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Research Article

Molecular Docking of some Neem Constituents with COX-2 and NOs: An *in silico* Study

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ABSTRACT

Introduction: *Azadirachta indica* is commonly known as Neem, is found abundantly in India. This plant is used in the treatment of infections, pain and wounds. **Methods:** The aim of present study to investigate *in silico* molecular docking study used for three phytoconstituents β-sitosterol(22,23-Dihydrostigmasterol, Stigmast-5-en-3-ol, β-Sitosterin), isomeldenin and nimbandiol from Azadirachtaindica to identify whether these compounds interact with the COX-2 and NOs. The structure of neem constituents were downloaded from Pubchem and structure of enzymes/ proteins were obtained from protein data bank. **Results:** Among all the compounds β-sitosterol and nimbandiol showed best docking score. **Conclusion:** Further cell culture-based studies might ensure the interaction of these constituents with COX-2 and NOs.

Key words: Neem, Docking COX-2, NOs, *Azadirachta indica* is, Infections, Pain, β-sitosterol, Isomeldenin, Nimbandiol.

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INTRODUCTION

The cyclo-oxygenase is a principal isoenzyme associated with biosynthesis of thromboxane and prostaglandins. Prostaglandins are autacoids which play vital role in various physiological and pathological events. The COX-1 is expressed in many tissues viz. stomach, kidney, brain, lungs, spleen etc., whereas, COX-2 is an inducible enzyme which is expressed during damage to tissues. The use of analgesics is associated with renal and gastrointestinal toxicities (due to COX-1 inhibition) which restrict the use of potent analgesics during pain and inflammation. Nitric oxide is a cell signaling entity which is known to induce inflammation. The biosynthesis of NO is due to activity of nitric oxide synthase enzyme. This enzyme bio transforms arginine into citrulline, as a result, NO is produced. NO is associated with over-expression of immune response by cytokine activated macrophages. The adverse effects associated with overproduction of NO include inflammation, vasoconstriction and tissue damage. NO is responsible for pathogenesis of inflammatory disorders of the gut, lungs and joint.

Man has been using medicine in form of herbs since ancient times.[1] Ayurveda includes compilation of bioactive herbs found in Indian sub-continent. The method of preparation doses and precautions are also mentioned for usage of these herbs.[2] Neem (*Azadirachta indica*) is native to India, Sri Lanka, Nepal and Bangladesh. It is well recognized for its various medicinal properties like antibacterial, antifungal, antidiabetic, sedative and contraceptive effect.[3] Neem is also associated with a dose dependent analgesic effect in experimental animal model. The mechanism of analgesic effect seems to be mediated by central as well as peripheral effects.[4,5] The present study aims to study *in silico* interactions of some Neem constituents with COX-2 and NOs enzymes.

Experimental

Software

Python 2.7- language was downloaded from www.python.com, Molecular graphics laboratory (MGL) tools and AutoDock 4.2 was downloaded from www.scripps.edu, Discovery Studio visualizer 4.1 was downloaded from www.accelerys.com.

Docking studies

Molecular docking was performed on Neem phytochemicals (β-sitosterol, isomeldenin and nimbandiol) against COX-2 and NOs. The structures of these phytochemicals were downloaded from PubChem. Protein structure files (PBD ID: 4COX for COX-2 and PBD ID:5UO1for NOs) were downloaded from PDB (www.rcsb.org/pdb) and edited by removing the hetero atoms with simultaneous adding of C terminal oxygen. For docking calculations, Gasteiger Marsili partial charges were assigned to the ligands, non-polar hydrogen atoms were merged and all torsions were allowed to rotate during docking. Active pockets were identified and ligplot of PDB. Computed atlas of surface topography of proteins server was used to cross-check the active pockets on the target protein molecules. The Lamarckian genetic algorithm was applied for energy minimization using default parameters. The docking results were visualized by Discovery Studio.

RESULTS

In the present study, docking was carried out on active sites of two target proteins 4COX and 5UO1 with β-sitosterol, isomeldenin and nimbandiol. Docking interactions of these targets with β-sitosterol, isomeldenin an dnimbandiol are presented in Figure 1-2 and Table 1-2. The results of docking analysis of COX-2 and NOs enzyme are listed in Table 1 and 2. After docking the ligand protein complex was saved in pdb format then subjected for analysis in the Accelrys Discovery Studio Visualizer. Docking studies showed that β-sitosterol as well as Isomeldenin has best docking scores for COX-2 and NOs. Interaction between the neem constituents with the COX-2 and NOs are represented in Figure 1 and 2.

DISCUSSION

Advances in computational techniques have helped bio information to screen potential drug molecules. The process of virtual screening has created a positive impact on the discovery and development of new drug. The process of virtual screening uses docking and scoring of each compound from a dataset. This technique based on prediction of binding

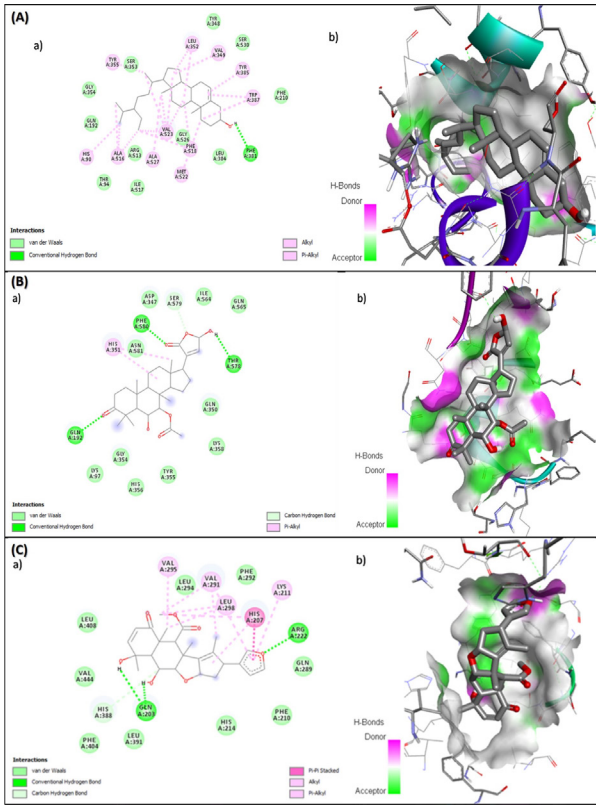

Figure 1: Docking interaction of some neem constituents with COX-2. β-sitosterol (b)isomeldenin (c)nimbandiol

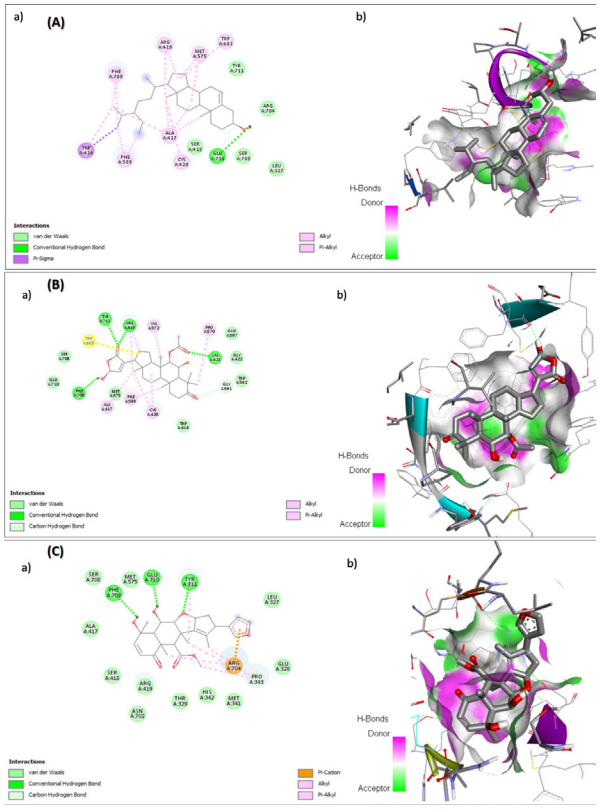

Figure 2: Docking interaction of some neem constituents with NOs. β-sitosterol (b)isomeldenin (c)nimbandiol

Table 1: Docking results of some neem constituents with COX-2.

COX-2	Binding Energy (k cal/mol)	Inhibition constant
β-sitosterol	-12.38	848.52pM
Isomeldenin	-7.24	4.89 μM
Nimbandiol	-6.97	7.76μM

Table 2: Docking results of some neem constituents with NOs.

NOs	Binding Energy (k cal/mol)	Inhibition constant
β-sitosterol	-10.6	16.36 μM
Isomeldenin	-10.24	30.06 nM
Nimbandiol	-8.74	31.06 nM

modes and binding affinities of each compound in the dataset. In the present studies, by using Auto dock, the three neem constituents (β-sitosterol, isomeldenin and nimbandiol) active site of the COX-2 and NOs enzymes. Docking studies showed that β-sitosterol as well as Isomeldenin has best docking scores for COX-2 and NOs. Some previous studies have established analgesic effect of neem on experimental animals.[6,7] Similarly analgesic effects of these constituents have been reported.[8-10] The outcomes of the present study establish a notable interaction between selected neem constituents and inflammatory mediators.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

pdb: Protein Data Bank; **COX-2:** cyclooxygenase; **NOs:** Nitric oxide synthase.

SUMMARY

The outcomes of the present study justifies the promising interactions of neem constituents against pain COX-2 and NOs.

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