FLAVONES FOR THE TREATMENT OF HEPATO-CELLULAR CARCINOMA: IN SILLICO STUDY

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ABSTRACT:

Hepatocellular carcinoma (HCC) is the third common type of cancer that accounts millions of cases per year. Docking is a powerful tool for the identification of possible mechanism between the protein and ligands. The aim of the study is to identify the possible mechanism and best inhibitors of protein from the flavones category. To carry out this work protein structures were download from the PDB database and three dimensional structures of ligands were downloaded from the Pub hem database. The docking was performed by using the iGEMDOCK software suit. The binding energies of ligands Aliening, Lute Olin, Nobile tin, Sinensetin and Tangeretin for the protein Aurora kinase was found to be -90.68, -95.73, -97.06, -99.67, -94.45 kcal/mol and for protein c-Kit -106.58, -109.07, -108.47, -105.06, -112.23kcal/mol and for protein Bcl-xL -113.49, -118.82, -109.12, -111.16, -101.27 kcal/molrespectively. The binding energies of the molecules are the sum of hydrogen bond, vanderwaal's and electrostatic energies that inversely linked to protein-ligand interaction. The binding energies showed the behavior of ligand against the protein. The result can be concluded on the basis of binding energies and docked poses of protein-ligand interactions. According to the result Lute Olin has maximum binding energy for Bcl-xL that showed the anti-apoptotic mechanism in HCC.

Keywords: Hepatocellular Carcinoma, iGEMDOCK, *in silico*, Binding energy, apoptosis

INTRODUCTION:

Hepatocellular carcinoma (HCC) has become the third most frequent cancer in the world, accounting for millions of new cases each year, due to poor prognosis and a lack of viable treatment options. Aurora kinase, proto-oncogenecKit (c-Kit), and B-cell lymphomaextra large (Bcl-xL) are all targets for various signaling pathways. Using the programmeiGEMDOCK, an attempt was made to dock these flavones with HCC targets such as Aurorakinase, c-Kit, and Bcl-xL *in silico*[1].

Aurora kinases are crucial for cell growth and are serine/threonine kinases. They are phosphor transferase enzymes that assist dividing cells in disseminating their genetic resources to their daughter cells. Aurora kinases, in particular, play an important role in cellular division by regulating chromatid segregation. Genetic instability, which is strongly linked to cancer, can be caused by defects in this segregation. All Aurora kinase members perform oncogenic functions in human malignancies, promoting cancer cell survival and proliferation through their mitotic activity. To far, three Aurora kinases have been discovered in mammalian cells. These three kinases have sparked great attention in the cancer research area due to their high expression patterns in many human malignancies, in addition to being implicated as mitotic regulators.Aurora kinases have three domains: N-terminal (39–139aa), kinase (250–300aa), and C-terminal (15–20aa). Between Aurora proteins, the kinase domain is highly conserved, with 71 percent, 60 percent, and 75 percent homology between AurA/B, AurA/C, and AurB/C, respectively. The Aurora kinase domain is made up of two lobes: a stranded N-terminal lobe and alpha helical C-terminal lobe connected by a hinge region that controls the active conformation. The Aurora kinase domain is made up of twelve conserved subdomains separated by insertion sites that are less conserved. A conserved residue at Thr288 (AurA), Thr232 (AurB), and Thr195 (AurC) in the C-terminal lobe of the kinase domain promotes a conformation shift associated with the acquisition of kinase activity[2].

The oncogene c-kit encodes the c-kit receptor (CD117), a transmembrane protein with tyrosine kinase activity. It belongs to the type III receptor tyrosine kinase family; additional tyrosine kinase receptors include macrophage colony-stimulating factor receptor, platelet-derived growth factor receptor, and flk2/flk3 receptor, among others. The white spot dominant gene's allele is the c-kit gene. The foetal brain cDNA collection was used to isolate human c-kit cDNA. The type I transmembrane glycoprotein c-kit receptor (CD117) belongs to the type III receptor tyrosine kinase family and has a relative molecular mass of 145kDa. The c-kit receptor is made up of 976 amino acids that are divided into an extracellular domain with 519 amino acids, a trans-membrane domain with 23 amino acids, and an intracellular tail with 433 amino acids that includes a juxta-membrane domain and a tyrosine kinase domain inserted by about 80 amino acid residues. C-kit signalling regulates red blood cell synthesis, lymphocyte proliferation, mast cell growth and function, melanin generation, and gamete formation, among other things. Overexpression of c-kit, a proto-oncogene, has been linked to primary liver cancer[3].

Bcl-xL is a member of the Bcl-2 family that exhibits anti-apoptotic function. It was initially discovered in 1993. Bcl-xL is thought to regulate apoptosis through influencing mitochondrial membrane permeability and regulating cytochrome c release. Bcl-2 is not detectable after partial hepatectomy-induced liver regeneration, but hepatocytes express Bcl-xL during the G1 phase, and hepatocyte growth factor dramatically promotes Bcl-xL expression and prevents large hepatocyte death. In vitro, genetic modification to introduce Bcl-2 into liver cancer cells partly inhibited Fas-induced apoptosis. As a result, we hypothesised that Bcl-xL is the functional anti-apoptotic protein of the Bcl-2 family in both hepatocytes and HCC cells[4].

Flavones are one of the most significant flavonoid subclasses. Flavones are found as glucosides in leaves, flowers, and fruits. Flavones may be found in celery, parsley, red peppers, chamomile, mint, and *Ginkgo biloba*. This group of flavonoids includes luteolin, apigenin, and tangeretin. The polymethoxylated flavones tageretin, nobiletin, and sinensetin are abundant in citrus fruit peels. They have a double bond between positions 2 and 3 of the C ring, as well as a ketone in position 4. The hydroxyl group in position 5 of the A ring is found in the majority of flavones from vegetables and fruits, while hydroxylation in other positions, most commonly in position 7 of the A ring or 3′ and 4′ of the B ring, varies depending on the taxonomic classification of the particular vegetable or fruit[5,6].

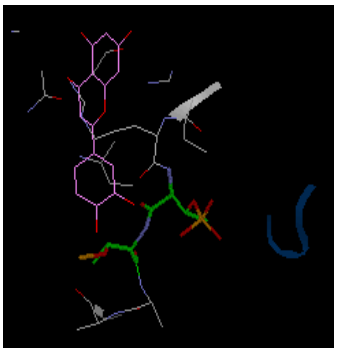
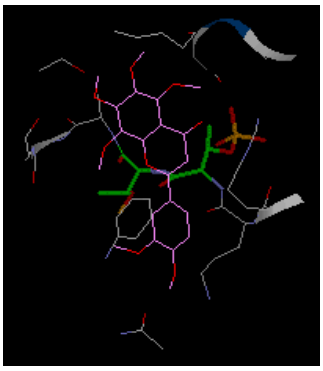
MATERIAL AND METHODS:

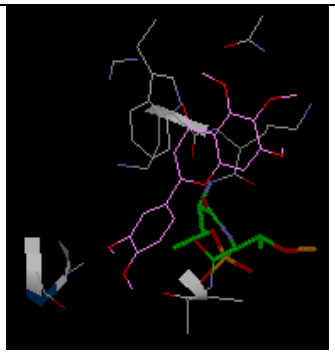
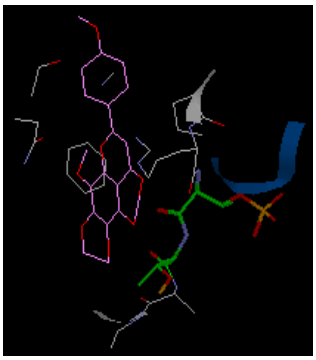
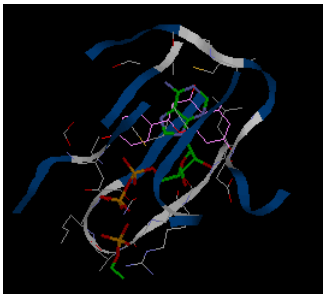
In-silico docking studies were carried out between the flavone molecules (ligand) and the target proteins Aurora kinase, proto-oncogene c-Kit (c-Kit), B-cell lymphoma-extra large (Bcl-xL) responsible for the pathogenesis of hepatocellular carcinoma by using the iGEMDOCK suit v 2.1. The three dimensional structure of target proteins i.e. Aurora kinase, c-Kit, and Bcl-xLhave been fetched from the Protein Data bank (https://www.rcsb.org) at 2.25A resolution for the *in-silico*molecular docking studies. The bioactive ligands or flavones were developed for the molecular docking profiling. The ligands were taken from the PubChem database. The three dimensional conformer of the molecules were utilized in Mol file (.mol) format for the protein ligand interaction by using the conversion software openbabel (https://openbabel.org).iGEMDOCK gives a virtual screening platform for *in-silico* docking analysis of pharmaceutical agents. iGEMDOCK gives the result in the form of electrostatic (E), hydrogen-bonding (H), and van der Waals (V) binding energies between proteins and ligand. iGEMDOCK infers pharmacological interactions and clusters the screening compounds for post-screening analysis based on these profiles and compound structures.Depending on these data and bioactive molecule structures, iGEMDOCK provides the interactions based on pharmacology and collect the ligands for the post screening analysis. Finally the iGEMDOCK ranks and visualizes the screening molecules by combining the pharmacological interactions and energy based scoring function of GEMDOCK.In docking accuracy setting the standard docking with population size of 200, generations of 70 and number of solutions of 2 was kept for genetic algorithm(GA) parameters. In docking scoring functions the ligand intra energy of hydrophobic and electrostatic preferences were kept on 1.00. The ligand with lowest energy is taken as the best inhibitor[7, 8, and 9].

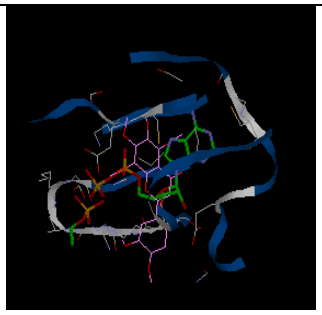
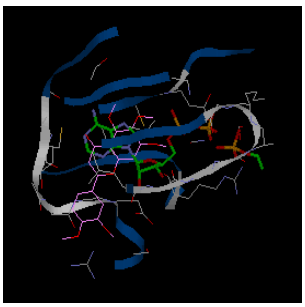
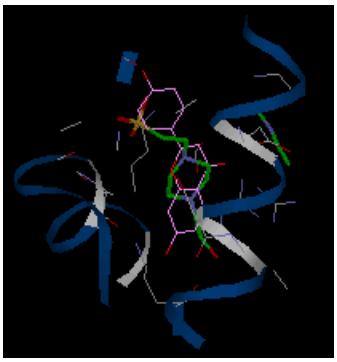
RESULT& DISCUSSION:

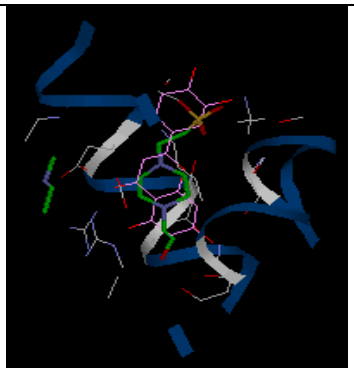
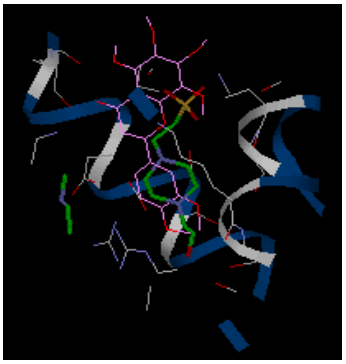
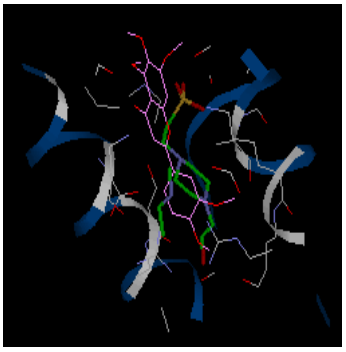
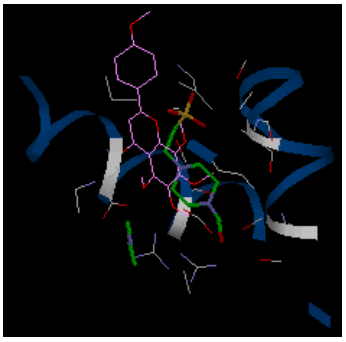
Cancer is a complicated disease defined by cellular homeostasis and functioning that persists. Cancer spreads due to uncontrolled cell proliferation and differentiation, as well as a lack of apoptotic functions, resulting in a huge rise in the number of malignant cells. Phytochemicals are non-nutrient bioactive molecules found in plants that can reduce the risk of many diseases. There are over 50,000 different phytochemicals in the plant kingdom. The presence of one or more aromatic benzene rings, as well as the capacity to be mono or poly hydroxylated, distinguishes phenolics. Furthermore, phenolics are perhaps the most prevalent antioxidants in the human nutrition[10, 11]. The docking score of ligand protein interaction was shown in Table 1 with their docked poses. The docking score of Apigenin, Luteolin, Nobiletin, Sinensetin and Tangeretin for Aurora kinases, c-Kit and Bcl-xL was found to be -90.68, -106.58, -113.49, -95.73, -109.07, -118.82, -97.06, -108.47, -109.12, 99.67, -105.06, -111.16, -94.45, -112.23, and -101.27 respectively. High docking score for the Bcl-xL protein suggest the anti-apoptotic effect of all the ligand with highest in the luteolin. Upregulation of Aurora kinases can lead to a disruption in cell division and proliferation, which can result in hepatocellular cancer. All flavones suppress Aurora kinas upregulation and hepatocellular proliferation. Overexpression of c-kit, a proto-oncogene, has been linked to primary liver cancer. The highest activity against the c-kit was seen in polymethoxy flavones tangeretin (-112.23) that down-regulate the c-kit expression. The key regulator of tissue homeostasis is apoptosis. It aids in the removal of damaged cells from normal tissues and maintains a healthy cell number in the context of physiologic cell proliferation and tissue healing. Bcl-xL is a member of the Bcl-2 family and contains anti-apoptotic properties. Bcl-xL is thought to regulate apoptosis through managing mitochondrial membrane permeability and regulating cytochrome-c release. In this docking investigation, all flavones inhibited Bcl-xL, a protein that controls apoptosis, to the greatest extent possible. Luteolin showed the maximum inhibition of Bcl-xL. The amino acid residues that bind to luteolin were H-S ASP 11, H-S-ARG 91, H-S ASP 11, H-S SER 25, H-S ARG 91 as shown in Table I.

Table I: Post screening analysis of Protein-Ligand interaction by iGEMDOCK suit

Protein(s) (PDB ID)	Ligands with docked poses	Total energy (kcal/mol)	V	H	E	Amino acid residue
Aurora Kinase (2W1C)	Apigenin 	-90.68	-73.79	-16.9	0	H-M TPO 288
	Luteolin 	-95.73	-64.05	-31.67	0	H-S ARG 255, H-M TRP 277, H-S ARG 286, H-M TPO 288
	Nobiletin 	-97.06	-92.06	-5	0	H-S TYR 334
	Sinensetin	-99.67	-88	-11.67	0	H-S GLN 177, H-S ARG 255, H-M TPO 288

						
	Tangeretin 	-94.45	-75.33	-19.12	0	H-S GLN 177, H-M TPO 288
c-KIT (1PKG)	Apigenin 	-106.58	-86.88	-19.71	0	H-S LYS 623, H-S GLU 640, H-M CYS 673, H-S ASP 677, H-M ASP 810
	Luteolin 	-109.07	-82.12	-26.95	0	H-S LYS 623, H-S GLU 640, H-M CYS 673, H-M ASP 810, H-S ASP 810, H-M MG 1481
	Nobiletin	-108.47	-96.53	-11.94	0	H-S LYS 623, H-M MG 1481

						
	Sinensetin 	-105.06	-103.36	-1.7	0	-
	Tangeretin 	-112.23	-98.94	-13.29	0	H-S LYS 623, H-M MG 1481
BCL-XL (4CIN)	Apigenin 	-113.49	-80.78	-32.71	0	H-S ASP 11, H-S ARG 91, H-S ASP 11, H-S ARG 91
	Luteolin	-118.82	-81.73	-37.09	0	H-S ASP 11, H-S-ARG 91, H-S ASP 11, H-S SER 25, H-S ARG 91

						
	Nobiletin 	-109.12	-99.48	-9.63	0	H-S LYS 87, H-S ARG 91
	Sinensetin 	-111.16	-86.57	-24.59	0	H-S ASP 11, H-S LYS 87, H-S ARG 91, H-S TRP 24, H-S ARG 91
	Tangeretin 	-101.27	-75.48	-25.79	0	H-A ASP 11, H-S LYS 87, H-S ARG 91, H-S ASP 11, H-S SER 14, H-S ARG 91, H-M EPE 1199

Molecular docking was performed by iGEMDOCK suit where V= Van der Waals bond energy, H= Hydrogen bond energy, E= Electrostatic bond energy; H-S denotes hydrogen bond with side chain, H-M denotes hydrogen bond with main chain, V-M denotes Van der Waals interaction with main chain, V-S denotes Van der Waals interaction with side chain. Rasmol viewer are used to visualize the docked poses (predicted poses in pink colour and native ligand in green colour)

CONCLUSION:

The process of apoptosis has piqued scientific curiosity in recent years. Apoptosis, like cell reproduction, is regulated by a network of positive and negative growth signals. According to current beliefs, malignant cells should be incapable of apoptosis and/or insensitive to death signals, allowing cancer to develop unrestrictedly. A growing number of studies have found that apoptosis plays a role in the growth and development of many cancers, although the pattern of apoptosis differs amongst tumours. The cellular aetiology of HCC, which is one of the most prevalent and malignant malignancies in the world today, is poorly understood. Apoptosis is uncommon in healthy liver tissues. As a result, the Aurora kinases, c-Kit, and Bcl-xL, are one of the most exciting targets in HCC treatment right now. The in-silico results are highly intriguing and promising for the creation of a new class of Aurora kinase, c-Kit, and Bcl-xL inhibitors with structurally distinct structures. These ligands or inhibitors, hopefully, will be effective in the treatment of HCC.

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