Insilico Molecular Docking and Molecular Dynamics Analysis of Quercetin Compound with Exploration of Anti-cancer Disease

Gunjan Tiwari[1], Vikesh Kumar Shukla*[2], Dr. Satyendra K. Rajput[3].

Gunjan Tiwari[1], Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India.

Vikesh Kumar Sukhla[2], Associate Professor, Department of Pharmaceutical Science, Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India.

Dr. Satyendra K. Rajput[3], Professor, Gurukul Kangri Deemed to Be University, Haridwar. India.

Author 1

Gunjan Tiwari,
Department of Pharmaceutics and DRA,
Amity Institute of Pharmacy,
Amity University, Noida, Uttar Pradesh.
Email: guntiwari.pharma2gmail.com
Mobile:9990054327

***Corresponding Author**

Vikesh Kumar Shukla,
Associate Professor,
Department of Pharmaceutical Science,
Amity Institute of Pharmacy,
Amity University, Noida, Uttar Pradesh.
Email: vkshukla@amity.edu, vikeshg2002@gmail.com

Abstract:

Quercetin, a natural anticancer drug, has shown promise in the treatment of ovarian, lung, and prostate cancer. Molecular dynamics simulations are an effective method for studying the structural and dynamic characteristics of biomolecules associated with cancer pathways in the context of cancer research. This paper presents a Molecular Dynamic Simulation study using GROMACS software with CHARMM35 forcefields to investigate the potential of the drug against different cancer-related proteins. RMSD was used to examine the stability, conformational changes, and dynamic behavior of proteins and complexes (Tyrosine Kinase Receptor (TKR)-Drug Quercetin Complex), while RMSF was utilized to measure the general fluctuation of proteins.

Keywords: Quercetin, Cancer, Anti-cancer, Molecular Dynamics, Molecular Docking, GROMACS, Tyrosine Kinase Receptor, RMSF.

Introduction:

Cancer has been viewed throughout history as one of the major health risks. It is now ranked as one of the world's worst illnesses. Approximately 18.1 million new cases of cancer and 10 million people's deaths caused by cancer worldwide. This pathology causes high economic impact on public and private health. A comprehensive and terrible medical disorder called cancer is defined by the body's abnormal cells growing and dividing out of control. Through the circulation, these abnormal cells can travel from tumors to other regions of the body. They can also surround tissues. Common treatment modalities including surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy and hormone therapy. In some cases, a combination of these treatments may be used. But it should have a lot of side effects. So, scientists have investigated natural drugs and especially plant products. Nowadays many anticancer medicines obtained from plants. Many studies have emphasized the beneficial effects of flavonoids in the daily diet and suggested that the consumption of flavonoid could be effective in reducing the cancer.

A natural flavonoid substance called Quercetin is widely distributed in a variety of fruits, vegetables, and grains. Due to its possible anti-cancer qualities, it has attracted a lot of attention in cancer research. Numerous biological actions, including as antioxidant, anti-inflammatory, and anticancer properties, are displayed by quercetin. The varied methods of action of quercetin against cancer are a result of its distinctive chemical structure, which is defined by a flavonoid backbone and many hydroxyl groups. Quercetin has powerful anti-cancer capabilities through a variety of pathways, according to studies. One of the main mechanisms is its capacity to stop cancer cells from growing and multiplying. By causing cell cycle arrest, quercetin can stop the division and proliferation of cancer cells. Additionally, it causes cancer cells to undergo apoptosis, a process of programmed cell death, which

eliminates them. In addition to inhibiting the proliferation of cancer cells, quercetin also has anti-inflammatory qualities that are helpful in the treatment and prevention of cancer. Cancer formation and progression are closely correlated with chronic inflammation, which quercetin can help to reduce. Quercetin fosters an environment that is less favorable for the development of cancer by preventing the synthesis of inflammatory chemicals and controlling the signaling pathways involved in inflammation. Quercetin has also been demonstrated to have strong antioxidant action. DNA damage and the emergence of cancer can be brought on by oxidative stress, which is brought on by an imbalance between the generation of Reactive Oxygen Species (ROS) and the body's antioxidant defenses. Quercetin is a potent antioxidant that may scavenge ROS and shield cells from oxidative damage, which lowers the likelihood that cancer will form. The capacity of quercetin to alter signaling pathways involved in the development of cancer is yet another fascinating feature of this compound. It may prevent some essential proteins and transcription factors from being activated, which may affect cell survival, growth, and angiogenesis. By concentrating on these signaling pathways, quercetin can stop the development of new blood vessels that facilitate the spread of cancer as well as the growth and survival of cancer cells. Additionally, it has been shown that quercetin has a chemo preventive effect, which means it can aid in preventing the development and spread of cancer. It can lessen DNA damage that can result in the development of cancer by inhibiting the activity of enzymes involved in the metabolism of carcinogens and the activation of procarcinogens. In addition, it has been demonstrated that quercetin supports DNA repair pathways, improving the cell's capacity to preserve genomic integrity and stop the buildup of mutations. Studies looking at how quercetin affects different forms of cancer have shown encouraging findings. For instance, quercetin has shown inhibitory effects on tumor development and metastasis in breast cancer. It has been demonstrated to stop the growth of cancer cells and cause cell death in cases of colorectal cancer. Along with other cancers, quercetin has shown promise in the treatment of ovarian, lung, and prostate cancer. While quercetin has a lot of promise for preventing and treating cancer, further study is necessary to completely understand how it works and how effective it is against various cancer types. Due to its potential for restricted absorption and dispersion within the body, quercetin's bioavailability presents another difficulty. Its bioavailability can be increased by the use of innovative delivery vehicles or combination with other substances, two strategies that are currently being investigated.

Quercetin can interact with various receptors in the body. These interactions with receptors contribute to its biological effects and potential health benefits while quercetin can interact

with several receptors like Estrogen receptors, Histamine receptors, Adenosine receptors, Tyrosine kinase receptors, Nicotinic acetyl receptors, Protein kinase receptors, Jak-stat receptors and Cyline dependent receptors. The underlying mechanisms through which quercetin exerts its anti-cancer effects are complex and involve the modulation of various signaling pathways and cellular processes.

Tyrosine kinase receptor interaction is one significant method via which quercetin affects cancer. The transmission of signals from the extracellular environment to the interior of the cell through these receptors is essential for controlling critical cellular functions such cell growth, proliferation, and survival. Several tyrosine kinase receptors that are typically dysregulated in cancer have been found to be inhibited by quercetin, which blocks the signaling pathways that support the growth of cancer.

Protein kinase receptors, which are enzymes that change proteins by adding phosphate groups, can likewise be impacted by quercetin. Phosphorylation is a fundamental step in several biological signaling cascades. Quercetin can interfere with the signaling pathways that are abnormally active in cancer by altering the activity of protein kinase receptors, which inhibits the development and survival of cancer cells.

Cyclin-dependent kinase receptors are a different group of receptors that quercetin has the ability to affect. These receptors have a role in controlling the cell cycle, which is how cells divide and multiply. Cancer frequently exhibits dysregulation of the cell cycle, which results in unchecked cell proliferation. According to research, quercetin inhibits the function of certain cyclin-dependent kinase receptors, causing cell cycle arrest and halting the growth of cancer cells.

The JAK/STAT signaling system, which is crucial for controlling cell proliferation, differentiation, and immunological responses, can also be impacted by quercetin. Different forms of cancer have been linked to dysregulation of this system. According to research, quercetin prevents the JAK/STAT pathway from activating, which prevents cancer cells from growing and surviving.

Molecular Docking:

In the area of cancer research and medication discovery, computational Molecular Docking has become a potent tool. It enables investigation of the interactions between ligands and target proteins related to cancer processes, offering significant knowledge for the development of innovative therapies. Molecular docking has the potential to transform the

creation of efficient cancer therapies by allowing for the investigation of binding poses and the calculation of binding affinities. Predicting and examining the binding interactions between a ligand and a target protein is the basic idea underlying molecular docking. The target protein is a biomolecule that is crucial to the initiation and progression of cancer, whereas the ligand is a tiny molecule, such as a therapeutic candidate. Understanding the precise connections between these molecules might make it easier to find prospective medication candidates that bind to the target protein with specificity and obstruct processes connected to cancer. A number of crucial processes are involved in the molecular docking procedure. The ligand is first prepared, having its shape optimized and receiving partial charges. The ligand will be in an energetically advantageous condition for subsequent docking simulations thanks to this preparation. Once the ligand is prepared, docking simulations are carried out to investigate the binding poses and determine the affinities for the ligand to bind to the target protein. Complex algorithms examine the conformational space of the ligand and the target protein during the docking calculations to find advantageous binding orientations. The algorithms assess the strength of the ligand-protein interactions by taking into account a variety of non-covalent interactions, including as hydrogen bonding, hydrophobic interactions, electrostatic contacts, and van der Waals forces. The total binding affinity between the ligand and the target protein is influenced by these interactions. Through quantification of the binding affinity of each ligand posture inside the binding site of the target protein, scoring functions play a critical role in molecular docking. These procedures calculate the binding energy and order the postures according to their anticipated affinities. The postures that scored the highest are examples of prospective medication candidates that might demonstrate robust binding to the target protein. Then, these candidates may be further looked into and empirically verified. Molecular docking has shown to be an effective method for cancer research. It enables scientists to find prospective therapeutic candidates that can target important proteins implicated in cancer processes in a selective manner. For instance, signal transduction pathways that regulate cell growth and proliferation depend heavily on protein kinases. By using molecular docking, scientists can find tiny compounds that attach to certain parts of these kinases, reducing their activity and perhaps preventing the spread of cancer. Additionally, molecular docking can help in the creation of medications that target particular cancer-related protein mutations or altered forms. Understanding the structural and functional ramifications of these changes may allow researchers to develop pharmacological candidates that will bind to the altered protein more potently, hence overcoming drug resistance and enhancing therapeutic efficacy. The capacity of molecular docking to hasten

the process of drug discovery is one of its major benefits. Large databases of compounds may be quickly tested using virtual screening approaches to find prospective therapeutic candidates that have the appropriate binding affinities and specificities for the target protein. Compared to conventional screening approaches, this enables researchers to concentrate their efforts on a smaller group of compounds, saving time and money. Additionally, molecular docking offers important insights into the molecular interactions and binding processes that underpin the onset and spread of cancer. Researchers can learn more about the structural and functional elements that contribute to the ligand's binding affinity by examining the binding poses of ligands inside the binding site of the target protein. This information can aid in the logical development of more effective and targeted cancer-related protein-targeting medications. Although molecular docking has made a substantial contribution to the development of anti-cancer drugs, it is crucial to recognize its limits. The quality of the input structures and the scoring methods used have a significant impact on the precision of molecular docking predictions. To confirm the accuracy of the findings, it is essential to experimentally validate the anticipated binding interactions. Additionally, molecular docking ignores any conformational changes brought on by interaction and treats the target protein and ligand as rigid entities. These restrictions can be overcome by integrating flexibility into the docking process using cutting-edge methods like molecular dynamics simulations.

Molecular Dynamics:

The study of biomolecular systems, especially those implicated in cancer, has been transformed by the computational modeling method known as Molecular Dynamics. Molecular dynamics gives a thorough knowledge of the dynamic behavior and structural characteristics of biomolecules by modeling the movements and interactions of atoms and molecules over time, offering insight on the underlying causes of cancer genesis and progression. The study of protein folding and dynamics as well as the investigation of drug binding and protein-ligand interactions are all included in the broad scope of molecular dynamics. The behavior of individual atoms and molecules as they move and interact in a dynamic environment may be seen by researchers using molecular dynamics simulations, which integrate classical physics concepts with computer techniques. Molecular dynamics simulations are an effective technique for studying the structural and dynamic characteristics of biomolecules associated with cancer pathways in the context of cancer research. Researchers can learn more about the fundamental mechanisms driving cancer by observing how proteins, nucleic acids, and other biomolecules move and interact with one another. The

capacity of molecular dynamics to capture the dynamic character of biomolecules is one of its main benefits. In contrast to experimental methods like nuclear magnetic resonance (NMR) spectroscopy or X-ray crystallography, which provide static structural models, molecular dynamics simulations enable the analysis of conformational changes and fluctuations in atomic locations throughout time. Understanding how biomolecules interact with one another and their surroundings is crucial for understanding the processes behind the development of cancer. A molecular dynamics simulation needs a number of things to work. First, the system of interest's initial atomic configuration is determined, frequently using data from experiments or computer simulations. The forces acting on each atom are then described by a series of mathematical equations that are applied to the system. These interactions, which include bound and non-bonded forces including covalent bonds, van der Waals forces, and electrostatic interactions, are accounted for by potential energy functions. The locations and velocities of the atoms are updated numerically over brief time intervals using the equations of motion, such as Newton's second law. The simulation advances over time by solving these equations iteratively, exposing the system's dynamic behavior. The resultant trajectory reveals details about the atoms' motion and interactions, enabling researchers to examine many desirable qualities. Investigating protein-ligand interactions is one of the main uses of molecular dynamics in the study of cancer. Researchers can learn more about the binding kinetics, binding affinity, and structural changes that take place upon binding by mimicking the binding of small compounds, such as therapeutic candidates, to target proteins implicated in cancer processes. This knowledge is essential for the logical development of treatments that can target and regulate cancer-related proteins with precision. Furthermore, protein-protein interactions, which are essential in cancer signaling pathways, can exhibit dynamic behavior that can be understood using molecular dynamics simulations. Researchers can discover important binding surfaces and find possible targets for blocking protein-protein interactions that promote cancer by examining the structural changes and fleeting interactions between proteins. Molecular dynamics simulations may depict the behavior of more substantial, complicated systems, such as cell membranes or protein complexes, in addition to analyzing individual biomolecules. These simulations shed light on the structure, behavior, and interactions between nearby molecules and biomolecular assemblies. To comprehend how cancer-related mutations or medication interactions impact membrane integrity and function, for instance, lipid bilayers and membrane proteins might be simulated. Additionally, the consequences of genetic changes on protein structure and function may be investigated using molecular dynamics simulations. Researchers can see how mutations affect a protein's

stability, dynamics, and interactions by putting them into the protein's atomic model. Understanding the genetic basis of cancer-related mutations and using this information to design tailored treatments can both be very beneficial. However great their promise, molecular dynamics simulations are not without difficulties. The time scales that may be examined are often in the nanosecond to microsecond range due to the computing complexity of modelling massive biomolecular systems. But improvements in computer power and algorithmic effectiveness have made it possible to run longer simulations and examine more intricate systems.

Methods and Materials:

Molecular Docking:

The docking study you described involves using computational methods to investigate the potential of quercetin as an inhibitor against different cancer-related proteins. The three-dimensional structure of the quercetin drug is docked into the active site of the target proteins, including Cyclin Dependent Kinase Receptor (CDKR), Jak Stat Receptor (JSR), Protein Kinase Receptor (PKR), and Tyrosine Kinase Receptor (TKR).

The crystal structures of these target proteins are obtained from the Protein Data Bank (PDB) using their respective PDB IDs. Both the compound (quercetin) and the proteins are prepared and protonated according to standard procedures to ensure accurate calculations.

The docking calculation yields results in the form of binding energies and interactions between the ligand (quercetin) and the receptor pocket of the target protein. These results can be further analysed using software like Discovery Studio (DSV) for interaction analysis.

Molecular Dynamics Simulation:

After analysing the results of the drug against all aforementioned anticancer target proteins, the best pose of Tyrosine Kinase Receptor (TKR) was chosen for the Molecular Dynamic Simulation study using GROMACS software with a CHARMM35 forcefield. The input file of the protein -ligand complex for equilibration and MD production was generated by using the charm-gui server. The system was solved using the TIP3 water model and NaCl ions with a 0.15 concentration for neutralization in the cubic periodic boundary condition. To remove bad contact from the initial structure, the system was minimized in five thousand steps using the steepest descent method and equilibrated in 125,000 steps with a 0.001 step size. After equilibration, the MD production was run for 20 nanoseconds.

Results and Discussions:

Molecular Docking:

To investigate the anticancer potential of the quercetin drug. The drug was docked into the active site of the receptor proteins Cyline Dependent Kinase Receptor (CDKR) (PDB id = 1GII), Jak Stat Receptor (JSR) (PDB id = 6SM8), Protein Kinase Receptor (PKR) (PDB id = 6JZ0), and Tyrosine Kinase Receptor (TKR) (PDB id = 3GQL), which act as molecular targets of cancer. All the proteins were downloaded, protonated, and prepared, and docking calculations were performed according to standard procedure. The best 2D interaction plot of every protein is shown in figure 1. The drug quercetin with s-score -5.9689 shows hydrogen bonding interactions with key residues Glu12, Glu81, and Val83 and hydrophobic interactions with Ile10, Val18, Ala31, Leu134, and Ala144 of the Cyline Dependent Kinase Receptor (CDKR) (**Figure 1. a**). The drug interacts with Jak Stat Receptor (JSR) important residues Arg879, Leu881, Pro960, and Asp1021 through hydrogen bonding, while Val889 and Leu1010 show π-π interactions. The binding energy of the drug-Jak Stat Receptor (JSR) complex is -5.8706 (**Figure 1. b**). The drug shows conventional hydrogen bond interactions with Leu718, Lys745, Met793, and π-π interactions with Val726,

Ala743, and Leu844 of the Protein Kinase Receptor (PKR). The binding energy of the Protein Kinase Receptor (PKR)-drug complex is -5.6152 (**Figure 1. c**). The drug interacts through conventional hydrogen bonding with Ile545, Glu562, Ala564, Asp641, and π-π stacked interactions with Phe642 of the Tyrosine Kinase Receptor (TKR). The same drug with binding energy -6.3524 also shows π-π interactions with Val492, Ala512, Leu630, and Ala640 (**Figure 1. d**).

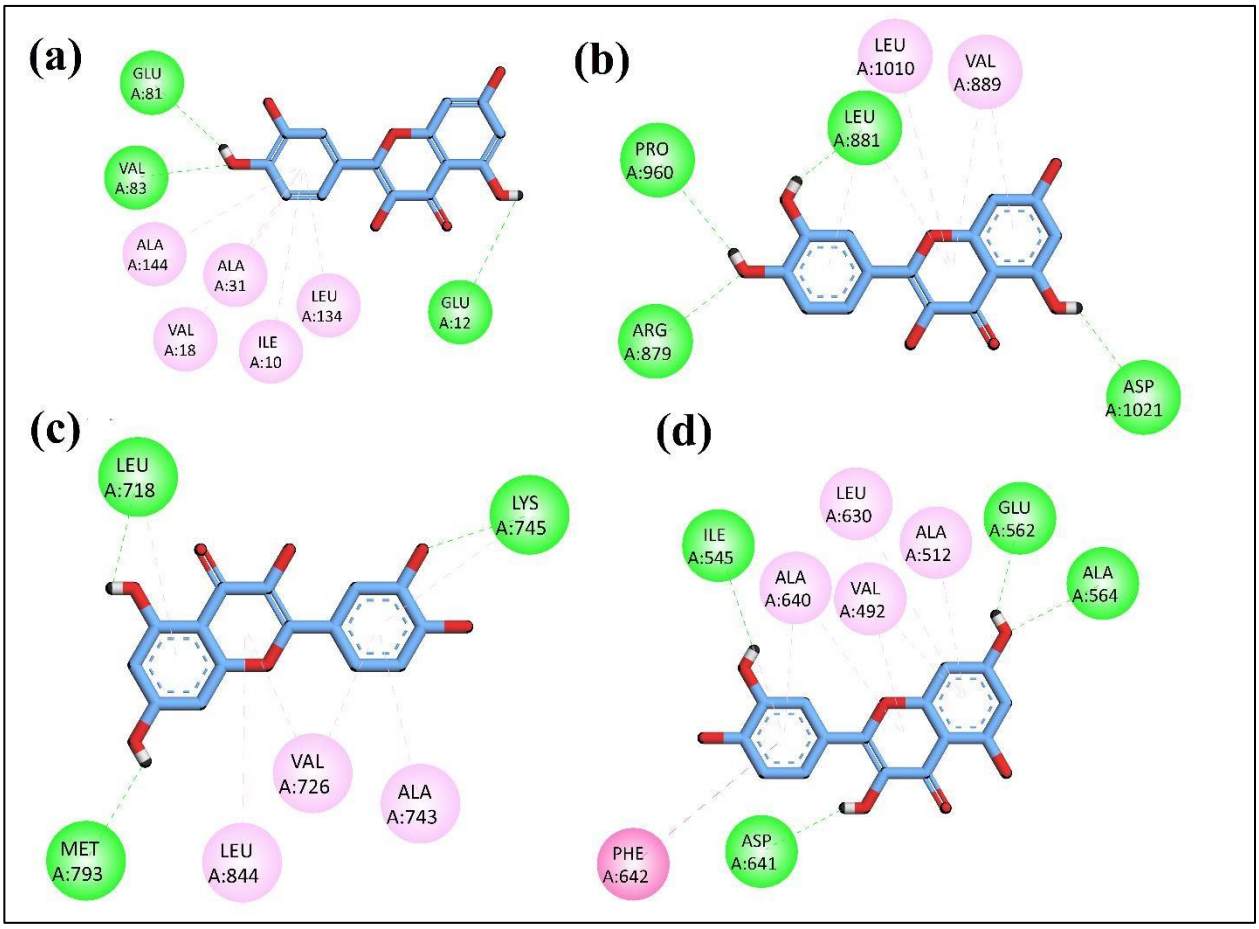

Figure 1. This is the 2D plot of interaction of drug Quercetin with (**a**) Cyline Dependent Kinase Receptor (CDKR), (**b**) Jak Stat Receptor (JSR), (**c**) Protein Kinase Receptor (PKR) and (**d**) Tyrosine Kinase Receptor (TKR)

Molecular Dynamics Simulation:

The Root Means Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) of the Molecular Dynamic Simulation were calculated using the GROMACS tool for the examination of system attributes. RMSD was used to examine the stability, conformational changes, and dynamic behavior of proteins and complexes (Tyrosine Kinase Receptor (TKR)-Drug Quercetin Complex), while RMSF was used to measure the general fluctuation of proteins and complexes. Using the initial structure as a reference frame, the backbone (bb) values of proteins and complexes were measured and analyzed. Proteins have a larger average RMSD value than complexes, indicating that drug binding reduced protein mobility

and dynamics. The RMSD value measured for proteins from the start to 10 ns is between 0.10 and 0.23, whereas complexes have a value between 0.10 and 0.23 (**Figure-2**).

The RMSF value explores the flexibility of protein and complex. High RMSF value is due to high flexibility and enlarges the receptor site which significantly influences the substrate-complex kinetics and affinity. Generally, the RMSF value of complex is slightly less than protein, which shows protein flexibility decrease after binding the drug. The average RMSF value of complex slightly is about 0.1. Most importantly the slight decrease shows that the drug is interacting with active positions (**Figure 3**).

Hydrogen bonding interactions play a very important role in the stability of the drug-TKR complex. To analyse and validate the stability of complexes, the dynamic of hydrogen bond pairs within 0.35 nm was performed. An average hydrogen bonding interaction between drug-TKR complexes is 3. The results show that in the initial 10 nm, when the number of hydrogen bonds reaches 4 or even 5, the complex shows less fluctuation. In the last 10 ns, the fluctuation of complexes became higher when the number of hydrogen bonds decreased to an average of 3 (**Figure 4**).

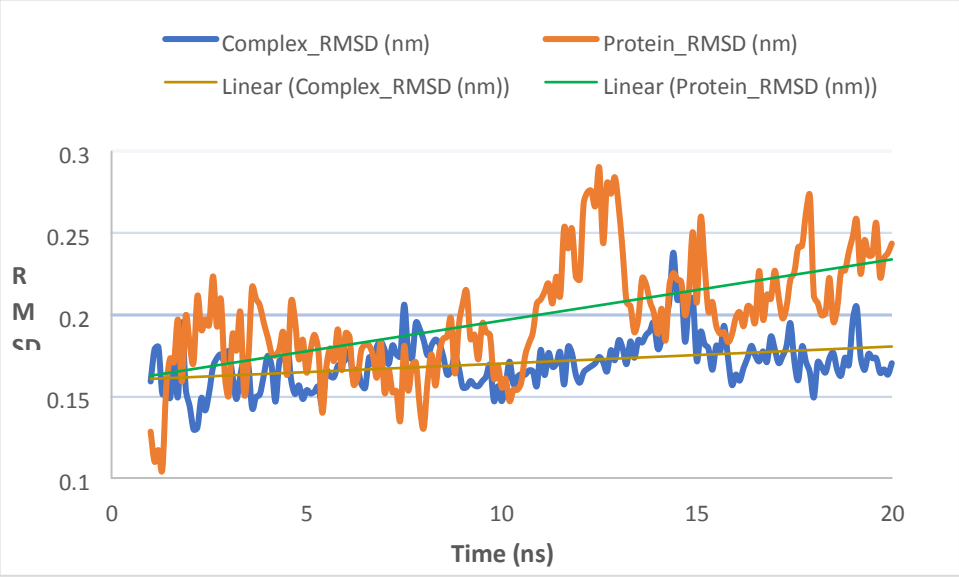

Figure 2. The Root Mean Square Deviation (RMSD) data for TKR of 20 ns. Complexes of TKR and drug are color blue while free proteins are shown in yellow.

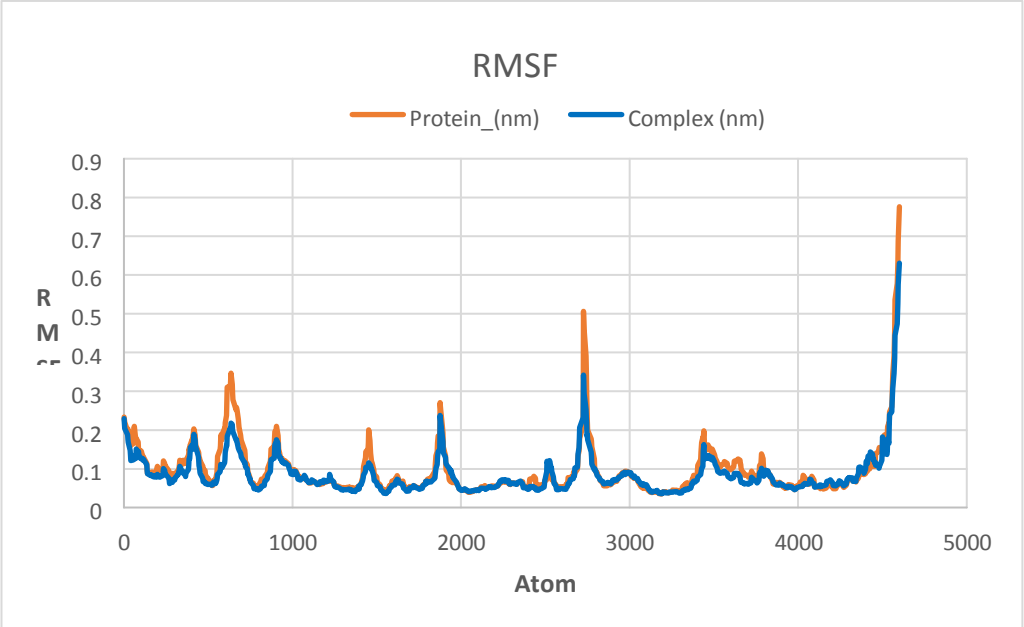

Figure 3. The Root Mean Square Fluctuations (RMSF) data for TKR of 20 ns. Complexes of TKR and drug are blue color while free proteins are shown in yellow.

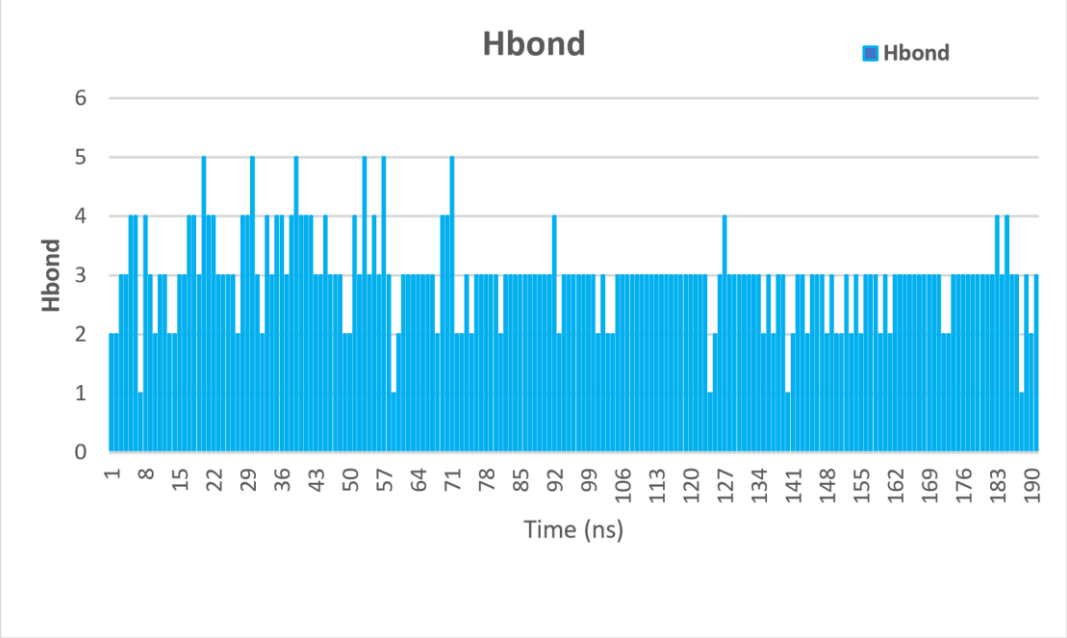

Figure 4. Hydrogen bonding interaction between drug and TKR (complex

Conclusion:

In this paper, the interactions of Cyline Dependent Kinase(CDK), Protein Kinase(PK), Jak-stat(JS), and Tyrosine Kinase(TK) Receptors with ligand(quercetin) were studied by both

Molecular Docking and Molecular Dynamics Simulation. According to the results pf molecular docking, quercetin were bound n the cavity of Cyline Dependent Kinase(CDK), Protein Kinase(PK), Jak-stat(JS), and Tyrosine Kinase(TK) Receptors. Hydrophobicity, hydrogen bonding and pi-pi interactions played a major role in the stability of ligand receptor complexes. The drug and receptors CDKR, JSR, PKR and TKR bonding values are 5.9689**,** 5.8706**,** 5.6152**,** 6.3524. Tyrosine Kinase receptor was found to be the strongest receptor to Quercetin.

Molecular Dynamics Simulation shows that Tyrosine Kinase Receptor (TKR) – Ligand (Quercetin) complexes were suitable within 20ns. Since ligand interact with TKR, better inhibition effect of cell growth. Affinity values of Quercetin -Tyrosine Kinase Receptor complex higher than other three receptors, which indicates that the binding energy of the Quercetin-Tyrosine Kinase Receptor (TKR) complex is the highest in the molecular docking results. In addition, the atom fluctuation curve shows that the interactions between Quercetin and Tyrosine Kinase Receptor stable within the simulation time. The result of molecular dynamics simulation are consistent with those of molecular docking, which further proves the accuracy of docking results.

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