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In Silico analysis of piperine, resveratrol, and vanillic acid on NF-κB p65 protein expression

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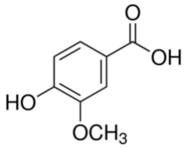
ABSTRACT

The NF-κB is family of transcription factor has important role in innate immunity and inflamation which regulate expression of both pro- and anti-inflammatory mediators. Dysregulation of NF-κB pathways results in severe diseases such as arthritis, immunodeficiency, autoimmunity, and cancer. Hence, a method of inhibiting NF-κB signaling has potential therapeutic application in cancer and inflammatory diseases. Naturally, obtain bioactive compounds such as piperine, vanillic acid and resveratrol have ample of scope in this area due to their safety and efficacy. In the present study, selected bioactive compounds investigated for their binding affinity with NF-κB p65 using AutoDock 4.2 software. Our obtained results revealed that piperine has better binding energy -7.41 Kcal/mol and IC_{50} activity 3.67 µmol/KI towards NF-κB p65 as compared to resveratrol and vanillic acid. These results could help to understand the type of interaction between bioactive compounds with NF-κB p65 binding sites.

Keywords: *Piperine, Vanillic acid, Resveratrol, NF-κB p65.*

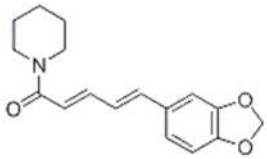
1. INTRODUCTION

Naturally, obtain bioactive compounds has plenty of scope in the area of inflammation due to their safety and efficacy. Vanillic acid is a benzoic acid derivative, which is an oxidized form of vanillin commonly used as flavoring agent [1]. It is also used as an intermediary compound in the synthesis of vanillin from ferulic acid.The highest quantity found in the roots of *angelica sinensis*, which is have been commonly used in traditional Chinese medicine for improvement of women's health, osteoarthritis, and inflammatory responses [2].



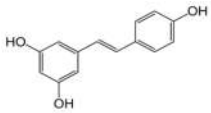
Struture 1. Vanillic acid (4-hydroxy-3-methoxybenzoic acid)

Piperine is a naturally obtain bioactive compound present in long pepper (Piper longum) and black pepper (Piper nigrum) [3]. It has been used in Indian traditional medicine and acts as an insecticide. Piperine possesses a widerange of biological effects such as antithyroid, hepatoprotective and antitumor anti-metastatic, antidepressant [4, 5].



Struture 2. Piperine (1-[5-(1, 3-Benzodioxol-5-yl)-1-oxo-2,4pentadienyl] piperidine)

Resveratrol is a naturally obtain polyphenol, found in several fruits and vegetables whereas abundant quantity found in grapes. Resveratrol possesses a wide range of biological effects such as cardio protective, antioxidant, and anti-inflammatory and analgesic [6].



Struture 3. Resveratrol (3, 5, 4-trihydroxy-trans-stilbene)

NF-κB has long been considered a prototypical proinflammatory signaling pathway, largely based on the activation of NF-κB by proinflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor α (TNFα), and the role of NF-κB in the expression of other proinflammatory genes including cytokines, chemokines, and adhesion molecules, which has been extensively reviewed elsewhere. However, inflammation is a complex physiological process and the role of NF-κB in the inflammatory response cannot be extrapolated from in vitro studies [7].

NF-κB refers to a family of transcription factors that has been highly conserved through evolution and is present in the cytoplasm of all cells. NF-κB has been called a "stress sensor" because its activity is induced by a wide variety of stimuli including tumor necrosis factor (TNF-α), PMA and other tumor promoters, cigarette smoke extract (CSE), lipopolysaccharide (LPS), oxidants, and pathogenic bacteria [8, 9]. The NF-κB family comprises five members: p50 (NF-κB1), p52 (NF-κB2), RelA (p65), RelB, and c-Rel. p50 and p52 are cleaved from inactive precursor proteins, p105 and p100, respectively, prior to translocation to the nucleus and playing a crucial role in inflammation and autoimmunity [10]. Here, the present work was conducted to find out the interactions between the NF-κB p65 with selected bioactive compounds to predict the possible pharmacological effect on inflammation.

2. EXPERIMENTAL SECTION

2.1. AutoDock. Auto Dock is a suite of automated docking tools (AutoDock 4.2). 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 [11]. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. AutoDock tools are used to prepare, run and analyze the docking simulations, in addition to modeling studies [12]. 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.

2.2. Protein Data Bank (PDB). The 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 [13]. The PDB is a key resource in areas of structural biologies, 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 the type of structure and assumed evolutionary relations; GO categorize structures based on genes [14].

2.3. 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 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 [15].

3. RESULTS AND DISCUSSION

In the molecular docking study of three bioactive compounds against NF-κB p65, and obtained results have revealed that hydrogen, van der waal, and electrostatic interactions are presented in Table 1.

3.1. Piperine docking interactions with NF-KB p65. Piperine showed better binding energy -7.41 Kcal/mol towards NF-κB p65 with the IC_{50} value of 3.67µmol/KI and having two H-bond interactions. It has shown electrostatic interactions with following aminoacids ARG:149, ILE:145, GLN:148, TYR:152, LYS:123, Van der waals interaction with ASP:151, ILE:130, GLY:150, LEU:126, GLU127 residues. Moreover, hydrogen bonding was with LYS: 123, TYR: 152, ARG: 84 (Figure 1). Hydrogen bond length found to be 2.14 and 1.94 Å along with the aromatic bond length of 3.70, 3.00, and 5.34 Å respectively (Table 1).

3.2. Vanillic acid docking interactions with NF-κB p65.The binding energy of vanillic acid for NF-κB p65 found to be -6.65 µmol/KI along with the IC_{50} value of 13.33 µmol/KI. Electrostatic interaction of Vanillic acid was ASP: 125, ARG: 124, CYS: 38, LYS: 37, VAL: 121, CYS: 120, LYS: 122. Van der waal interaction along with TYR: 36. Moreover, hydrogen bonding was with CYS: 120, LYS: 37, LYS: 122, ASP: 125 (Figure 2). Hydrogen bond length found to be 1.88, 1.87, 2.19, and 1.66 Å along with the aromatic bond length of 4.75 and 4.63 Å respectively (Table 1).

3.3. Resveratrol docking interactions with NF-κB p65. Resveratrol has a binding energy of -5.95 Kcal/mol towards NF-KB p65 along with IC_{50} of 43.79 µmol/KI. Electrostatic interaction of resveratrol were found to be LYS:123, TYR:152, ASP:151, GLN:148, ILE:145, ARG:149, van der waal interaction along with GLY:150, LEU:126, ILE:130 and hydrogen interaction with LYS:123, TYR:152, ASP:151, ARG:84 125 (Figure 3). Hydrogen bond length found to be 1.97, 2.40, and 1.70 Å along with the aromatic bond length of 0.86, 5.88, 5.44, 3.70, 3.00 Å respectively(Table 1).

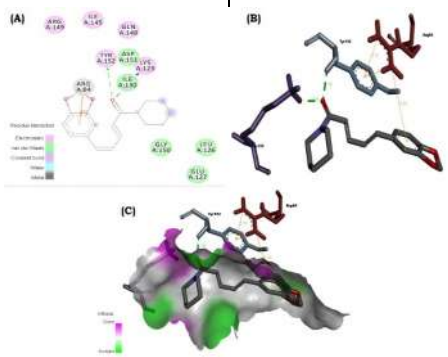


Figure 1. A) represents 2D interactions of Piperine, B) represents 3D interactions formed by the compound Piperine, whereas C) Represents Surface area interactions of compound piperine.

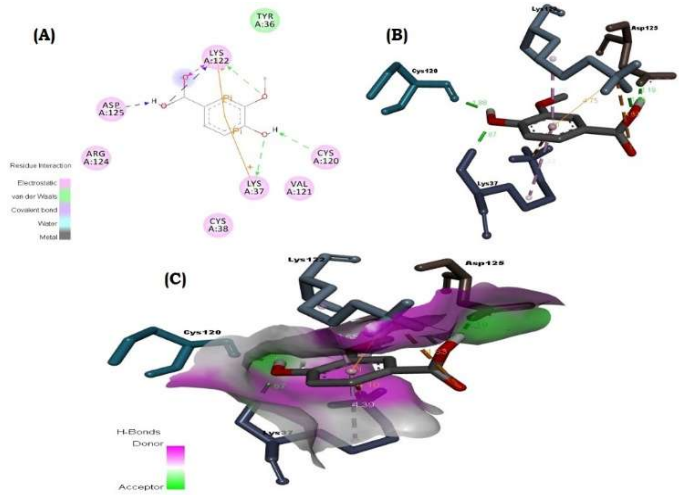


Figure 2. A) Represents 2D interactions of vanillic acid, B) Represents 3D interactions formed by the compound vanillic acid, whereas C) Represents Surface area interactions of compound vanillic acid.

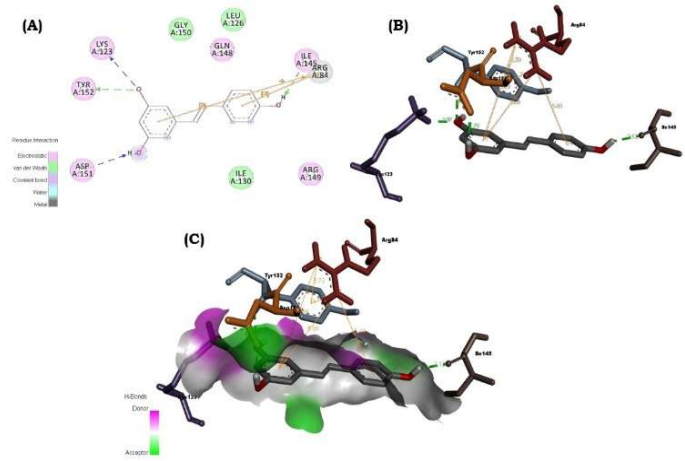


Figure 3. A) Represents 2D interactions of Resveratrol, B) represents 3D interactions formed by the compound Resveratrol, whereas C) Represents Surface area interactions of compound Resveratrol.

Table 1. Protein ligand interaction with NF-κB p65.

No	Compounds	Protein ligand interaction						
		Electrostatics	Van der Waals	H bond	H Bond length (Å)	Aromatic Bond length (Å)	Binding Energy Kcal/mol	IC_{50} Value µmol/KI
1.	**Piperine**	ARG:149, ILE:145, GLN:148, TYR:152, LYS:123	ASP:151, ILE:130, GLY:150, LEU:126, GLU127	LYS:123, TYR:152, ARG:84	2.14, 1.93	3.70 3.00 5.34	-7.41	3.67
2.	**Vanillic acid**	ASP:125, ARG:124, CYS:38, LYS:37, VAL:121, CYS:120, LYS:122	TYR:36	CYS:120, LYS:37, LYS:122, ASP:125	1.88 1.87 2.19 1.66	4.75 4.63	-6.65	13.33
3.	**Resveratrol**	LYS:123, TYR:152,ASP:151, GLN:148, ILE:145, ARG:149	GLY:150, LEU:126, ILE:130,	LYS:123, TYR:152, ASP:151, ARG:84	1.97 2.40 1.70	0.86 5.88 5.44 3.70 3.00	-5.95	43.79

4. CONCLUSIONS

In this study piperine, vanillic acid and resveratrol were investigated using AutoDock 4.2 software to determine the most potent compound as an NF-κB p65 inhibitor. Docking studies of these three compound shown a good correlation between biding free energy (Kcal/mol) and IC 50 value (µmol/KI) against NF-kB p65 protein expression**.** Among all selected compounds, piperine has shown comparable residue interaction, binding free energy and IC 50 value than vanillic acid and resveratrol. Therefore, this study could demonstrate piperine as an effective ligand for NF-κB p65 protein expression.

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