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ER Stress Proteins Can be an Effective Target for Epicatechin in Triple Negative Breast Cancer – An *in-silico* Approach

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Abstract

Triple-negative breast cancers (TNBC) are more aggressive and faster when compared to other types of breast cancers. Targeted therapy is not available for TNBC due to the lack of receptors. Due to various cellular stress and oxidative stress, the accumulated misfolded or unfolded proteins induce endoplasmic reticulum stress that further activates an unfolded protein response (UPR). Plants are the source of several potent and effective medicines. Epicatechin is a polyphenol that is extensively present in fruits and vegetables and has a wide range of pharmacological uses. *In silico* docking studies have proven to be an important tool to facilitate the structural diversity of natural products to be harnessed in an organized manner. Here, we attempted using *in silico* docking to check whether epicatechin interacts with selected endoplasmic reticulum (ER) stress proteins in triple-negative breast cancer cells. ASK1 and JNK showed good interaction with epicatechin among the various ER stress proteins. The results from *in silico* docking between epicatechin and ER stress proteins showed that the epicatechin could regulate the oncoprotein expression in stress conditions. Thus, epicatechin may be used as a therapeutic agent against ER stress proteins which play a major role in the development of cancer.

Keywords: Epicatechin, ER stress proteins, unfolded protein response, triple-negative breast cancer, *in silico* docking

Introduction

Breast cancer is the most widely diagnosed cancer in women worldwide and is the second leading cause of cancer-related death. This deadly disease is diagnosed for more than one million people worldwide and is responsible for more than 400,000 deaths per year(1). The development of breast cancer is a multi-stage process involving multiple types of cells, and its prevention remains challenging. The absence of estrogen and progesterone receptors and the absence of HER2 overexpression define triple-negative breast cancers (TNBCs). These cancers are a heterogeneous subtype of breast cancer that has a poor prognosis. Compared to other types, these cancers are very aggressive because they lack receptors, many treatments, including hormonal therapy, is not successful (2). So patients with TNBC do not benefit from chemotherapy-based treatments. Improvement in treating TNBC appears to be a major obstacle (3).

The endoplasmic reticulum (ER) is a complex cellular organelle that is responsible for membrane-bound and secreted protein folding and post-translational processing. Disturbances

in normal functions of the endoplasmic reticulum lead to an evolutionarily conserved cell stress response, the unfolded protein response, which ultimately causes cell death if the ER dysfunction is extreme or prolonged. Disturbances in normal functions of the endoplasmic reticulum lead to an evolutionarily conserved cell stress response, the unfolded protein response (UPR), which ultimately causes cell death if the ER dysfunction is extreme or prolonged(4). The key issue with any strategy to block cell death caused by ER stress lies in the multitude of parallel pathways that can lead to downstream cell death mechanisms. Intrinsic tumor stresses, such as oncogenic activation, and extrinsic stresses exerted by the environment of the tumor increase the levels of misfolded proteins in the ER, causing UPR pathways to activate. In many forms of cancer, like breast cancer, the cancer environment is hypoxic and acidic and the UPR is highly active. While sustained UPR activation is linked to apoptotic signaling induction, this apoptotic switch is bypassed by cancer cells and the UPR is used to promote proliferation and metastasis. In addition, recent evidence suggests that UPR activation, in addition to aiding tumor growth, may also restrict the efficacy of chemotherapy by leading to the development of chemo-resistance. Studies of the genes and gene products involved in ER-initiated cell death are therefore required to fully validate drug discovery targets (5).

Medicinal plants play important part in traditional medicines since ancient times. The drug discovery processes have identified phyto-chemicals for new leads(6). Epicatechin is a polyphenol commonly found in cocoa, dark chocolate, red wine, and tea. The main biological characteristics of epicatechin include antioxidant, antibacterial, anti-inflammatory, anticancer, and cardioprotective activity (7).

Molecular docking may be a method of anticipating the ligand's preferred receptor orientation (Protein) to form a stable complex. The optimal orientation is used by using scoring functions to determine the degree of association or binding affinity between ligand and protein. Usually, docking is applied to anticipate drug candidates' binding orientation toward protein targets to predict drug affinity and activity. So, docking plays a significant role in the process of drug design and discovery (8). The main objective of molecular docking is to simulate the molecular identification mechanism computationally and achieve optimized conformation to reduce the free energy of the overall system. The way a brand new drug is developed can be a very challenging process. Modern drug discovery is especially primarily based *on in-silico*–and chemical biological approaches. The use of computer-aided techniques in the method of drug discovery and development is gaining rapid acceptance, implementation, and appreciation (9).

The aim of our study was mainly focused to identify a suitable target in triple-negative breast cancer using *in silico* methods to validate the action of epicatechinon the selected ER stress proteins.

Materials and Methods

Molecular docking (schrodinger – maestro version11.8)

Maestro is the graphical user interface (GUI) for almost all computational programs in Schrodinger, such as Desmond, Epik, Glide, Impact, LigPrep, MacroModel, Phase, Prime, QikProp, Qsite, SiteMap, and Strike. It includes resources for viewing, creating, and manipulating chemical structures; preparing, arranging, and storing structures and related data; and setting up, uploading, recording, and visualizing measurement results on structures (10). We selected ER stress proteins namely ASK1, IRE1, P4HB, TRAF2, JNK, BCL-XL, BCHE, VCP, HMOX1, and DGC4, and did docking studies to identify the best target.

Ligprep

LigPrep 6.4 version can be a solid set of software designed to organize prime quality, all

3D atom structures for vast quantities of drug-like molecules, starting with 2D or 3D structures in SDF or maestro format. The simplest use of LigPrep generates a single, low-energy, 3D structure with correct chiralities for any successfully processed input with numerous ionization states, tautomers, stereochemistry, and ring conformations, and removes the molecular exploitation of various parameters like molecular weight or specified numbers and types of functional groups present.

Protein preparation

The protein processing facility carries out the final stages of protein preparation for Glide use. Only heavy atoms constitute a regular PDB structure file. Hence, before use in Glide calculations, hydrogen must be added which uses an all-atom force field. Also essential to the results produced by Glide is the charge status of protein residues. Until running a protein preparation job, certain preliminary preparation tasks don't need to be automated. The facility for the processing of proteins consists of two components: preparation and refinement. After ensuring chemical correctness, the preparation section adds hydrogen and neutralizes side chains that are not close to the binding cavity and which are not involved in salt bridges. The refinement portion performs the restrained impact minimization of the co-crystallized complex, which reorients side-chain hydroxyl groups and soothes possible steric clashes. The protein preparation panel is used to develop jobs that perform these tasks. The protein is rendered under the wizard of the protein preparation.

Qikprop

QikProp 5.8 version is fast, accurate, and easy to predict the ADME properties like absorption, distribution, metabolism, and excretion. QikProp forecasts the physically essential descriptors of organic molecules and their pharmaceutically soothing properties. In addition to predicting the properties of compounds, QikProp provides ranges for comparing the properties of specific compounds with 95% of known drugs.

Active site prediction using site map

The position of the primary binding site on a receptor such as a protein is also identified from a co-crystallized complex structure. Efforts to design better ligands for these receptors can profit from the knowledge of complement interactions between the ligands and receptors, and the extension of the ligands into the adjacent region which promotes binding, by identifying the role of the neighbouring molecules of active sites.

Glide

Glide (Grid-based ligand docking with Energetics) version 8.1 looks for beneficial interactions between one or more ligand molecules and receptor molecules, typically a macromolecule. Glide uses a hierarchical set of filters to scan for potential ligand locations within the receptor's active site region. Glide is operated in a rigid or flexible docking mode; the conformation for each input ligand is created automatically later. Through a comprehensive conformational scan, conformational versatility is treated in glide; increased by heuristic screen easily removes unsuitable conformations, such as formations with long-range internal hydrogen bonds.

Finally, the reduced poses are graded again using the proprietary scoring method of Schrodinger's GlideScore. GlideScore is predicated on ChemScore but ChemScore does include a steric-clash concept that adds buried polar terms developed by Schrodinger to penalize electrostatic mismatches and change alternative terms:

GScore = 0.065* vdW + 0.130* Coul + Lipo + Hbond + Metal + Rewards + RotB + website. The binding affinity is predicted by GlideScore.

Running and monitoring jobs

For each product, Maestro has panels

for the preparation and submission of jobs. Appropriate products and activities should be chosen from the application menu and its submenus for using these tables. In the row, set the necessary options, and then press Start to open the Start dialogue box and set options to run the job. The monitor panel is the control panel or tracking work progress and task pause restarts or kills. The text pane displays different output information from the active pause, restart, stop, destroy and update buttons. Upon completion of a tracked task, the results are incorporated into the project according to the settings used to start the work.

Results and Discussion

Molecular docking analysis of active compound epicatechin against various ER stress proteins namely ASK1, IRE1, P4HB, TRAF2, JNK, BCL-XL, BCHE, VCP, HMOX1, and DGC4 helped to explore targets for binding of the selected drug with anti-breast cancer activity. More negative glide scores and energy findings suggested that the epicatechin with differing amino acid interaction sites suggested more binding for ER stress proteins. A higher negative value of glide score and glide energy shows more binding affinity. The docking results showed, with all selected ER stress proteins epicatechin showed a high glide value. The results obtained in the *in-silico* studies of the target ER stress proteins with active phyto-compound epicatechin are shown in table 1.

Table 1. *In silico* docking results of ligand epicatechin against selected ER stress proteins

S.No	Protein	Glide score	Glide energy	H-Bond
	ASK1	-7.997	-46.068	6
	IRE1	-7.086	-45.269	4
	P4HB	-4.502	-27.973	2
	TRAF2	-6.934	-35.227	4
	JNK	-7.763	-39.99	3
	BCL – XL	-5.24	-37.227	2
	BCHE	-7.152	-41.147	4
	VCP	-7.16	-37.163	3
	HMOX1	-6.758	-40.542	3
	DGC4	-6.254	-37.462	3
Ligand - Epicatechin (Pubchem Id – 72276)				

ASK1 is Apoptosis signal-regulating kinase 1 also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5). It activates c-Jun N-terminal kinase and p38 in response to stresses such as oxidative stress, endoplasmic reticulum stress, and calcium influx. ASK family plays a key role in cancer and neurodegenerative diseases (11).Figure 1 shows the interaction of epicatechin with ASK1 and depicts the ligand interaction and surface view of epicatechin with ASK1. Theepicatechin ligandwithASK1showeda glidescorevalueof -7.997 and a glide energyvalue of -46.06 KJ. A strong interaction in terms of hydrogen bonding could be observed between epicatechin and ASK1 with six hydrogen bonds.

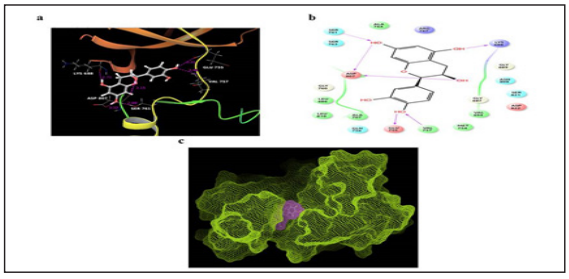

Figure 1. a)H-bond interaction between active compound epicatechin and ASK1 b) Ligand interaction of epicatechin and ASK1 c) Surface view of epicatechin with active site of target ASK1

IRE1 is known as the ER-trans-membrane protein Inositol-Requiring Enzyme 1, an integral component of the UPR pathway that is critical for sensing and responding to ER stress (12).The interaction between the ligand epicatechin and IRE1

occurred through four hydrogen bonds. The epicatechin ligand with IRE1 showeda glide score value of -7.086 and a glide energy value of -45.26 KJ. The interaction of epicatechin with IRE1 was shown in figure 2.

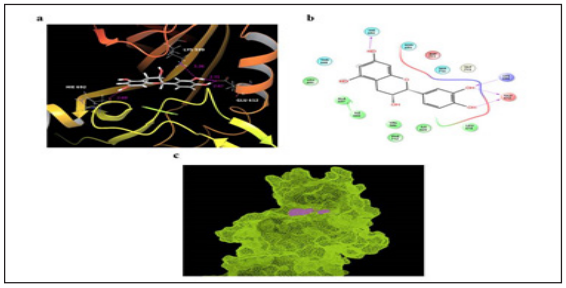

Figure 2.a)H-bond interaction between active compound epicatechin and IRE1 b) Ligand interaction of epicatechin and IRE1 c) Surface view of epicatechin with active site of target IRE1

P4HB **(**prolyl 4-hydroxylase subunit beta) is the gene that encodes the beta subunit of prolyl 4-hydroxylase, a highly abundant multifunctional enzyme that belongs to the protein disulfide isomerase family**.**It can act as a chaperone that inhibits the aggregation of misfolded proteins in a concentration-dependent manner (13). The representation of docked epicatechin at the P4HB binding sites is shown in figure 3. Theepicatechin ligandwithP4HB showeda glidescorevalueof -4.502 anda glide energyvalue of -27.97 KJ.

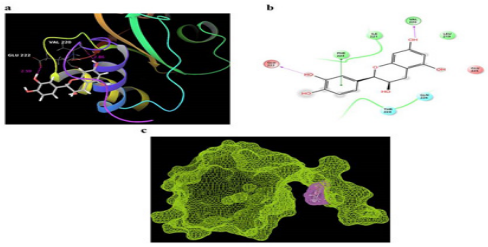

Figure 3.a)H-bond interaction between active compound epicatechin and P4HB b) Ligand interaction of epicatechin and P4HB c) Surface view of epicatechin with active site of target P4HB

Figure 4 shows the interaction of epicatechin with TRAF2. The interaction between the ligand epicatechin and TRAF2 occurred through four hydrogen bonds. The epicatechin ligand with TRAF2 showed a glide score value of -6.934 and a glide energy value of -35.22KJ. TRAF2 is known as TNF (Tumor Necrosis Factor) receptor-associated factor 2 is a protein encoded by the TRAF2 gene in humans. TRAF2 can bind and oligomerize caspase-12, and thus its cleavage and activation. Caspase-12 activation then encourages apoptosis as a response to ER stress (14).

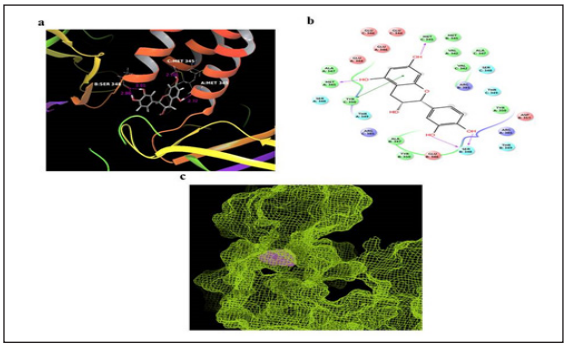

Figure 4.a)H-bond interaction between active compound epicatechin and TRAF2 b) Ligand interaction of epicatechin and TRAF2 c) Surface view of epicatechin with active site of target TRAF2

The interaction between the ligand epicatechin and JNK occurred through three hydrogen bonds. Theepicatechin ligandwithJNK showeda glidescorevalueof -7.763 anda glide energyvalueof -39.99 KJ. JNK abbreviated as c-Jun N-terminal kinase is one of the major signaling cassettes of the mitogen-activated protein kinase (MAPK). Activation of JNK is a common response to many forms of stress and is known to influence cell-death machinery through the regulation of the BCL2 family protein (15). Figure 5 indicates the hydrogen interaction between target residues and ligands.

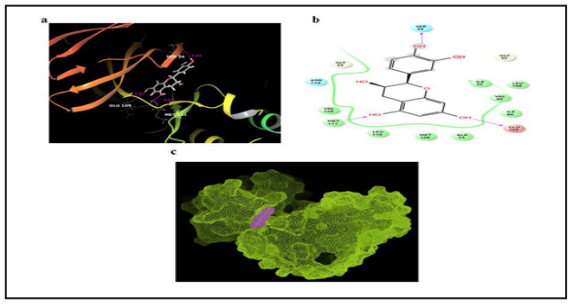

Figure 5. a) H-bond interaction between active compound epicatechin and JNK b) Ligand interaction of epicatechin and JNK c) Surface view of epicatechin with active site of target JNK

BCL-XL is the B-cell lymphoma-extra-large encoded by the BCL2-like 1 gene. A core feature of the BCL-2 family of proteins is the aggregation of stress signaling networks, regulating cell death, homeostasis of calcium, UPR, and autophagy (16). Figure 6 demonstrates the epicatechin interaction with the arrangement of the BCL-XL proteins. The interaction between the ligand epicatechin and the BCL-XL took place through two hydrogen bonds. The BCL-XL epicatechin ligand showed a glide score value of -5.24 and a glide energy value of -37.22 KJ.

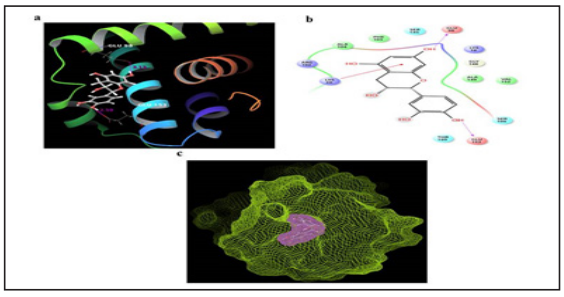

Figure 6.a) H-bond interaction between active compound epicatechin and BCL-XL b) Ligand interaction of epicatechin and BCL-XL c) Surface view of epicatechin with active site of target BCL-XL

Figure 7 shows the interaction of epicatechin with BCHE. The interaction between the ligand epicatechin and BCHE occurred through four hydrogen bonds. The epicatechin ligand with BCHE showed a glide score value of -7.152 and a glide energy value of -41.14 KJ. BCHE is defined as butrylcholinesterase and it is an unspecific enzyme of cholinesterase which hydrolyses many different esters based on choline. It is encoded by the BCHEgene (17).

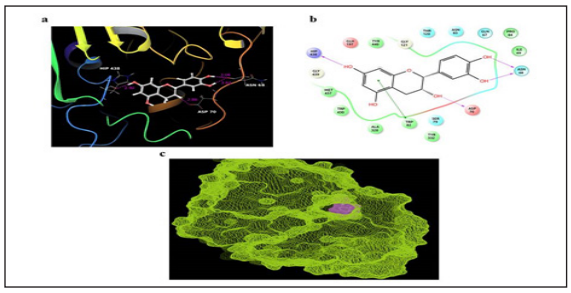

Figure 7. a) H-bond interaction between active compound epicatechin and BCHE b) Ligand interaction of epicatechin and BCHE c) Surface view of epicatechin with active site of target BCHE

Figure 8 represents the interaction of epicatechin with VCP. The interaction of the ligand epicatechin and VCP was mediated by three hydrogen bonds with a glide score of -7.16 and a glide energy value of -37.16 KJ. VCP is known as valosin-containing protein which is an AAA family hexameric ATPase involved in several cellular functions including protein degradation by the ubiquitin-proteasome (UPS) system (18).

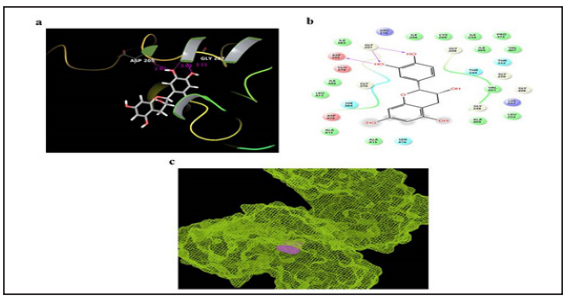

Figure 8.a) H-bond interaction between active compound epicatechin and VCP b) Ligand interaction of epicatechin and VCP c) Surface view of epicatechin with active site of target VCP

HMOX1 called heme oxygenase 1 is a protein-coding gene. It plays a crucial role in protecting the cells and the inductive enzyme involved in the spectrum of human disease (19). Figure 9 represents the interaction of epicatechin with HMOX1 through three hydrogen bonds with a glide score valueof -6.758 and a glide energy value of -40.54 KJ.

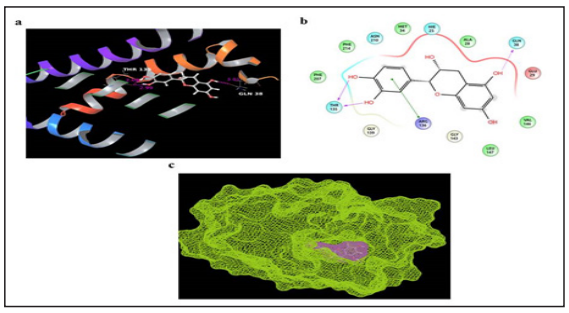

Figure 9. a) H-bond interaction between active compound epicatechin and HXOM1 b) Ligand interaction of epicatechin and HXOM1 c) Surface view of epicatechin with active site of target HXOM1

DGC4 is the dystrophin-glycoprotein complex 4 (20). Epicatechin showed strong interaction with DGC4 and glide score value -6.254 and glide energy value -37.46 KJ as shown in Figure 10. The interaction was through three hydrogen bonds.

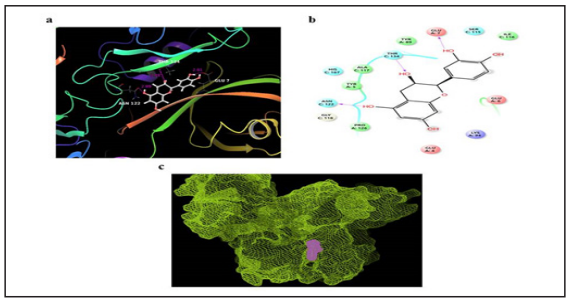

Figure 10. a) H-bond interaction between active compound epicatechin and DGC4 b) Ligand interaction of epicatechin and DGC4c) Surface view of epicatechin with active site of target DGC4

Docking studies are valuable tools that help us to exploit the structural diversity of theinteraction between protein and ligand which plays an important role in structural drug design. The results of the docking studies showed that epicatechin exhibited good interaction with all the ER stress proteins analyzed.Predicting the binding modes and affinities of compounds when interacting with a protein-binding site lies at the center of drug design based on structure. Drug design starts with the hypothesis that modulation could have good therapeutic utility for a particular biological target. Molecular docking is widely used to suggest binding modes for the protein-inhibitor. Most docking algorithms can build several possible structures, but they often require a means for each structure to score (21). Studies with epicatechin and ER tension proteins were done *in silico* docking. Among the various ER stress proteins, ASK1 and JNK have good interaction with epicatechin. The spontaneous interaction of epicatechin with ASK1 and JNK indicated that epicatechin had the potential to disrupt the natural integrity of ASK1 and JNK and promotes apoptosis in triple-negative breast cancer.

Epicatechin in cancer provides a good option for antineoplastic or cancer prevention. It causes apoptosis via mitochondria, by altering the tumor cells' nuclei, DNA fragmentation, and cell arrest. Reactive oxygen species and other free radical species are now known to be a major factor in the onset of oxidative stress, which in turn causes cellular damage and may eventually result in carcinogenesis. The epicatechin has exhibited a greater chemopreventive effect against oxidative damage (22).

Conclusion

Currently, people have more drug options for the chemoprevention of breast cancer, while biological prevention has been recently developed to improve patients' quality of life. Progress in the treatment of TNBC remains animportant challenge. Thecomputational methods are employed to assess the anticancer potential of drugs or chemicals. *In silico* docking revealed that the natural compound

epicatechin exhibited better binding energy with IRE1 and BCHE among the nine different ER stress proteins tested. The results thus indicate epicatechin can be exploited effectively targeting triple-negative breast cancer. Further, *in vitro* and *in vivo* experimental studies need to be done for the validation of the results.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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Abbreviations:

vdW = van der Waals interaction energy

Coul = Coulomb interaction energy

Lipo = Lipophilic-contact plus phobic-attractive term

Hbond = Hydrogen-bonding terminally a reward)

Rewards = various reward or penalty terms

RotB = Penalty for freezing rotatable bonds website = Polar interactions in the active site