Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 560 - 569

Research Article **Bio Informatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INSILICO ANALYSIS ON THE EFFECT OF RUTIN BIOFLAVONOID AND CHEMOTHERAPEUTIC DRUG CYCLOPHOSPHAMIDE ON NUCLEAR FACTOR KAPPA-B PROTEIN EXPRESSION

B.REVATHI MANI*[1] AND R.VENKATESWARI[2]

[1] Department of Biochemistry, Queen Mary's College, India.
[2] Department of Biochemistry,Vel's University, India.

ABSTRACT

Cancer is medically termed as a malignant neoplasm characterized by uncontrolled growth of abnormal cells. Lung cancer is one of the commonest malignant neoplasms all over the world. Lung cancer is initiated by activation of oncogenes or inactivation of tumor suppressor genes. NF-kB p65 nuclear expression is an early and frequent phenomenon in the pathogenesis of lung cancer. It has been found that NF-kB activation plays a vital role in lung cancer pathogenesis and also identified as molecular link between inflammation and cancer. Rutin is a natural citrus bioflavonoid glycoside found in buckwheat, the leaves and petioles of Rheum species, and asparagus. The scavenging activity of rutin was strongest when compared with other flavonoids such as quercetin and naringin. The present study was undertaken to show the activity of natural therapeutic flavonoid rutin inhibiting the activity of NF-kB in lung cancer. Docking study of rutin was performed against NF-kB activity compared with the already existing chemotherapeutic agent Cyclophosphamide. The study showed a strong interaction between the rutin flavonoid with the hydrogen bonds of the NF-kB protein with a negative score inhibiting the expression of NF-kB which leads to chronic inflammation and progression of lung cancer.

KEYWORDS: **NF-kB, Rutin, Lung cancer, bioflavonoid, docking, cyclophosphamide**

B.REVATHI MANI
Department of Biochemistry, Queen Mary's College, India.

INTRODUCTION

Cancer harms the body when damaged cells divide and multiply uncontrollably to form lumps or masses of tissue called tumors except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream. Tumors can invade and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign.[1] Lung cancers are a result of smoking. However approximately 25% of lung cancer cases worldwide are not attributable to tobacco use. It accounts for over 300,000 deaths each year.[2] Nuclear factor-kB NF-kB, initially discovered as a transcription factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin, has since been shown to be expressed ubiquitously in the cytoplasm of all types of cells.[3] NF-kB translocates to the nucleus only when activated, where it regulates the expression of more than 200 genes that control inflammation and cell growth.[4] The role of inflammation in the early pathogenesis of lung cancer provide a rationale for targeting NF-kB activation in therapeutic and preventive approaches to this neoplasm. There is an increasing level of interest in developing inhibitors of NF-kB for novel chemoprevention and treatment strategies of human cancers. A number of lines of evidence suggest that chronic inflammation contributes to the process of lung carcinogenesis through activation of a number of molecular pathways, including NF-kB.[5] Current theories suggest that the inflammation and oxidative stress induced by smoking lead to proteolytic imbalance and progressive lung structural derangement, with disease susceptibility being controlled by inherited variations in protective or inflammatory genes.[6] NF-kB activation has been associated with the initiation and progression of several human cancers.[7] and is considered a molecular link between chronic inflammation and cancer development. [8]

Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cancer is a disease that is often characterized by too little apoptosis. Cancer cells typically possess a number of mutations that have allowed them to ignore normal cellular signals regulating their growth and become more proliferative than normal. Under normal circumstances damaged cells will undergo apoptosis, but in the case of cancer cells mutations may have occurred that prevent cells from undergoing apoptosis. In these cases there is no check on the cellular proliferation and consequently the disease can progress to the formation of tumors. In many cases these tumors can be difficult to kill, as many cancer treatments rely on damaging the cells with radiation or chemicals and mutations in the apoptotic pathway often produce cells that are resistant to this type of attack. Understanding how apoptosis is regulated in cancer is therefore crucial in the development of treatments for this disease.[9] Cyclophosphamide is an antineoplastic compound that is chemically related to nitrogen mustard. Cyclophosphamide, a nitrogen mustard compound is a member of the group of cytostatic alkylating agents. Its actions lead to splitting of the DNA molecule as well as crossed linking of DNA's double helix which interferes with DNA replication and RNA transcription.[10] Hepatic metabolism of cyclophosphamide by the microsomal P450 mixed function oxidase system forms hydroxycyclophosphamide.[11]

This metabolite, or its tautomeric aldehyde, aldophosphamide, split spontaneously to form two other cytostatic compounds, phosphoramide mustard an acrolein. Phosphoramide mustard has the DNA as its main target, while acrolein affects proteins. Cyclophosphamide, a bifunctional alkylating compound, is the most widely used oxazaphosphorine in the treatment of many neoplastic diseases: breast carcinoma, acute lymphoblastic leukaemia, non-Hodgkin's lymphoma and a variety of bone and soft tissue sarcomas. Rutin is one of the phenolic

compounds found in the invasive plant species *Carpobrotus edulis* and contributes to the antibacterial and antioxidant properties of the plant. Rutin, also called rutoside, quercetin-3-O-rutinoside and sophorin, is the glycoside between the flavonol quercetin and the disaccharide rutinose α-L-rhamnopyranosyl-1→6-β-D-glucopyranose.[12] Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenicity, anticarcinogenicity, and anti-aging, among others, originate from this property. Natural products phenolic substances, terpenoids, fatty acids etc. have played a significant role in human disease therapy and prevention.[13] The emergence of Bioinformatics has provided a platform to explore diseases at the molecular level using Computational techniques. Insilico methods are mainly harnessed to reduce the time, cost and risk associated with Drug Discovery. Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.[14] Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. AutoDock is a molecular modeling simulation software. It is especially effective for Protein-ligand docking. AutoDock 4 is available under the GNU General Public License. AutoDock Vina is available under the Apache license. AutoDock consists of two main programs: AutoDock for docking of the ligand to a set of grids describing the target protein; AutoGrid for pre-calculating this grids.[15]

MATERIALS AND METHODS

(i) Uniprot

UniProt is a comprehensive, high-quality and freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from the research literature. The UniProt/Swissprot Knowledgebase UniProtKB is the central access point for extensive curated protein information, including function, classification, and cross-reference. It consists of two sections: UniProtKB/Swiss-Prot which is manually annotated and is reviewed and UniProtKB/TrEMBL which is automatically annotated and is not reviewed. The UniProt Reference Clusters UniRef databases provide clustered sets of sequences from the UniProtKB and selected UniProt Archive records to obtain complete coverage of sequence space at several resolutions while hiding redundant sequences. [16]

http://www.uniprot.org/

(ii) PDB

The Protein Data Bank PDB is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations. The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived i.e., secondary databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorize structures based on genes.[17]

http://www.rcsb.org/pdb/home/home.do

(iii)PFam

The Pfam database contains information about protein domains and families. Pfam-A is the manually curated portion of the database that contains over 10,000 entries. For each entry a protein sequence alignment and a hidden Markov model is stored. These hidden Markov models can be used to search sequence databases with the HMMER package written by Sean Eddy. Because the entries in Pfam-A do

not cover all known proteins, an automatically generated supplement is provided called Pfam-B. Pfam-B contains a large number of small families derived from clusters produced by an algorithm called ADDA.[18]
http://pfam.sanger.ac.uk

(iv)Active site Prediction

Q-SiteFinder is a new method of ligand binding site prediction. It works by binding hydrophobic CH3 probes to the protein, and finding clusters of probes with the most favourable binding energy. These clusters are placed in rank order of the likelihood of being a binding site according to the sum total binding energies for each cluster. Pocket-Finder works by scanning a probe of radius 1.6 angstroms along all gridlines of a grid resolution 0.9 angstroms surrounding the protein. The probe also scans cubic diagonals. Grid points are defined to be part of a site when the probe is within range of protein atoms followed by free space followed by protein atoms. Since the protein is scanned in seven directions, each grid point can be defined to be part of a site up to seven times. Grid points are only retained if they are defined to be part of a site at least five times. Pocket-Finder uses the same interface as Q-SiteFinder. Q-SiteFinder is twice as effective as Pocket-Finder in generating predicted sites that map accurately onto ligand coordinates. It also generates predicted sites with the lowest average volumes of the methods examined in this study. Unlike pocket detection, the volumes of the predicted sites appear to show relatively low dependence on protein volume and are similar in volume to the ligands they contain. Restricting the size of the pocket is important for reducing the search space required for docking and de novo drug design or site comparison.[19]
http://www.modelling.leeds.ac.uk/qsitefinder/

(v) Pubchem

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information NCBI, a component of the National Library of Medicine, which is part of the United States National Institutes of Health NIH. PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. The American Chemical Society tried to get the U.S. Congress to restrict the operation of PubChem, because they claim it competes with their Chemical Abstracts Service.[20]
http://pubchem.ncbi.nlm.nih.gov/

(vi) Autodock

Auto Dock is a suite of automated docking tools. The software is used for modelling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structures. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools are used to prepare, run and analyze the docking simulations, in addition to modeling studies. Auto Dock is the most cited docking software because it is very fast, it provides high quality predictions of ligand conformations and good correlations between inhibition constants and experimental ones.
http://autodock.scripps.edu/resources/tools

RESULTS AND DISCUSSION

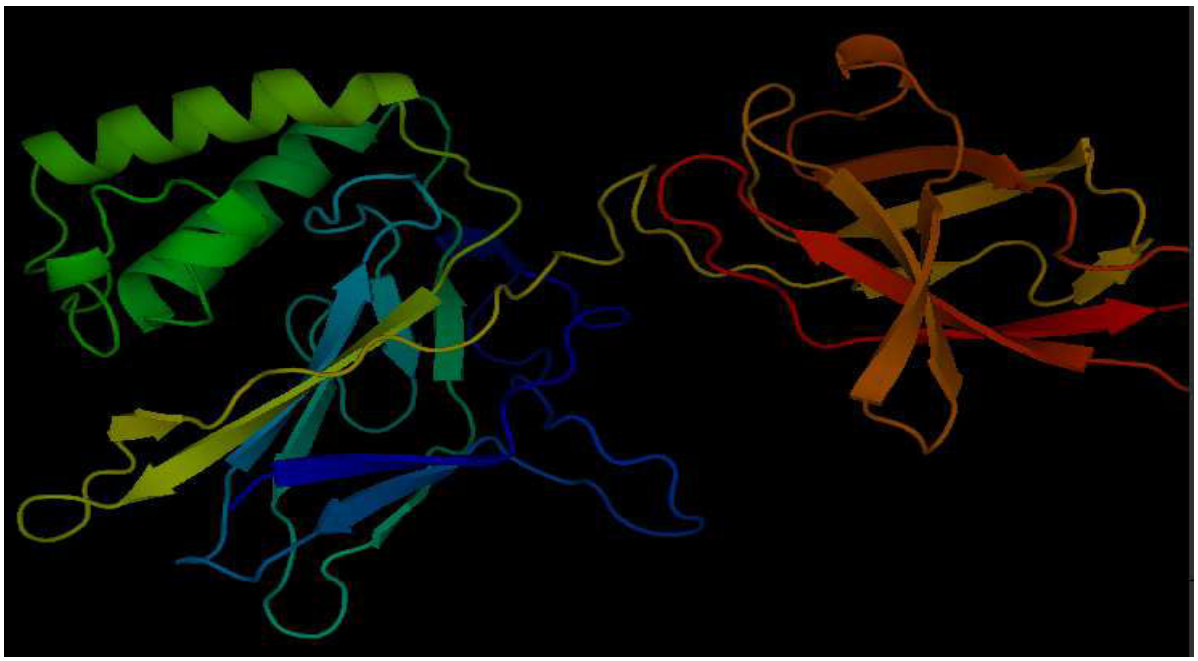
(i) Sequence Retrieval

The protein sequence of NF-KB was retrieved from UNIPROT protein sequence database with the Accession No: P19838

(ii) Structure Retrieval

The 3-Dimensional Structure of NF-KB was retrieved from PDB Database, its PDB ID is 1SVC P chain.

Figure 1
3-D Structure of Nuclear Factor Kappa Protein

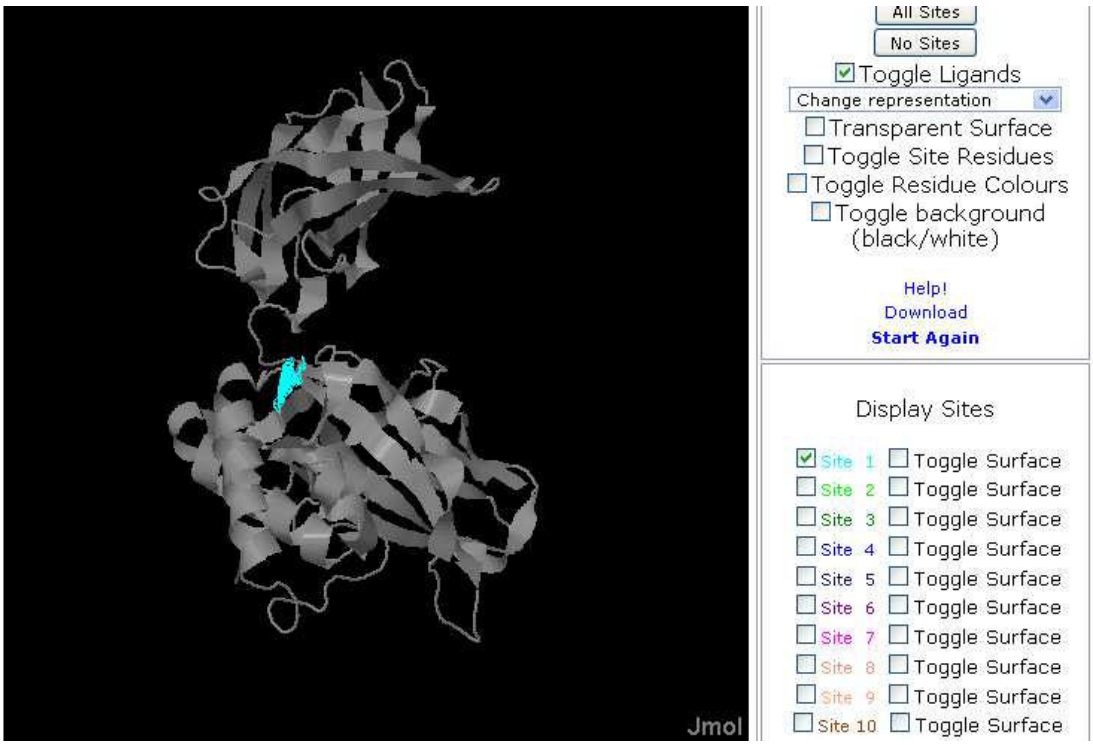


PFam Results
Domains were predicted in NF-KB using PFam Domain analysis database and predicted domain is Rel homology domain RHD and the region is 44-242

(iii) Q-site finder
After obtaining the final model, the possible binding sites of NF-KB were searched using Q-SiteFinder. Ten binding sites were obtained.
THR102,ASN103,GLY104,LYS105,ASN106,HIS108,LEU109,HIS110,LEU154,
GLN204,THR205,GLU207,MET208,ASP209, VAL212, VAL213

(iv)Inhibitors
The Structure of inhibitors were retrieved from Pubchem database and converted to PDB format using OPEN BABEL converter.

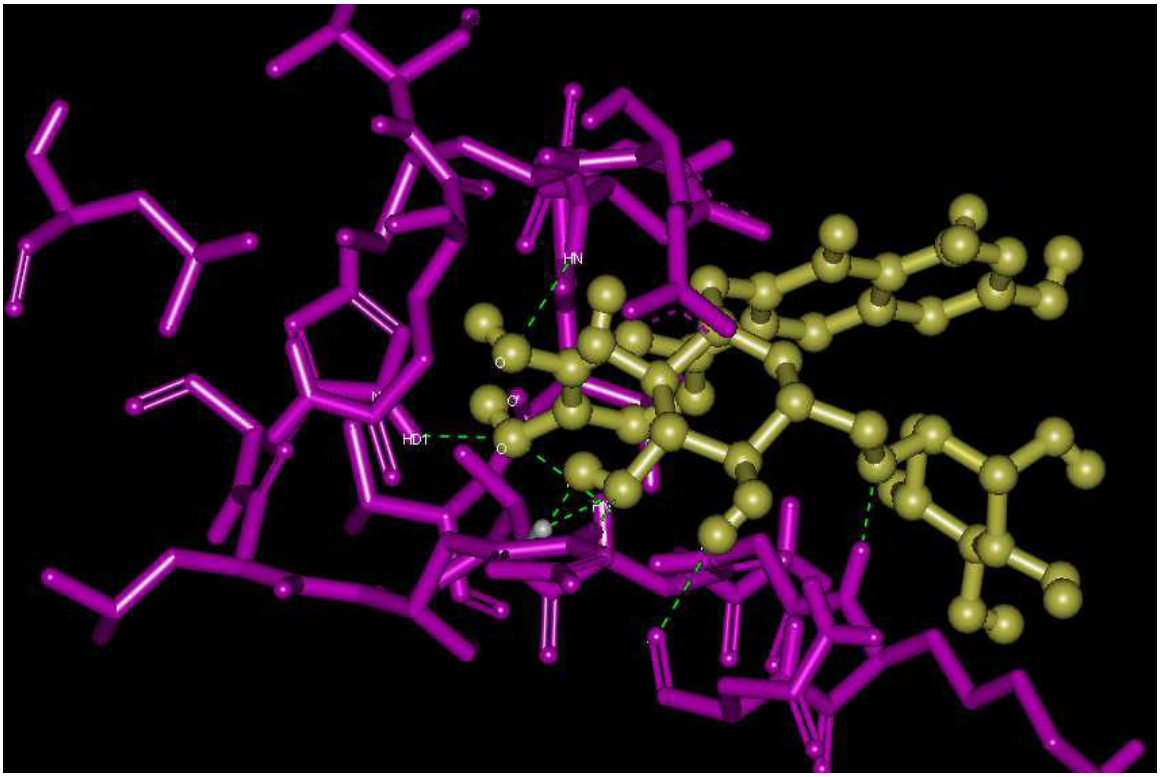
Figure 2
Binding Sites of Nuclear Factor Kappa Protein Using Q-Site Finder



(v) Docking the inhibitors with the Active Site of NF-KB

The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities.[21] The natural flavonoid Rutin and already existing Chemotherapeutic drug cyclophosphamide was docked with NF-KB using the Lamarckian Genetic AlgorithmLGA provided by the AutoDock Program, version 4.1. Polar hydrogens were added to the receptor, kollaman Charges were assigned and salvation parameters were added with the "Addsol" option in AutoDock. For the inhibitors charges of the Gasteiger type were assigned. The internal degree of freedom and torsions were defined using the "Ligand torsions" menu option of AutoDock. The grid maps representing the protein were calculated using the "AutoGrid" option. The protein was centred on the geometric centre prior to docking. Docking simulations were carried out with an initial population of 50 individuals, and a maximum number of 25,000 energy evaluations were used as the docking parameters for obtaining the final docked structures. Autodock uses interaction maps for docking. Prior to the actual docking run, these maps are calculated by the program autogrid. For each ligand atom type, the interaction energy between the ligand atom and the receptor is calculated for the entire binding site which is discretized through a grid and in addition to returning the docked structure, AutoDock also calculates an affinity constant for each ligand-receptor configuration. The best ligand-receptor structure from the docked structures was chosen based on lowest energy and minimal solvent accessibility of the ligand. The NF-KB and Rutin shows 9 hydrogen bonds with the docking score of -3.32 Kcal/mol shown in table 1 and NF-KB and Cyclophosphamide shows only 2 hydrogen bonds with the docking score of -6.95 Kcal/mol shown in table 2.

Figure 3
Hydrogen bond Interaction between Active site of NF-KBpink colour and Rutin yellow colour visualized using Weblab molecular visualization tool



A bond is formed between two atoms by overlapping the atomic orbitals. This overlap of atomic orbitals to form molecular orbitals occurs only at certain distances between the atom. When the amino acid residues of the active site is closer, then the interactions is much higher than the other sites. On the basis of the available evidence, when a hydrogen atom lies between two atoms having high negativity it shows a unique property of forming a bond or bridge between them, holding one of the atoms by a covalent bond and other by purely electrostatic forces. The electrostatic bond has a strength of about 21KJ/mol.

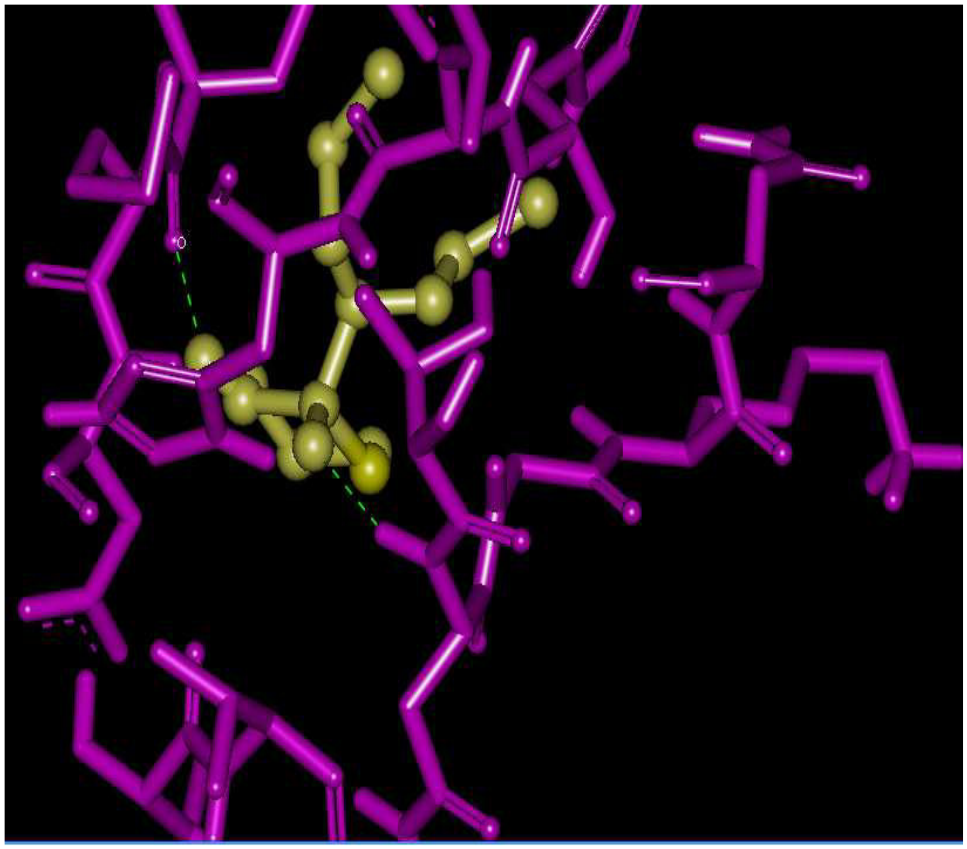
Table 1
Results showing the Atoms and aminoacids interact with atom of Rutin and the docking energy

NF-KB		RUTIN	DISTANCE A	DOCKING SCORE KCal/mol
RESIDUE	ATOM	ATOM		
ASP 209	HN	O	2.26	
HIS 110	HD1	O	1.99	
THR 102	HG1	O	2.0	
THR 102	HG1	H	2.31	
HIS 108	HD1	O	1.84	-3.32
VAL 212	O	H	1.84	
ASN 103	HN	O	2.41	
ASN 106	O	H	2.41	
LYS 105	HN	O	1.80	

Hydrogen bonding is very important when the electronegative atoms are O, F, N. These three elements are negative enough for the necessary attraction to exist. The effectiveness is due to their high electronegativity and their small size. Many reports indicate that flavonoids can access intracellular locations, because of their benzyl structures, justifying their ability to attenuate oxidative stress induced by diverse stimuli. The chemical structure of rutin may contribute to its direct antioxidant properties.[22]

Figure 4

Hydrogen bond Interaction between Active site of NF-KB pink colour and Cyclophosphamide yellow colour visualized using Weblab molecular visulaisation tool



Binding energy is correlated with the probability of affinity and stable bound between ligand and its receptor. [23] Binding energy values may also predict the bioactivity value for a ligand to the corresponding receptor. Commonly, receptor gained from the protein data bank already docked with a native ligand, which has a specific binding energy value. If the binding energy value of subjected ligand is lower than that of the native ligand, then it may predict that the subjected ligand is very active. So, according to the final docked structure, the hydrogen bond interaction between NFkB and rutin has 9 hydrogen bonds with 8 oxygen atoms, 3 nitrogen atoms. Thus the hydrogen atom has strong interaction between oxygen and nitrogen because of their high electronegativity. The conformation of the active site is well suited to accommodate the ligand for the best interaction with lowest energy when compared with cyclophosphamide, which has 2 hydrogen, 2 oxygen and 1 nitrogen with docking score of -6.95 kcal/mol. These results show the least interaction between the ligand and the protein. The present study reveals that the rutin, a natural flavonoid is a potent inhibitor of the NFkB protein when compared with the chemotherapeutic drug cyclophosphamide.

Table 2
Results showing the Atoms and aminoacids interact with atom of cyclophosphamide and the docking energy

NF-kB		CYCLOPHOSPHAMIDE	DISTANCE	DOCKING SCORE KCal/mol
RESIDUE	ATOM	ATOM		
GLU 207 ASN 103	O HN	H O	2.27 2.26	- 6.95

CONCLUSION

Cyclophosphamide is a chemotherapy drug used to treat lung cancer, but it has various side effects such as hemorrhagic cystitis, bone marrow suppression, potentially causing transitional cell carcinoma, nephrotoxicity and acute myeloid leukemia. Rutin, a natural compound has minimum side effects when compared to cyclophosphamide and it has antioxidant and anticancer activity. Rutin induced cell cycle arrest and apoptosis in leukemia cells in vitro and in vivo. Analysis of the receptor/ligand complex models generated after successful docking of the Flavonoid was based on the parameters such as hydrogen bond interactions, binding energy and orientation of the docked compound within the active site. The docking poses are ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses may be exported. The present study strongly suggest that rutin shows better interaction (9- Hydogen bonds) with the lung cancer drug target NF-kB when compared with the interaction (2-Hydrogen) between cyclophosphamide with the protein NF-kB. Hence, rutin a bioflavonoid can be used as an anticancer agent for lung cancer. Therefore, these results may offer therapeutic advantages in the treatment and prevention of human lung cancer. This study will also help to resolve major controversies on the optimum intake of antioxidants, pro oxidant effects and safety and make possible of nutrition and public health recommendations on the use of the antioxidant.

REFERENCES

1. http://www.medicalnewstoday.com/info/cancer-oncology/
2. Sophie Sun, Joan H. Schiller & Adi F. Gazdar, Lung cancer in never smokers — a different disease. Nature Reviews Cancer, 7: 778-790, (2007).
3. Sen R, Baltimore D, Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. Cell,47:921–928, (1986).
4. Aggarwal BB, Nuclear factor-kappaB: the enemy within. Cancer Cell, 6:203–208, (2004).
5. Ballaz S, Mulshine JL, The potential contributions of chronic inflammation to lung carcinogenesis. Clin Lung Cancer, 5:46–62, (2003).
6. Anderson GP, Bozinovski S, Acquired somatic mutations in the molecular pathogenesis of COPD. Trends Pharmacol Sci.,24:71–76 (2003).
7. Garg AK, Hortobagyi GN, Aggarwal BB, Sahin AA, Buchholz TA, Nuclear factor-kappa B as a predictor of treatment response in breast cancer. Curr Opin Oncol., 15:405–411 (2003).
8. Karin M, Greten FR, NF-kappa B: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol., 5:749–759 (2005).

9. Richetti SK, Blank M, Capiotti KM, et al., Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. Behavioural Brain Research, 2171:10–15 (2011).
10. Beecher GR, Overview of dietary flavonoids: nomenclature, occurrence and intake. Journal of Nutrition, 13310: 3248S–3254S (2003).
11. Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman A, McLennan MT, "Nitrogen mustard therapy". JAMA, 132 (3): 126–132 (1946).
12. Lucci, Mazzafera, "Rutin synthase in fava d'anta: Purification and influence of stressors". Canadian journal of plant science, 89 (5): 895–902 (2009).
13. Cook N C, Samman S, Flavonoids-Chemistry, metabolism,cardioprotective effects, and dietary sources. Nutr. Biochem., 7: 66-76 (1996).
14. Lengauer T, Rarey M, "Computational methods for biomolecular docking". Curr. Opin. Struct. Biol., 6 (3): 402–6 (1996).
15. http://autodock.scripps.edu/
16. Uniprot C, "The Universal Protein Resource UniProt in 2010". Nucleic Acids Research 38 Database issue: D142–D148 (2009).
17. Berman HM, "The Protein Data Bank: a historical perspective". Acta Crystallographica Section A: Foundations of Crystallography, A64 (1): 88–95 (2008).
18. Heger A, Wilton CA, Sivakumar A, Holm L, "ADDA: a domain database with global coverage of the protein universe". Nucleic Acids Research 33 Database issue: D188–D191 (2005).
19. Laurie AT, Jackson RM, Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites. Bioinformatics, 21: 1908-1916 (2005).
20. "PubChem and the American Chemical Society". *Reshaping Scholarly Communication*. USA: University of California.
21. Antioxidant Activities of Flavonoids. Department of Environmental and Molecular toxicology. Oregon State University. Available at: http://lpi.oregonstate.edu/fw00/flavonoid.html
22. Istyastono EP, Docking studies of curcumin as a potential lead compound to develop novel dipeptydyl peptidase-4 inhibitors. Indones J Chem., 9:123–6 (2009).
23. Kartasasmita RE, Herowati R, Harmastuti N, Gusdinar T, Quercetion derivatives docking based on study of flavonoids interaction to cyclooxygenase-2. Indones J Chem., 9:297–302 (2009).