. In some studies, more than one of the lectin-based techniques were used to validate the observed changes in glycosylation patterns [160].

2.4.1 Lectin microarray technologies

Lectin microarray technologies have further increased the use of lectins to detect cancer biomarkers [161,162]. In this technique, multiple lectins are spotted on a micro slide and are used to detect the differential expression of membrane glycoproteins isolated from tumors [163]. In a previous study, lectin array was used to detect differential expression of fucosylated proteins in serum of patients at different stages of cancer and in the serum of healthy controls [164]. Further, glycan profiling using different lectins was used to establish differences in glycan expression between ovarian cancer cells that were the sensitive and resistant to specific drugs [165]. This further expanded the application of lectin microarray to speculate the outcome of chemotherapy.

Modifications in glycosylation profiling associated with invasive breast cancer were identified using TFD-binding lectins such as ABL, SBA, jacalin, ricin and Bauhinia purpurea lectin [166,167]. Another lectin microarray approach was used to demonstrate the variation in α-2-macroglobulin glycosylation in serum from colorectal cancer patients and healthy individuals [168]. Further, Li et al., demonstrated the use of lectin microarray coupled with mass spectrometric analyses to identify the significantly altered glycans in gastric cancer [169].

2.4.2 Lectin histochemistry

Lectin histochemistry has been used to differentiate normal and transformed tissues based on alterations in glycosylation patterns [170]. It also became possible to identify the particular stage of cancer by using the specific lectins; for instance, α2,3-linked lectin, *Maackia amurensis* and α2,6-linked lectin *Sambucus nigra* agglutinin both of which bind to sialic acid have been used to identify the grades of cervical neoplasia on the basis of expression of sialic acid [171]. Likewise, SNA has been proposed as a prognostic probe in identifying the differential expression of sialic acid in in situ invasive ductal carcinoma as compared with the stage 0 breast cancer tissue [172]. In another study, lectins with different carbohydrate specificities such as WGA, PHA-L, UEA-I, HPA, GNA were used to detect changes in glycosylation patterns that may be associated with colorectal cancer progression [173]. Also, Con A and UEA-I have been shown to be effective in distinguishing between the histological grades of mucoepidermoid carcinoma [174].

2.4.3 Lectin Blotting

Lectin blotting is a method similar to western blotting wherein instead of antibodies, lectins are used to detect the specific glycoconjugates. The method has been used to identify the differential patterns of glycosylated proteins expressed in cancers such as cervical cancer, ovarian cancer and prostate cancer [175-177]. Lectin blot analysis using *Pinellia pedatisecta* agglutinin was used to identify the differential glycosylation patterns in leukemia cells, solid tumor cells including the H1299 lung cancer cells and liver cancer cells such as Huh7 cells, PLC cells and Bel 7404 cells [178].

2.4.4 Enzyme-linked lectin assay (ELLA)

ELLA is based on the same principle as ELISA but a lectin is used instead of an antibody. In a previous study, direct ELLA with PNA was used to demonstrate the increased levels of serum TF-ag in uterine cervical cancer patients as compared to normal controls. The study further evaluated the

differential levels of TF-ag in serum of patients, pre and post radiotherapy [179]. In another study, UEA-1, a fucose-specific lectin was used in ELLA to demonstrate a remarkable increase in the serum levels of prostate specific antigen in prostate cancer patients as compared to patients with benign prostatic hyperplasia [180].

More recently, sandwich ELLA was used to measure the increased serum O-glycosylated protein levels in patients with different stages of breast cancers as compared to normal individuals [181]. In a slightly different context, biotinylated-AAL and the Fab portion of anti-haptoglobin antibody were used in a sandwich ELLA to identify fucosylated haptoglobin as a marker for pancreatic cancer [182].

Aberrant glycosylation has almost always been associated with disease progression and is considered as a reliable hallmark of carcinogenesis. However, using direct ELLA, reduced levels of sialylation and fucosylation of cytosolic glycoproteins were observed in cancerous cervical tissues when compared with normal tissue samples from the same patient [183].

2.5 IMMUNOMODULATORY EFFECTS OF LECTINS

Lectins are capable of exerting immunomodulatory effects either directly by specifically binding to glycans that are present on the immune cells or indirectly by interacting with the non-immune cells. Such interactions can generate efficient immune responses against infections and tumors. In recent years, the therapeutic potential of lectins that exert immunomodulatory effects are being extensively studied. More importantly, some of the dietary lectins have been shown to stimulate proliferation of lymphocytes, thereby modulating several immune functions. The dietary lectins affect the immune cells cytokine production and can polarize the immune response towards certain effector functions. Con A, pokeweed mitogen and PHA are well-known examples of lectins with immunomodulatory effects. Certain lectins such as the ABL and lectin from Musca domestica pupa have also been shown to modulate the immune response, *in vivo* [184].

Interaction of plant lectins with the carbohydrate moieties present on the immune cell surface trigger signal transduction resulting in the secretion of cytokines. While lectins such as Artin M, cramoll isolated from *Cratylia mollis*, BanLec and garlic lectin induced a Th1 immune response by stimulating the cells to produce Th1 cytokines such as IFN-γ and IL-12; others such as ricin and ScLL, a lectin isolated from *Synadenium carinatum* have been reported to induce a Th2 response [8]. Certain other lectins such as ML-1, visum album coloratum, viscum articulatum, Con A, Artin M, BanLec and tarin, isolated from taro have been demonstrated to exhibit potent immunomodulatory effects, *in vivo*, using different models.

There are numerous reports to emphasize the significance of fungal lectins as potent modulators of immune response. Among the edible mushrooms, the lectin isolated from *Agrocybe cylindracea* was shown to induce proliferation of mouse spleenocytes [185]. Further, the lectin stimulated the macrophages to produce increased levels of nitric oxide [186]. Likewise, the lectin isolated from *Volvariella volvacea,* edible mushroom, induced the mouse splenic lymphocytes to produce IL-2 and IFN-γ thereby leading to a Th1 response [187]. Two other lectins isolated from the mushroom, *Tricholoma mongolicum* instigated the macrophages to produce nitrite ions thus inducing a Th1 response. More importantly, the *in vivo* activation of mouse macrophages resulted in inhibition of tumor growth [188].

More importantly, increased levels of lectins in edible sources such as seeds, cereals, beans, grains and nuts have been found to be detrimental to humans and animals. Lectins are known to bind to the epithelial surface of digestive tract leading to immune allergic reactions and anti-nutritional effects [189]. Further, consumption of lectin-rich foods can also lead to leptin resistance thereby increasing the risk of obesity [190]. When injected into animals, lectins were shown to cause local necrosis and hemorrhages in the stomach, intestinal wall and other organs [191].