ISSN No:-2456-2165

Lectin Therapy: A Way to Explore in Order to Inhibit the Binding of COVID-19 to these Host Cells

Fouad AKIF
Centre Régional des Métiers de l'Éducation et de la Formation Souss Massa, B.P. 106 My. Abdellah Av. Inezegane, Morocco.
Glycobiology Laboratory, Faculty of Medicine, Free University of Brussels, 808 Lennik route, 1070 Brussels, Belgium.

Abstract:- When COVID-19 appeared in November 2019 in the city of Wuhan, in central China, no epidemiologist had predicted such a pandemic. Currently, no vaccine or drug is available, the treatment with azithromycin and hydroxychloroquine remains limited because it does not specifically target the viral pathogen. Most enveloped viruses express glycoproteins on their surface; lectins are proteins that specifically bind to glycosylated residues, many of which are proposed as a treatment for viral infections. Several antiviral lectins are successfully used against hepatitis C, influenza A / B, herpes, Japanese encephalitis, HIV and the Ebola virus. In this review, we will highlight the various viral infections treated with lectin-based drugs, as well as their modes of administration. In addition, and in order to inhibit the binding of COVID-19 to these receptors, we propose the use of some lectins as antiviral therapeutic agents, either by blocking receptors (glycoproteins) of the virus at the level of host cells or by masking the glycoproteins of the viral envelope.

Keywords:- *COVID-19, Lectins, Drugs, Receptors, Glycans, Glycoproteins.*

I. INTRODUCTION

Lectins are proteins or glycoproteins of non-immune origin having at least two binding sites which recognize a specific sequence of carbohydrate residues [1]. Lectins can be isolated from a wide range of natural sources, namely: bacteria, yeasts, fungi, higher plants [2], [3], [4], [5], [6], [7], [8], [9]. The physiological functions of lectins are still conjectural, however their role in growth [10], their intervention in cell adhesion and recognition [11], [12], phagocytosis [13], fertilization [14], ontogenesis [15], their modification during differentiation, their function in the secretion phenomenon and proteolytic stability [16] have been demonstrated. By their strict specificity for different glycans, lectins have become a first choice tool for the biochemical [17] and histochemical [18] characterization of cellular glycoconjugates. Lectin-glycoprotein interactions are the basis of many biological phenomena such as the adhesion of viruses to the surfaces of host cells. Many lectins inhibit replication of viruses by interacting with viral envelope glycoproteins [19], [20], [21], [22], [23]. Inhibition of this type of interaction by lectins will allow to develop new antiviral therapies. In this review, we highlight whatever lectins have been used for their ability to block the access of certain viruses in their host cells. In addition, we propose some lectins that can potentially be used as a possible therapy for Covid-19.

II. LECTINS AS ANTIVIRAL DRUGS

Any viral therapy relies on the early inhibition of viral penetration in these target cells, and the choice of inhibitors, the identification and characterization of the molecules that block the entry of the Virus are essential. The spread of a virus and the progression of the disease depend on the direct interactions between the virus and the host cell receptors [24], these receptors are often glycoprotein in nature. The glycoproteins of the viral envelope are highly glycosylated, the lectins have binding properties to these carbohydrates involved in antiviral activity, thus blocking the interaction between the glycoproteins of the viral envelope and cell surface molecules involved in the entry of the virus. Targeting glycans of viral envelope proteins is a promising approach in the development of antiviral therapies. In addition, lectins are powerful tools for better understanding the first stages of the entry of viruses into host cells. Several lectins isolated from natural sources inhibit microbial and viral pathogens in vitro and in vivo [25], [26], [27], [28], [29], [30]. These lectins specifically bind to glycoproteins in the viral envelope, thereby blocking entry of the virus into host cells [31], [32], [33], [34], [35], [36]. In this review, we outline some viral infections treated with lectins.

- *Hepatitis C virus (HCV)*

Hepatitis C virus (HCV) is a linear, single-stranded RNA virus of positive polarity, with an icosahedral capsid and an envelope. The glycoproteins E1 (gp31) and E2 (gp70) are membrane proteins used in the composition of viral envelopes. Like type I transmembrane proteins, they have an N-terminal domain and an hydrophobic C-terminal region for anchoring in the endoplasmic reticulum and related structures. The 27 N-terminal amino acids of E2 constitute the hypervariable region, exposed on the surface of E2 and site of one of the main neutralizing epitopes of the virus [37]. E1 and E2 can form two types of complexes: E1 / E2 heterodimers made up of native proteins and stabilized by non-covalent interactions (productive pathway); heterogeneous aggregates linked by disulfide bridges and interacting with chaperone molecules (non-productive pathway), which could play a role in negative

regulation of viral particle formation and replication [38], [39]. An early interaction between the surface of the envelope glycoproteins and the glycosaminoglycans located on the surface of target cells could be involved in the recognition and cellular tropism of the virus [40]. Envelope glycoproteins are involved in cell recognition and the interaction of viral particles with the receptor molecule (s) and the penetration of the virus into the cell. They play a role after internalization, in particular in the undressing of enveloped particles and in the assembly of virions. An early interaction between the surface of the envelope glycoproteins and the glycosaminoglycans located on the surface of target cells could be involved in the recognition and cellular tropism of the virus [40]. Plant or microbial lectins are known to exhibit potent antiviral activities against viruses with glycosylated surface proteins. Studies of Kachko et al., [41], have demonstrated that the lectin *Galanthus nivalis* (GNA) can be a better agent therapeutic due to the specificity for hepatitis C virus glycoproteins. GNA binds specifically to the disaccharide Mannoseα (1-3) terminal Mannose. Other studies have shown that lectins recognizing mannosylated residues have an affinity grade for hepatitis C virus glycoproteins like lectin *Griffithsin sp.*, (GRFT) which specifically binds to α (1,2) mannobiose = (α-D-Man-1 → 2-D-Man, 2-O-α-D-Mannopyranosyl-D-mannopyranose) [42] and the lectin *Cymbidium agglutinin* (CA) which recognizes N-linked oligosaccharides with high mannose content [43].

- *Influenza A / B virus*

Influenza viruses belong to the family of *Orthomyxoviridae*, represented by three genera, viruses A, B and C. Only influenza A and B viruses are responsible for influenza. These are enveloped viruses whose genome consists of a segmented single-strand negative RNA (8 segments). Influenza A viruses are also classified into subtypes, depending on the nature of the hemagglutinin (HA or H) and neuraminidase (NA or N) surface glycoproteins.

The H1N1 viruses preferentially bind to sialic acid α-2,3-galactose β-1,3-N-acetyl galactosamine, this trissacharide is recognized by the lectin *Maackia amurensis* (MAA); while H3N2 viruses have specificity for sialic acid α, 2,6-galactose β1,4-N-acetyl glucosamine which is recognized by the lectin *Sambucus nigra* (SNA) [44]. The virus-sialic acid α, 2,6-galactose β1,4-N-acetyl glucosamine interaction is facilitated by the methyl groups of neuraminic acid [45].

- *Herpes virus*

It is a double stranded DNA virus in which there are two types, the Herpes simplex virus type 1 (HSV-1) and the Herpes simplex virus type 2 (HSV-2). It can be the cause of l infection commonly called herpes. The jackfruit lectin (JFL) of *Artocarpus heterophyllus* has been shown to have inhibitory activity in vitro with a cytopathogenic effect against the herpes simplex virus type 2 (HSV-2) [46]. JFL specifically binds to viral glycoproteins with terminal disaccharides Galactoseβ (1, 3) N-acetyl galactosamine. Other terminal mannose-specific lectins have shown great antiviral activity of herpes, namely *Griffithsin* (GRFT) [47], *Cyanovirin N* (CV-N) [48], *Typhonium divaricatum* (L.) Decne [49], and *Polygonatum odoratum* (POL) [50].

- *Japanese encephalitis virus*

The Japanese encephalitis virus (JEV) is an enveloped virus, the viral genome, represented by a positive single-stranded RNA, is enclosed in a protein capsid. The outer envelope is formed by a glycoprotein (E) which constitutes the protective antigen [51], and formed by two potential glycosylation sites [52], which are important for fixation, fusion, penetration, cellular tropism, virulence and attenuation of the virus [53]. The JEV outer envelope glycoproteins are highly mannosylated.

Griffithsine (GRFT) is a lectin specific for glycoproteins carrying the terminal oligomannose residues α-D-Mannoseα (1-2)D-Mannose,2-O-α-D-Mannopyranosyl-D- mannopyranose. Hassan et al., [54], evaluated the antiviral activity (in vitro and in vivo) of GRFT against infection with the Japanese encephalitis virus (JEV); this study suggested that GRFT prevents JEV infection at the entry phase by targeting the virus, and that this lectin is an antiviral agent with a potential application in the development of therapies against JEV or other flavivirus infections.

- *Coronavirus*

Coronaviruses have been known in the veterinary community since the 1930s. In 2003, the identification of a coronavirus as the etiological agent of Severe Acute Respiratory Syndrome (SARS), circulating in a pandemic manner [55]. In 2012, a new human coronavirus, MERS-CoV, emerged in the Middle East. It is responsible for a pathology similar to SARS [56]. These coronaviruses are characterized by a single stranded, unsegmented RNA genome; they are the largest genomes of RNA virus listed to date. Yohichi Kumaki et al., [57] have shown that the lectin Agglutinin *Urtica Dioica* (UDA) binds to N-acetylglucosamine-like residues present on glycosylated envelope glycoproteins, thus inhibiting the binding of SARS-CoV to host cells. The elimination of the MERS-CoV virus has been successfully achieved, using the lectin *Galanthus nivalis agglutinin* (GNA), which recognizes the disaccharide mannoseα (1-3) Mannose terminal of the glycoprotein of the viral envelope [58]. The surface glycoprotein (S) of the severe acute respiratory syndrome coronavirus (SARS-CoV) facilitates its penetration into host cells, by binding to the angiotensin 2 converting enzyme (ACE2) [59], [60], [61]. SARS-CoV and SARS-CoV 2 differ from each other only by 25% with regard to viral proteins [62], in addition SARS-CoV 2 also binds the enzyme of angiotensin 2 conversion [63], [64]. Protein S of SARS-CoV has several N-glycosylated surface sites rich in mannose, hybrid N-glycans and complex N-glycans [65]. Glycoprotein S is the gateway to the virus, and a major target for antiviral drugs such as lectins, which it is proposed to be used to prevent binding of COVID-19 to these target cells. Among the lectins which can optionally bind to the glycoprotein S, there may be mentioned: *Canavalia ensiformis* (Con A), *Pisum sativum* (PSA), *Lens*

culinaris (LCA), *Vicia faba agglutinin* (VFA), *Vicia cracca* (VCA), *Onobrychis viciifolia* (OVA) and *Lathyrus sativus* (LEC).

➢ *Human immunodeficiency viruses (HIV)*

The viral envelope glycoproteins play an essential role in the phenomenon of its intrusion into host cells. In the case of HIV-1, the surface envelope glycoproteins gp120 and gp41 are highly glycosylated, and play a key role in the fusion of the virus to infected cell membranes [66]. Indeed, powerful anti-HIV actions are attributed to lectins with specific recognition for mannose (Man) and / or N-acetylglucosamine (GlcNAc) [67]. Lectins with antiretroviral activities have been identified and isolated from animals [68], plants [69], microorganisms [70] and are used as natural anti-HIV products. Many lectins have been researched as natural anti-HIV products, namely: *griffithsin* (GRFT), *actinohivine* (AH), *concanavalin-A* (ConA), *cyanovirin-N* (CV-N), *microvirin* (MVN) and *banana lectin* (BanLec).

The *griffithsin* lectin (GRFT): extracted from the red alga *Griffithsia sp.*, Is a lectin with six oligosaccharide binding sites recognizing the terminal oligomannose residues α-D-Mannoseα(1-2)-D-Mannose, 2 -O-α-D-Mannopyranosyl-D-mannopyranose [71], [72], [73], [74], [75]. This lectin inhibits the binding of HIV-1 to these host cells. GRFT inhibits the CD4-dependent glycoprotein (gp) 120, which is rich in mannose-type glycans.

Actinohivine (AH): Is an anti-HIV lectin, isolated from *actinomycetes* of the genus *longispora albida* [76]. HA specifically binds to glycoproteins with several mannose-rich glycans [77]. The low toxicity of AH is explained by its extremely high specificity for glycoproteins having a high amount of glycans, and more specifically a high amount of mannose, as is the case for gp120 of HIV. Furthermore, AH does not fix certain human glycoproteins, which however are provided with numerous molecules of mannose because they do not have the same structure as gp120. Thus AH is developed as a microbicide preventing transmission and infection by HIV.

Concanavalin-A (ConA): Among the first lectins discovered [78], which has a great affinity for the mannosylated glycan chains. Its inhibitory power of the human immunodeficiency virus type 1 (HIV-1) has been demonstrated by Pashov et al [79] *Concanavalin A* (ConA) behaves like neutralizing antibodies, which do not interact directly with the CD4 of gp120, but rather with the later stages of access of the virus in these target cells. The binding of ConA to viral envelope glycoproteins is less sensitive to mutations in glycosylation sites, due to its great affinity for mannose residues.

Cyanovirin-N (CV-N): It is extracted from cultures of cyanobacteria (blue-green algae) *Nostoc ellipsosporum* [80]. Low concentrations of natural CV-N inactivate irreversibly various strains of the human immunodeficiency virus (HIV) type 1 as well as strains of HIV type 2. The antiviral activity of this lectin is due to its high affinity for the mannose-rich glycoproteins of the envelope of gp120 viral surface.

Microvirin (MVN): It is a lectin isolated from the *cyanobacterium Microcystis aeruginosa*, it is capable of inhibiting HIV-1 infection, and also inhibits the formation of syncytium between T cells persistently infected by HIV-1 and uninfected CD4 (+) T cells [81]. This lectin specifically binds to surface glycoproteins carrying mannose α (1-2) mannose disaccharides. Therefore, MVN can be considered a useful lectin for potential microbicidal use due to its broad and potent antiviral activity.

Banana lectin (BanLec): It is a lectin isolated from the banana fruit, *Musa acuminata* [82]. This lectin binds to glycoproteins with a high content of mannosylated carbohydrates, such as the human immunodeficiency virus type 1 (HIV-1). BanLec inhibits HIV-1 infection by binding to the glycosylated viral envelope and blocking cell entry; it is used as an antiviral microbicide to prevent the sexual transmission of HIV-1 [83], [84].

Generally, these lectins contain multiple glycan binding sites allowing them to form multivalent interactions with gp120; such interactions give lectins the ability to neutralize different strains of HIV-1 and HIV-2.

➢ *Ebola virus*

Ebola is a negative-stranded RNA virus from the *Filoviridae* family, which is the cause of fatal hemorrhagic fevers. The envelope glycoproteins are highly glycosylated (GP1 and GP2), they ensure binding to receptors (GP1) and fusion of the host-virus cell membrane (GP2). Surface glycoproteins (GP) are highly glycosylated, and are rich in mannosylated N-glycans [85]. *Banana lectin* (BanLec), has been used as an antiviral agent because it binds to the mannose-rich glycans present on viral surface glycoproteins, thus exerting anti-Ebola virus effects by inhibiting both entry and transcription / virus replication [86]. In addition, other authors have used the lectin *Amaryllis* (HHL), which binds only to α-mannose residues; and prove the effectiveness of the administration of physiological doses of Mannose-binding lectin (MBL) products with high concentrations to individuals infected with the Ebola virus [87].

III. HOW TO ADMINISTER THESE LECTINS?

For lectins to be used as drugs, they must obey three main physiological conditions: (1) must not be toxic (2) must not be degradable by proteolytic enzymes (3) and do not cause immunogenicity. The purpose of choosing the general or systemic route is for the lectin used as an antiviral (the active ingredient) to pass through the bloodstream and to reach its site of action intact. Studies of macaques with simian immunodeficiency virus (SIV) have shown that the use of the gel *cyanovirin-N* lectin prevents rectal transmission of SHIV in macaques (88); the same lectin inhibits viral development in mice affected by the Zairian strain of Ebola virus (Ebo-Z), after its administration by subcutaneous injection [85]. *Griffithsin*

(GRFT), a lectin of the Griffithsia species, inhibits the replication of the human immunodeficiency virus-1 (HIV-1) by intraperitoneal injection [89]. Other lectins have been used by intranasal administration to prevent respiratory infections in the case of infections with viruses of the Coronavirida family [90], and of influenza A [91].

IV. CONCLUSION

Lectins have shown great potential to inhibit, prevent multiple infections, and / or improve the general health of people with viral infections. The covid-19 envelope glycoprotein S is rich in mannose, hybrid, and complex N-glycans. This glycoprotein is the key to the viral life cycle and a major target for antiviral drugs such as lectins. The choice of these lectins must imperatively respect the specificity of the glycoproteins of the viral envelope. In order to prevent the binding of COVID-19 to these target cells, it would be appropriate to use lectins whose binding specificity is mannose or / and hybrid and complex N-glycans. Therefore, we propose the following lectins as a therapeutic agent because of their specificities for the Covid-19 glycoproteins: *Canavalia ensiformis* (Con A), *Pisum sativum* (PSA), *Lens culinaris* (LCA), *Vicia faba agglutinin* (VFA), *Vicia cracca* (VCA), *Onobrychis viciifolia* (OVA) and *Lathyrus sativus* (LEC). Given the number of lectins used as therapeutic agents against several viruses, it is not surprising that others can be used as covid-19 drugs.

ACKNOWLEDGMENTS

The precious contributions of my biology students from the Regional Center for Education and Training Professions Sous Massa (group 2019-2020), as well as those of the chemist Rachid Drissi el bouzaidi, are gratefully acknowledged.

REFERENCES

[1]. I.J. Goldstein, RC. Hughes, M. Monsigny, T. Osawa, N. Sharon. What should be called a lectin? *Nature* 285: 66, 1980.
[2]. N. Sharon and H. Lis. Lectins: Cell agglutination and sugar specific proteins. *Science* 177: 949-959, 1972.
[3]. Lis H., Sharon N. The biochemistry of plant lectins phytohemagglutinins. *Ann Rev Biochem* 42: 541-574, 1973.
[4]. H. Lis and N. Sharn. Lectins in higher plants. The biochemistry of plants 6, Marcus A., Stumpf P.K. and Conn EE (eds) p: 371-447, 1981.
[5]. G.L.Nicolson. the interactions of lectin with animal cell surfaces. *Int Rev Otol*. 39: 89-190, 1974.
[6]. J.C Brown and R.C Hunt. Lectins. *Int. Rev. Cytol*. 52: 227-267, 1978.
[7]. I.J. Goldstein, C.C. Ayes. The lectins: carbohydrate-binding proteins. *Adt. Carbohydr Biochem* 35: 127-340, 1978.
[8]. D. Simpson, D. Thorne, H. Loh. Lectins: endogenous carbohydrate binding proteins from vertebrate tissues: fonctional role in recognition processes. *Lift. Sci* 22: 727-748, 1978.
[9]. I.E. Liener, N. Sharon, I.T. Goldstein. The lectins. Properties, functions and applications in biology and medicine. Academic Press,Orlando, 1986.
[10]. C.G. Gahmberg and S. Hakomori. Altered growth behavior malignant cells associated with changes in externally labelled glycoprotein and glycolipid. *Proc. Natl. Acad. Sci*. USA 70: 3329-3333, 1973.
[11]. H. Rauvala, W.G. Carter, S. Hakomori. Studies on cell adhesion and recognition. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (glycosidases and lectins) and fibronectin. *J. Cell. Biol.* 88: 127-137, 1981.
[12]. S.H. Barones. Soluble lectins: A vew class of extra-cellular proteins. *Science* 223: 1259-1264, 1984.
[13]. R. Goldman. Effet of Concanavalin A on phagocytosis by macrophage. *FEBS Letters* 46: 209-213, 1974.
[14]. F. Rosati and R.D. Santis. Role of the surface carbohydrates in sperme-egg interaction in Coina intestinalis . *Nature* 283: 762-764, 1980.
[15]. G.M. Mbamalij and S.E. Zalik. Endogenous β-D-galactoside binding lectin during the expansion of the yolk sac in the developing chick embryo. *Roux's Arch Dev Bio*. 1196: 176-184, 1987.
[16]. K. Olden, R.M. Pratt, K.M. Yamada. Role of carbohydrates in protein secretion and turnover effects of tunicamycin on the major cell surface glycoprotein of chick embryo fibroblasts. *Cell* 13: 461-473, 1978.
[17]. I.Y. Yoshitaka, Yoshihiro. Purification, characterization and cDNA cloning of a novel lectin from the jellyfish Nemopilema nomurai. *Biochemistry and Molecular Biology*. Volume 156, Issue 1, Pages 12-18, 2010.
[18]. F. Akif and Genten F., Restoration of the glycoconjugates expression in the ventral prostate of androgen-deprived mice by dihydrotestosterone: A glycohistochemical study. *International Journal of Innovation and Applied Studies.* Vol. 16 No. 1, pp. 19-24, 2016.
[19]. F. Helle, C. Wychowski, N. Vu-Dac, K. R. Gustafson, C. Voisset, and J. Dubuisson, "Cyanovirin-N inhibits hepatitis C virus entry by binding to envelope protein glycans," *The Journal of Biological Chemistry* vol. 281, no. 35, pp. 25177–25183, 2006.
[20]. Y. Sato, K. Morimoto, T. Kubo et al., "Entry inhibition of influenza viruses with high mannose binding lectin ESA-2 from the red alga Eucheuma serra through the recognition of viral hemagglutinin," *Marine Drugs.* vol. 13, no. 6, pp. 3454–3465, 2015
[21]. V. Tiwari, S. Y. Shukla, and D. Shukla, "A sugar binding protein cyanovirin-N blocks herpes simplex virus type-1 entry and cell fusion," *Antiviral Research*, vol. 84, no. 1, pp. 67–75, 2009.
[22]. Y. Kumaki, M. K. Wandersee, A. J. Smith et al., "Inhibition of severe acute respiratory syndrome coronavirus replication in a lethal SARS-CoV BALB/c mouse model by stinging nettle lectin, Urtica dioica agglutinin," *Antiviral Research*, vol. 90, no. 1, pp. 22–32, 2011.

[23]. B. Hoorelbeke, D. Huskens, G. Férir et al., "Actinohivin, a broadly neutralizing prokaryotic lectin, inhibits HIV-1 infection by specifically targeting high-mannose-type glycans on the gp120 envelope," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 8, pp. 3287–3301, 2010.
[24]. D.S. Dimitrov. Virus entry: molecular mechanisms and biomedical applications. *Nat. Rev. Microbiol*. 2 (2), 109-122, 2004.
[25]. I.C. Michelow, M. Dong, B.A. Mungall, L.M. Yantosca, C. Lear, X. Ji, M. Karpel, C.L. Rootes, M. Brudner, G. Houen, et al. A novel L-ficolin/mannose-binding lectin chimeric molecule with enhanced activity against Ebola virus. *J. Biol. Chem*.285:24729–24739, 2010.
[26]. I.C. Michelow, C. Lear, C. Scully, L.I. Prugar, C.B. Longley, L.M.Yantosca, X. Ji, M. Karpel, M. Brudner, K. Takahashi, et al. High-dose mannose-binding lectin therapy for Ebola virus infection. *J. Infect. Dis*. 203:175–179, 2011.
[27]. P. Meuleman, A. Albecka, S. Belouzard, K. Vercauteren, L. Verhoye, C. Wychowski, G. Leroux-Roels, K.E. Palmer, J. Dubuisson. Griffithsin has antiviral activity against hepatitis C virus. Antimicrob. Agents Chemother. 55:5159–5167, 2011.
[28]. M.D. Swanson, H.C. Winter, I.J. Goldstein, D.M. Markovitz. A lectin isolated from bananas is a potent inhibitor of HIV replication. *J. Biol. Chem.* 285:8646–8655, 2010.
[29]. K.B. Alexandre, E.S. Gray, H. Mufhandu, J.B. McMahon, E. Chakauya, R.B. O'Keefe, R. Chikwamba, L. Morris. The lectins griffithsin, cyanovirin-N and scytovirin inhibit HIV-1 binding to the DC-SIGN receptor and transfer to CD4+ cells. *Virology* 423: 175–186, 2012.
[30]. D. Huskens, D. Schols. Algal lectins as potential HIV microbicide candidates. *Marine Drugs*. 10:1476–1497, 2012.
[31]. A. Kachko, S. Loesgen, S. Shahzad-Ul-Hussan et al., "Inhibition of hepatitis C virus by the cyanobacterial protein microcystis viridis lectin: Mechanistic differences between the high-mannose specific lectins MVL, CV-N, and GNA," *Molecular Pharmaceutics*, vol. 10, no. 12, pp. 4590–4602, 2013.
[32]. L. S. M. Ooi, W.-S. Ho, K. L. K. Ngai et al., "Narcissus tazetta lectin shows strong inhibitory effects against respiratory syncytial virus, influenza A (H1N1, H3N2, H5N1) and B viruses," *Journal of Biosciences*, vol. 35, no. 1, pp. 95–103, 2010.
[33]. B. Nixon, M. Stefanidou, P. M. M. Mesquita et al., "Griffithsin protects mice from genital herpes by preventing cell-to-cell spread," *Journal of Virology*, vol. 87, no. 11, pp. 6257–6269, 2013.
[34]. Y. Luo, X. Xu, J. Liu et al., "A novel mannose-binding tuber lectin from Typhonium divaricatum (L.) Decne (family Araceae) with antiviral activity against HSV-II and anti-proliferative effect on human cancer cell lines," *Journal of Biochemistry and Molecular Biology*, vol. 40, no. 3, pp. 358–367, 2007.
[35]. E. Keyaerts, L. Vijgen, C. Pannecouque et al., "Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle," *Antiviral Research*, vol. 75, no. 3, pp. 179–187, 2007.
[36]. M. R. Boyd, K. R. Gustafson, J. B. McMahon et al., "Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development," *Antimicrobial Agents and chemotherapy*, vol. 41, no. 7, pp. 1521–1530, 1997.
[37]. Farci P, Alter HJ, Wong DC, et al. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. *Proc Natl Acad Sci* USA; 91: 7792–7796, 1994.
[38]. J. Dubuisson, H.H Hsu, R.C Cheung, H.B Greenberg, D.G Russell, C.M Rice. Formation and intracellular localization of hepatitis C virus glycoprotein complexes expressed by recombinant vaccinia and Sindbis viruses. *J .Virol*. 68: 6147–60, 1994.
[39]. V. Deleersnyder, A. Pillez, C. Wychowski, et al. Formation of native hepatitis C virus glycoprotein complexes. *J. Virol*. 71: 697–704, 1997.
[40]. F. Penin, C. Combet, G. Germanidis, P.O Frainais, G. Deleage, J.M Pawlotsky. Conservation of the conformation and positive charges of hepatitis C virus E2 envelope glycoprotein hypervariable region 1 points to a role in cell attachment. *J Virol* 75: 5703–5710, 2001.
[41]. A. Kachko, S. Loesgen, S. Shahzad-Ul-Hussan et al., "Inhibition of hepatitis C virus by the cyanobacterial protein microcystis viridis lectin: Mechanistic differences between the high-mannose specific lectins MVL, CV-N, and GNA," *Molecular Pharmaceutics*, vol. 10, no. 12, pp. 4590–4602, 2013.
[42]. Y. Sato, K. Morimoto, T. Kubo et al., "Entry inhibition of influenza viruses with high mannose binding lectin ESA-2 from the red alga Eucheuma serra through the recognition of viral hemagglutinin," *Marine Drugs*, vol. 13, no. 6, pp. 3454–3465, 2015.
[43]. C. Bertaux, D. Daelemans, L. Meertens et al., "Entry of hepatitis C virus and human immunodeficiency virus is selectively inhibited by carbohydrate-binding agents but not by polyanions," Virology, vol. 366, no. 1, pp. 40–50, 2007.
[44]. R.J Connor, Y. Kawaoka, R.G Webster, J.C Paulson. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology* 205:17–23, 1994.
[45]. G. Lamblin, M. Lhermitte, A. Klein , J. Perini, P. Roussel. Diversité des chaînes glycanniques des mucines bronchiques humaines et défense antimicrobienne de la muqueuse bronchique. *Med. Sci*. 7 : 1031-1040, 1991.
[46]. N. Wetprasit, W. Threesangsri, N. Klamklai, and M. Chulavatnatol, "Jackfruit lectin: Properties of mitogenicity and the inhibition of herpesvirus infection," *Japanese Journal of Infectious Diseases*, vol. 53, no. 4, pp. 156–161, 2000.
[47]. B. Nixon, M. Stefanidou, P. M. M. Mesquita et al., "Griffithsin protects mice from genital herpes by

preventing cell-to-cell spread," *Journal of Virology*, vol. 87, no. 11, pp. 6257–6269, 2013.
[48]. V. Tiwari, S. Y. Shukla, and D. Shukla, "A sugar binding protein cyanovirin-N blocks herpes simplex virus type-1 entry and cell fusion," *Antiviral Research*, vol. 84, no. 1, pp. 67–75, 2009.
[49]. Y. Luo, X. Xu, J. Liu et al., "A novel mannose-binding tuber lectin from Typhonium divaricatum (L.) Decne (family Araceae) with antiviral activity against HSV-II and anti-proliferative effect on human cancer cell lines," *Journal of Biochemistry and Molecular Biology*, vol. 40, no. 3, pp. 358–367, 2007.
[50]. Y. Yang, H.-L. Xu, Z.-T. Zhang et al., "Characterization, molecular cloning, and in silico analysis of a novel mannose-binding lectin from Polygonatum odoratum (Mill.), with anti-HSV-II and apoptosis-inducing activities," *Phytomedicine*, vol. 18, no. 8-9, pp. 748–755, 2011.
[51]. H. Sumiyoshi, C. Mori, I. Fuke, K. Morita, S. Kuhara, J. Kondou, Y. Kikuchi, H. Nagamatu, A. Igarashi. Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. *Virology* 161:497–510, 1987.
[52]. K. Dutta, P.N Rangarajan, S. Vrati, A. Basu. Japanese encephalitis: pathogenesis, prophylactics and therapeutics. *Curr Sci* 98:326, 2010.
[53]. B.D Lindenbach, H-Ju Thiel, C.M Rice. Flaviviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology. Lippincott-Raven, Philadelphia, pp 1108–1109, 2007.
[54]. Z. A Hassan, C. Li. Ishag, L. Huang et al., "Griffithsin inhibits Japanese encephalitis virus infection in vitro and in vivo," *Archives of Virology*, vol. 158, no. 2, pp. 349–358, 2013.
[55]. C. Drosten, S. Günther, W. Preiser, S. van der Werf, H.-R. Brodt, S. Becker, and al. Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. *N Engl J Med* 348: 1967-1976, 2003.
[56]. A.M. Zaki, S. van Boheemen, T.M Bestebroer, A.D.M.E Osterhaus, R.A.M Fouchier. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *N Engl J Med.* 367: 1814-1820, 2012.
[57]. T. Mori, B. R. O'Keefe, R. C. Sowder II et al., "Isolation and characterization of Griffithsin, a novel HIV-inactivating protein, from the red alga Griffithsia sp.," *The Journal of Biological Chemistry*, vol. 280, no. 10, pp. 9345–9353, 2005.
[58]. P. Schult-Dietrich, S. Büttner, B. Dilmaghani, D. Lohmann, P.C. Baer, U. Dietrich, H. Geiger. Lectin Affinity Plasmapheresis for Middle East Respiratory Syndrome-Coronavirus and Marburg Virus Glycoprotein Elimination. *Blood Purif*; 46:126–133, 2018.
[59]. T.M. Gallagher, Buchmeier M. Coronavirus spike proteins in viral entry and pathogenesis. *Virology* 279: 371–374, 2001.
[60]. W. Li, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC et al,. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426: 450–454, 2003.
[61]. G. Simmons, Zmora P, Gierer S, Heurich A, Pohlmann S., Proteolytic activation of the SARS-coronavirus spike protein: cutting enzymes at the cutting edge of antiviral research. *Antiviral Res* 100: 605–614, 2013.
[62]. F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, Y. Hu, Z.W.Tao, J.H. Tian, Y.Y. Pei et al., A new coronavirus associated with human respiratory disease in China. *Nature* 579: 265–269, 2020.
[63]. R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Guo, Q. Zhou. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. *Science* 367: 1444–1448, 2020.
[64]. P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579: 270–273, 2020.
[65]. G. Ritchie et al,. Identification of N-linked carbohydrates from severe acute respiratory syndrome (SARS) spike glycoprotein. *Virology* 399, Issue 2,257-269, 2010.
[66]. T. Mizuochi, M. W. Spellman, M. Larkin, J. Solomon, L. J. Basa, and T. Feizi, "Carbohydrate structures of the human-immunodeficiency-virus (HIV) recombinant envelope glycoprotein gp120 produced in Chinese-hamster ovary cells," *Biochemical Journal*, vol. 254, no. 2, pp. 599–603, 1988.
[67]. J. L. Davis, M. Fei, and L. Huang, "Respiratory infection complicating HIV infection," *Current Opinion in Infectious Diseases*, vol. 21, no. 2, pp. 184–190, 2008.
[68]. J.-H. Wang, J. Kong, W. Li et al., "A β-galactose-specific lectin isolated from the marine worm Chaetopterus variopedatus possesses anti-HIV-1 activity," *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, vol. 142, no. 1-2, pp. 111–117, 2006.
[69]. W. J. Peumans, W. Zhang, A. Barre et al., "Fruit-specific lectins from banana and plantain," *Planta*, vol. 211, no. 4, pp. 546–554, 2000.
[70]. H. Chiba, J. Inokoshi, M. Okamoto et al., "Actinohivin, a novel anti-HIV protein from an actinomycete that inhibits syncytium formation: Isolation, characterization, and biological activities," *Biochemical and Biophysical Research Communications*, vol. 282, no. 2, pp. 595–601, 2001.
[71]. T. Mori, B.R. O'Keefe, R.C. Sowder, S. Bringans, R. Gardella, S. Berg, P. Cochran, J.A. Turpin, R.W. Buckheit, J.B. McMahon, et al. Isolation and characterization of griffithsin, a novel HIV-inactivating protein, from the red alga Griffithsia sp. *J. Biol. Chem.* 280:9345–9353, 2005.
[72]. T. Moulaei, S.R. Shenoy, B. Giomarelli, C. Thomas, J.B. McMahon, Z. Dauter, B.R. O'Keefe, A. Wlodawer. Monomerization of viral entry inhibitor griffithsin elucidates the relationship between multivalent binding to carbohydrates and anti-HIV activity. *Structure* 18:1104–1115, 2010.

[73]. N.E. Ziolkowska, B.R. O'Keefe, T. Mori, C. Zhu, B. Giomarelli, F. Vojdani, K.E. Palmer, J.B. McMahon, A. Wlodawer. Domain-swapped structure of the potent antiviral protein griffithsin and its mode of carbohydrate binding. *Structure* 14:1127–1135, 2006.
[74]. N.E. Ziolkowska, S.R. Shenoy, B.R. O'Keefe, J.B. McMahon, K.E. Palmer, R.A. Dwek, M.R. Wormald, A. Wlodawer. Crystallographic, thermodynamic, and molecular modeling studies of the mode of binding of oligosaccharides to the potent antiviral protein griffithsin. *Protein Sci.* 67: 661–670, 2007.
[75]. N.E. Ziolkowska, S.R. Shenoy, B.R. O'Keefe, A. Wlodawer. Crystallographic studies of the complexes of antiviral protein griffithsin with glucose and N-acetylglucosamine. *Protein Sci*. 16:1485–1489, 2007.
[76]. J. Inokoshi, H. Chiba, S. Asanuma, A. Takahashi, S. Omura, and H. Tanaka, "Molecular cloning of actinohivin, a novel anti-HIV protein from an actinomycete, and its expression in escherichia coli," *Biochemical and Biophysical Research Communications*, vol. 281, no. 5, pp. 1261, 2001.
[77]. H. Tanaka, H. Chiba, J. Inokoshi et al., "Mechanism by which the lectin actinohivin blocks HIV infection of target cells," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 106, no. 37, pp. 15633–15638, 2009.
[78]. I.J. Goldstein, RC. Hughes, M. Monsigny, T. Osawa, N. Sharon. What should be called a lectin? *Nature* 285: 66, 1980.
[79]. A. Pashov, S. MacLeod, R. Saha, M. Perry, T.C. VanCott, T. Kieber-Emmons. Concanavalin A binding to HIV envelope protein is less sensitive to mutations in glycosylation sites than monoclonal antibody 2G12. *Glycobiology* 15 (10): 994-1001, 2005.
[80]. M. R. Boyd, K. R. Gustafson, J. B. McMahon et al., "Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development," *Antimicrobial Agents and chemotherapy*, vol. 41, no. 7, pp. 1521–1530, 1997.
[81]. D. Huskens, G. Férir, K. Vermeire et al., "Microvirin, a novel α(1,2)-mannose-specific lectin isolated from Microcystis aeruginosa, has anti-HIV-1 activity comparable with that of cyanovirin-N but a much higher safety profile," *The Journal of Biological Chemistry*, vol. 285, no. 32, pp. 24845–24854, 2010.
[82]. V.L. Koshte, et al.,. Isolation and characterization of BanLec-I, a mannoside- binding lectin from Musa paradisiac (banana). *Biochem. J.* 272 (3), 721-726, 1990.
[83]. M.D. Swanson, et al., A lectin isolated from bananas is a potent inhibitor of HIV replication. *J. Biol. Chem.* 285 (12), 8646-8655, 2010.
[84]. M. D. Swanson, D. M. Boudreaux, L. Salmon et al., "Engineering a therapeutic lectin by uncoupling mitogenicity from antiviral activity," *Cell* vol. 163, no. 3, pp. 746–758, 2015.
[85]. L.G. Barrientos, B.R. O'Keefe, M. Bray, A. Sanchez, A.M. Gronenborn, M.R. Boyd. Cyanovirin-N binds to the viral surface glycoprotein, GP1,2 and inhibits infectivity of Ebola virus. *Antiviral Res* 58: 47–56, 2003.
[86]. E.M. Cove´s-Datson, J. Dyall, L.E. DeWald, S.R. King, D. Dube, M. Legendre et al. Inhibition of Ebola Virus by a Molecularly Engineered Banana Lectin. *PLoS Negl Trop Dis* 13 (7): e0007595. https://doi.org/10.1371/journal. pntd.0007595, 2019.
[87]. M. Brudner, M. Karpel, C. Lear, L. Chen, L.M. Yantosca, C. Scully, I.C. Michelow. Lectin-Dependent Enhancement of Ebola Virus Infection via Soluble and Transmembrane C-type Lectin Receptors. *PLoS ONE* 8(4), e60838. doi:10.1371/journal.pone.0060838, 2013.
[88]. C.C. Tsai, et al., Cyanovirin-N gel as a topical microbicide prevents rectal transmission of SHIV89.6P in macaques. *AIDS Res. Hum. Retroviruses* 19 (7), 53-541, 2003.
[89]. J.C. Kouokam, Lasnik A.B., Palmer K.E., Studies in a murine model confirm the safety of griffithsin and advocate its further development as a microbicide targeting HIV-1 and other enveloped viruses. *Viruses* 8 (11), 2016.
[90]. B.R. O'Keefe et al., Broad-spectrum in vitro activity and in vivo efficacy of the antiviral protein griffithsin against emerging viruses of the family Coronaviridae. *J. Virol*. 84 (5), 2511-2521, 2010.
[91]. D.F. Smee et al., Treatment of influenza A (H1N1) virus infections in mice and ferrets with cyanovirin-N. *Antivir. Res*. 80 (3), 266-271, 2008.