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In Silico Molecular Docking Studies of Rutin Compound against Apoptotic Proteins (Tumor Necrosis Factor, Caspase-3, NF-Kappa-B, P53, Collagenase, Nitric Oxide Synthase and Cytochrome C)

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Abstract Rutin as a flavonoid compound contains many flavonoids having antitumor properties. Therefore, the present study was aimed to dock rutin compound with apoptotic proteins like TNF, Caspase-3, NF-Kappa-B, P53, Collagenase, Nitric Oxide Synthase and Cytohrome C by AutoDock software. The docking scores were highest in Nitric oxide synthase (-3.68 kcal/mol) followed by Tumor Necrosis Factor (-3.22 kcal/mol), Caspase-3 (-2.95 kcal/mol), Collagenase (-2.47 kcal/mol), Cytochrome C (-2.31 kcal/mol), NF-kappa-B (-1.8 kcal/mol) and P53 (-0.32 kcal/mol). The Log P value and lower hydrogen bond counts, confirming the ability of rutin compound for binding at the active sites of the receptor was determined by the *in silico* method. The potential drug candidate can further be validated by wet lab studies for its proper function.

Keywords: *Rutin, AutoDock and Apoptotic proteins*

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1. Introduction

Apoptosis or Programmed Cell Death is an evolutionarily conserved and extremely synchronized form of cell death to facilitate the deletion of redundant, infected, injured or malformed cells during the normal life span in various biological systems which is an essential course of action in maintaining homeostasis in multicellular organisms. It is usually implicated in embryogenesis, metamorphosis, immune system and normal adult tissue remodeling as well as in a number of pathological disorders such as cancer, autoimmunity and degenerative diseases. Generally cancer cells themselves are more prone to undergo apoptosis and a comprehensive understanding of the molecular pathways that regulate apoptosis will assist in investigating novel cancer chemotherapeutic targets [1] which in turn would offer new opportunities for the discovery and development of drugs [2].

Flavonoids, the polyphenolic compounds act as the major nutritional constituents of plant-based food as habitual and folkloric medicine worldwide [3,4]. Rutin, a common dietary flavonoid with a wide range of pharmacological activities is present in many plants, fruits, vegetables and red wine [5,6,7]. Different studies have represented the biological effects of rutin, such as anti-oxidative, anti-inflammatory, antihypertensive, anti-carcinogenic, cytoprotective, anti-platelet, antithrombic, anti-diabetic, anti-adipogenic, neuroprotective, hormone therapy and cardioprotective activities [8,9,10].

In the present investigation, to study the physco chemical properties of the apoptoticproteins, to carry out docking of seven different kinds of apoptotic proteins *viz*., Tumor Necrosis Factor (TNF-alpha), Caspase-3, NF-kappa-B p105 subunit, Cellular Tumor Antigen p53, 72 kDa Type IV Collagenase, Nitric Oxide Synthase and Cytochrome C oxidase subunit 2.

2. Materials and Methods

2.1. Preparation of Protein Structure

The protein information was obtained from Swissprot and the apoptotic protein structures were obtained from RCSB Protein Databank (www.rcsb.org/pdb/home/home.do). The hydrogen atoms were added to the target protein molecules after removing the water molecules for docking. The 3D structures of the proteins were visualized using RASMOL.

2.2. Preparation of Ligand Structure

Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual confirmations in which binding may occur. These are commonly called binding modes [11]. ChemSketch

developed by advanced chemistry development, inc., (http;//www.acdlabs.com) was used to construct structure of rutin. Using draw mode of ChemSketch, the ligands were generated and the three dimensional optimization were done and then saved in .mol file and TORSDOF was used in calculating the changes in free energy caused by the loss of torsional degrees of freedom using binding. After all the above conditions are set and the ligand was saved in "pdbq" format.

2.3. Preparation of Receptors

The receptor file used by AutoDock must be in "pdbqs" format which is pdb plus "q" charge "s" salvation parameters: AtVol, the atomic fragmental volume and AtSolPar, the atomic salvation parameter which are used to calculate the energy contributions of desolvation of the receptors, *ie.*, macromolecules by ligand binding was also calculated using Open Babel.

2.4. Preparation of Grid Parameter File

The grid parameter file tells AutoGrid the types of maps to compute, the location and extent of those maps and specifies pair-wise potential energy parameters. In general, one map is calculated for each element in the ligand plus an electrostatic map. Self-consistent 12-6 Lennard Jones energy parameters equilibrium internuclear separation and the energy well depth are specified for each map based on types of atoms in the macromolecule. If we want to model hydrogen bonding, this is done by specifying 12-10 instead of 12-6 parameters in "gpf" format. The grid parameter were set using AutoGrid.

2.5. Starting AutoGrid and AutoDock

AutoGrid and AutoDock must be run in the directories where the macromolecules, gpf, dpf files, ligand and parameter files are to be found.

2.6. Analysing the Docking Results

The key results in a docking log are the docked structures found at the end of each run, the energies of these docked structures and their similarities to each other. The similarities of docked structures are measured by computing the RMSAD between the coordinates of the atoms. The docking results consists of the PDBQ of the Cartesian coordinates of the atoms in the docked molecule along with the atate variables that describes this docked conformation and position and this was done by PyMol.

3. Results and Discussion

In the present study, the interaction between Tumor Necrosis Factor (TNF-alpha), Caspase-3, Nuclear Factor NF-kappa-B p105 subunit, Cellular Tumor Antigen p53, 72 kDa Type IV Collagenase, Nitric oxide Synthase and Cytochrome C oxidase subunit 2 were studied to explore the binding mode, docking study was performed using AutoDock with PyMol tool. Apoptotic protein structures were derived from PDB Database and used as a target for docking simulation. The ligands were created and prepared for docking studies using ChemSketch. The structure of the ligands obtained from ChemSketch is given in Figure 1. The deduction of ligand-binding sites is the initial step for normal drug discovery. Here the Q-site finder predicted the active site of the apoptotic proteins precision as shown in Figure 2. As most of the amino acid resumes in the active site are hydrophobic, they are the main contributions to the receptor-ligand interactions.

3.1. Details of Docking Interaction

To study the binding mode of rutin compound in the binding site of apoptic proteins, intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the protein-inhibitor complexes. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target proteins. Several potential inhibitors have been identified through the docking simulation. The binding affinity of the apoptotic proteins with the rutin compound was measured by kcal/mol. The docking scores were highest for Nitric oxide synthase with -3.68 kcal/mol with the stronger interaction followed by Tumor Necrosis Factor (-3.22 kcal/mol), Caspase-3 (-2.95 kcal/mol), Collagenase (-2.47 kcal/mol), Cytochrome C (-2.31 kcal/mol) Nuclear Factor NF-kappa-B (-1.8 kcal/mol) and the least score was found in p53 (-0.32 kcal/mol) as shown in the Table 1 and Figure 3. Likewise, hydrogen bond formation was good in all the seven proteins, when docked with rutin. The hydrogen bond formation was high in Caspase 3 with 6 hydrogen bond formation, followed by TNF and Collegenase with 4 hyrogen bond formation, NF-kappa-B with 3 hydrogen bond formation, p53 and Nitric Oxide Syntase with 2 hydrogen bonf formation and the least was observed in Cytochrome C with 1 hydrogen bond formation. Similar type of studies with Qucertin, Fucoidan and Resveratrol compounds were also performed by Ashok and Sivakumari (2015) [12], Manimaran *et al*. (2015) [13], Muthukala *et al*. (2015) [14] and Rajesh *et al*. (2016) [15]. The protein-ligand interaction plays an important role in structural-based designing [16].

Drug discovery is most prominent process in current days and that begin with target and lead discovery, followed by lead optimization and pre-clinical *in vitro* and *in vivo* studies to recognize the potent compounds for which assure the main criteria for drug development [17]. To develop a drug through an *in vivo* and *in vitro* methods take long time and with high expenditure [18,19]. Computational drug discovery can help in identifying potent drugs molecules and targets *via*., bioinformatics tools. They can also be used to evaluate the target structures for possible binding/active sites, generate active drug molecules, check for their dynamic and kinetic properties, the docking studies of these molecules with the target molecules will help us to know the affinity and efficacy of developed molecule and we rank them according to their binding affinities [20]. The molecules which are showing better activity can be modified and built to get good activity towards the target molecules, and further the molecules are optimized to improve binding

characteristics. The use *in silico* methods will help us in all aspects of drug discovery today and forms the importance of structure-based drug design. There are plenty of programs which are helping us to build an active drug molecule. Meanwhile, high-performance computing, data management software and internet are helping us to generate high quality data generated complex data and also transformation of huge complex biological data into accessible knowledge in current trends to discover a novel drug molecules [21,22].

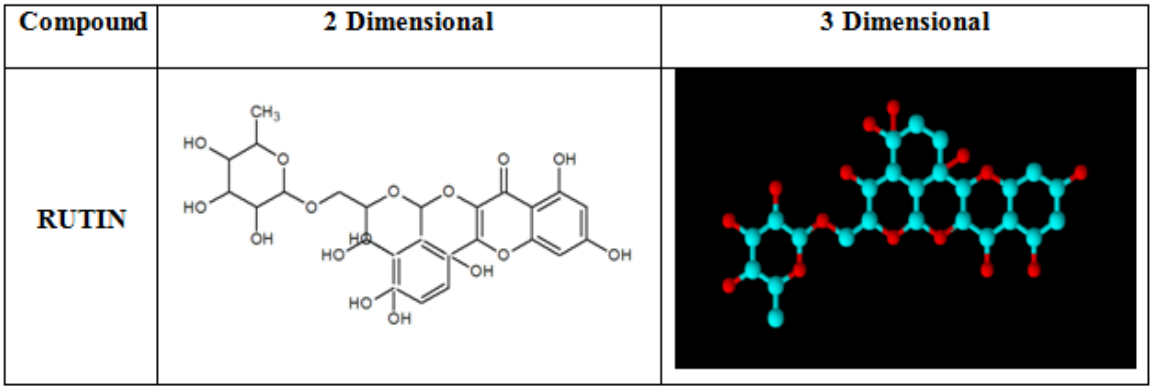

Figure 1. 2D and 3D structure of Rutin

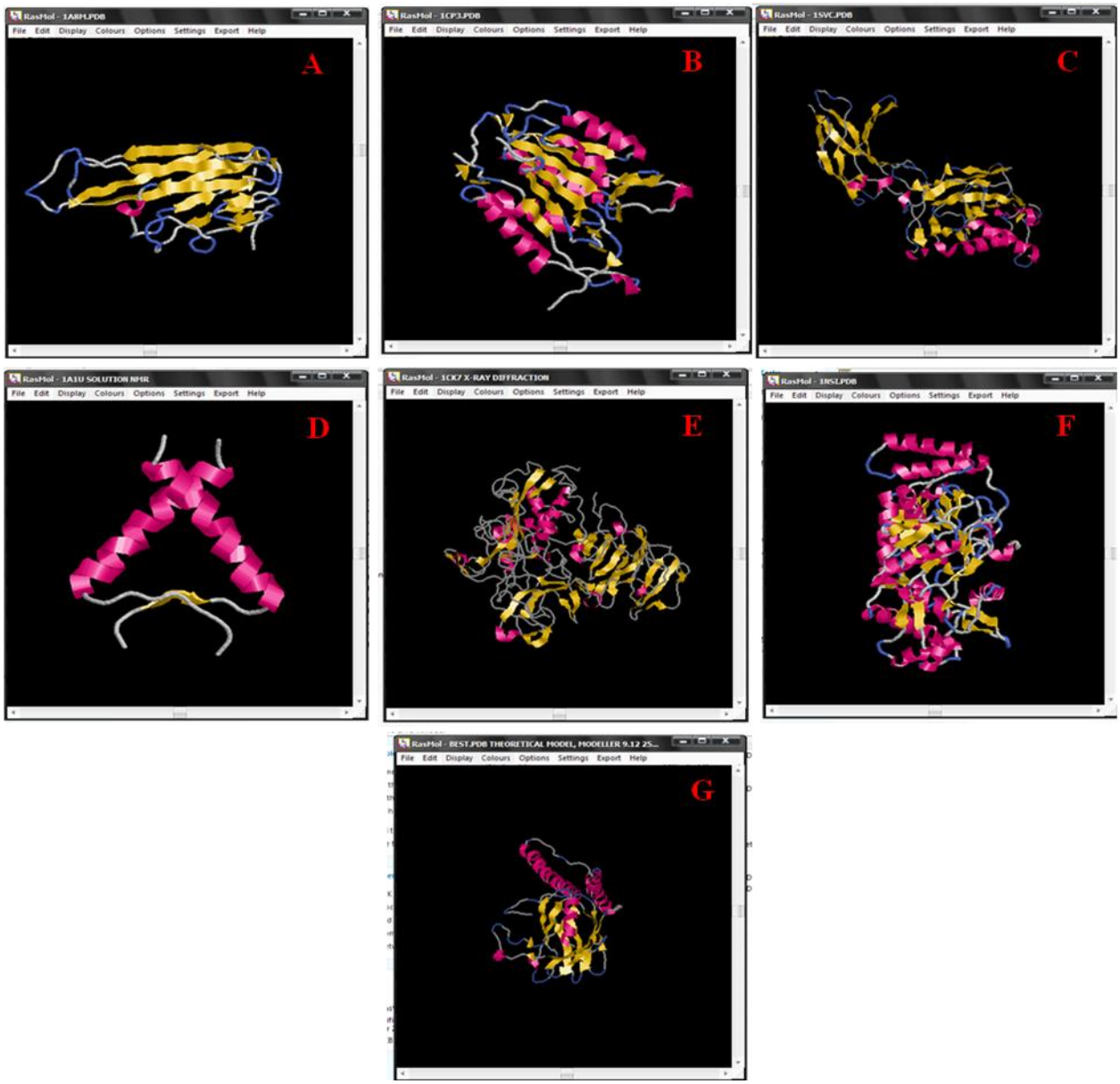

Figure 2. The active sites of apoptotic proteins have the amino acid sequences are as follows: A - TNF-alpha protein, B - Caspase-3, C - NF-kappa-B, D - P53, E – Collagenase, F - Nitric Oxide Synthase, G – Cytochrome C

Table 1. Docking score and number of hydrogen bonds formed between the proteins and rutin compound

S. No.	APOPTOTIC PROTEINS	RUTIN	
		DOCKING SCORE (KCal/mol)	H-BOND
1	Tumor necrosis factor	-3.22	4
2	Caspase-3	-2.95	6
3	Nuclear factor NF-kappa-B p105 subunit	-1.8	3
4	Cellular tumor antigen p53	-0.32	2
5	72 kDa type IV collagenase	-2.47	4
6	Nitric oxide synthase, inducible	-3.68	2
7	Cytochrome C	-2.31	1

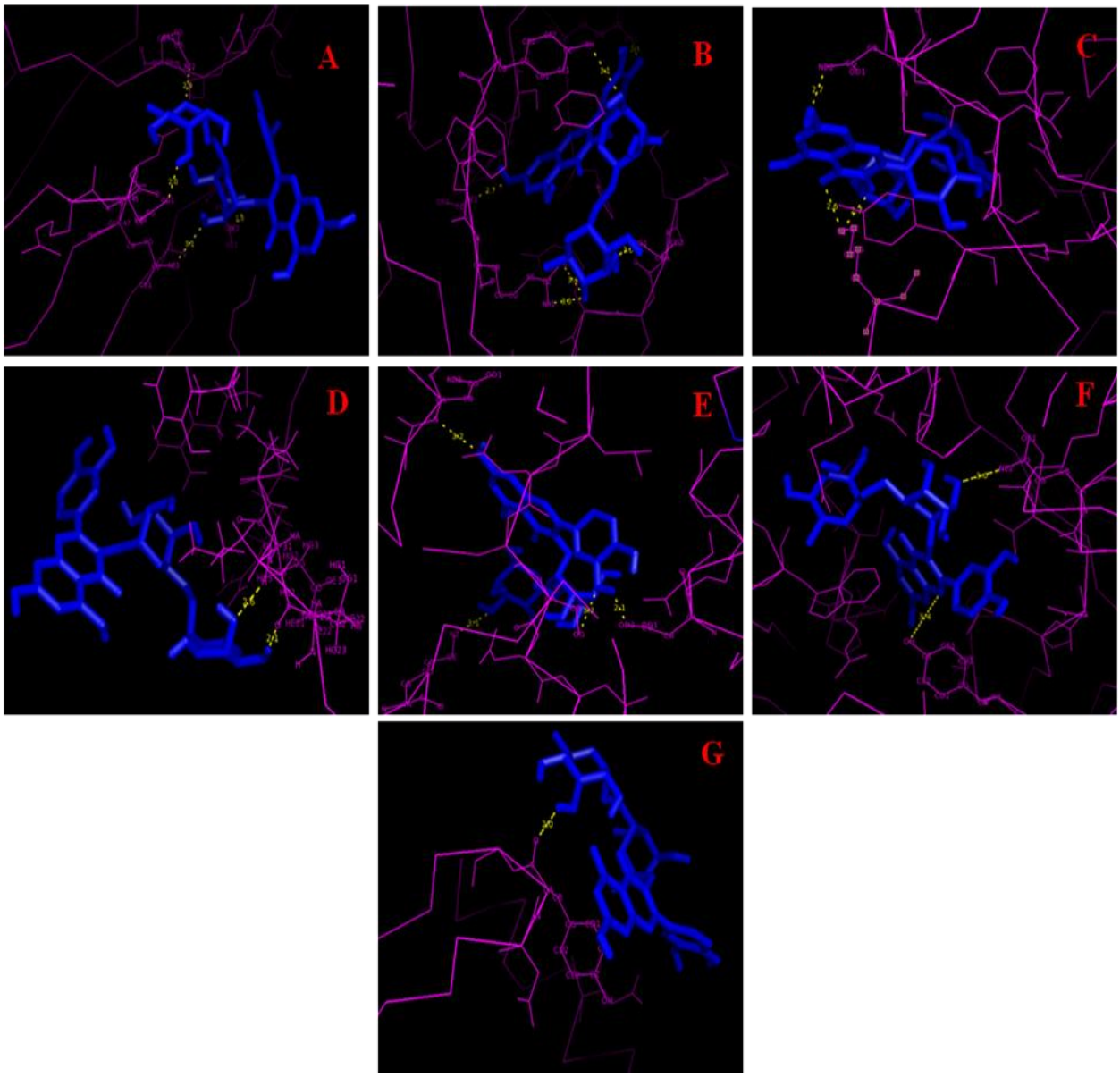

Figure 3. Pictorial representation of Docked Complex using PYMOL tool where, Protein is in pink color, Rutin in blue color and the H-Bond is indicated by yellow color dots: A - TNF-alpha protein, B - Caspase-3, C - NF-kappa-B, D - P53, E – Collagenase, F - Nitric Oxide Synthase, G – Cytochrome C

Apoptosis is a tightly regulated and at the same time highly efficient cell death program which requires the interplay of a multitude of factors. The components of the apoptotic signaling network are genetically encoded and are considered to be usually in place in a nucleated cell ready to be activated by a death-inducing stimulus [23-31]. Apoptosis is well identified biological response exhibited by cells after suffering DNA damage and is a useful marker for screening compounds for subsequent development as possible anti-cancer agents [32]. Apoptosis provides a number of clues with respect to effective anticancer therapy, and many chemotherapeutic agents reportedly exert their antitumor effects by inducing apoptosis in cancer cells [33].

The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures [34]. Ligand binding is the key step in enzymatic reactions and, thus,

for their inhibition. Therefore, a detailed understanding of interactions between small molecules and proteins may form the basis for a rational drug design strategy [35-39]. The unit of Glide Score is Kcal/mol and it includes ligand-protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, pi stacking interactions, RMSD and desolvation [40]. Molecular docking, both structure-based and ligand-based, has become a powerful and inexpensive method for searching a novel lead compound. Molecular docking has been successful in discovering novel anticancer compounds against several protein targets, such as BCR-ABL tyrosine kinase, Chk1, FKBP, protein tyrosine phosphatase (PTP) Caspase-3, Caspase-9, Cytochrome-C, NF-kappa-B, β-Actin, Transferrin, Plasminogen, BCL-2 and EGFR as well.

The docking result showed that there exists a binding interaction between each protein and rutin ligand, which was validated by the formation of hydrogen bond between the proteins and the ligand. Lipinski rule also suggests ruitn as the best therapeutic drug. The results clearly depicts that there is an interaction between the ligand and proteins. Hence, the *in silico* molecular docking studies suggests that rutin can be utilized as a potential and green therapeutic agent to treat various diseases.

4. Conclusion

In this study, the molecular docking was carried out to explore the binding interaction of rutin compound with apoptotic proteins and to correlate its docking score with the activity of rutin compound. The results are helpful for designing and developing a novel drug that has better inhibitory activity against several types of cancers. From this study we conclude that rutin compound is one of the best phytochemical anticancer agent. This potential drug candidate awaits further validation by wet lab studies for its proper function as an anticancer drug.

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