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In silico interaction of catechin with some immunomodulatory targets: A docking analysis

Aditya Ganeshpurkar[1,2] and Ajay Saluja[*1,3]

[1]Faculty of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat

[2]Shri Ram Institute of Technology- Pharmacy, Jabalpur, Madhya Pradesh, India

[3]AR College of Pharmacy, Vallabh Vidyanagar, Gujarat

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The use of plant products as immunomodulator has a long history. For eternal era, plant, mineral and animal products are used as drugs for the treatment of various diseases. The present-day synthetic compounds find their leads in natural products. The process of immunomodulation amends the immune system of an individual by prying with its usual functions. Research of immunomodulators from natural sources has been extensively studied for modulation of immune system along with protection and prevention of diseases. Catechin, a flavonoid has been investigated for its potential anti-inflammatory effects. Analgesic effects have been observed for catechin in experimental animals. The present study is focused on exploring the *in silico* interaction of catechin with some chemokines and inflammatory targets. In this study, catechin was docked with tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2). Docking studies revealed the interaction of catechin with these targets. The result of present study provides insight for the discovery of novel molecules for immunomodulation and treatment of inflammatory disorders. Findings from the present study show that catechin interact with several chemokines and inflammatory mediators. Further studies quantitative structure activity relationship and quantitative structure property relationship on catechin and associated flavonoids are necessary to develop and establish studies which may serve a stepping stone for the development of novel and safe immunomodulator. Catechin, can, therefore, can be considered as a candidate for development of an immunomodulatory agent.

Keywords: Catechin, immunomodulatory agent, inflammation, cytokines, nitric oxide

Introduction

The immune system is a comprehensive system consisting of various cellular and humoral mediators that are responsible for defending the body against numerous environmental challenges. Chemokines released from macrophages play a vital role in such processes[1]. In the presence of bacterial endotoxin lipopolysaccharide, macrophage tends to generate various types of mediators like interleukins (IL-1β, IL-6) and TNF-α[2]. Along with nitric oxide (NO), these mediators play an essential role in the progression of inflammation[3-4]. Immunomodulators are the agents that amend the activity of immune system and have dual effects. A few of them may stimulate the immune system at a lower level while the others may have inhibitory effects on standard or activated immune response[5]. The phenomena of immunosuppression and immunostimulation are often desired to control the normal functioning of immune system. Immunomodulators from a natural source can serve as an alternative to 'conventional chemotherapy' mainly when there is need to activate host defence mechanism during innate impaired immune response[6]. From centuries, medicinal plants are an integral part of health care system[7]. Many synthetic compounds used as drugs find their structural similarity with natural compounds[8]. Flavonoids are one of the principal classes of natural products that find multiple roles for the promotion of human health[9]. Catechin is an important flavonoid that is abundantly found in plants. Studies have established anti-inflammatory[10], and analgesic[11]. In a study, catechin inhibited nitric and COX-2 enzyme production[12]. It also suppressed production of IL-1β[13] and TNF-α[14]. The present study aims to explore the *in silico* interaction of catechin with interleukins, TNF-α and NOs.

*Author for correspondence:
Tel: 91-2692-230788
akspharmacy@yahoo.com, adityaganeshpurkar@gmail.com

Materials and Methods

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.

Protein Preparation

The three-dimensional crystalline structures of 5 targeted diabetic proteins (Table 3) were retrieved from the Protein Data Bank (http://www.rcsb.org/). The retrieved protein were TNF-α (PDB ID: 2AZ5), IL 1β (PDB ID: 2NVH), IL 6 (PDB ID: 1P9M), iNOs (PDB ID: 1NSI) and COX-2 (PDB ID: 1CVU). The coordinates of the structures were complexed with water molecules, and other atoms which are responsible for increased resolution and therefore the water molecules and het-atoms were removed using Discovery Studios and saved in .pdb format.

Ligand Selection and Energy Minimization

The 2D and 3D chemical structures of ligand (catechin) which were available on PubChem database were retrieved (http://pubchem.ncbi.nlm.nih.gov/). All the .sdf and .mol files obtained from PubChem were converted into .pdb files using the Marwin Sketch (http://www.chemaxon.com/marvin/ sketch/index.jsp). The ligand was energy minimized using Marwin Sketch which resulted in 10 different energy conformers. Among all, the conformer showing lowest binding energy was selected and saved in .pdb format.

Docking Analysis

The docking analysis of catechin was carried out using the Autodock Tools (ADT) v1.5.4 and autodock v4.2 program; (Autodock, Autogrid, Autotors, Copyright-1991e2000) from the Scripps Research Institute, http://www.scripps.edu/mb/olson/doc/autodock. To run autodock, we used a searching grid extended over ligand moieties, Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and nonpolar hydrogens were merged with the carbons, and the internal degrees of freedom and torsions were set. Citrus flavonoids were docked to all the target protein complexes with the molecule considered as a rigid body. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic maps, were computed with a grid spacing of 0.375. The search was carried out with the Lamarckian genetic algorithm; populations of 100 individuals with a mutation rate of 0.02 have been evolved for 10 generations. The remaining parameters were set as default. A root mean square deviation (RMSD) tolerance for each docking was set at 2.0. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on RMSD values, with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most reliable solution.

Results

The five crystal structures of proteins were retrieved from PDB. A docking program was performed to simulate the binding mode to identify the precise binding sites on various immunomodulatory targets. Docking studies were carried out on active sites of five target proteins 2AZ5, 2NVH, 1P9M, 1NSI and 1CVU with catechin. Figure 1-4 indicates the interaction of catechin with

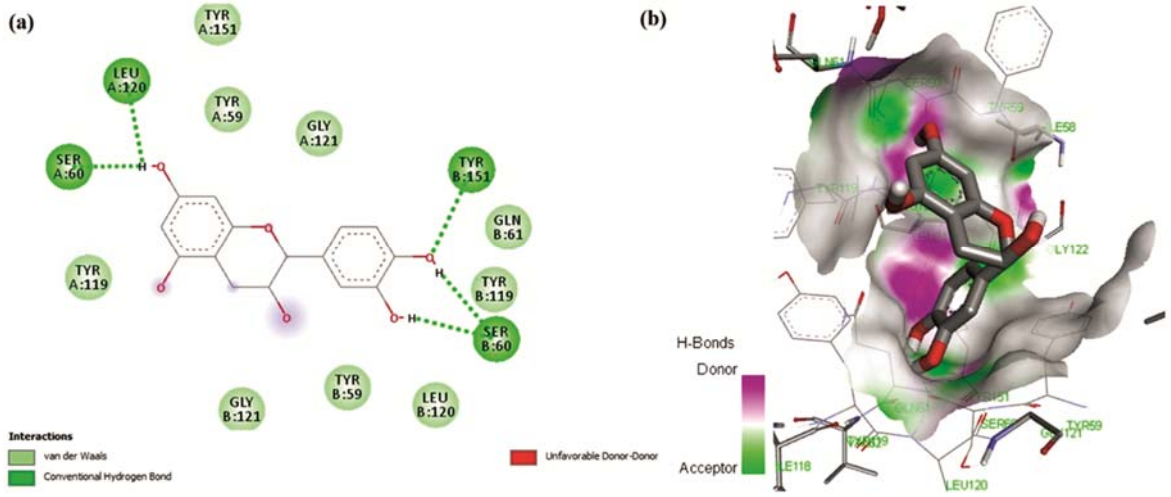

Fig. 1 — Molecular docking studies of catechin against TNF-α [(a) 2D-interactions (b) 3D-interactions].

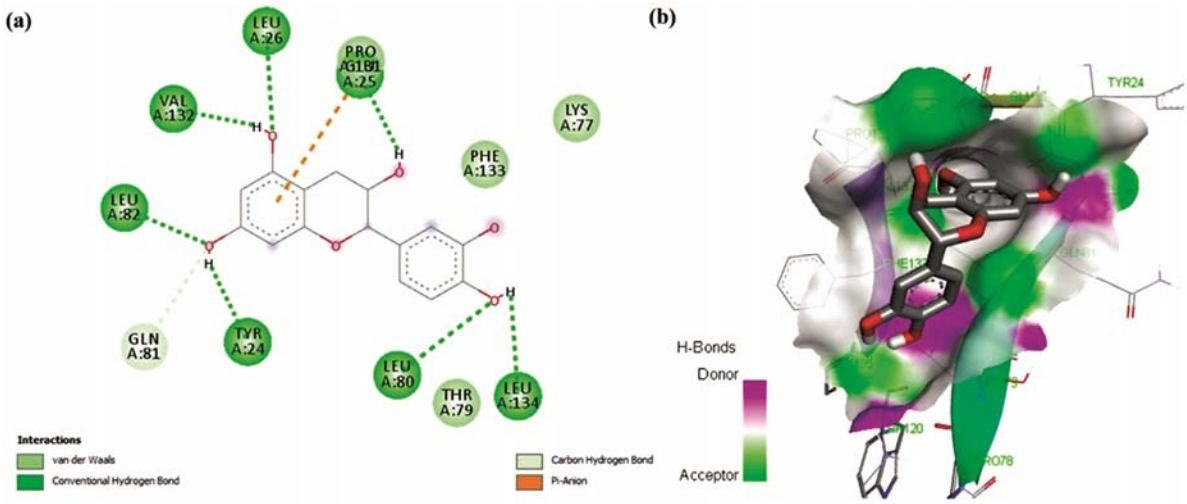

Fig. 2 — Molecular docking studies of catechin against IL-1β [(a) 2D-interactions (b) 3D-interactions].

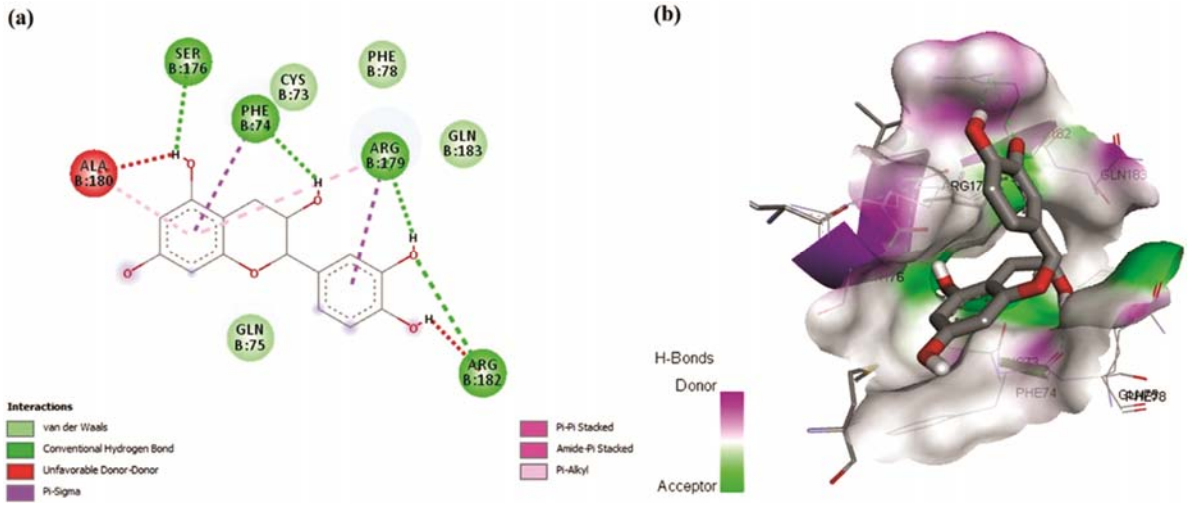

Fig. 3 — Molecular docking studies of catechin against IL-6 [(a) 2D-interactions (b) 3D-interactions].

the active pocket of immunomodulatory targets which demonstrated minimum binding energy with objectives via non-covalent interaction. The binding energy ranged from -5.79 to -7.50 kcal/mol. Binding energy for TNF-α was -5.79 kcal/mol, interleukin 1β -7.50 kcal/mol, interleukin 6 -6.20 kcal/mol, inducible nitric oxide synthase -7.44 kcal/mol. The docking of catechin with TNF-α revealed 'hydrogen bond' as an interacting force. Bonding at serine 60 and leucine 120 on backbone chain A and serine 60 and tyrosine 151 on backbone chain B were hydrogen bonded with a hydroxyl group. In case of IL-1β oxygen of hydroxyl group was bound to tyrosine 24, leucine 26, leucine 80, leucine 82, valine 132 and leucine 134 of A chain. With proline 131, catechin revealed the existence of Pi- anion bond. Interaction of catechin was full of multiple bond. Hydrogen bonding was observed at phenylalanine 74, serine 176, arginine 179 and arginine 182. With glycone as well as aglycone moiety, there was π-π interaction with phenylalanine 74 and arginine 179. Concerning interleukin-6, two hydroxyl groups of benzopyrone nucleus (viz. OH at C-3 and OH at C-5) demonstrated hydrogen bonding type interactions with phenylalanine 74 and serine 176 respectively. Arginine at 179 and 182 position represented hydrogen bonding interactions. Apart from displaying hydrogen interactions, phenylalanine 74 and arginine 179 at B chain demonstrated crucial interaction. Additionally, arginine at 179 also revealed π-alkyl bond. Less favoured interactions at alanine 120 of B chain were also seen. Multiple interactions were observed in docking of catechin with NOS. Hydrogen bonding was seen at serine 418, leucine 705 and

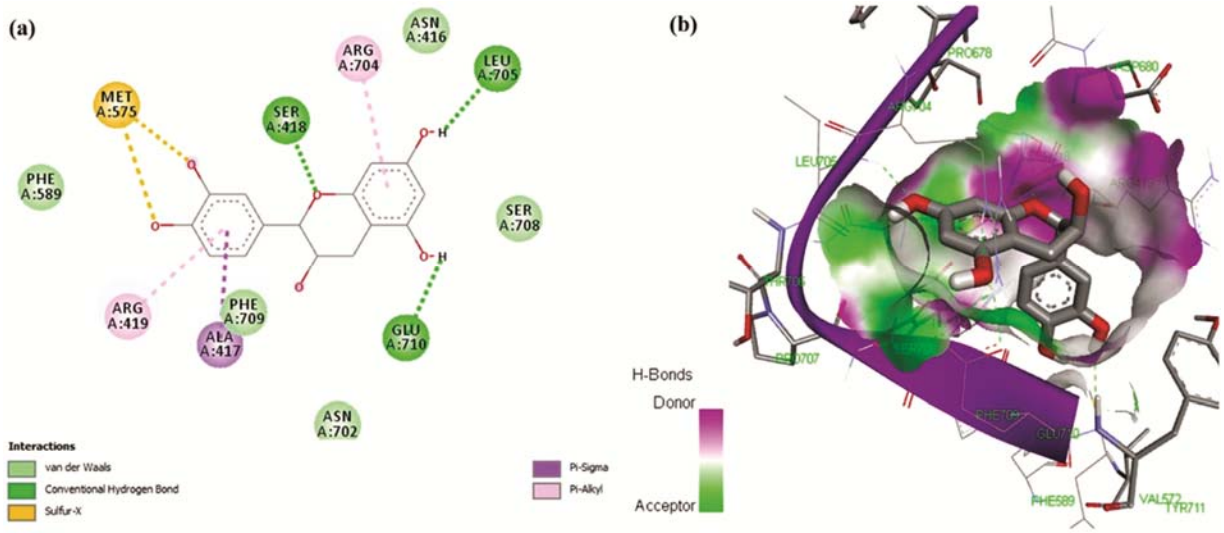

Fig. 4 — Molecular docking studies of catechin against NOs [(a) 2D-interactions (b) 3D-interactions].

glutamine 710 at A chain. With catechin (ring B), catechin interacted with methionine 575 by sulfide bond. A π- π interaction of arginine 174 with ring B of catechin was notable. Arginine 419 and arginine 714 interacted with A and C ring of catechin via the π-alkyl bond.

Discussion

Monocytes and activated macrophages produce tumour necrosis factor-alpha (TNF-α). TNF-α is designed by the biological system for paracrine signalling. TNF-α binds to TNF receptor. The various cellular response produced by TNF-α is due to binding of TNF-α to TNFR1 or TNFR2 receptors. The death-inducing activity of TNFR1 is due to the presence of 80 amino acid containing 'death domain' present at carboxyl terminal. Bonding at serine 60 and leucine 120 on backbone chain A and serine 60 and tyrosine 151 on backbone chain B were hydrogens bonded with a hydroxyl group. On the contrary, the 6,7-dimethyl-3-[(methyl{2-[methyl ({1-[3-(trifluoromethyl)phenyl]- 1h-indol-3 yl}methyl) amino]ethyl}amino)methyl]-4h-chromen-4-one present in actual PDB demonstrated binding at tyrosine 59 and tyrosine 119 located at B chain and glycine 121 at A chain [15]. Binding of capsazepine to 2AZ5 showed similarity with catechin. Both molecules revealed interactions at serine 60 and leucine 120. These docking studies are supported by some *in vivo* /'studies wherein catechin treatment decreased TNF-α expression[16].

Cytokines are cellular signalling molecules that intensify the local and systemic immune response. Thus, an increase in the levels of interleukins is considered as an increase in 'immune response.' Cytokines are essential for stimulation of T and B lymphocytes. T-helper lymphocytes (Th) divide and form Th1 and Th2 cells. Th1 are accountable for pro-inflammatory cellular immunity and express IL-2 whereas Th2 cells secrete IL-6 and facilitate humoral immunity[17-18]. IL-1β with IL-6 and TNF-α is responsible for producing hyperalgesia[19]. In the event of injury, interleukin-1β supports the expression of interleukin-6[20]. Drugs acting against TNF-α and proinflammatory interleukins like IL-1β and IL-6 seems to be useful against psoriasis, Crohn's disease, rheumatoid arthritis with decreased production of COX-2[21]. IL-1β is expressed in various cells during acute and chronic inflammation. Two isoforms of interleukins viz. IL-1α and IL-1β are observed. However, expression of IL-1β gene is spontaneously seen. The binding site on A chain of IL-receptor includes some amino acid residues viz. 11, 13-15, 20-22, 27, 29-36, 38, 126-131, 147 and 149[22]. In the present docking study, catechin interacted with some amino acid residues of IL-1 receptor next to aforementioned active sites which include tyrosine 24, leucine 26 and valine 132. The binding energy of interaction between catechin and IL-1 receptor was -7.50. The phenomenon of the interaction of IL-1β with the IL-1 receptor is the first pace involved in the interaction. Blocking or prevention of such interaction is the first step in the development of antagonist. In an experiment, catechin analogue (epigallocatechin 3-gallate) inhibited the response of IL-1 expression[23-25]. Thus, binding of catechin with some of the crucial

residues of this site may depict one of its anti-inflammatory mechanisms of action.

Interleukin 6 (IL-6) is another proinflammatory cytokine[26]. IL-6 is secreted by macrophages and T-cells and aids in the initiation of the immune response after burns trauma and injury. Cell-surface type I cytokine receptor complex comprising CD126 (IL-6Rα chain) and CD130 (signal-transducing component gp130. IL-6 interacts with its receptor and activates CD130 and IL-6R proteins resulting in the formation of a complex resulting in receptor activation. Due to IL-6 receptor activation inflammation and the autoimmune response is activated which tend to be involved in the pathogenesis of rheumatoid arthritis[27]. The phenomenon of binding of catechin especially at arginine 179 of IL-6 receptor could be a reason for its anti-inflammatory effect. Such a prediction is supported by specific biological studies[10,28-29] where catechin prevented of expression of IL-6.

Inducible nitric oxide synthase (iNOS) synthesises nitric oxide from L-arginine[30]. The expression of TNF-α, IL-1 follows iNOS production. It is abundantly found in macrophages and monocytes at the site of infection or inflammatory disease[31]. The biosynthesis of nitric oxide (NO) prevents bacterial growth and retards inflammation (due to suppression of proliferation of T cells)[32]. Overproduction of (NO) and 'reactive nitrogen intermediates' is associated with the progression of many inflammatory diseases[33]. The embarrassment of this enzyme may play a vital role in anticipation of inflammation. There are many natural products which have been studied for inhibition of inducible iNOS; for instance, flavonoids from *Tanacetum microphyllum*, especially ermanin and 5,3'-dihydroxy-4'-methoxy-7-methoxycarbonylflavonol were most potent inhibitors of this enzyme[34]. In NOs, the arginine urea group forms a bidentate interaction with Glu-377 adjacent to the active site. It is a site of larger competitive inhibitors that may inhibit NOS[35]. Catechin retained all the primary interaction shown by co-crystallized NOS inhibitor (PDB ID: 5UO1). Catechin kept the critical interaction with porphyrin ring; this porphyrin ring plays a vital role in catalytic enzyme mechanism. In the present study, *in silico* docking studies revealed inhibition of NOS by catechin.

Conclusion

In the present study, we carried out docking studies on catechin to various inflammatory and immunomodulatory targets, with the purpose to study and analyse *in silico* interaction of former on later. The docking scores and analysis of the interactions of catechin suggest the ability of catechin to bind to multiple targets involved in inflammation and immunomodulation. Catechin interacted with various chemokines and inflammatory mediators' viz. TNF-α, IL1β, IL6, iNOs and COX-2. With each target, catechin demonstrated a noteworthy affinity for binding. Findings from the present study show that catechin may interact with several chemokines and inflammatory mediators. Further studies on catechin and associated flavonoids are necessary to develop and establish QSAR and QSPR studies that may serve a stepping stone for the development of novel, efficient and safe immunomodulator.

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