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Patterns of interaction of major active components of the blue green algae *Spirulina fusiformis* against chosen orphan nuclear receptors: an *in silico* study

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Abstract : Objective: Hepatotoxicity is a metabolic disorder, caused due to oxidative stress which leads to liver damage. Non -steroidal anti-inflammatory drugs like diclofenac are the agent for hepatotoxicity. The aim of our study is to predict the inhibitory effect of ligands like 3'-hydroxy-4'-methoxydiclofenac, 3'-hydroxydiclofenac, (3Z)-phycocyanobilin, beta carotene, vitamin B12 and diclofenac sodium with the receptors like apo human pregnane X receptor, nuclear bile acid receptor FXR, constitutive androstane receptor and LXR. Molecular docking techniques are used in this experiment to predict the interacting residue and hydrogen bond of ligand-protein complex.Patch dock server is used to dock ligand with a receptor. The 3D structure of the docked complex is analyzed using PyMol molecular viewer. Vitamin B12 showed significant interaction with all the receptor compared to other ligand. We predicted that vitamin B12 will have good effectiveness against hepatotoxicity; its potential effects can be further studied *in vivo* models.
Keywords: Hepatotoxicity, docking, diclofenac, vitamin B12.

Introduction

A hepatotoxicity cause severe metabolic disorder which occurs due to multi-dimensional function, oxidative stress and xenobiotics leading to distortion of its function and even death[1]. It is caused by the hepatotoxic chemical which induces lipid peroxidation, mitochondrial dysfunction and also causes oxidative damages in liver[2],[3]. Some of the examples for hepatotoxic chemicals are Acetaminophen, Alipidem, Diclofenac, Disulfiram, Ibuprofen, Pirprofen, Salicylic acid, Tacrin, etc[3]. The drugs available for liver damages are known to cause serious side effect, to overcome these problem researchers are now working on the herbal drug to prevent hepatotoxicity[2],[4].The antidiabetic drug troglitazone was banned from the market as it is reported to cause hepatotoxicity[5].

Diclofenac and Troglitazone is the most harmful hepatotoxic chemical. These Chemicals are metabolized by cytochrome P450 3A4 in human and 3A1 in rat[6]. They form quinine intermediates which bind to the protein and nucleic acid. Diclofenac further cause oxidative stress by oxidative reacting species via redox recycling[7],[8]. Diclofenac is a non-steroidal anti-inflammatory drug which was discovered in the late 1970s.

Diclofenac on long term use will lead to increase in liver function test[9]. This all together results in cell death, necrosis of liver cell and end in severe liver damage[10].

A numerous number of the plant have possessed to cure the hepatotoxicity, but there is a lack of scientific evidence to prove the properties of those plants[4]. Spirulina is a natural protein which has low calorie, low fat and it is a cholesterol-free protein with carbohydrate, phytonutrient, antioxidant, all amino acids[11]. Chemist uses the method of molecular docking to discover new drugs in less time and cost[12]. Autodock[13] is good molecular docking software which helps in predicting the binding site of ligand-protein interaction[14]. The aim of the present study is to predict the inhibitory effect of ligands like 3'-hydroxy-4'-methoxydiclofenac, 3'-hydroxydiclofenac, (3Z)-phycocyanobilin, beta carotene, vitamin B12 and diclofenac sodium with the receptors like apo human pregnane X receptor, nuclear bile acid receptor FXR, constitutive androstane receptor and LXR alpha. This docking study will able to predict the effectiveness of Spirulina.

Materials and Methods

Receptor designing

PDB structure of receptors ApoHumanPregnane X Receptor, Nuclear bile acid receptor FXR, Constitutive androstane receptorand LXRalpha were obtained from RCSB (Research Collaborator for Structural Bioinformatics) protein data bank (http://www.rcsb.org/pdb/home/home.do). Table 1 shows the PDB Id of each receptor. The protein is prepared for docking by adding hydrogen molecule and removing water molecule.

Table 1: Used receptors for molecular docking

S.No.	Receptor	PDB Id.
1	Apo Human Pregnane X Receptor	1ILG
2	Nuclear bile acid receptor FXR	1OSH
3	Constitutive androstane receptor	1XNX
4	LXRalpha	5AVI

Active site prediction

PDB file of residues was submitted to 3dligand site server (http://www.sbg.bio.ic.ac.uk/3dligandsite/) which predicts their ligand binding site. The residues of binding site are predicted by converting CASP8 and homologous structures.

Ligand designing

The canonical smile of the ligands like 3'-hydroxy-4'-methoxydiclofenac, 3'-hydroxydiclofenac, (3Z)-Phycocyanobilin, beta carotene, vitamin B12 and diclofenac sodium were retrieved from PubChem database (http://pubchem.ncbi.nlm.nih.gov). The Canonical smile of ligands was pasted in Corina Molecular Network (https://www.molecular-networks.com/online_demos/corina_demo) to obtain their PDB structure[15]. Table 2 shows the details of each ligand and Figure 1 shows the molecular structure of ligand.

Table 2: Characteristics of the Ligand

Molecule Name	Molecule formula	Molecular weight (g/mol)	PubChem compound Id.
3'-Hydroxy-4'-methoxydiclofenac	$C_{15}H_{13}Cl_2NO_4$	342.17402	129615
3'-hydroxydiclofenac	$C_{14}H_{11}Cl_2NO_3$	312.14804	112230
(3Z)-Phycocyanobilin	$C_{33}H_{38}N_4O_6$	586.67802	5280816
Beta carotene	$C_{40}H_{56}$	536.87264	5280489
Vitamin B12	$C_{63}H_{88}CoN_{14}O_{14}P$	1355.365177	16212801
Diclofenac sodium	$C_{14}H_{10}Cl_2NNaO_2$	318.130469	5018304

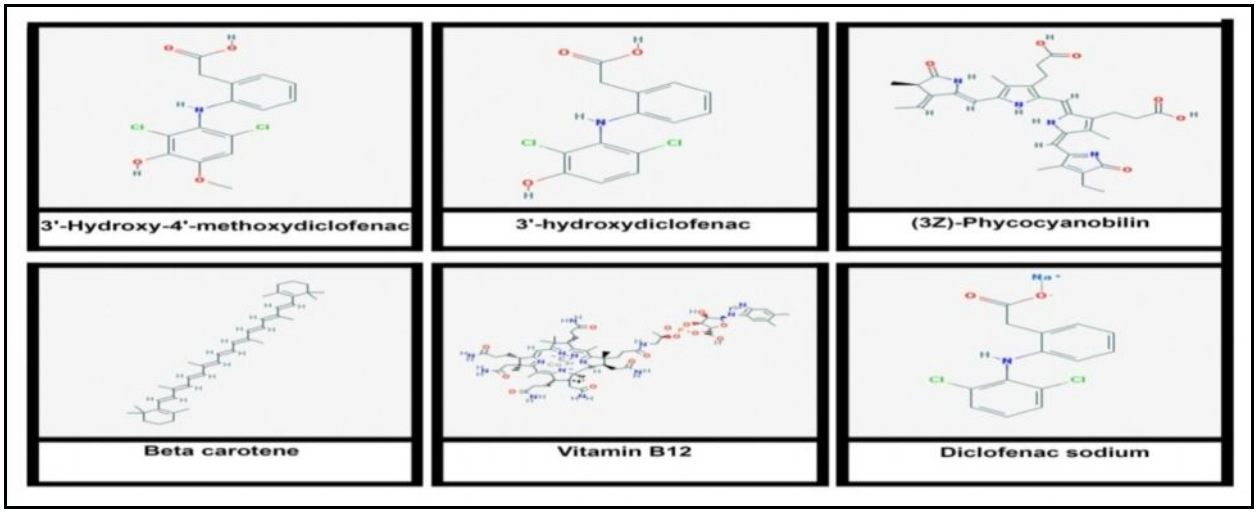


Figure 1: Molecular structure of Ligands

In silico docking

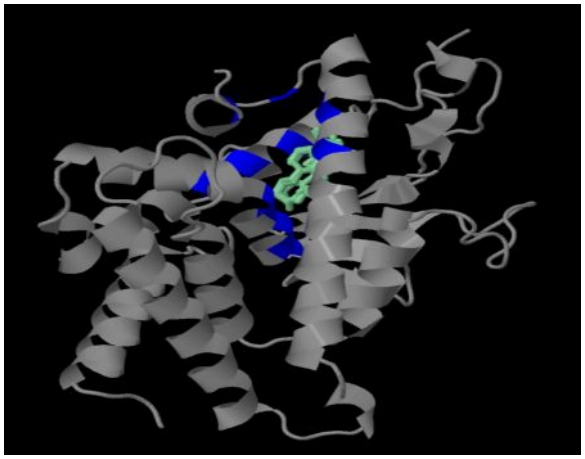
The PDB files of receptor and ligand were submitted to PatchDock automatic server for molecular docking (http://bioinfo3d.cs.tau.ac.il/PatchDock/).The results were retrieved from the user's e-mail address.

Analyses of Docked complex:

The PyMOL software helps in studying the Intermolecular interaction. The Structure of docked complex is analyzed using PyMOL software. Active site and length of the hydrogen bond is labeled using PyMOL software

Results

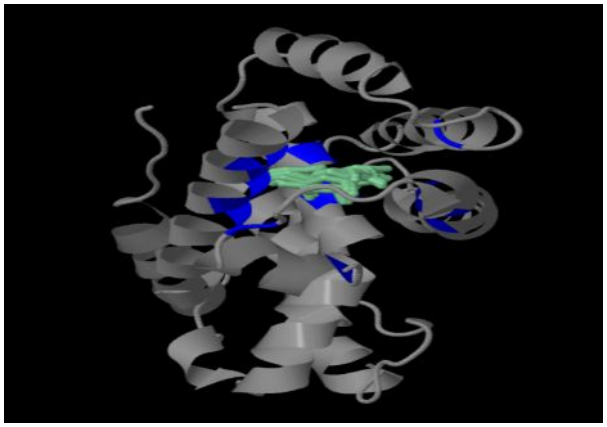
The predicted active site residue of 1ILG are MET-243, SER-247, MET-250, PHE-251, PHE-281, CYS-284, GLN-285, PHE-288, HIS-407, LEU-411, PHE-420, MET-425. Figure 2 represents the predicted active site residue of 1ILG.



Predicted binding site (blue) Other residues (grey)

Figure 2: Active site of 1ILG

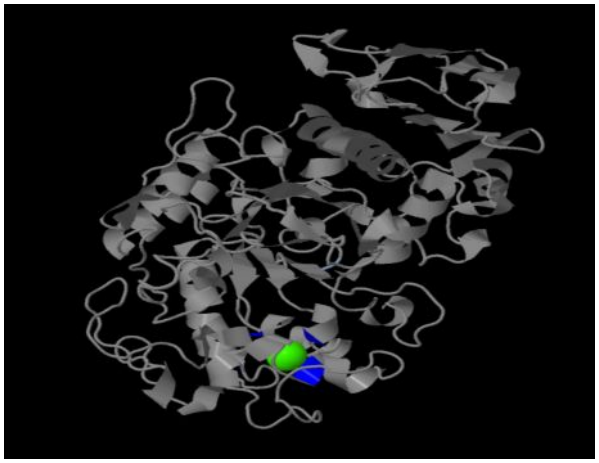
The predicted active site residue of 1OSH areLEU-291, MET-294, ALA-295, HIS-298, MET-332, PHE-333, SER-336, TYR-365, HIS-451, MET-454, LEU-455, PHE-465, TRP-473. Figure 3 represents the predicted active site residue of 1OSH.



Predicted binding site (blue) Other residues (grey)

Figure 3: Active site of 1OSH

The predicted active site residue of 4X9Y are ASN-100, ARG-158, ASP-167, HIS-201. Figure 4 represents the predicted active site residue of 4X9Y.



Predicted binding site (blue) Other residues (grey)

Figure 4: Active site of 4X9Y

The active site of 5AVI was not able to predict as there are no insufficient homologous structures with ligands bound identified.

Table 3: Score, ACE and interacting residues 1ILG receptor with ligands

Receptor	Ligand	Score	Area	ACE	Bonds		
					Atom	Residue	Length
1ILG	3'-Hydroxy-4'-methoxydiclofenac	4468	494.50	-174.54	O21	GLN-285	3.1
					O2	SER-247	2.9
					O9	SER-247	2.6
	3'-hydroxydiclofenac	4192	467.00	-159.67	NIL		
	(3Z)-Phycocyanobilin	6900	858.50	-463.61	N6	GLU-309	3.4
					O35	LYS-226	2.1

	Beta carotene	6448	866.40	-292.25	NIL		
	Diclofenacsodium	3866	467.30	-264.43	O10	TYR-328	2.8
	Vitamin B12	8178	1152.90	-955.88	O87	ALA-312, THR-311	3.0
					H	SER-208	2.5
					O58	GLU-309, LEU-308	3.4
					N45	PHE-315	3.4

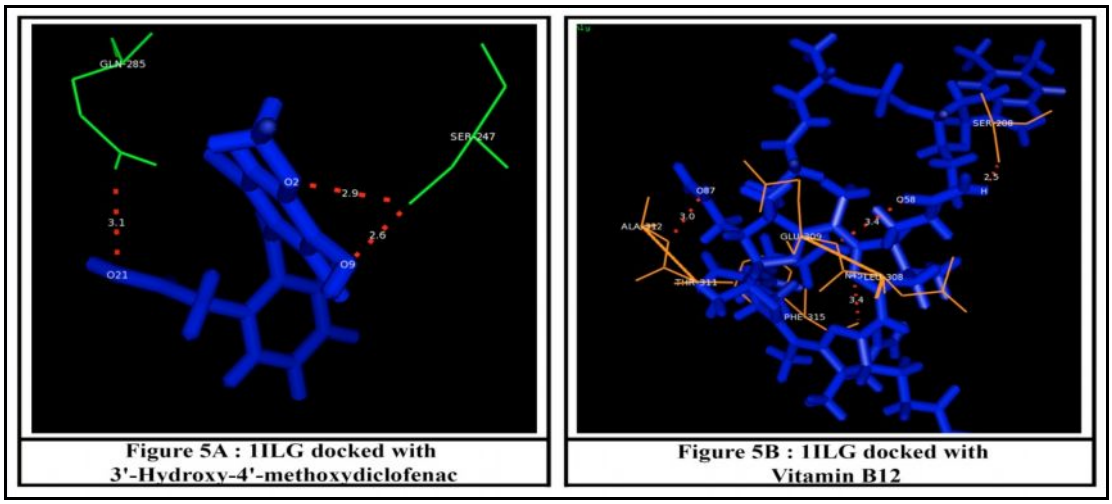


Figure 5A : 1ILG docked with 3'-Hydroxy-4'-methoxydiclofenac

Figure 5B : 1ILG docked with Vitamin B12

Figure 5: Docking of 1ILG with ligands

The docked complex of 1ILG with 3'-Hydroxy-4'-methoxydiclofenac (Figure 5A) and Vitamin B12 (Figure 5B)showed high interacting residue and high hydrogen bond compared to the docked complex of all other ligands (Table 3). The interacting residues and bond length of 1ILG with 3'-Hydroxy-4'-methoxydiclofenac are GLN-285:- 3.1, SER-247:- 2.6 and SER-247:-2.9 respectively (Figure 5A).The interacting residues and bond length of 1ILG with Vitamin B12 are ALA-312 and THR-311:- 3.0, SER-208:- 2.5, GLU-309 and LEU-308:-3.4 and PHE-315:-3.4 respectively (Figure 5B).

Table 4: Score, ACE and interacting residues 1OSH receptor with ligands

Receptor	**Ligand**	**Score**	**Area**	**ACE**	**Bonds**		
					Atom	**Residue**	**Length**
1OSH	3'-Hydroxy-4'-methoxydiclofenac	4776	522.80	-191.94	O20	TYR-373	3.4
					H28	TYR-373	2.7
					H28	SER-336	2.5
	3'-hydroxydiclofenac	4380	476.40	-178.46	O10	HIS-298	2.9
					O10	SER-336	2.1
	(3Z)-Phycocyanobilin	6088	857.00	-422.27	NIL		
	Beta carotene	6004	718.50	-140.53	NIL		
	Diclofenacsodium	4708	506.04	-162.31	O9	TYR-373	2.5
	Vitamin B12	8112	1060.60	-427.35	H	GLN-383	1.5
					O70	LYS-343	3.0
					H	LYS-343	0.8
					H	MET-269	1.5

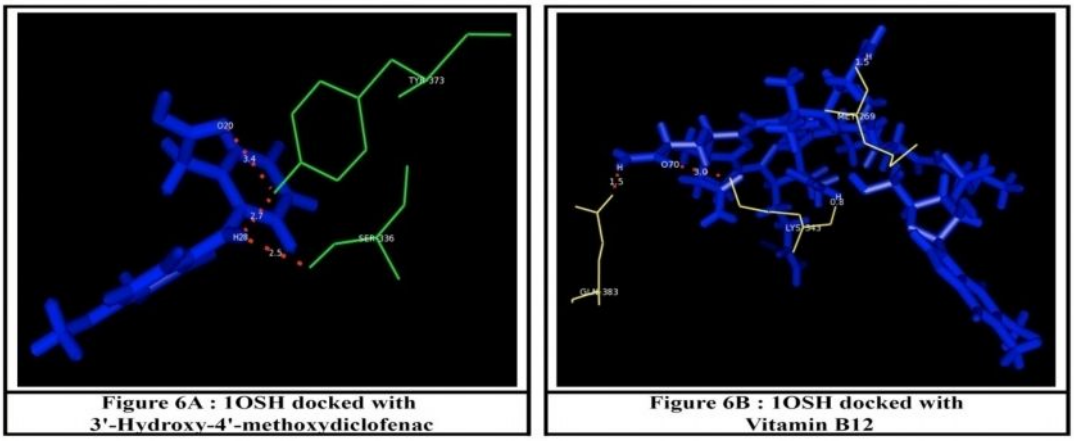


Figure 6A : 1OSH docked with 3'-Hydroxy-4'-methoxydiclofenac

Figure 6B : 1OSH docked with Vitamin B12

Figure 6: Docking of 1OSH with ligands

The docked complex of 1OSH with 3'-Hydroxy-4'-methoxydiclofenac (Figure 6A) and Vitamin B12 (Figure 6B) showed high interacting residue and high hydrogen bond compared to the docked complex of all other ligands (Table 4). The interacting residues and bond length of 1OSH with 3'-Hydroxy-4'-methoxydiclofenac areTYR-373:-3.4, TYR-373:-2.7 and SER-336:-2.5 respectively (Figure 6A). The interacting residues and bond length of 1OSH with Vitamin B12 are TYR-373:-2.5, GLN-383:-1.5, LYS-343:-3.0, LYS-343:-0.8 and MET-269:-1.5 respectively (Figure 6B).3'-hydroxydiclofenac showed few interacting residue.

Table 5: Score, ACE and interacting residues 1XNX receptor with ligands

Receptor	Ligand	Score	Area	ACE	Bonds		
					Atom	Residue	Length
1XNX	3'-Hydroxy-4'-methoxydiclofenac	4420	463.90	-173.83	H35	PHR-171	2.7
	3'-hydroxydiclofenac	4384	461.10	-173.35	NIL		
	Beta carotene	6672	819.00	-243.00	NIL		
	(3Z)-Phycocynobilin	6172	803.40	-228.67	O35	PHE-317, LEU-318	2.6
	Diclofenacsodium	4658	498.60	-171.05	NIL		
	Vitamin B12	7104	1202.20	-370.25	O21	LYS-321	2.6
					O21	ARG-316	3.1
					N73	ASN-258	2.9
					H	GLN-265	1.1

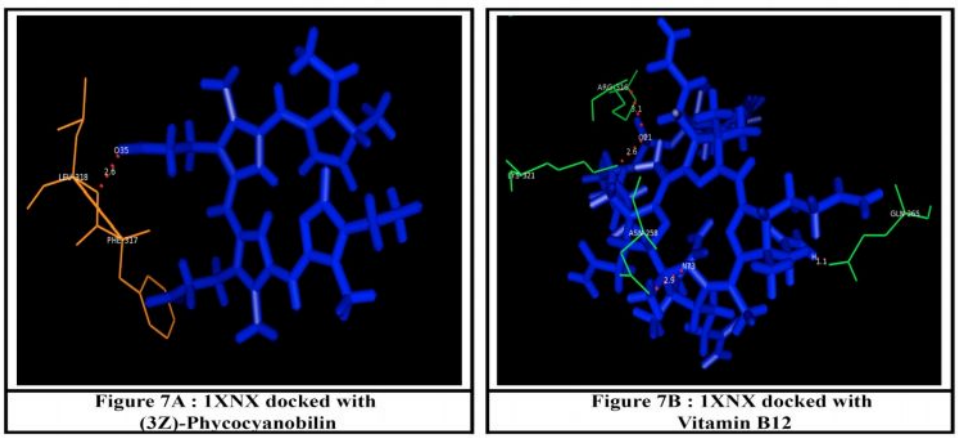


Figure 7A : 1XNX docked with (3Z)-Phycocyanobilin

Figure 7B : 1XNX docked with Vitamin B12

Figure 7: Docking of 1XNX with ligands

The docked complex of 1XNXwith (3Z)-Phycocyanobilin (Figure 7A) and Vitamin B12 (Figure 7B) showed high interacting residue and high hydrogen bond compared to the docked complex of all other ligands (Table 5). The interacting residues and bond length of 1XNX with (3Z)-Phycocyanobilin are PHE-317 and LEU-318:2.6 respectively (Figure 7A). The interacting residues and bond length of 1OSH with Vitamin B12 are LYS-321:-2.6, ARG-316:-3.1, ASN-258:-2.9 and GLN-265:-1.1respectively (Figure 7B).The interacting residues and bond length of 1XNX with 3'-Hydroxy-4'-methoxydiclofenac are PHR-171:-2.1.3'-hydroxydiclofenac, beta carotene and Diclofenac sodium showed no interacting residues.

Table 6: Score, ACE and interacting residues 5AVI receptor with ligands

Receptor	Ligand	Score	Area	ACE	Bonds		
					Atom	Residue	Length
5AVI	3'-Hydroxy-4'-methoxydiclofenac	4760	535.70	-227.55	O20	THR-302	3.4
	3'-hydroxydiclofenac	3974	452.40	-239.44	O9	SER-264	3.2
					H28	THR-302	2.4
	(3Z)-Phycocyanobilin	6068	891.30	-348.61	H51	SER-422	1.3
					H69	SER-422	2.4
					N6	SER-422	3.0
					H58	SER-422	0.6
	Beta caroten	7812	1038.80	-398.05	NIL		
	Diclofenacsodium	4350	495.50	-231.53	H26	THR-302	2.8
					O9	SER-264	3.1
	Vitamin B12	10046	1402.50	-346.95	H	GLU-334	0.8
					O21	SER-422	3.0
					O29	PHE-426	3.4

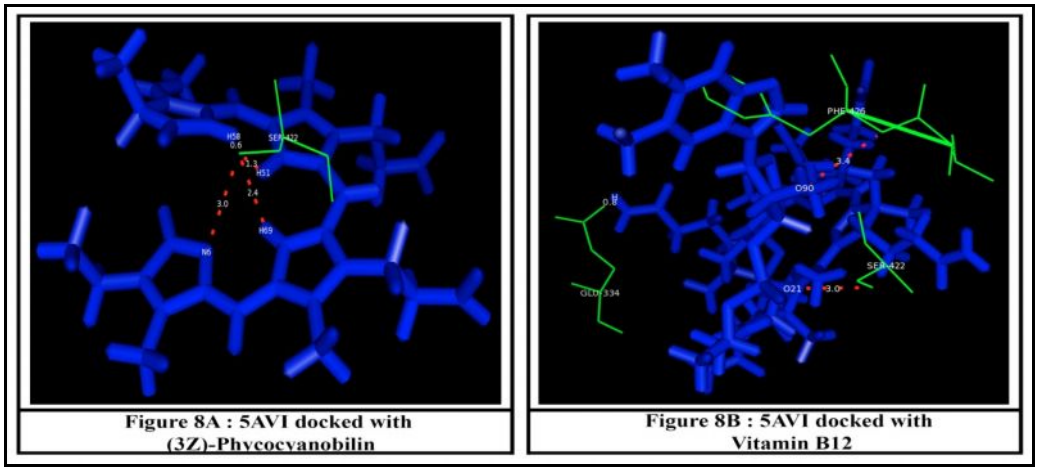


Figure 8A : 5AVI docked with (3Z)-Phycocyanobilin

Figure 8B : 5AVI docked with Vitamin B12

Figure 8: Docking of 5AVI with ligands

The docked complex of 5AVI with (3Z)-Phycocynobilin (Figure 8A) and Vitamin B12 (Figure 8B) showed high interacting residue and high hydrogen bond compared to the docked complex of all other ligands (Table 6). The interacting residues and bond length of 5AVI with (3Z)-Phycocyanobilin are SER-422:- 1.3, 2.4, 3.0 and 0.6 respectively (Figure 8A). The interacting residues and bond length of 5AVI with Vitamin B12 are GLU-334:-0.8, SER-422:-3.4 and PHE-426:-3.4respectively (Figure 8B).

Discussion

Spirulina was proved to have the protective effect against lead induced hepatotoxicity[11]. Spirulina was also demonstrated to have anti-inflammatory activity and protective role against toxicants byVo, Ryu, and Kim (2013)[16]. In our study, the docked complex Vitamin B12 showed high interaction and hydrogen bond with all

the receptors followed by 3'-Hydroxy-4'-methoxydiclofenac and (3Z)-phycocyanobilin. The other ligands like 3'-hydroxydiclofenac and Diclofenac sodium showed few interacting residues and few hydrogen bonds with receptors. But the docked complex of beta carotene showed no interacting residues and hydrogen bonds with all the receptors.

Conclusion

The docked complex of vitamin B12 is observed to have the high inhibitory effect compared to the docked complex of other compounds. Our present study predicts that vitamin B12 has high effectiveness in the treatment of hepatotoxicity whichcould be further proved by gene expression studies.

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