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RESEARCH PAPER

An *in-Silico* approach to evaluate the binding efficacy and stability profile of MWCNT entangled rutin for breast cancer treatment

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ABSTRACT

Objective(s): Cancer is one of the most devastating diseases and ranks second in a total number of deaths caused globally. In recent years there is a steady increase in breast cancer (BC) incidence due to several etiological factors. Due to indiscriminate drug delivery and the lack of target specificity, current cancer therapies can cause life-threatening side effects. The present research emphasises targeting the rutin-loaded onto carbon nanotubes (CNTs) for breast cancer treatment. Further, rutin-loaded multi-walled carbon nanotube (MWCNT) and conjugated with folic acid is the focus of our research against breast cancer.

Materials and Methods: Intermolecular interaction studies between rutin (PubChem CID 5280805) and the target protein folate receptor (PDB ID 4LRH) via Autodock Vina programme and PyRx tool and molecular dynamics simulation studies were performed.

Results: The docking score was found to be -8.7 Kcal mol^{-1}. In comparison, that of the standard chemotherapeutic drug 5-Fluorouracil was -5.9 Kcal mol^{-1}. Molecular dynamic studies were performed via Desmond for 100ns. The root-mean-square deviation (RMSD) value of the ligand remained stable, root-mean-square fluctuation (RMSF) values have been observed to be stable throughout the simulation time.

Conclusion: Based on these promising results, rutin-loaded CNTs can be further evaluated for their efficacy against breast cancer preclinically.

Keywords: *Breast cancer, Folate receptor, Molecular docking, Molecular dynamics, Rutin*

How to cite this article

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INTRODUCTION

Cancer is one of the most disturbing diseases and poses a significant challenge to effective treatment. The disease being the second leading cause of death globally, affected nearly 19.3 million people and caused 10 million deaths in 2020 *i.e.*, almost one in six deaths have occurred due to cancer. Cancer cases have significantly increased over the years owing to rapid urbanization and direct/indirect exposure to carcinogens; mortality, however has reduced due to a better understanding of cancer biology, enhanced diagnosis, and therapeutics. Breast cancer (BC) was the most diagnosed type of cancer, with 2.26 million cases in 2020, followed by lung (2.21 million cases), colon and rectum (1.93 million cases), prostate (1.41 million cases), skin (1.20 million cases), and stomach (1.09 million cases). Breast cancer has taken 685,000 lives, with the incline incidences needing immediate action to enhance the diagnosis and treatment protocols [1-4].

Molecular subtypes of BC include Luminal A – higher expression of estrogen (ER)/ progesterone (PR) receptors and less expressed human epidermal growth factor 2 (HER2) receptors, these

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tumours tend to have a slow growth rate (Ki-67 labelling index of <14%) and mostly show good prognosis often with endocrine therapy alone [5]; Luminal B – Higher ER expression and lower PR and HER2 expression, this type of BC tend to have a poor prognosis when compared to Luminal A type (Ki-67 labelling index of ≥14%); HER2 positive subtype shows high expression of HER2/erB2 receptors, the tumours have higher growth rate than luminal subtypes (Ki-67 labelling index of ≥14%); Basal-like or triple-negative breast cancer (TNBC) has neither of the receptors expressed rendering it hard to target this subtype and is framed the most aggressive with high recurrence rate and poor prognosis (Ki-67 labelling index of ≥14%) [6].

Current cancer treatment protocols include chemotherapy, surgical intervention, and hormonal therapy, among others. Chemotherapy is known for the systemic side effect it causes due to the indiscriminate delivery of the drug towards both normal cells as well as the neoplasm [7], adding on to it the tumour heterogenicity, breast cancer stem cells and the microenvironment of a tumour increases the chemotherapy failure rates further. Multidrug resistance (MDR), a condition with a lack of tumor size reduction and a decline in therapeutic response post-initiation of chemotherapy, is also a factor rendering the therapy inefficient. Active drug targeting and the use of natural phytoconstituents for the treatment in combination is a promising alternative being explored to control the tumour while causing less adverse effects and side effects [8, 9].

Cancer cells show overexpression of certain types of receptors which could pave the way for actively targeting them [10], TNBC leads to over expression of Folate Receptor α (FRα) while it does not express any other receptors like Estrogen, Human epidermal growth factor receptor, Progesterone, etc. which are generally overexpressed in different types of BC [11]. The overexpression of receptors can be taken advantage in actively and selectively targeting the cancer cells with the aid of nanoparticles.

Nanotechnology in medicine is widely being researched as an efficient drug delivery system that selectively and actively targets cancer cells while enhancing drug retention at the site of action [12]. Certain types of nanoparticles have also been shown to overcome multidrug resistance (MDR), Nanoparticles like carbon nanotubes, liposomes, dendrimers, micelles, viral nanocarriers, quantum dots are some of the nanocarriers [13]. These nanocarriers offer flexibility in their formulation, such as surface modification which can be made use of to pinpoint the drug to the site of action [14].

Liposomes are amphiphilic molecules having both hydrophobic and hydrophilic groups, liposomes are being actively used to treat ovarian cancer. Dendrimers are branched nanostructures containing functional groups actively used in the treatment as a microbicide and an antiviral formulation. Quantum dots consist of a shell and a core with a polymer coating and a stabilizing molecule, these are being used actively in diagnostics as fluorescent contrast agents.

Carbon nanotubes are of multiple types (single-walled carbon nanotube, double-walled carbon nanotube, and multi-walled carbon nanotube) [15]. They constitute a single graphene sheet that folds to form a hollow core in turn creating a tube-like structure. Owing to the high drug-loading efficiency, chemical stability, and surface modification of CNTs, it makes the most favourable as nanocarriers. Surface functionalization and tagging the CNTs with chemical moieties like transferrin or folic acid have been shown to aid in active and site-specific delivery of the drug (since folate receptors are highly expressed in TNBC) [16, 17].

Phytochemicals have continuously been an interest of research due to their natural availability and lesser toxic effects; literatures have proven the scope of various herbs in cancer inhibition or as chemo-preventives [18-20]. A naturally occurring flavonoid rutin, a glycoside of rutinose and quercetin, has shown promising pharmacological effects, including neuroprotection, antiproliferative, anti-carcinogenic, and anti-oxidative stress [21].

In silico study is a novel method of combining mathematical modelling and simulation to accelerate the rate of drug development. The drug discovery process is time-consuming and costly, ranging from 8-15 years, with expenses reaching billions of dollars. Computer-assisted drug discovery, a combination of homology modelling, molecular docking, virtual screening or virtual high-throughput screening, quantitative structure-activity relationship, and 3D pharmacophore mapping in general aid in the acceleration of the drug discovery rate. Encapsulating rutin in a carboxylic functionalized multi-walled carbon nanotube (MWCNT) and tagging folic acid is the focus of the current research against breast cancer.

Experimental

Drug-likeness and bioactive compound screening

The SMILES code of Rutin were submitted for input in an online drug-likeness test by the Swiss Institute of Bioinformatics: absorption, distribution, metabolism, and excretion test or SwissADME (http:// www.swissadme.ch/index.php). The SMILES codes were submitted to the Way2Drug PASS Online website (http://www.way2drug.com/

PASSOnline) for screening bioactivity of carboxylic functionalized MWCNT with and Rutin.

Protein preparation

X-Ray crystallographic structure of the target proteins was derived from the Protein Data Bank (PDB); before analysis, the proteins were prepared, *i.e*., the existing ligands, if any, were removed while adding polar hydrogens in BIOVIA Discovery Studio Visualizer version 4.0 software (Accelrys Software Inc., San Diego, CA), missing residues were further added in Swiss-PDB Viewer v4.1.0 finally the resulting protein was subjected to molecular docking [22].

Molecular docking

Ligand molecules carboxylic functionalized MWCNT, Rutin and folic acid were downloaded from PubChem database and used for molecular docking. The intermolecular interaction of carboxylic functionalized MWCNT with rutin and folic acid and rutin's molecular interaction with the identified target proteins were examined via molecular docking studies. Ligand being rutin and the identified target proteins were estrogen receptor, progesterone receptor, epidermal growth factor receptor (EGFR), EGFR subtype ERB1, and folate receptor. PyRx 0.8 tool and AutoDock Vina program were used to accomplish the molecular docking study. Polar hydrogen and partial charges were added to the 3D structures via PyRx software. Autodock Vina was used to calculate binding energy based on the binding energy at the active binding site. Obtained scores RMSD of each interaction was obtained while Discovery Studio Visualizer was used to obtain the 2D and 3D images of the interactions [23].

Molecular dynamics

The ligand-receptor complex was subjected to molecular dynamics simulation using DESMOND v3.6 package [24]. The molecular dynamic simulation analysed the binding stability, confirmation, and interaction modes of the rutin with folate receptor (4LRH). The best confirmations were then chosen based on the interactions and dynamical features of the complex. Furthermore, using the pre-set SCP water model, a water model was developed at 10Å units of the orthorhombic period boundary. Further, the electric charges were neutralized by adding the necessary amount of counter ions, and before the molecular dynamic simulation process began, the system decreased its energies through the heating and equilibrium process. The system production step lasted 100 ns, with time steps of 0.001ps; 300K temperature and 1 atmospheric pressure were used with the Nose-Hoover method and the NPT (isothermal-isobaric) ensemble.

RESULTS AND DISCUSSION

Molecular Docking

Molecular docking analysis was carried out to analyse the intermolecular binding profile of the carboxylic functionalised MWCNT (COOH_ MWCNT) with rutin and folic acid; and the binding profiles of the selected target protein Folate receptor (PDBID: 4LRH) RCSB Protein Data Bank (http://www.rcsb.org/pdb), and the drug Rutin and the standard drug 5-fluorouracil (5-FU). Molecular docking analysis was carried out via Autodock Vina. The binding energy of COOH_ MWCNT with rutin was found to be -7.9 Kcal $mol^{-1,}$ while that of COOH_MWCNT with folic acid was found to be -10.9 Kcal mol^{-1} 2D interactions can be seen in the Fig. 1 and 2. Binding scores of rutin

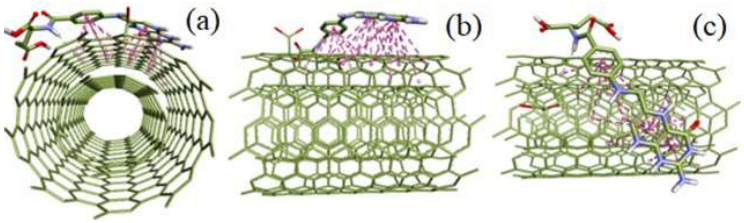

Fig. 1. Three images showing the interaction of COOH_MWCNT with Folic acid

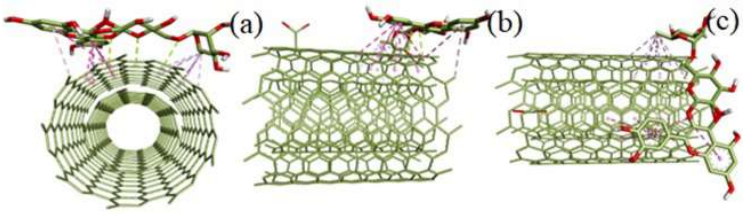

Fig. 2. Three images showing the interaction of COOH_MWCNT with Rutin

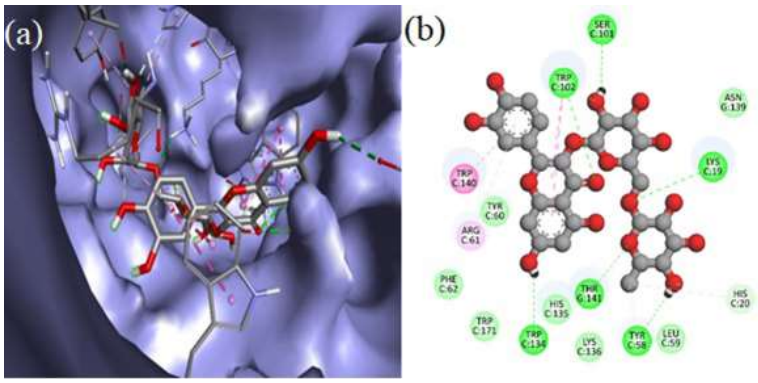

Fig 3. The interaction between rutin (CID: 5280805) and 4LRH. Left side represents 3D (a); and right side represents 2D complex protein-ligand interaction (b)

with folate receptor was found to be -8.7 Kcal mol^{-1}, while that of 5-fluorouracil and Folate receptor was found to be -5.9 Kcal mol^{-1}.

Interpretation of protein-ligand interactions

The binding interactions between the ligands and the folate receptor was visualised via Biovia Discovery Studio Visualizer Tool. Rutin (CID: 5280805) showed better interaction with the folate receptor (PDB ID: 4LRH**),** the interaction showed the formation of six conventional hydrogen bonds with LYS C:19, TYR C:58, SER C:101, TRP C:102, TRP C:134, THR G:141; seven van der waals bond LEU C:59, TYR C:60, PHE C:62, HIS C:135, LYS C:136, ASN G:139, TRP C:171. One Pi-Pi interaction was seen with TRP C:140; one Pi-alkyl bond was formed with ARG C:61; and one carbon hydrogen bond with HIS C:20 as depicted in Fig. 3.

Molecular dynamics simulation

Protein-ligand docking despite its successful application allows only the visualization of a static view of the binding interaction of the protein and the selected ligand, while molecular dynamics (MD) simulate the atoms of particles in the framework as a component of time with incorporation of Newton's situations of movements. Rutin and folate receptor protein were subjected for molecular dynamics simulation for 100 ns, and the obtained results were interpreted. Molecular dynamics trajectories were analysed using root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of receptor atoms, to interpret the fluctuations and stability of protein-ligand complex.

RMSD, a significant parameter to analyse the equilibration of MD trajectories and denotes the protein-ligand complex stability throughout the simulation, structural confirmation variations were assessed by plotting the RMSD of the protein backbone atoms against time. Initially, the complex RUT-4LRH showed variations in backbone RMSD till 20 ns raining from 1.2 to 1.4nm. Stable conformation was attained between the time 21ns and 85ns with no considerable deviations. At the final stage, from 20ns to 60ns the complex again showed variations. The RMSD of the RUT-4LRH complex is depicted in Fig. 4.

Root Mean Square Fluctuation RMSF is a parameter which signifies the flexibility and stability of protein-ligand complex systems during simulation. After binding with a ligand, changes in the behaviour of amino acid residues of the

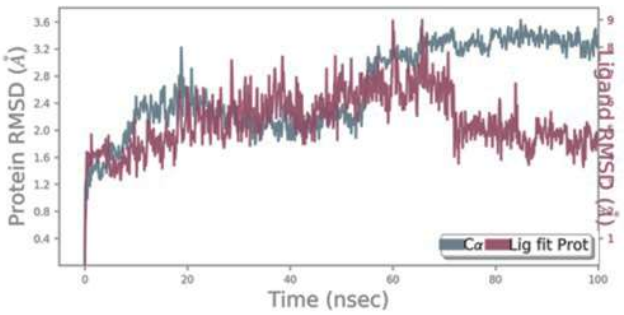

Fig. 4. RMSD study plot of RUT-4LRH for 100 ns molecular dynamic simulation

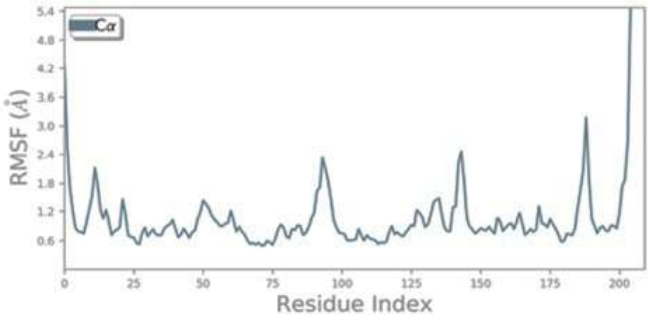

Fig. 5. RMSF study plot of RUT-4LRH for 100 ns molecular dynamic simulation

target protein were analysed using root mean square fluctuation. RMSF values for Cα atoms of the protein were calculated and plotted with respect to the residues. In the RUT-4LRH complex, the amino acid residues showed no fluctuations throughout the entire simulation period and the obtained graph was observed to be mostly stable. The amino acids of 4LRH which interacted with RUT in molecular docking also showed low fluctuation values during molecular dynamic simulation. The binding of the ligand did not stimulate any major effects on the flexibility of the protein as seen in Fig. 5.

Rutin interacted with 4LRH throughout the simulation by forming hydrogen bonds, hydrophobic interactions, ionic interactions and water bridges. An interaction fraction of 1.90 was observed with amino acid residue TRP140 through hydrophobic bonding mediated by a water bridge suggesting this interaction was maintained throughout the simulation period, likewise the interaction fraction of 1.30 with TRP102, 1 with SER101, 1 with ARG61, 0.75 with HIS135, and 0.5 with LEU59 respectively were observed as depicted in Fig. 6. A schematic of detailed ligand atom interactions with the protein residues is depicted in Fig. 7. The interaction with TRP140 occurred for 64% of the simulation period and the interaction with TRP102 occurred for 50% of the simulation period in the selected trajectory.

CONCLUSION

With the steady rise in breast cancer incidence among women globally, effective and efficient cancer therapy is the need of the hour. Active and selective targeting of the breast cancer cells with a phytoconstituent loaded onto a nanocarrier is a promising way for efficient therapy which is the main interest of the current research. This study looked at the molecular docking and molecular dynamics studies of rutin with folate receptor (FR) in comparison with the standard drug. Folate receptor targeting is gaining traction

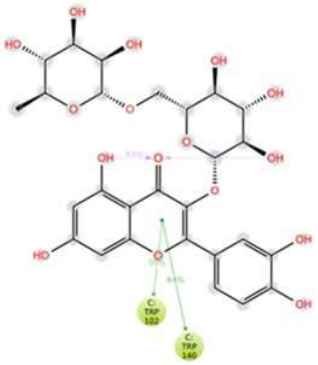

Fig. 7. Ligand protein contacts

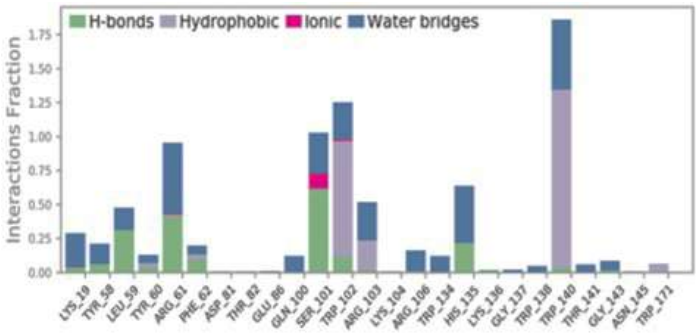

Fig. 6. Amino acid residues of protein interactions with the ligand

due to its overexpression in cancers like the breast, lungs, ovaries, etc. Targeting the FR using phytoconstituents shown to have anticancer properties might pave way for effective therapy with reduced side effects. Molecular docking investigation of rutin with 4LRH showed better interaction scores than the standard. The molecular dynamics simulation also showed favourable results depicting rutin might effectively treat breast cancer.

ETHICS APPROVAL

Not applicable.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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