BOERHAAVIA DIFFUSA IN CANCER THERAPY – AN INSILICO ANALYSIS

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ABSTRACT

Cancer is a leading cause of death worldwide and it is characterized by uncontrolled growth of cells in the body. Apoptosis or programmed cell death plays a major role in normal cell development and tissue homeostasis and cancer cells do lack this property. The Bcl-2 family of proteins regulates apoptotic pathway by the interaction of pro apoptotic and anti apoptotic proteins. They maintain a balance between newly forming cells and old dying cells. Over expressed anti apoptotic Bcl-2 will alter the ratio of pro and anti apoptotic proteins, resulting in the prevention of apoptosis. Elevated levels of apoptosis-inhibitory protein Bcl-2 has frequently been detected in many forms of human cancer. Therefore, Bcl-2 appears to be a relevant target for cancer therapy. Plants have been used for treating various diseases since ancient times. *Boerhaavia diffusa* is an important medicinal plant used in various human ailments, including cancer, diabetes, hepatoprotective and anti-inflammatory effects. However, most of the reports in traditional medicine are yet to be validated through scientific studies. The present study focuses on identifying phytocompounds in *Boerhaavia diffusa* having anticancer potential to effectively inhibit the action of Bcl- 2 protein. Insilico docking analysis are well adapted for screening of phytocompounds so as to find the inhibitory or promotive role of potent lead molecules against respective targets. Thirty seven ligands of various confirmations were docked to target protein Bcl-2 using Discovery studio 3.5. The most effective ones were identified through docking score and interaction energy.

Key words: Boerhaavia diffusa, Cancer, Phytocompounds, Molecular docking, Bcl-2, Discovery studio 3.5

INTRODUCTION

Cancer is one the most deadly disease caused due to abnormal growth of the cells in our organs. It is characterized by uncontrolled proliferation of cells, resulting in the formation of lumps or growths [1]. Complex and dynamic nature of cancer is a major stumbling block in the treatment of disease. There are several reasons for cancers and the leading cause is impaired apoptotic pathway [2]. Apoptosis or programmed cell death is an ordered cellular mechanism resulting in the natural death of cells after a period of physiological activity. Genes that control apoptosis have a profound impact on cancer [3]. Up regulation or down regulation of these genes causes loss of balance between cell death and cell division resulting in the formation of cancer. [4] Dysregulation of apoptosis not only preserves malignant cells, but also the normal cells which are bearing mutations. The capacity of cancer cells to restrict apoptosis and to continue proliferation is one of the major targets of cancer therapy development. Bcl-2 (B cell lymphoma 2) protein is the first identified apoptotic regulator which is encoded by Bcl2 gene [5]. This family contains pro-apoptotic and anti-apoptotic proteins that regulate the cell cycle by eliminating mutated and damaged cells. The pathway is controlled by the interaction between anti apoptotic and pro apoptotic proteins, leading to the release of cytochrome *c* from the mitochondrial membrane, the activation of caspase cascade and, finally, to the execution of apoptosis. When Bcl-2 levels are high, apoptotic promoting proteins cannot form pores in the mitochondrial membrane, thus prevents the release of cytochrome c and caspase cascade which contribute to the formation of cancer. Elevated levels of apoptosis-inhibitory protein Bcl-2 has frequently been detected in many forms of human cancer. Therefore, Bcl-2 appears to be a relevant target for cancer therapy [6].

Since prehistoric time, plants have been used for treating various diseases. Natural products are used to cure different ailments, including cancer without causing toxicity. The majority of modern drugs used to control cancer cells have been developed from natural products. *Boerhaavia diffusa* commonly known as 'Punarnava' is a tropical herbaceous plant being used in various Ayurvedic treatments since ancient times [7]. The various parts of the plant are reported to have anticancer, antidiabetic, hepatoprotective and anti-inflammatory effects. A large number of secondary metabolites have been identified from this plant which includes rotenoids, flavanoids, alkaloids, triterpenoids, carbohydrates, proteins, and glycoproteins [8].

Computer-aided drug design (CADD) has become one of the most successful ways to screen a ligand's capability as a drug. This method predicts the predominant binding mode of a ligand within the targeted receptor

protein within a short time period [10]. The main purpose of the study is to identify the interaction of phytocompounds in *Boerhaavia diffusa* with the target protein Bcl 2 thereby inhibiting the it's activity and promoting apoptosis which helps in the development of cancer therapies.

MATERIALS AND METHODS

Identification and Preparation of target protein

Bcl-2 protein was chosen as target receptor due its significant role in human cancers. The increased level of this protein has been reported in various malignancies. Therefore Bcl-2 is an important therapeutic target in cancer treatment. The three dimensional structure of Bcl-2 was retrieved from the Protein Data Bank (PDB) (http://www.pdb.org/) with PDB ID 1G5M [11]. The NMR structure of this human anti apoptotic protein was prepared using 'Prepare protein' option in Discovery studio 3.5. The protein was prepared by removing crystallographic water molecules and heteroatoms. Hydrogen atoms were added to correct the chemistry of protein. Energy minimization was performed by employing CHARMm force field. It is a program for macromolecular dynamics. The prepared target was further used for docking analysis.

Active site analysis

The binding sites of the receptor protein 1G5M was predicted using Discovery studio 3.5 based on 'Receptor cavity method'. The amino acids responsible for binding the various ligands are seen in these binding regions. Based on this protocol active sites of the target receptor were obtained and they were chosen for docking analysis.

Identification and preparation of Phytocompounds

The important phytocompounds with pharmacological or biological activity present in *Boerhaavia diffusa* were identified by extensive literature survey. The structures of ligands were downloaded in sdf format from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) [12]. It is a public database which provides information on various properties of molecules such as chemical formula, molecular weight, Xlogp, compound structure, hydrogen bond donor and acceptor. The ligands were prepared using 'Prepare ligand' option in Discovery studio 3.5. Preparation was carried out by energy optimization and adding hydrogen atoms. The ligands of various confirmations were generated based on different energy values.

Evaluation of drug likeliness and Toxicity prediction

The drug likeliness of the selected active phytochemicals was analyzed using Lipinski's and Veber rules. The rules are molecular weight < 500 daltons, number of hydrogen bond donors <5 and number of hydrogen bond acceptors < 10,calculated water partition coefficient (CLOGP) < 5 [12].The ligands passing the Lipinski properties were taken for docking studies.

Molecular docking using Discovery studio 3.5

Molecular docking analysis of target protein Bcl-2 and phytochemicals of *Boerhaavia diffusa* was carried out using CDOCKER docking protocol of Discovery studio 3.5. CDOCKER (CHARMm-based DOCKER) is a molecular dynamics based docking algorithm. It uses the CHARMm family of force fields and offers full flexibility to ligand including dihedrals, angles and bonds. Docking helps to predict best binding compounds based on various scoring functions. For each active site, ligands were docked to obtain best interaction poses. The top poses along with CDOCKER energy and interaction energy were calculated.

RESULTS AND DISCUSSION

Protein Preparation and Active site prediction

The receptor anti apoptotic Bcl-2 protein retrieved from PDB has 166 amino acids. The energy of protein was minimized using DS 3.5 protocol. The potential energy (kcal/mol), van der waals

energy (kcal/mol) and electrostatic energy (kcal/mol) of the receptor molecule was minimized. The energy levels before minimization and after minimization are given in table (1). Minimized receptor structure is more stable and it is ready for performing docking analysis. The active site prediction was done by receptor cavity method and has identified nine active sites. The receptor ligand interaction studies were performed in these each active site to get the various poses of interaction.

TABLE1: ENERGY VALUES OF TARGET PROTEIN BEFORE AND AFTER MINIMIZATION

Target Protein Bcl-2	Force field	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)
Before minimization	CHARMm	-4167.77	-444.79	-4720.58
After minimization	CHARMm	-9226.12	-889.807	-96197.06

Ligand selection and Screening

Sixteen phytomolecules were selected from *Boerhaavia diffusa* for the interaction studies. The molecular structures of these ligands were retrieved from PubChem Chemical database. The preparations of these ligands were done by removal of water molecules and addition of hydrogen bonds. After preparation, 42 ligands of various confirmations were obtained. The screening of these ligands was done using 'Lipinski's and Veber rule which calculates molecular weight, log P, number of hydrogen bond donors, and acceptors. Beta-Sitosterol, Ursolic acid, n-Hentriacontane, Tetracosanoic acid, Eicosanoic acid, Hexadeccanoic acid and 1-triacontanol were failed in the screening process. Lipinski properties of the active phytocompounds were tabulated in Table (2). The screened compounds such as Boeravinone (A-F), Coccineone B, Coccineone E and Eupalitin were taken for the further analysis.

TABLE 2: LIPINSKI AND VEBER PROPERTIES OF THE ACTIVE PHYTOCOMPOUNDS

Sl. No	Compound Name	Pubchem ID	Molecular weight [g/mol]	Molecular formula	XlogP3	H-Bond Donor	H-bond Acceptor
1	Boeravinone A	14018346	326.30016	$C_{18}H_{14}O_6$	2.9	2	6
2	Boeravinone B	14018348	312.27358	$C_{17}H_{12}O_6$	2.4	3	6
3	Boeravinone C	13940642	344.31544	$C_{18}H_{16}O_7$	2.3	3	7
4	Boeravinone D	15081178	342.29956	$C_{18}H_{14}O_7$	2.6	3	7
5	Boeravinone E	11537197	328.27298	$C_{17}H_{12}O_7$	2	4	7
6	Boeravinone F	12004175	326.2571	$C_{17}H_{10}O_7$	2.7	3	7
7	Coccineone B	44420939	298.247	$C_{16}H_{10}O_6$	2	3	6
8	Coccineone E	12004176	344.31544	$C_{18}H_{16}O_7$	2.3	2	7
9	Eupalitin	5748611	330.28886	$C_{17}H_{14}O_7$	2.8	3	7
10	Beta-Sitosterol	222284	414.7067	$C_{29}H_{50}O$	9.3	1	1
11	Ursolic acid	64945	456.70032	$C_{30}H_{48}O_3$	7.3	2	3
12	n-Hentriacontane	12410	436.83986	$C_{31}H_{64}$	16.4	0	0
13	Tetracosanoic acid	11197	368.63672	$C_{24}H_{48}O_2$	10.7	1	2
14	Eicosanoic acid	10467	312.5304	$C_{20}H_{40}O_2$	8.5	1	2
15	Hexadeccanoic acid	985	256.42408	$C_{16}H_{32}O_2$	6.4	1	2
16	1-triacontanol	68972	438.81268	$C_{30}H_{62}O$	14.9	1	1

Docking using Discovery studio 3.5

The molecular docking analysis of the target protein Bcl 2 and thirty seven phytocompounds was performed using CDOCKER protocol of Discovery studio 3.5. Docking studies on selected molecules gives variety of interaction between target and ligand. The measure of interaction is determined by the energy values, number of hydrogen bonds and binding energy thus providing a balanced confirmation. The phytocompounds Boeravinone D, Boeravinone E, Boeravinone F exhibited good CDOCKER energy and interaction energy. The final docked results are given in Table (3).The number of hydrogen bonds, bond length, amino acids and binding energy of Boeravinone D, Boeravinone E, Boeravinone F were calculated. The results are shown in Table (4). Out of Boeravinone F, Boeravinone E and Boeravinone D, Boeravinone F is showing greater CDocker score, interaction energy with four hydrogen bonds and low binding energy. The interaction of these ligands and the aminoacids involved are described in Table (5). The hydrogen bond interaction with Boeravinone F and the receptor Bcl-2 is shown in Fig.1 and the bond length was shown in Fig.2

TABLE 3: DOCKING RESULTS OF LIGANDS WITH BCL-2 TARGET

Sl. No	Molecule name	PubChem ID	Active site	-CDOCKER Energy	-CDOCKER interaction energy
1	Boeravinone A	14018346	2	30.0447	42.4052
2	Boeravinone B	14018348	2	28.4926	40.5003
3	Boeravinone C	13940642	1	25.2158	43.4242
4	**Boeravinone D**	**15081178**	**9**	**39.1019**	**47.9985**
5	**Boeravinone E**	**11537197**	**2**	**39.2981**	**48.7553**
6	**Boeravinone F**	**12004175**	**9**	**48.8799**	**48.7689**
7	Coccineone B	44420939	1	28.2234	41.463
8	Coccineone E	12004176	2	13.5773	35.7737
9	Eupalitin	5748611	1	24.2751	44.4226
10	Beta-Sitosterol	222284	2	-50.256	28.9896

TABLE 4: HYDROGEN BOND INTERACTION

Sl. No	**Molecule Name**	**Hydrogen Bonds**	**Distance**	**Amino acids**
1	Boeravinone F 12004175	A:ARG26:HH12 -12004175:O7 A:ARG26:HH22- 12004175:O7 A:ARG106:HE - 12004175:O5 A:ARG106:HH2-112004175:O5	1.7225 2.0201 2.1631 1.9238	ARG 106 ARG 26
2	Boeravinone D 15081178	A:ARG26:HH12 - 15081178:O6 A:ARG106:HE - 15081178:O7 A:ARG106:HH21 - 15081178:O7	1.6576 2.47199 1.73645	ARG 106 ARG 26
3	Boeravinone E 11537197	A:ASN11:HD21 - 11537197:O6 A:THR7:HN - 11537197:O3 A:THR7:HN - 11537197:O2	1.82733 2.03023 2.39988	ASN11 THR7

TABLE 5: BINDING ENERGYOF LIGANDS

Sl.No	**Molecule Name**	**Binding Energy (kcal/mol)**	**Protein Energy (kcal/mol)**	**Complex Energy (kcal/mol)**
1	Boeravinone F 12004175	-149.0782	-4788.0668	-5004.8544
2	Boeravinone D 15081178	-159.4543	-4788.0668	-4991.2962
3	Boeravinone E 11537197	-144.5763	-4788.0668	-4984.6713

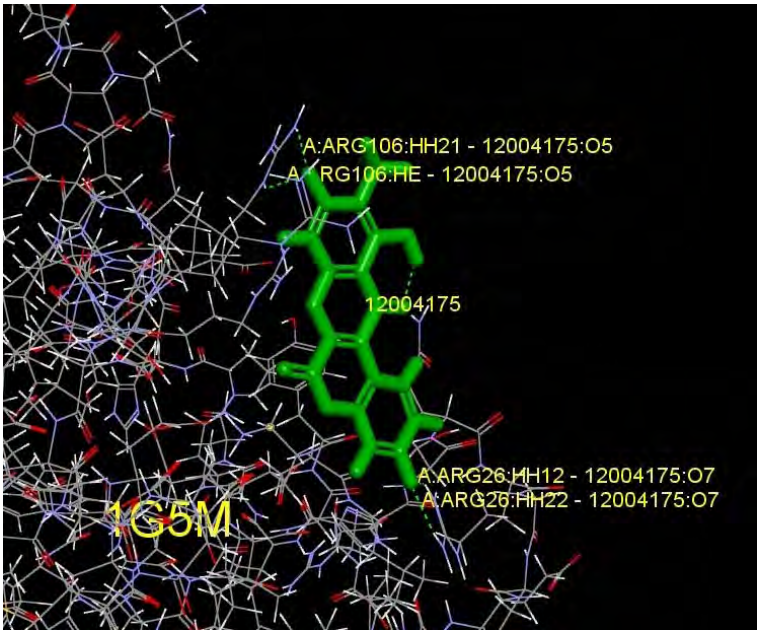

Fig.1 Hydrogen bond interactions between Bcl2 and Boeravinone F

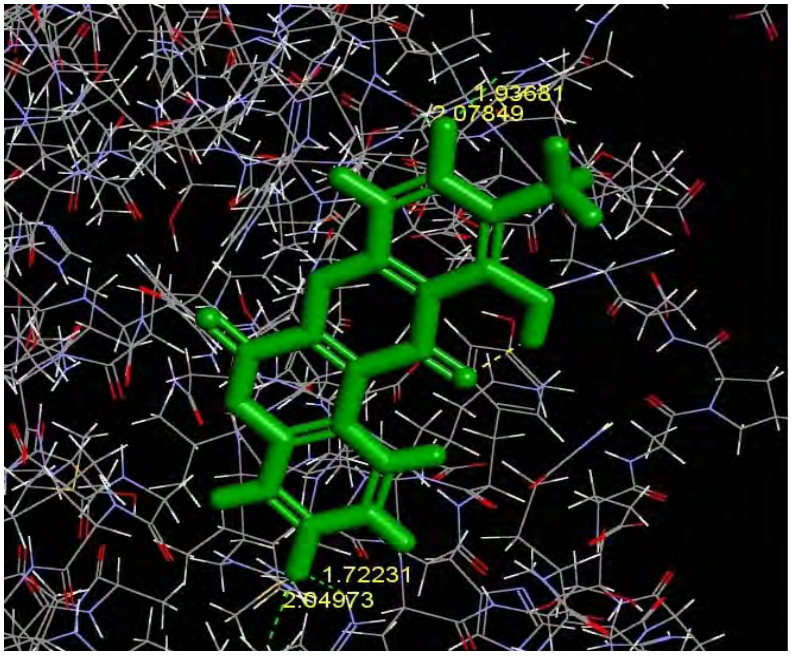

Fig.2 Hydrogen bond length between Bcl2 and Boeravinone F

CONCLUSION

In recent years designing and developing herbal drugs based on ethanobotanical and traditional systems of medicine is gaining more importance [14]. The various biomolecules from medicinal plants have been identified as effective in cancer therapy. These lead molecules can be used for developing effective novel drugs. Insilico molecular docking helps to study the protein ligand interaction. From the current molecular analysis Boeravinone F has good interaction and an inhibitory effect on receptor molecule. So it is concluded that Boeravinone F exhibits potent anticancer activity and might be considered as a lead compound for the development of potentially useful drugs that inhibit the target Bcl2 protein their by regulating apoptosis. For complete analysis of mechanism further invitro and invivo studies are required. Hence computational analysis will be a major contribution to identify new lead molecules and have opened a new pave in the development of potential drugs.

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