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EVALUATION OF ANTI-VIRAL ACTIVITY OF BANLEC AGAINST HEPATITIS C VIRUS

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ABSTRACT

BanLec is a mannose binding lectin derived from *Musa acuminata* (banana). It is known to have drug potential against HIV and current studies are working on its efficacy against AIDS. In this study, using *in silico* methods, the binding efficiency of BanLec against Hepatitis C virus has been tested and positive interactions with BanLec have been observed. Furthermore, mutational analysis using bioinformatics tools in BanLec show interesting and more tentative results with Hepatitis C virus.

KEYWORDS: Banana lectin, mannose-binding lectin, in silico studies, HCV, liver cirrhosis.

INTRODUCTION

Hepatitis C Virus (HCV)

Hepatitis C virus infection is one of the major death-causing infections globally (Marinho *et al.*, 2014). HCV-related complications are significant medical concerns all over the world, especially in the United States. Recent data show that about 350,000 deaths occur globally per annum as a result of HCV-related complications (Lynch & Wu, 2016). It has been recently documented that more than 185 million chronic HCV infected patients (Lynch & Wu, 2016) are present worldwide; in the US alone 4.6 million people are affected by HCV (Zhang *et al.*, 2016).

The World Health Organization (WHO), in 1999, reported an estimation of 3 percent prevalence globally with 170 million people affected by HCV (WHO, 1999). A study conducted from 1998-2002 shows that HCV infection is spreading rapidly in Pakistan. Out of 16,400 patients tested for HCV infection, 751 were found to be +HCV Ab (Muhammad & Jan, 2005). Additionally, it was also revealed that 1.8 percent seroprevalence was observed in male blood donors in Karachi, Pakistan (Akhtar *et al.*, 2004). Reports from Cairo, Egypt, show high HCV prevalence in the region (28%). HCV infection rates in Yemen and Saudi Arabia were significantly low with 2.1% and 1.8% respectively (Theodore & Jamal, 2006).

Long-term HCV infection leads to liver cancer (hepatocellular carcinoma) and cirrhosis which lead to more than 15,000 deaths per annum in the United States. At present, the main cause of liver transplantation in the US is HCV (Zhang *et al.*, 2016).

HCV belongs to the family Flaviviridae which also includes pestiviruses- bovine viral diarrhea virus and hog cholera virus, and flaviviruses like yellow fever virus and dengue virus. Hepatitis C virus infections in most cases lead to chronic hepatitis. The rate of this association is getting high annually. On an estimated value, every year 150 million patients of hepatitis are infected with HCV all around the globe, with a yearly rate of approximately 3-4 million additions of new infections (WHO, 2013).

HCV is also considered to be the major etiologic agent of non-A, non-B hepatitis acquired by community on the basis of transfusion (Weiner *et al.*, 1992). The transfusion is most usually acquired through direct blood-blood contact, most of which become chronic hepatitis leading to liver carcinoma, cirrhosis and other fatal liver conditions (Lynch & Wu, 2016). The acute HCV infections, around 30% of the total, however, are resolved easily. Work-related HCV infections are commonly reported by medical and other health care staff due to accidental needle pricks from infected individuals (Liu *et al.*, 2006).

For scientific purposes, tracking down this entire transmission of HCV from donor to recipient is next to impossible given the time of transmission, symptoms in recipient and further medical diagnosis. Once inside the body, the virus replicates very quickly with the help of an error-prone viral RNA polymerase resulting in the production of a genetically different group of HCV (Ribeiro *et al.*, 2012). In each infected person, viral variants differ from the donor. It is very difficult to conduct a thorough study on the transmitted variants themselves primarily because patients are generally

unaware of the condition when it is still an acute infection (D'Arienzo *et al.,* 2013).

The HCV envelope glycoproteins, E1/E2, identified from invitro translation studies (Houghton *et al.,* 1991)and vaccine expression (Choo *et al.,* 1991) are reported to be the principle means of target cell attachment and entry. Experimental results performed by Weiner *et al.,* 1992, suggested that the hypervariable (HV) domain of the envelope glycoprotein E2 of HCV is where the human immune system targets.

Banana Lectin

Banana (*Musa acuminata*) lectin, BanLec, is an MBL that has been extensively shown to have drug potential against HIV by blocking the entry of HIV due to its attachment with HIV-1 envelope protein (gp120), thus stopping its replication. Currently, many studies are conducted on the treatment efficiency of AIDS with lectins including BanLec (Mishra *et al.,* 2016). BanLec also has the potential to bind to all enveloped viruses including Hepatitis C virus.

BanLec is capable of recognizing high mannose moieties thus making it a highly effective receptor of glycosylated virus surfaces (enveloped viruses). In case of HIV, BanLec has repeatedly been shown to be a potential inhibitor by binding to the mannose rich structures of the HIV-1 glycoprotein-120, GP-120, blocking its entry (Acharya*et al.,* 2015). Furthermore, BanLec has been acting as a successful vaginal microbicide for *in vivo* macaque vaginal and rectal transmission models, though public safety concerns remain unanswered (Swanson *et al.,* 2010). BanLec binds to 3-*O*-α-D-glucopyranosyl internal units. It also has the potential to bind to the reducing ends of α1, 3-linked glucosyl oligosaccharides [e.g., laminaribiose (LAM) and its higher homologs, laminaridextrins] (Meagher, *et al.,* 2005).

Banana lectin was first isolated by Koshte *et al.,* from *Musa paradisiaca* in 1990. It was during a study of different food items triggering various antibody responses in humans that they accidentally stumbled upon the unique binding of IgG4 to banana. Their piqued interest in this non-specific binding led them to purify the compound attached to IgG4 from banana using gel filtration method on Sephadex G-75 column. The elution of the binding compound, however, was largely recovered with mannose sugar, indicating the binding compound to be a lectin. Thus, they named this mannose-binding lectin from banana as BanLec-I. Following this discovery many more experiments were conducted on purification and analysis of BanLec, all alluding to the fact that BanLec is mannose-specific. Along with inducing production of IgG4 antibodies in humans, BanLec is also reported to be a T-cell mitogen.

Following its isolation and characterization to be homodimeric and mannose specific, further experiments on BanLec revealed that it has a total weight of approximately 30 kDa, each subunit being 14–15 kDa with 141 amino acid residues. Sequence comparison of BanLec with other lectins showed that it related to Jacalin because of the similar β-prism I fold in structure Goldstein *et al.* in 2001 explored the sugar binding properties of BanLec and showed that BanLec interacts with branched chain α-glucans and α-mannans. Each subunit of BanLec consists of two primary carbohydrate binding sites. This property is rare for a monocot plant; in fact, banana lectin is the only lectin from the group of β-prism I fold lectins that exhibits two primary sugar binding sites for every subunit).

BanLec and lectin from closely related species, plantain, have the novel characteristic of recognizing internal α1, 3- linked glucosyl residues which occur in linear polysaccharides (nigeran and elsinan). These lectins are reported to be the first plant lectins to do so, thus, indicating that plant lectins do have the potential to recognize internal α1,3- linked glucosyl residues (Singh *et al.,* 2014). Bananas of normal ripeness have shown to have no lectin but with increasing ripeness of banana, lectin also increased. However, in overripe bananas, BanLec production decreased again (Clendennen & May, 1997).

BanLec Structure and Binding Specificity

BanLec belongs to the family of jacalin related lectins, commonly known as JRLs and exists as a homodimer of ~30 kDa molecular mass, each monomer an approximate of 15 kDa (Swanson *et al.,* 2010). BanLec, as all the members of JRL family has a characteristic β-prism 1 structure comprising of three unique Greek key turn motifs wherein Greek keys 1 and 2, containing a G*XXX*D binding motif, are directly involved in binding with carbohydrates and Greek key 3 loop though, does not contain the G*XXX*D binding motif, determines the lectin specificity thereby aids in ligand binding (Swanson *et al.,* 2010). Hence, BanLec has two primary binding sites for sugars per subunit unlike other lectins whose second carbohydrate binding site is believed to have disappeared due to divergent evolution (Singh *et al.,* 2005).Revealed by molecular cloning studies, BanLec has been shown to have similar sequences with other JRL family members and also has the same overall 3-D folding pattern (Gupta *et al.,* 2008).

BanLec, in general, recognizes internal α-1, 3-mannosyl/glucosyl units, terminal α-D-mannosyl/glycosyl units and reducing terminal β-1, 3-glucosyl units (for example, the polysaccharide Laminarin from Laminariadigitata, a brown alga) (Balzarini*et al.,* 1991).

Like other JRL family members, each homodimer unit of BanLec has twelve β strands set in a β-prism-I fold. Also, proposed by Khan *et al*., 2013, the distinct ribbon-shaped structure of BanLec, each monomer subunit consisting of a single Trp amino acid residue at 10^{th} position. An important discovery made from the X-ray

crystallography of BanLec showed that it contains a second site for carbohydrate binding per subunit of BanLec (Meagher *et al.,* 2005). Moreover, the amino acid residues in this second site are common to other JRL family members. This study signals to the presence of a sub-group of JRL called mannose-specific jacalin-related lectins (mJRL), where two primary carbohydrate-binding sites are present for the same kind of sugar residue (Gupta *et al.,* 2008).

Mutational study of BanLec showed that a single amino-acid substitution, replacement of histidine 84 with a threonine, preserves its broad-spectrum antiviral potency at the same time considerably reduces its mitogenicity. Glycocluster assays, NMR spectroscopy and X-ray crystallography, demonstrated that loss of mitogenicity is very much interrelated with loss of pi-pi stacking among aromatic amino acids H84 and Y83, which removes a wall separating two carbohydrate binding sites, thus thinning down the possibilities of multivalent interactions (Swanson *et al.,* 2015).

The current study has tried to evaluate the stronger binding of BanLec with terminal mannose residues through site-directed mutagenesis *in-silico*. Using GLYCAN server (Jo & Im, 2013) it has been demonstrated that terminal mannose residues are also present in the glycoproteins of other pathogenic viruses besides HIV, including Ebola virus, Influenza virus, Dengue, SARS CoV and HCV. Many anti-viral lectins have already been demonstrated to bind to the mannose-rich envelopes of these viruses. The objective addressed in this study was to find a mutagenic BanLec sequence which can act as a more potent antiviral drug by evaluating the potential of BanLec to bind to HCV glycoproteins (vital in binding with their host cells) both in native and mutated states.

Detailed Methodology

To achieve the predefined goals of the current study, bioinformatics tools, techniques and softwares were extensively utilized. Various tools and databases like Protein Data Bank (Berman *et al.,* 2000), Uniprot (Uniprot, 2010), BLAST (Altschul *et al.,* 1990), ClustalW (Larkin *et al.,* 2007), and GLYCAN (Jo & Im, 2013) were utilized to collect data about protein and glycan sequences and structures. RasMol (Sayle & Milner-White, 1995) and other structure visualization programs like SPDBV (Guex & Peitsch, 1997), Jmol and Chimera (Peterson *et al.,* 2004) were utilized to analyze the modeled structure of proteins. Docking has been performed through, ZDock, an online Docking Server. PDBsum (Laskowski *et al.,* 1997) was majorly visited along with Contact Map Analysis to analyze the protein-protein interactions among BanLec and viral envelop proteins. The sequences and structures were obtained from RCSB PDB.

Table 1: Some viral glycoproteins and their complement receptors in human body.

No.	Virus	Glycoprotein	Human Receptor
1	HIV	Gp-120	CD-4
2	Ebola	Gp-1/2	DC-SIGN
3	HCV	E1-E2	CD-81
4	SarsCoV	S-Gp	ACE-2
5	Dengue	Gp-E	DC-SIGN

Data Collection and Tabulation

RCSB PDB- For the collection of available structures of BanLec and viral proteins of HCV as well as viral receptors in human body (Berman *et al.,* 2000).

UniProt- For the retrieval of sequences of BanLec and viral proteins of HCV (Uniprot, 2010) whose experimental structures were absent in RCSB PDB.

Because MBLs also have the ability to bind with hexoses other than mannose, no sugar moiety was removed during the processing of viral glycoprotein PDB files. However, during mutation and further analysis of the interactions, BanLec-NAG (N-acetyl glucosamine) complexes were screened out. Only BanLec-MAN (mannose) interactions with high probabilities were selected and proceeded for analysis. Presence of terminal mannose on the viral surface was also determined using GLYCAN(Jo & Im, 2013).

Next to ensure homology and conservation of receptor-binding site location in all isolates and subtypes of HCV, on average 10-15 different isolates were selected from UniProt and PDB (for available structures) and multiple alignment was performed using ClustalW(Larkin *et al.,* 2007). The resultant showed that all isolates of HCV had tentative conservations in their receptor-binding sites. However, the minor differences in positions of the receptor-binding sites in different subtypes of a virus is due to slight sliding of sequences in order to align them properly during each sequence's folding. No modeling of structure was required during analysis. All structures used in the study were experimental and were downloaded from RCSB-PDB (Berman *et al.,* 2000).

Preparing files for Docking

Using PDB Editor, water molecules and ligands (antibodies, viral proteins etc.) other than sugars were removed. The overlapping chain IDs of amino acid residues and sugars had to be changed prior to docking for PDBSUM analysis.

Docking

Docking was performed through online Docking Servers mainly ZDOCK (Pierce *et al.,* 2014) to predict the preferred orientation of BanLec to viral protein when bound to each other to form a stronger and more stable complex. Top 10 complexes were downloaded for interaction analysis. Docking servers like PatchDock (Schneidman-Duhovny *et al.,* 2005), ClusPro (Kozakov *et al.,* 2017), Hex, and AutodockVina (Stephen *et al.,* 2004; Trott & Olsen, 2010) were also utilized for comparing the results of docking. Docking of BanLec with HCV glycoprotein (refer Results section) was succeeded with docking of the same viral glycoprotein with their complement human receptors (refer Results section).

Analysis

The docking results were analyzed using PDBSUM (Laskowski *et al.,* 1997). The summary of interaction of HCV glycoprotein with BanLec is tabulated in Results and Discussion section. Furthermore, a summary of interaction of HCV glycoprotein with its natural human receptor is provided for comparison and selection of best interaction. Except for GLYCAN server diagrams (Tables 2), Chains A and B in every figure depict BanLec, the rest depict viral glycoprotein.

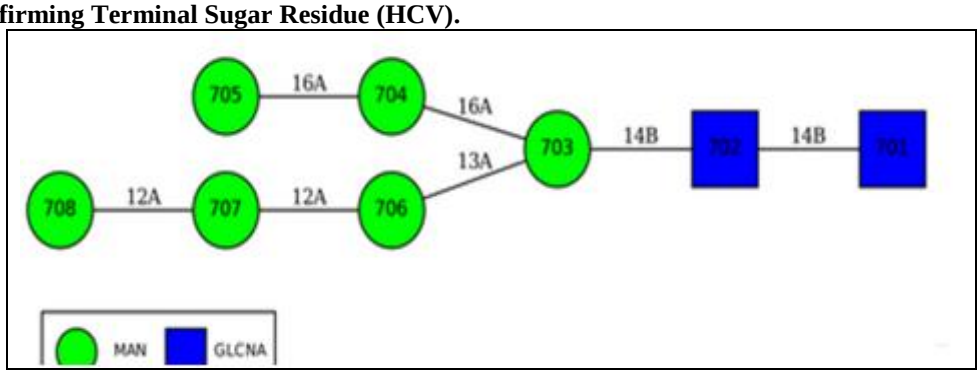
Mutations

For evaluation of the differences in interactions between the non-mutated BanLec-Viral protein models and the mutated BanLec-Viral protein models, mutant structures of BanLec were generated *in silico* using SPDBV. Before performing mutations, terminal positions of viral glycoprotein sugars were confirmed using GLYCAN server (Tables 2).

Glycan Reader Sequence Graph

- Blue circle: D-Glucose
- Blue Square: *N-Acetylglucosamine* (*N-acetyl*-D-*glucosamine*, or GlcNAc, or *NAG*) is a monosaccharide and a derivative of *glucose*.
- Green circle: D-Mannose

Table 2: Confirming Terminal Sugar Residue (HCV).



Mutations were performed in the binding pockets and in the binding sites of BanLec as observed from non-mutated BanLec result interactions. Mutation was carried out using the following categories.

1. Neutral Polar amino acids [Serine (Ser)]
2. Positive Polar amino acids [Histidine (His)]
3. Negative Polar amino acids [Glutamate (Glu)]
4. Hydrophobic amino acids [Phenylalanine (Phe)]

From each category, one amino acid was randomly selected as mentioned in parenthesis above. Excluding the group from which the target residue belonged to, remaining three categories of amino acids were used. For instance, if the target residue was a threonine (belongs to Neutral Polar amino acid category), it was mutated with His, Glu and Phe. Of the BanLec residues interacting with viral glycoproteins, 8 residues with greater interaction value were selected for mutation. Each residue was further mutated to 3 different amino acids following the above parameter.

The effects of mutations were analyzed using PDBSUM, RasMol and SPDBV. The summary of interaction of HCV glycoprotein with mutant BanLec is tabulated in Results section.

RESULTS

Although previous studies allude to the fact that BanLec is a mannose-binding lectin and this study supports it, the results also show that BanLec binds, largely in some cases, to *N-Acetylglucosamine* (NAG)also. This is in accordance to carbohydrate-binding studies performed by Goldstein *et al*. in 2001. However, only BanLec interactions with mannose (MAN) and beta mannose (BMA) of the terminal positions (Tables 2) are taken into consideration here. A list of BanLec residues interacting with mannose sugar of HCV viral proteins is tabulated below.

Table 3: List of Mannose-interacting BanLec residues in case of HCV.

BanLec							
Sr.No.	**Residues**	**Position**	**Chain**	**Sr.No.**	**Residues**	**Position**	**Chain**
1	ALA	17	A	**16**	ASP	41	A
2	PHE	18	A	**17**	SER	58	A
3	GLY	15	A	**18**	HIS	84	B
4	HIS	54	A	**19**	VAL	88	B
5	TYR	55	A	**20**	PHE	131	B
6	GLY	56	A	**21**	GLY	128	A
7	GLY	57	A	**22**	GLY	129	A
8	GLY	127	A	**23**	SER	16	A
9	GLY	129	A	**24**	ALA	117	B
10	ASN	82	B	**25**	TYR	72	B
11	TYR	83	B	**26**	LEU	73	B
12	GLY	85	B	**27**	VAL	74	B
13	ALA	86	B	**28**	SER	95	B
14	THR	96	B	**29**	ILE	116	B
15	ASN	97	B	**30**	ALA	118	B

In case of Hepatitis C virus (Table 4), BanLec interacts the most with MAN 706-708 (complex 1), total 256, 3.51% of which is H-bonded, (Fig.1) followed with MAN 704-705 (complex 1), total 224, 1.78% H-bonded, (Fig.2). BanLec also interacts with BMA (Table 4) but the values of interactions are notably few. In comparison with HCV/CD-81 interactions (Table 5-6), the highest value is observed when CD-81 interacts with MAN 706-708 (total 224, 1.78% of which is H-bonded). The result is quite good considering that BanLec has interactions with the same sugar plus the total number of interactions is higher including H-bonded ones. A summary of the interactions of BanLec with HCV glycoprotein is given below.

Table 4: Summary of the interactions of HCV E1-E2 Complex with BanLec using PDBsum Server.

Proteins Docked	Complex	HCV Sugar	HCV Chain	HCV Res No.	BanLec Chain	No. of H-bonds	No. of Non-Covalent bonds	Total No. of interactions
BanLec and HCV E1-E2 Complex	1	BMA	C	703	B	1	20	21
		MAN		704-705	A,B	5	175	180
				706-708	A,B	9	247	256
		NAG		701-702	B	1	36	37
				709-710	B	0	66	6
				712	B	1	70	71
	2	BMA		703	B	1	54	55
		NAG		701-702	B	5	294	299
		MAN		704-705	A,B	7	91	98
	3	NAG	D	701	B	0	37	37
				702	A	4	111	115
	4	BMA	C	703	A	1	39	40
		MAN		704-705	A,B	4	220	224
				706-708	A,B	5	131	136
		NAG		701-702	A	2	72	74
				709-710	A	6	232	238
	5	NAG		701	A	0	69	69
				713	B	1	166	167
	6	BMA		703	B	0	4	4
		MAN		704-705	B	0	6	6
				706-708	B	6	152	158
	7	BMA		703	B	0	4	4

		MAN		704-705	B	3	62	65
				706-708	B	7	198	205
		NAG		709-710	A	4	140	144
				712	A	1	46	47
	8	BMA		703	A	0	1	1
		MAN		704-705	A,B	2	126	128
				706-708	A	4	55	59
		NAG		709-710	B	2	139	141
	9	BMA		703	B	1	11	12
		MAN		706-708	A,B	7	245	252
		NAG		701-702	B	1	59	60
				709-710	B	2	159	161
				712	B	1	45	46
	10	BMA		703	B	1	22	23
		MAN		704-705	B	1	74	75
				706-708	B	4	75	79
				709-710	B	3	146	149
				712	B	2	82	84
MAN: Mannose, NAG: N-acetyl glucosamine, BMA: Beta Mannose								

Table 5: Sugars involved in HCV GP E1-E2 and CD-81 Interaction using PDBsum Server.

Proteins docked	Complex	HCV GP-E2 sugar	HCV GP-E2 Res No.	HCV GP-E2 Chain	CD-81 Chain	No. of H-bonds	No. of Non-bonded contacts	Total No. of interactions
HCV GP-E2 and CD-81	1	NAG	706	C	A	1	28	29
	2	NAG	706		A	1	72	73
	5	NAG	701		A	1	56	57
			702		A	0	78	78
			701-702		A	1	134	135
		BMA	703		A,B	1	46	47
		MAN	704-705		A,B	1	71	72
			706-708		A,B	2	178	179
	6	NAG	709-710		A	1	95	96
	7	NAG	701		A	0	105	105
			702		A	0	45	45
			701-702		A	0	60	60
		BMA	703		A,B	6	44	50
		MAN	704-705		B	3	101	104
			706-708		A,B	4	220	224
	9	NAG	701		A	0	54	54
			702		A	2	96	98
	10	NAG	701-702		A	0	86	86
			709-710		B	3	70	73
			712		A	2	33	35
		BMA	703		A,B	2	35	37
		MAN	704-705		B	5	94	99
			706-708		B	2	60	62
MAN: Mannose, NAG: N-acetyl glucosamine, BMA: Beta Mannose Note: All interactions of HCV GP and CD-81 chain having more than 15 A° distance have been eliminated								

Table 6: Common HCV GP E1-E2 sugars involved in HCV GP E1-E2 & BanLec and HCV GP E1-E2 & CD-81 Interactions.

HCV GP-BanLec Complex	Sugar	HCV Res No.	HCV GP-CD-81 Complex	HCV Chain
1,2,4,6-10	BMA	703	5, 7, 10	C, D
1,2, 4,6-10	MAN	704-705	5, 7, 10	
1, 4,6-10		706-708	5, 7, 10	
1,2	NAG	701-702	5, 7, 9, 10	
1,4,7,8,9,10		709-710	6, 10	
1,7,9,10		712	10	
3,5		701	5,7,9	
3		702	5,7,9	
MAN: Mannose, NAG: N-acetyl glucosamine, BMA: Beta Mannose				

HCV E1/E2- BanLec Interactions

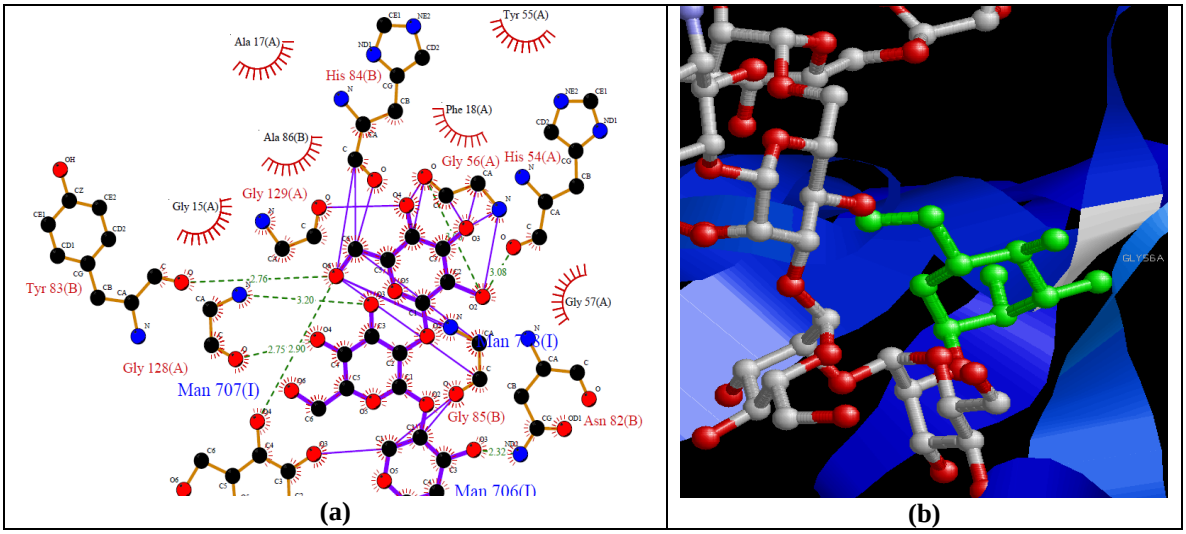


Figure 1: Complex 1 MAN 706-708. (a) PDBsum diagrammatic view of HCV sugar mannose in interaction with different BanLec monomer A & B residues. Green dotted lines indicate H-bonds whereas light purple lines indicate non-bonded contacts. (b) 3D representation of MAN (green) residue fitted in BanLec pocket (white patch denotes one of the interacting BanLec residues). Sugar residues in Ball & Stick style and BanLec shown in Ribbon style using RasMol.

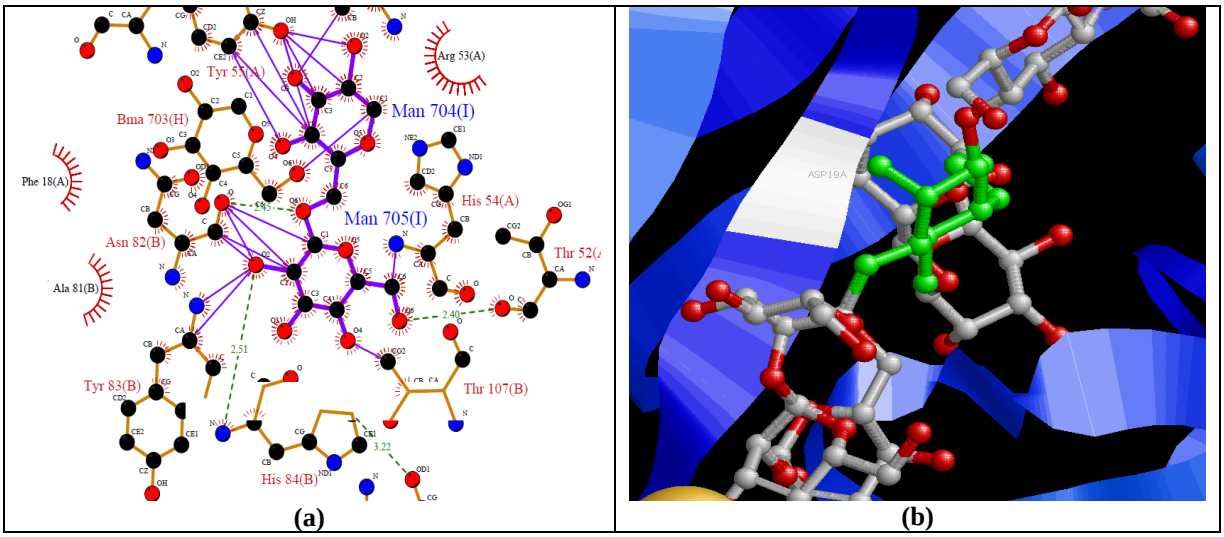


Figure 2: Complex 4 MAN 704-705. (a) PDBsum diagrammatic view of HCV sugar mannose in interaction with different BanLec monomer A & B residues. Green dotted lines indicate H-bonds whereas light purple lines indicate non-bonded contacts. (b) 3D representation of MAN (green) residue fitted in BanLec pocket using RasMol.

Mutation Interactions for HCV E1-E2 complex and BanLec

Mutational analysis in BanLec-HCV (Table 7) presents interesting results. A very significant rise in interactions is observed when BanLec residue PHE 18 (A chain) is mutated to HIS (Table 7; Fig.3), with a total of 306 interactions, 3.59% of which is H-bonded. Replacing PHE 18 (A chain) with Glu (Table 7; Fig.4) results in 289 interactions (3.46% H-bonded), replacing TYR 55 (A chain) with PHE (Table 7; Fig.5) gives 279 interactions (4.65%) followed by GLY 56 (A chain) replacement with SER results in a total of 260 interactions (3.46% H-bonded) (Table 7; Fig.6) all of which are higher than non-mutated BanLec/HCV interactions (Table 4).

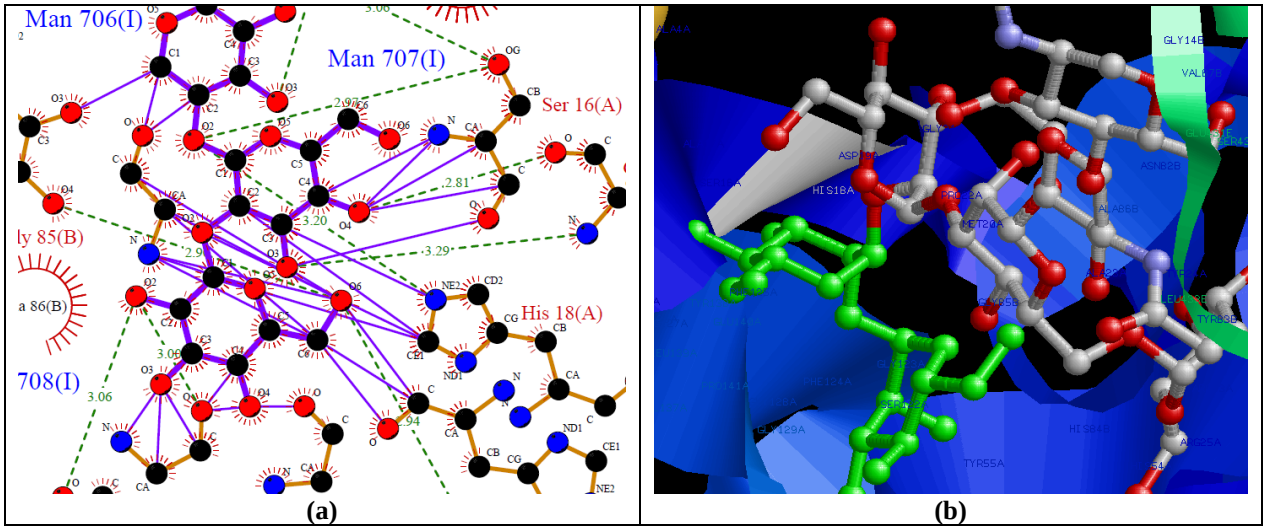


Figure 3: Complex 1 MAN 706-708 Residue Phe 18 (A) to His. (a) PDBsum diagrammatic view of mutated BanLec residue in interaction with HCV gp sugar residues. Green dotted lines indicate H-bonds whereas light purple lines indicate non-bonded contacts. (b) 3D representation of MAN (green) residue fitted in BanLec pocket using RasMol.

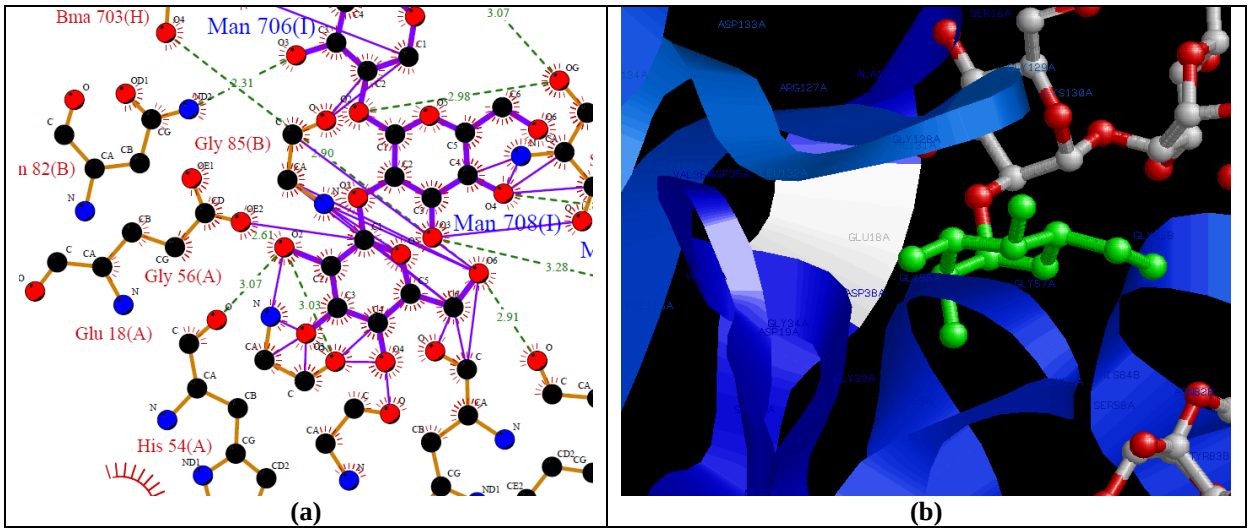


Figure 4: Complex 1 MAN 706-708 Residue Phe 18 (A) to Glu. (a) PDBsum diagrammatic view of mutated BanLec residue in interaction with HCV gp sugar residues. Green dotted lines indicate H-bonds whereas light purple lines indicate non-bonded contacts. (b) 3D representation of MAN (green) residue fitted in BanLec pocket using RasMol.

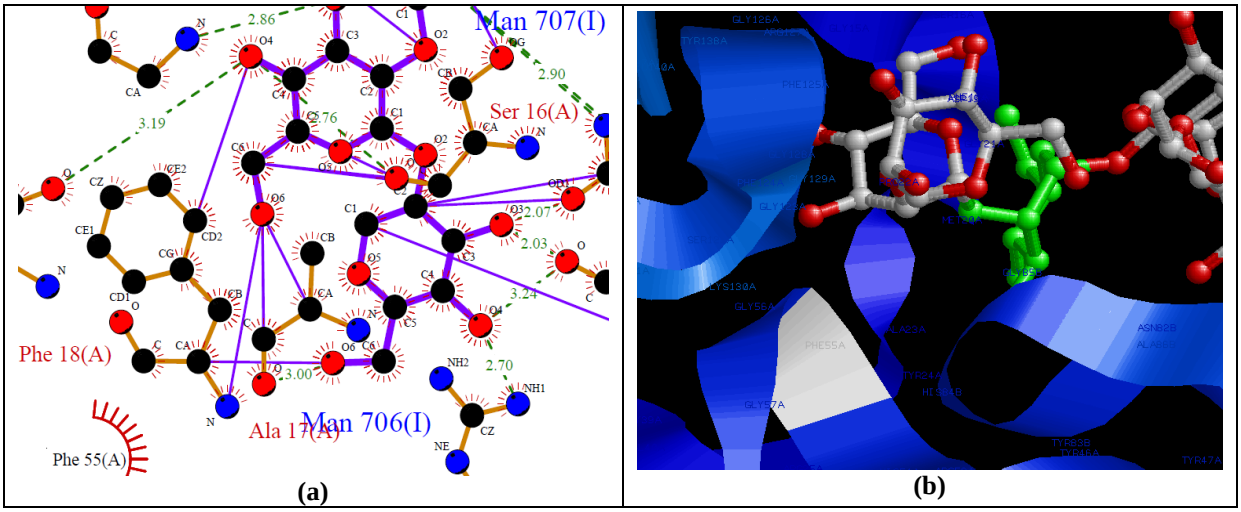


Figure 5: Complex 1 MAN 706-708 Residue Tyr 55 (A) to Phe. (a) PDBsum diagrammatic view of mutated BanLec residue in interaction with HCV gp sugar residues. Green dotted lines indicate H-bonds whereas light purple lines indicate non-bonded contacts. (b) 3D representation of MAN (green) residue fitted in BanLec pocket using RasMol.

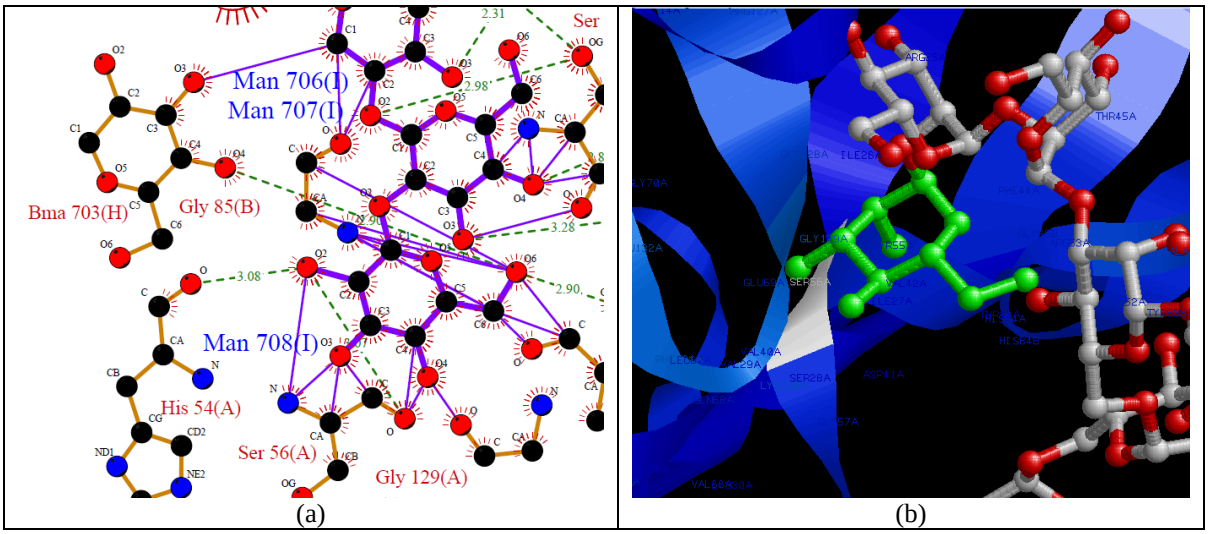


Figure 6: Complex 1 MAN 706-708 Residue Gly 56 (A) to Ser. (a) PDBsum diagrammatic view of mutated BanLec residue in interaction with HCV gp sugar residues.(b) 3D representation of MAN (green) residue fitted in BanLec pocket using RasMol.

Table 7: Summary of the interactions of HCV E1-E2 Complex with Mutant BanLecusing PDBsum Server.

Proteins Docked	Complex	HCV E1-E2 Sugar	HCV Chain	HCV Res No.	BanLec Residue & Chain	Mutant BanLec Residue	No. of H-bonds	No. of Non-Covalent bonds	Total No. of interactions
Mutant BanLec And HCV E1-E2 Complex	1	MAN	C	704-705	His 84 B	Ser	4	220	224
					His 84 B	Glu	5	78	83
					His 84 B	Phe	5	172	177
					Phe 131 B	Glu	7	153	160
					Phe 131 B	His	8	155	163
				706-708	Gly 56 A	Ser	9	251	260
					Gly 56 A	Glu	7	146	163

					Gly 56 A	His	7	146	163
					Phe 18 A	Glu	10	279	289
					Phe 18 A	His	11	295	306
					Tyr 55 A	Glu	9	245	254
					Tyr 55 A	His	9	77	86
					Tyr 55 A	Phe	13	266	279
	6			706-708	Ser 95 B	His	8	196	204
					Ser 95 B	Phe	6	164	170
					Val 74 B	His	9	169	178
MAN: Mannose									

DISCUSSION

BanLec is unique in having two sugar binding sites as opposed to just one in other lectins. According to crystal structure experiment performed by Meagher et al., 2005, binding site I, which is common in all other lectins, is composed of residues Gly 14, Gly 15, Lys 130, Phe 131 and also the side chain carboxylic acid of Asp 133 while equivalent residues for these in binding site II consists of residues Gly 59, Gly60, Asp35, Val36, and the side chain of Asp38 (Meagher *et al.,* 2005).Our findings show analogous results for Gly 15 (a conserved amino acid in the Gly-Gly loop) which can be seen interacting with MAN 706-708 of HCV (Table 4; Fig.1) and Phe 131 with MAN 704-705 (Table 4; Fig.2) suggesting that these sugar moieties were preferably inserted in the binding site I. Another conserved residue Gly 129 (Meagher *et al.,* 2005) was observed to interact with HCV complex 2 MAN 706-708 (Table 4; Appendix Fig.31).

Conversely, interactions of Ala 17 with HCV MAN 706-708 (Table 4; Fig.1) also imply that these sugar groups bind with the residues of binding site I in accordance with an experiment performed by Singh *et al*., in 2005, where they analyzed that the binding site I of BanLec consisted of two loops: loop 14-17 and loop 129-133, respectively, located on Greek key I (Singh *et al.,* 2005). Likewise, Gly 57 in its interaction with HCV MAN 706-708 (Table 4; Fig.1) and Gly 57 &Ser 58 with complex 1 MAN 704-705 (Table 4; Appendix Fig.28) is of particular interest as it indicates that the carbohydrates slotted themselves in binding site II (made up of loop 57-61 and loop 34-38) (Singh *et al.,* 2005). This again is consistent with the experimental conclusions made by Singh *et al.,* in 2005.

Moreover, a specific loop (83-86) is notably important as it serves as a common secondary site between the two binding sites of BanLec (Singh et al., 2005). In agreement with the experiment performed by Singh *et al*., in 2005, the observed binding interactions of Tyr 83, His 84 and Ala 86 with HCV MAN 704-705 (Table 4; Fig.2) and interactions of Tyr 83, Gly 85 and Ala 86 with MAN 706-708 (Table 4; Fig.1), respectively, imply that these sugar groups also bind with the common secondary site.

Based on our findings, mutation of BanLec Phe 131 reduces the total interaction value with HCVMAN 704-705 in complex 1 (Table 7) than in wild form (Table 4; Appendix Fig.60-61)presumably because it interferes with the binding site I on Greek key I. We assume that interference with the binding site I (via mutation) is not recommended in the above mentioned case.

Other tentative results in case of HCV were observed when His 84 (complex 1, MAN 704-705) was substituted with Ser (Table 7; Appendix Fig.57) and Gly 56 (complex 1, MAN 706-708) with Ser (Table 7). Likewise, prospective mutant BanLec with Ser 95 (in case of complex 6, MAN 706-708) substituted with His or Phe (Table 7; Appendix Fig.62-63) show better interactive results than wild (Table 4) with former being the most interactive. This indicates prospective potential of a mutant BanLec against HCV. On the other hand, mutation of BanLec His 84 to Glu (Table 7; Appendix Fig.58) in case of complex 1, MAN 704-705, reduces the affinity the most under the parameters of this study. Similarly, in complex 1 MAN 706-708, replacement of Tyr 55 with His severely causes reduction in overall binding interactions (Table 7; Appendix Fig.65) in comparison to wild BanLec (Table 4).

Thus, under the scrutiny of the *in silico* experiment performed in this study, BanLec's potential affinity for MAN 706-708 in case of HCV (Table 4; Fig.1) can be clearly established. Furthermore, mutation of PHE-18 (A chain) of BanLec with HIS has a significantly high potential against HCV (Table 7; Fig.3). Developing BanLec medication based on the previous and recent findings may potentially eradicate the problems of viral infections with increasing efficacy, less toxicity and lesser rates of drug resistance.An engineered/chimeric BanLec with specific site directed mutagenesis, notably in case of HCV, has a great prospective to act as a potential antiviral drug.

With designing of every new drug against viruses, scientists have to majorly take into consideration the side effects that come with it and maximum effort has to be put to minimize the adverse effects. One of the properties of an ideal drug along with being completely efficient and cost effective is to have zero side effects to the body. Designing drugs in such a way that 100% binding affinity with the ligand is achieved, is not enough for

practical use, though, it is a highly desired property. Despite the fact that 100% efficacy, binding affinity and zero side effects in a particular drug is not possible to achieve, that does not mean we should not strive towards it. Therefore, with time new drugs are designed that have better efficiency yet lesser side effects than the previous drugs. The current medications that are involved in antiretroviral therapy and highly active antiretroviral therapy bring with them a load of adverse effects, some of which may be fatal to the body. Each antiretroviral agent comes with its own side effects which may or may not be overcome with other antiretroviral medication. Thus, in highly active antiretroviral therapy (HAART) against HIV/AIDS, a combination of different classes of antiretroviral medications is given to minimize adverse effects (Montessori *et al.,* 2004). A few of the most common antiviral drugs used nowadays is discussed below.

A class of HAART medication is the reverse transcriptase inhibitors which is further divided into two groups - Nucleoside reverse transcriptase inhibitors (NRTIs) and non- nucleoside reverse transcriptase inhibitors (NNRTIs). Nucleoside reverse transcriptase inhibitors (NRTIs) are considered the backbone of the antiretroviral drug classes used in HAART (Montessori *et al.,* 2004). This class includes azidothymidine (AZT), dideoxycytidine (ddC), didanosine (ddI), tenofovir, abacavir (ABC) and stavudine (d4T) of which AZT is the prototype. NNRTIs include efavirenz (EFV), nevirapine (NVP) and delavirdine (DLV) of which nevirapine (NVP) is the prototype (Margolis *et al.,* 2014).

AZT, also commonly known as zidovudine, is the first and most common anti-HIV drug. It is an analogue of thymidine and works by successfully inhibiting the HIV's reverse transcriptase. As a result, the synthesis of DNA copy of the viral RNA is halted sometime during its production (Mitsuya *et al.,* 1990).

Zalcitabine (dideoxycytidine, ddC) also follows the same mechanism of action. It blocks the enzyme reverse transcriptase required to make double-stranded DNA to be integrated into the host infected cell. Zalcitabine, trade name Hivid, is no more sold since 2006 (HIVID, 2006). Another NRTI drug, Stavudine (d4T) is reported to be a highly potent HIV-1 replication inhibitor (Margolis*et al.,* 2014). Similarly, didanosine (ddI) is still very much in use today as a combination regimen along with Zidovudine (Broder, 2010).

Despite all these increasingly successful medications, it is well known that antiretroviral drugs have severe side effects (Chen *et al.,* 2013). In case of hepatitis C virus (HCV), interferons (IFN) used for the treatment of acute and chronic liver disease are associated with a variety of adverse effects (Manns *et al.,* 2006). Side effects induced by IFNs include neuropsychiatric disorders, flu-like symptoms, bone marrow depression and autoimmune syndromes. Due to the immunomodulatory properties of IFN, autoantibody destruction has been frequently reported in the thyroid (Manns *et al.,* 2006) along with antiphospholipid antibodies and antibodies to pancreatic islet cells and adrenal cortex (Wesche *et al.,* 2001). Moreover, IFN therapy may also aggravate other autoimmune diseases such as autoimmune hepatitis and diabetes (Manns *et al.,* 2006).

Ribavirin, a well-known medication against HCV, is used side by side with IFN therapy. It has been reported that anemia is the signature side effect of ribavirin (Afdhal *et al.,* 2004) which can result in persistent shortness of breath, fatigue and low quality life scores (Manns *et al.,* 2001). Though treatment of anemia is possible with erythropoietin but it is quite an expensive treatment. Along with the high expenditure of treatment, adverse side effects and constant resistance of HCV to drugs, designing alternative and yet more efficient drugs with less toxicity needs to be emphasized (Manns *et al.,* 2006)

In light of the above discussion, BanLec gives a glimpse of hope in its potential to act as a future drug against these deadly viruses. According to a statement made by Michael D. Swanson in 2010, the problem with viruses, such as HIV, HCV and Ebola, becoming resistant to ART medications can be overcome with BanLec (Swanson *et al.,* 2010). As BanLec binds to the sugar moieties present in different places of viruses, it will probably take several mutations for the virus to get around BanLec and become resistant (Swanson *et al.,* 2010).

Previous experimental reports indicate that mutant BanLec, H84T, decreases replication of HCV as well as HIV. The manner in which this decrement was observed is dose-dependent Similar results were also observed in case of Influenza virus whose hemagglutinin bears high-mannose type N-linked glycans. H84T BanLec has shown to be actively inhibitory against case study 1918 H1N1 and H5N1 pandemic influenza viruses (avian). A mutation of His 84 with Thr considerably reduces BanLec's mitogenicity yet preserving its antiviral potential and it also protected the experimental mice from getting flu (Swanson *et al.,* 2015).

Swanson *et al.,* in 2010, also report that the anti-HIV activity of banana lectin is comparable to anti-HIV drugs maraviroc and T-20 and also to Griffithsin and snowdrop lectin (Swanson *et al.,* 2010). Even a minimal amount of success in developing BanLec drug has the potential to save millions of lives (Swanson *et al.,* 2010).

CONCLUSIONS

BanLec has exhibited positive interactions with HCV glycoproteins. Specific mutations in BanLec apparently show better results with HCV under the parameters of this study. This research displays BanLec's potential affinity for the viral sugar codes and may very well aid in

the processing of efficient BanLec drug against fatal virus HCV.

Future perspective

Bananas being rich in potassium have plenty of well-known health benefits. Though many lectins have antiviral activity, their potential, specificity and toxicity vary on broad spectrum. Unique antiviral lectins like BanLec that have specificity towards high-mannose glycoprotein surfaces of enveloped viruses may prove to be highly successful against deadly viruses like HIV, Ebola and HCV. Along with being an excellent candidate to glycobiologists for glycoprotein research studies, BanLec's therapeutic potential can be of utmost importance. Recombinant BanLec and mutants may further develop the cause provided that they are orally safe for humans and animals. BanLec's resistance to mucosal surface binding and proteolytic degradation are areas that could be further explored for drug designing (Singh *et al.,* 2014).

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