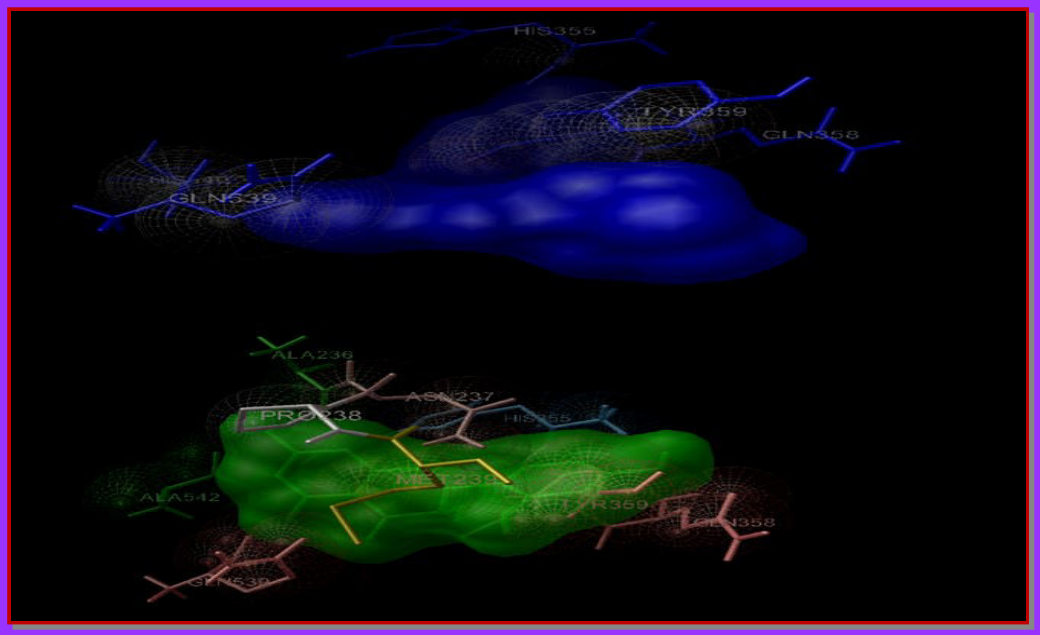

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Docking studies: *In silico* lipoxygenase inhibitory activity of some commercially available flavonoids

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Abstract

This study deals with the evaluation of the cyclooxygenase inhibitory activity of flavonoids using *in silico* docking studies. In this perspective, flavonoids like morin, naringenin, taxifolin, esculatin, daidzein, genistein, scopoletin, galangin and silbinin were selected. Azelastine, a known lipoxygenase inhibitor was used as the standard. Docking results showed that all the selected flavonoids showed binding energy ranging between -3.9 kcal/mol to -3.4 kcal/mol when compared with that of the standard (-3.7 kcal/mol). Intermolecular energy (-5.7 kcal/mol to -4.6 kcal/mol) and inhibition constant (1.3 mM to 3.4 mM) of the ligands also coincide with the binding energy. Morin contributed better lipoxygenase inhibitory activity because of its structural parameters.

Introduction

Drug design is an important tool in the field of medicinal chemistry where new compounds are synthesized by molecular or chemical manipulation of the lead moiety in order to produce highly active compounds with minimum steric effect (Cavasotto and Abagyan, 2004). A huge breakthrough in the process of drug design was the development of *in silico* method to predict about the therapeutic efficacy of the molecule (Saleshier et al., 2011; Khokra et al., 2011).

AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Schames et al., 2004). Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy (Cosconati et al., 2010).

Inflammation is a common process which precedes the destruction of cells leading to various unrelated disorders like, cancer, diabetes, Alzheimer's disease, Parkinson's disease, heart diseases, stroke, arthritis, multiple sclerosis, etc. (Brash, 1999). Inflammation persists when the immune system is continuously activated and this chronic inflammation leads to continued destruction of cells and thus leads to chronic diseases (Cotter et al., 1995).

Inflammatory mediators are soluble, many of which may be regarded as local hormones and play a key role in the orchestratrion of the inflammatory response. These inflammatory mediators are mainly tissue products such as histamine, serotonin, prostanoids, leukotrienes, platelet activating factor, bradykinin, neuropeptides, cytokines, lipoxins, chemokine and interferons. Lipoxins are the products of lipoxygenases and chemically conjucated trihydroxyl tetracenes (Mayes and Botham, 2003).

Lipoxygenases are a family of non-heme iron containing enzymes that catalyze the oxygenation of polyenic fatty acids such as arachidonic acid to corresponding lipid hydroperoxide products including leucotrienes, lipoxins, hydroxyl eicosatetraenoic acids (HETEs) (Vadivu and Lakshmi, 2008; Yu et al., 2007). Three different lipoxygenases insert oxygen into the 5, 12 and 15 positions of arachidonic acid, giving rise to hydroperoxides of eicosatetraenoic acids (HPETEs) (Orak et al., 2010).

The stereochemistry of binding of the flavonoids on lipoxygenase has not yet been characterized. In the present study, the structural models of the ligands in the lipoxygenase binding sites has been carried out, which may facilitate further development of more potent anti inflammatory agents.

Materials and Methods

Software required

The following software were downloaded- Python 2.7-language (www.python.com), Cygwin, Python 2.5 (www.cygwin.com), Molecular Graphics Laboratory tools, AutoDock 4.2 (www.scripps.edu), Discovery Studio Visualizer 2.5.5 (www.accelerys.com), Molecular Orbital Package and Chemsketch (www.acdlabs.com). Online Smiles Translation was carried out using cactus.nci.nih.gov/translate/.

Docking methodology

We employed the LGA for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first builds a population of individuals (genes), each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation and rotation and the local search algorithm then performs energy minimizations on a user-specified proportion of the population of individuals. The individuals with the low resulting energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian because every new generation of individuals is allowed to inherit the local search adaptations of their parents.

An extended PDB format, termed as PDBQT file was used for coordinate files which includes atomic partial charges. AutoDock tools was used for creating PDBQT files from traditional PDB files (Madeswaran et al., 2011). Crystal structure of lipoxygenase enzyme was downloaded from the Brookhaeven protein data bank (Figure 1).

The flavonoid ligands like morin, naringenin, taxifolin, esculatin, daidzein, genistein, scopoletin, galangin, silbinin and azelastine were built using Chemsketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies. The optimized ligand molecules were docked into refined lipoxygenase model using "LigandFit" in the AutoDock 4.2 (Goodsell et al., 1996; Figure 2).

The preparation of the target protein 3D3L (unbound target) with the AutoDock tools software involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gasteiger charges are calculated for each atom of the macromolecule in AutoDock 4.2 instead of Kollman charges which were used in the previous versions of this program. Three-dimensional affinity grids of size 277 × 277 × 277 Å with 0.6 Å spacing were centered on the geometric center of the

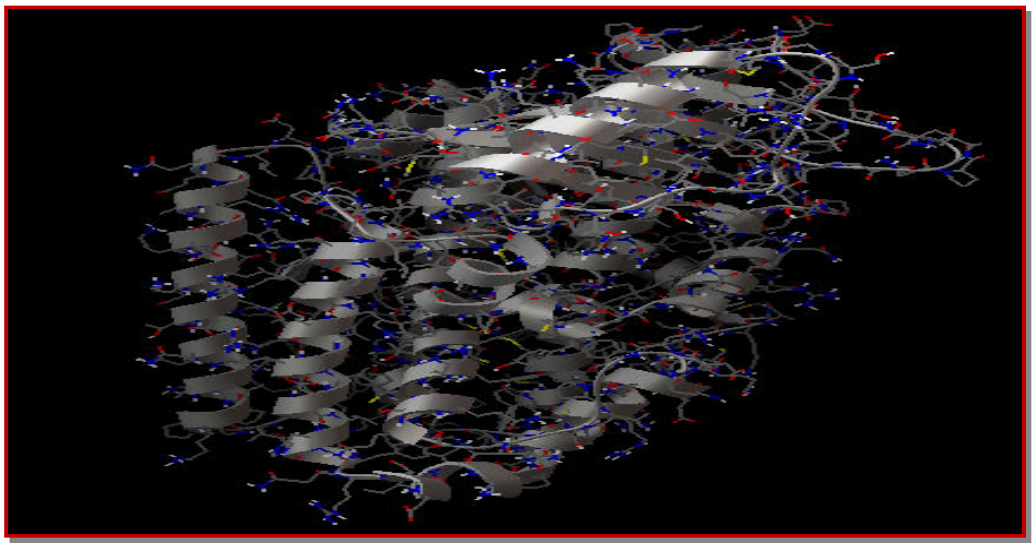

Figure 1: Lipoxygenase enzyme from RCSB (3D3L)

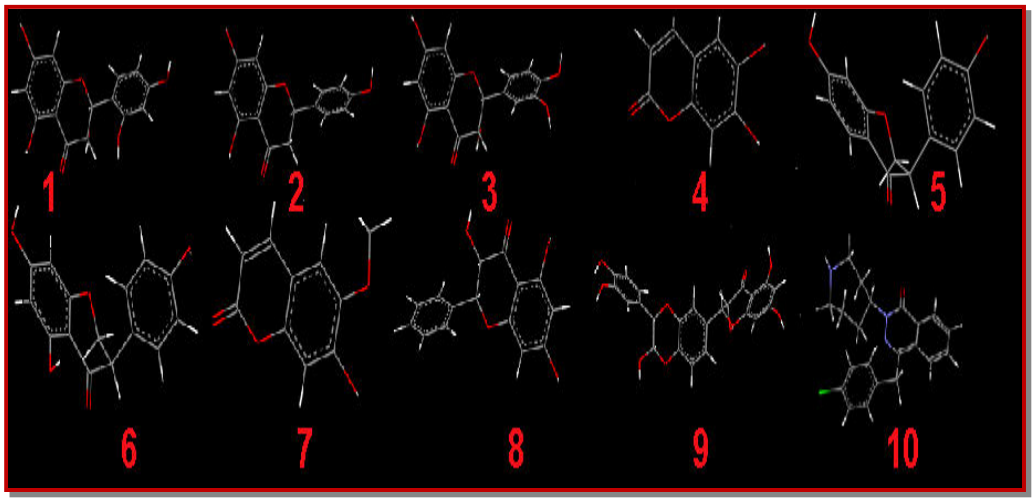

Figure 2: The optimized ligand molecules (1 morin, 2 naringenin, 3 taxifolin, 4 esculatin, 5 daidzein, 6 genistein, 7 scopoletin, 8 galangin, 9 silbinin and 10 azelastine)

target protein and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all possible atom types in a protein. Additionally, an electrostatic map and a desolvation map were also calculated (Konc et al., 2011).

Rapid energy evaluation was achieved by precalculating atomic affinity potentials for each atom in the ligand molecule. In the AutoGrid procedure, the target enzyme was embedded on a three dimensional grid point (Umamaheswari et al., 2011). The energy of interaction of each atom in the ligand was encountered.

We have selected important docking parameters for the LGA as follows: Population size of 150 individuals, 2.5 million energy evaluations, maximum of 27,000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on an individual in the population was set to 0.06. Unbound target 3D3L and unbound ligands were both treated as rigid.

AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates (Chang et al., 2010). AutoDock tools provide various methods to analyze the results of docking simulations such as, conformational similarity, visualizing the binding site and its energy and other parameters like intermolecular energy and inhibition constant. For each ligand, ten best poses were generated and scored using AutoDock 4.2 scoring functions (Park et al., 2006).

Results and Discussion

In silico docking study, was carried out to identify the inhibiting potential of selected flavonoids against lipoxygenase enzyme. In this study 9 different flavonoids were selected for the *in silico* docking studies. Lead optimization of the selected compounds was done by computation of druglikeness properties. The druglikeness scores of the compounds were evaluated with the help of Lipinski's rule. The docking studies were performed by the use of AutoDock 4.2. In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that the compound has higher activity.

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses (Sivakumar et al., 2011; Zhang et al., 2008). Docked pose of lipoxygenase enzyme with the ligands azelastine and morin clearly demonstrated the binding positions of the ligand with the enzyme (Figure 3). Binding energy of the individual compounds were calculated using the following formula,

Binding energy = A + B + C - D

Where, A denotes final intermolecular energy + Wandervalls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol), B denotes final total internal energy (kcal/mol), C denotes torsional free energy (kcal/mol), D denotes unbound system's energy (kcal/mol)

Most of the flavonoids have anti inflammatory properties. Therefore the consumption of flavonoids could be appropriate in medical conditions involving inflammation. Flavonoids are the excellent anti-

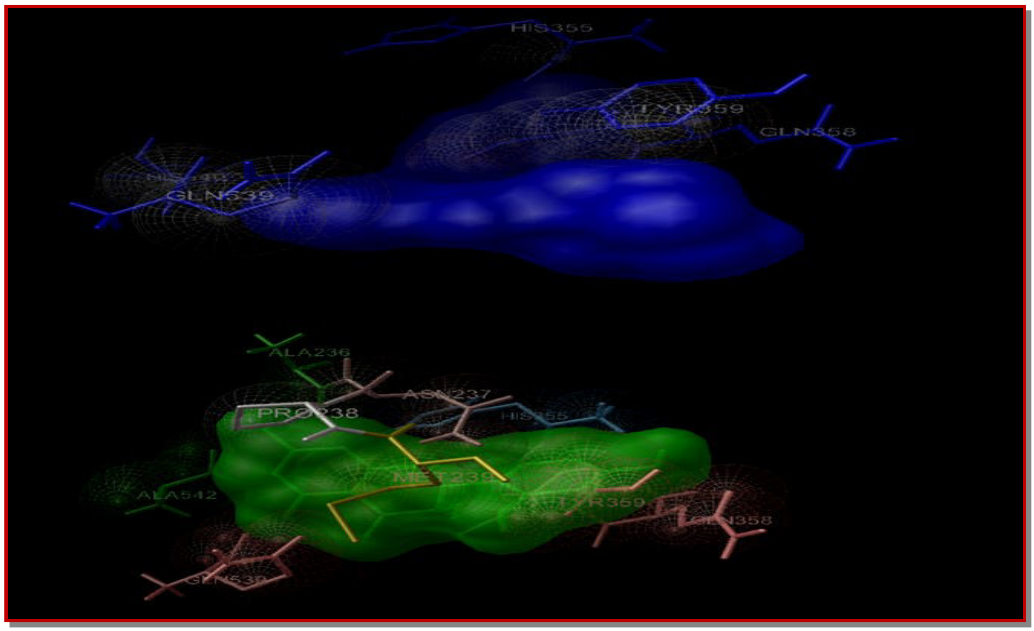

Figure 3: Docked pose of lipoxygenase enzyme (3D3L) with azelastin and morin

oxidants when compared to other compounds. Extracts from onion and different flavonoids activate the cellular antioxidant system.

Analysis of the receptor/ligand complex models generated after successful docking of the flavonoids was based on the parameters such as hydrogen bond interactions, π-π interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site (Azam et al., 2011). As a general rule, in most of the potent anti inflammatory compounds, both hydrogen bond and π-π hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity.

The binding sites of the Azelastin was found to be HIS 355, TYR 359, GLN 358, GLN 539 and HIS 540. The potential binding sites of the Morin was found that, ALA 236, PRO 238, ASN 237, MET 239, HIS 255, GLN 358, TYR 359, GLN 539, and ALA 542. This proves that the effective binding sites are present in the selected flavonoid Morin when compared with the standard Azelastine. It proves that the ability of inhibiting the lipoxygenase enzyme by the selected compound.

Flavonoids showed binding energy ranging between -3.9 kcal/mol to -3.4 kcal/mol (Table I). From the selected flavonoids Morin had showed better binding energy (-3.9 kcal/mol) when compared to the standard Azelastine (-3.7 kcal/mol). This proves that morin consist of potential lipoxygenase inhibitory binding sites when compared to the standard. Genistein showed binding energy -3.4 kcal/mol which indicates that the lesser potential of inhibiting lipoxygenase enzyme.

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. Inhibition constant is directly proportional to binding energy. Theoritical IC_{50} was calculated with the help of AutoDock 4.2. Flavonoids showed inhibition constant ranging from 1.3 mM to 3.4 mM (Table II). Morin had better inhibition constant (1.3 mM) when compared to the standard (1.9 mM). This implies that the Morin were found to be higher activity against lipoxygenase enzyme when compared to Azelastine. We found a decrease in inhibition constant of all the selected flavonoids with a simultaneous decrease in the binding energy. Genistein showed inhibition constant value 3.4 mM, which proves that the higher concentration is required for the inhibition of the lipoxygenase enzyme. Furhter it indicates that, the potency of the compound.

Intermolecular energy is also directly proportional to binding energy. We found a decrease in intermolecular energy of all the selected compounds with a simultaneous decrease in the binding energy. Flavonoids showed intermolecular energy ranging between -5.7 kcal/mol to -4.6 kcal/mol (Table III). Morin had better intermolecular energy (-5.7 kcal/mol) when compared to the standard (-4.6 kcal/mol). This result further proved that morin consist of better lipoxygenase inhibitory activity compared to the standard.

Based on the docking studies, the lipoxygenase inhibitory activity of the selected compounds was found to be decreased in the order of morin, azelastine, daidzein, naringenin, esculatin, galangin, scopoletin, taxifolin, silbinin and genistein. All the flavonoids possess binding sites with lipoxygenase enzyme. But,

Table I

Binding energies of the compounds based on their rank

Compounds	Binding energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Morin	-3.9	-3.9	-3.9	-3.8	-3.8	-3.8	-3.8	-3.8	-3.8	-3.8
Naringenin	-3.6	-3.6	-3.6	-3.5	-3.5	-3.3	-3.3	-3.1	-3.2	-3.2
Taxifolin	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5
Esculatin	-3.6	-3.6	-3.6	-3.6	-3.6	-3.5	-3.5	-3.5	-3.4	-3.4
Daidzein	-3.7	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.2
Genistein	-3.4	-3.3	-3.2	-3.0	-2.9	-2.9	-2.9	-2.9	-2.8	-2.8
Scopoletin	-3.6	-3.5	-3.5	-3.5	-3.5	-3.3	-3.3	-3.3	-3.3	-3.2
Galangin	-3.6	-3.6	-3.5	-3.5	-3.5	-3.3	-3.2	-3.2	-3.2	-3.2
Silbinin	-3.5	-3.1	-3.0	-3.0	-2.9	-2.7	-2.6	-2.5	-2.0	-1.7
Azelastine	-3.7	-3.7	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.4

Table II

Inhibition constant of the compounds based on their rank

Compounds	Inhibition constant of the compounds based on their rank (mM)									
	1	2	3	4	5	6	7	8	9	10
Morin	1.3	1.4	1.5	1.6	1.7	1.7	1.7	1.7	1.8	1.8
Naringenin	2.2	2.2	2.2	2.9	2.9	3.8	3.9	5.0	4.3	4.3
Taxifolin	2.7	2.7	2.7	2.7	2.7	2.8	2.8	2.8	2.9	3.0
Esculatin	2.4	2.4	2.4	2.4	2.5	2.5	2.6	2.6	3.1	3.1
Daidzein	2.0	3.1	3.1	3.2	3.2	3.2	3.2	3.3	3.3	4.2
Genistein	3.4	4.1	4.5	6.8	7.1	7.2	7.3	8.2	8.6	8.7
Scopoletin	2.4	2.6	2.6	2.6	2.7	3.6	3.6	3.8	3.9	4.3
Galangin	2.4	2.5	2.6	2.6	2.6	3.7	4.2	4.9	4.5	4.5
Silbinin	3.0	5.2	6.5	6.7	7.0	11.3	12.1	15.3	36.7	57.3
Azelastine	1.9	2.0	2.9	2.7	2.7	2.7	2.7	2.7	2.8	2.9

Table III

Intermolecular energies of the compounds based on their rank

Compounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Morin	-5.7	-5.7	-5.7	-5.6	-5.6	-5.6	-5.6	-5.6	-5.6	-5.5
Naringenin	-4.8	-4.8	-4.8	-4.7	-4.7	-4.5	-4.5	-4.3	-4.4	-4.4
Taxifolin	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.3	-5.2
Esculatin	-4.2	-4.2	-4.2	-4.2	-4.2	-4.1	-4.1	-4.1	-4.0	-4.0
Daidzein	-4.6	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.3	-4.1
Genistein	-4.6	-4.5	-4.4	-4.2	-4.1	-4.1	-4.1	-4.0	-4.0	-4.0
Scopoletin	-4.2	-4.1	-4.1	-4.1	-4.1	-3.9	-3.9	-3.9	-3.9	-3.8
Galangin	-4.8	-4.7	-4.7	-4.7	-4.7	-4.5	-4.4	-4.3	-4.4	-4.4
Silbinin	-5.8	-5.5	-5.4	-5.4	-5.3	-5.0	-5.0	-4.9	-4.3	-4.1
Azelastine	-4.6	-4.6	-4.4	-4.4	-4.4	-4.4	-4.4	-4.4	-4.4	-4.5

only the morin showed better binding interactions and docking parameters (binding energy, inhibition constant, intermolecular energy) than the other selected flavonoids and the standard.

On the basis of the above study, morin possess potential lipoxygenase inhibitory binding sites and docking parameters compared to that of the standard. This may be attributed due to the differences in the position of the functional groups in that compound. Further development of morin and their derivatives could be a potential drug candidate for the lipoxygenase inhibition in future.

Conclusion

These results indicate that from the selected flavonoids,

morin have better binding sites and interactions with lipoxygenase enzyme.

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Conflict of Interest

Authors declare no conflict of interest.

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