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

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Forms eternal era, plant, mineral and animal products are used as drugs for the treatment of various diseases. The use of medicinal plants for immunomodulation has a long history. The modern medicinal compounds find their leads in natural products. Immunomodulation amends the immune system of an individual by prying with its usual functions. Discovery of immunomodulators from natural sources has been comprehensively made to modulate the immune system to prevent diseases. Hesperidin has been investigated for its potential anti-inflammatory effects. Hesperidin demonstrated analgesic effects in experimental animals. The present study is focused on exploring the *in silico* interaction of hesperidin with some chemokines and inflammatory targets. In this study, hesperidin was docked with TNF-α, IL-1β, IL-6, and NOs. Docking studies revealed the excellent interaction of hesperidin with these targets. The result of this work provided an insight into the discovery of novel molecules for immunomodulation and treatment of inflammatory disorders. Additional studies on hesperidin and associated flavonoids are necessary to establish its safety. Hesperidin, can, therefore, can be considered as a candidate for development of an immunomodulatory agent.

Keywords: Cytokines, Hesperidin, Immunomodulatory, Inflammation, Nitric oxide

The immune system is a comprehensive network that defends the body against invading pathogens. A breakdown in the functioning of the immune system makes it react in an inappropriate manner[1]. The damage to immune system poses a biological burden on the body by altering the immunological profile (due to allergy or autoimmunity)[2]. Thus, it seems necessary to modulate the immune system during the event of immunosuppression or immunostimulation. Both these classes of synthetic drugs (*i.e.* immunostimulants and immunosuppressants) act by redefining the functions of immune cells either by suppressing or stimulating their activities[3]. However, these drugs have long-term adverse effects like persistent immunosuppression, general weakness, alopecia, *etc*.

The phenomenon that immune responses are modulated to alleviate diseases has existed in many forms of traditional medicine beliefs, with plants being used in such systems to promote health and to maintain the body's resistance against infections by potentiating immunity[4]. Some of these plants are specifically stimulatory or suppressive, and normalize or modulate pathophysiological processes, and are thereby termed 'immunomodulatory'[5,6].

Flavonoids are one of the important phytoconstituents having varied biological effects on living systems. Owing to their antioxidant effects, flavonoids are acknowledged for hepatoprotective, nephroprotective and cardio-protective effects[7,8]. Flavonoids also interfere with the functioning of pro-inflammatory cytokines and chemokines. Hesperidin is citrus flavonoids[9,10]. It is acknowledged for prevention of capillary fragility. Hesperidin administration (orally) in rats significantly allayed irradiation-induced inflammation[11]. The modification of lymphocyte composition in the intestinal epithelium was observed due hesperidin[12]. Hesperidin treatment in mice significantly suppressed levels of TNF-α[13], IL-1β and IL-6[14] in mice. There was a decrease in the expression of NOs[15]. Based on the above evidence, the present study aims to explore *in silico* interaction of hesperidin with interleukins, TNF-α, and NOs.

Materials and methods

Software

Python 2.7-language was downloaded from www.python.com[16]. Molecular graphics laboratory (MGL) tools and AutoDock 4.2[17] was downloaded

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from www.scripps.edu, Discovery Studio visualizer 4.1[18] was downloaded from www.accelerys.com. Calculations were performed on Windows 8.0 Operating System.

Protein preparation

The three-dimensional crystalline structures of 4 targeted proteins were retrieved from the Protein Data Bank (http://www.rcsb.org/). The retrieved protein was TNF-α (PDB ID: 2AZ5), IL-1β (PDB ID: 2NVH), IL-6 (PDB ID: 1P9M) and NOs (PDB ID: 1NSI). 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.

Docking analysis

Docking studies were performed to analyze interactions of hesperidin with immunomodulatory targets[19]. The three-dimensional crystalline structures of 4 proteins were obtained from Protein Data Bank (http://www.rcsb.org/). These protein were TNF-α (PDB ID: 2AZ5), IL 1β (PDB ID: 2NVH), IL-6 (PDB ID: 1P9M) and NOs (PDB ID: 5UO1). The structurally refined protein .pdb files were converted to. pdbqt files using grid module of autodock tools 1.5.6. Charges were assigned to the ions to the proteins manually wherever necessary. The 2D and 3D chemical structures of hesperidin (Molecular formula: $C_{28}H_{34}O_{15}$; Molecular weight 610.57 g/mol) was retrieved (http://pubchem.ncbi.nlm.nih.gov/). These .sdf and .mol files obtained from PubChem were converted into .pdb files using Marwin Sketch (http://www.chemaxon.com/marvin/sketch/index.jsp). These .pdb files were converted to .pdbqt using ligand preparation module of autodock tools 1.5.6. The docking analysis of hesperidin was carried out using the Autodock tools (ADT) v1.5.4 and autodock v 4.2 programs. Hesperidin was docked to all the target protein complexes with the molecule considered as a rigid body. 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 ten generations. The remaining parameters were set as default. The Docked structure was then visualised using Discovery Studio 2016 for obtaining the binding interactions.

Results

The four crystal structures of proteins were retrieved from protein databank. A docking was performed to identify the precise binding sites on various immunomodulatory targets. Molecular docking is an effective approach that helps to envisage the principal 'binding modes' of the ligand with the 'protein/receptor/enzyme' having known 'three-dimensional structure'.

In the present study, docking was carried out on active sites of four target proteins 2AZ5, 1ITB, 1P9M and 5UO1 with hesperidin. Docking interactions of these targets with hesperidin are presented in (Figs. 1-4).

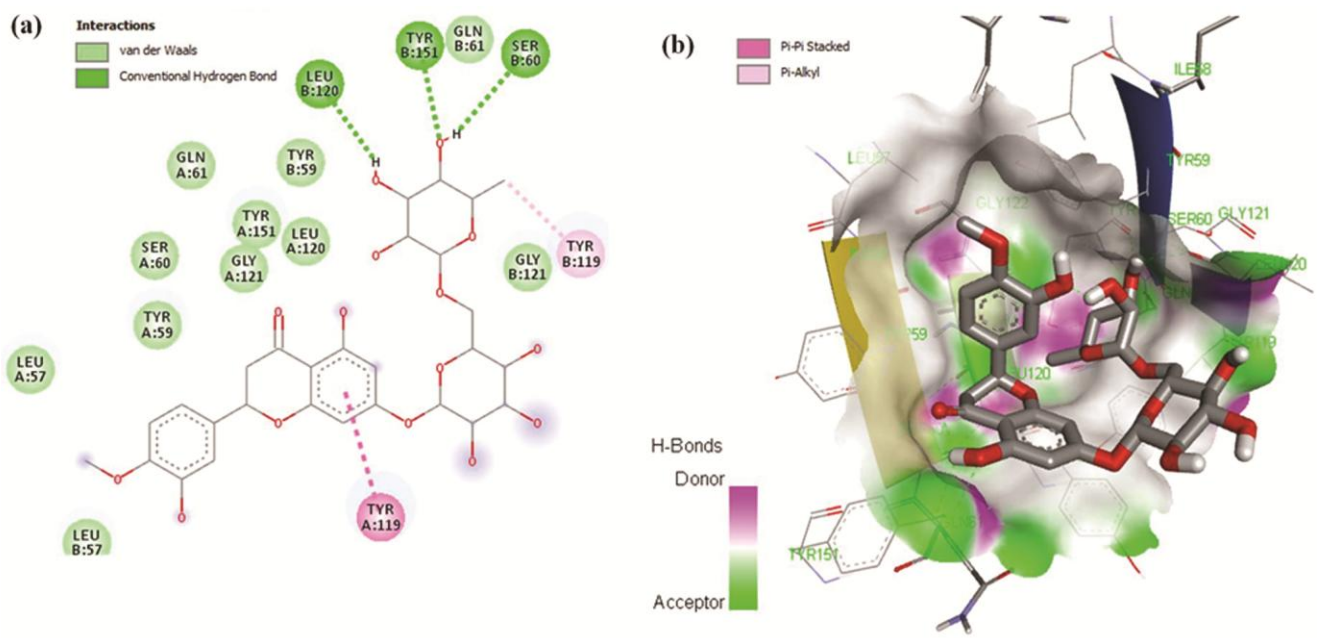

Fig. 1 — Molecular docking studies of hesperidin against TNF-α. A - 2D-interactions, B - 3D-interactions

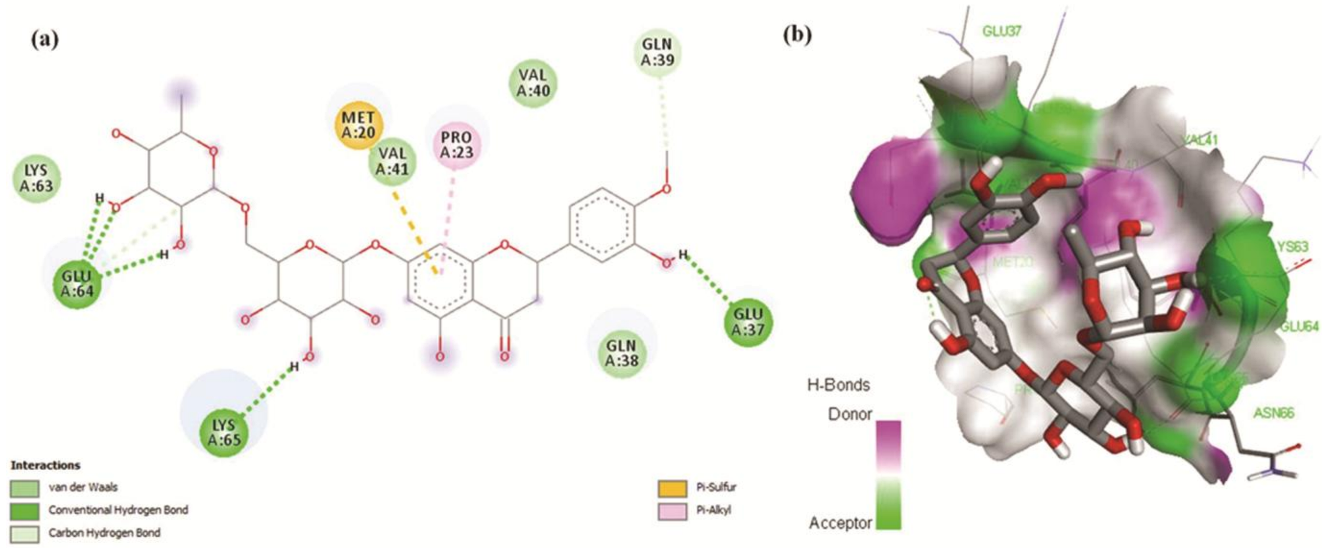

Fig. 2 — Molecular docking studies of hesperidin against IL-1β. A - 2D-interactions, B - 3D-interactions

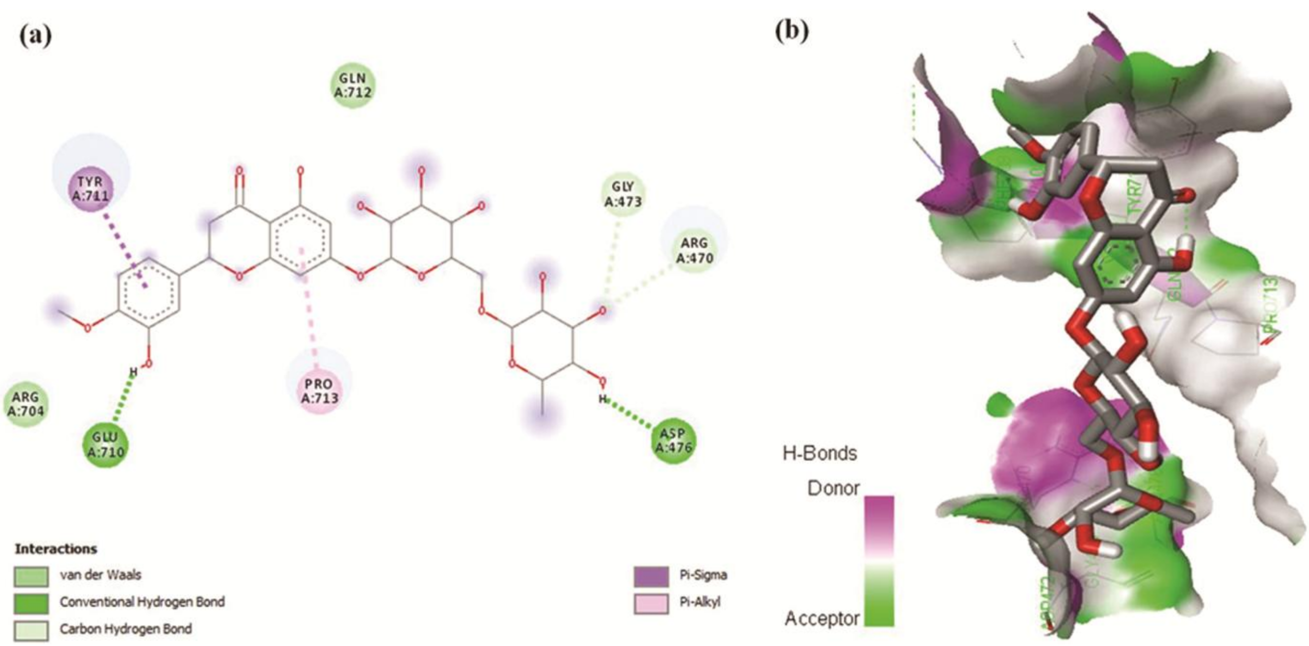

Fig. 3 — Molecular docking studies of hesperidin against IL-6. A - 2D-interactions, B - 3D-interactions

The binding energy of hesperidin for TNF-α was −6.96 kcal/mol, IL-1β −6.64 kcal/mol, IL-6 −7.07 kcal/mol, and for NOs −6.83 kcal/mol. Multiple interactions were observed with the binding of hesperidin to TNF-α. Serine 69 (1.36 Å), leucine 120 (1.46 Å) and Tyrosine 151 (1.34 Å) from B chain of TNF-α interacted with glycone part of hesperidin via hydrogen bonding. π-alkyl interaction at Tyrosine 119 (A chain) (1.39 Å) and π- π interaction at Tyrosine 119 (B chain) (1.37 Å) were observed. In case of IL-1β, glutamic acid 37 (1.45 Å), 64 (1.21 Å) and lysine 65 (1.55 Å) from (A chain) demonstrated hydrogen binding. Methionine 20 (A chain) (1.52 Å) revealed π-sulphur interaction and proline 23 (A chain) (1.52 Å) showed π-alkyl interaction. Hesperidin interacted with IL-6 in multiple ways. Hydrogen binding at methionine 67 (1.45 Å), glutamic acid 172 (1.49 Å), serine 176 (1.23 Å) and arginine 179 (B chain) (1.53 Å) was seen. At arginine 179 (B chain) (1.57 Å) π-alkyl interaction with hesperidin aglycone was observed. An unfavorable donor-donor interaction at alanine 180 (B chain) (1.46 Å) was seen. With respect to NOS, hydrogen bonding, π-sigma interaction, π-alkyl interaction, carbon-hydrogen bond, and vender Waal interaction

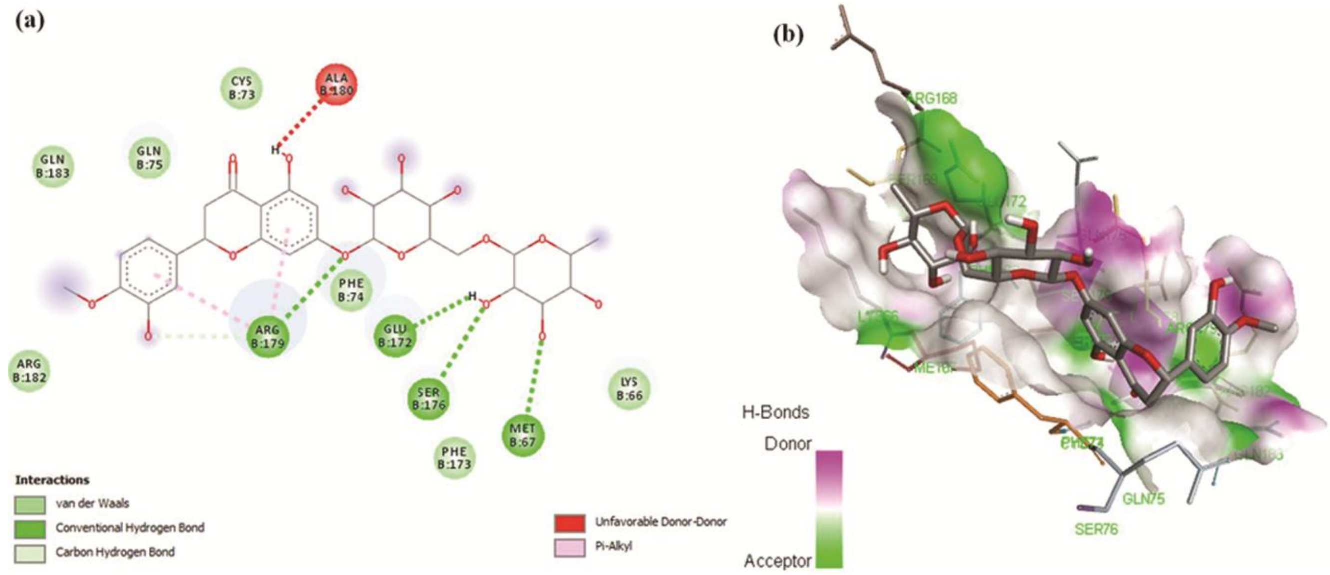

Fig. 4 — Molecular docking studies of hesperidin against NOs. A - 2D-interactions, B - 3D-interactions

were observed. Hydrogen bonding was seen at aspartic acid 476 (1.07 Å) and glutamic acid 710 (A chain) (1.23 Å). van der Waal interation was seen at glutamine 712 (A chain) (1.52 Å). Carbon -hydrogen binding was observed at arginine 470 (1.48 Å) and glycine 473 (A chain) (1.49 Å). π-sigma interaction was observed at tyrosine 711 (1.13 Å) whereas π-alkyl interaction was seen at proline 713 (A chain) (1.59 Å).

Discussion

Macrophages play a key function in the production of inflammatory cytokines and chemokines (*e.g.* TNF-α and pro-inflammatory interleukins). TNF-α, early recognized as an endotoxin-induced glycoprotein, play a pivotal role in proliferation, migration, differentiation and cell death. The cellular events *viz*. inflammation, infection and malignant conditions are observed due to binding of TNF-α binding to TNF-α receptors. Over-expression of TNF-α is responsible for the progression of pathological consequences *viz*. rheumatoid arthritis, ankylosing spondylitis, psoriasis and Crohn's disease[20]. Thus, the drugs that act against TNF-α could be effective in the management of above mentioned inflammatory condition[21]. Many studies proved the inhibitory role of hesperidin in decreased expression of TNF-α. Hesperidin decreased TNF-α expression on vascular cell adhesion molecule-1 which further prevented association of monocytes to endothelium[22]. Similar effects were observed over other experimental models[23]. The docking of hesperidin with TNF-α receptor represents a noteworthy interaction which could be responsible for its inhibition (as observed from previous *in vitro* studies)[13].

Various interactions with inflammatory and immune cells are arbitrated by a class of proteins termed as interleukins. Interleukins function to support 'cell growth, differentiation, and functional activation'.

IL-1β and IL-6 are two important interleukins. They are produced by macrophages, T-cells and bone marrow stromal cells. IL-1β (human leukocyte pyrogen/lymphocyte mitogen) is an important mediator to evoke an immune response. IL-1β contributes towards the progression of pain, inflammation and cell apoptosis[24]. Over-expression of IL-1β is responsible for the progression of osteoarthritis, rheumatoid arthritis[25] and type 2 diabetes[26]. Therefore, the blockade of IL-1β and IL-1 receptors is an important strategy to suppress inflammation and associated inflammatory disorders. The binding site on A chain of IL-1β receptor includes some residues *viz*. 11, 13-15, 20-22, 27, 29-36, 38, 126-131, 147, and 149[27]. The binding energy of interaction between hesperidin and IL-1β receptor as observed from docking study was −6.64. Hesperidin interacted with some residues of IL-1 receptor next to aforementioned active sites which include methionine 20, proline 23, glutamic acid 37. In many experiments, hesperidin has been studied for its possible inhibitory effect on IL-1 receptor and decreased expression of IL-1β. In a study, hesperidin administration in rats (intoxicated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a hepatotoxin) caused a decrement in the levels of IL-1β[28]. Many other studies also demonstrated such effect[29]. Thus, binding of hesperidin with some of the crucial residues on IL-1 β receptor may depict one of its effects as anti-inflammatory action.

IL-6 is another proinflammatory cytokine[30]. IL-6 is secreted by macrophages and T-cells. It mediates the immune response after burns, trauma , and injury. Overproduction of IL-6 is associated with progression of rheumatoid arthritis[31] and inflammatory bowel disease[32]. IL-6 is a hexameric structure with interlocking assembly[33]. The site I interface of the structure is surrounded by phenylalanine residue. Arginine 179 and lysine 171, by virtue of hydrogen bond, confine the specificity. In the present study, the interaction of hesperidin with arginine 179 proves blockade activity on the IL-6 receptor and thus, down-regulation of IL-6 could be predisposed due to this interaction. This interaction may also unfold anti-inflammatory mechanism of hesperidin in many animal studies[34].

NOs is responsible for the production of nitric oxide from the substrate l-arginine[35]. NOs bind calmodulin and contain haem. The activity of this enzyme increases in 'response' to lipopolysaccharide and cytokines. NOS finds an important role in numerous physiological and pathophysiological conditions, *viz*. regulation of blood pressure, infection, inflammation and progression of malignancies[36]. The secretion of nitric oxide is increased in cytokine-activated macrophages[37]. Nitric oxide, with respect to the immune system, regulates the growth, activity , and fatality of lymphocytes, macrophages, neutrophils, mast cells, antigen presenting cells, natural killer cells and antigen-presenting cells[38]. Increased nitrite accumulation is observed during arthritis[39]. Thus, a decrement in the activity of this enzyme may play a vital role in anticipating the inflammation. Some natural products have been tested for their potential NOS inhibitory activity. Extracts of Acanthopanax senticosus[40], Feijoa sellowiana[41], and Latycodon grandiflorum significantly inhibited the activity of NOs[42]. Hesperidin was studied for inhibitory effect on lipopolysaccharide-induced over-expression of inducible nitric oxide synthase in mouse macrophage. Hesperidin in the test dose (10-30 µM) caused a significant decrease in nitric oxide production. In present docking studies, the interaction of hesperidin with tyrosine 711 and glutamine 712 suggest its possible interaction with NOs. Altogether, hesperidin altogether with other flavonoids (rutin[43], catechin[44]) showed immunomodulatory potential.

Conclusion

In the present study, we carried out docking studies on hesperidin on 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 hesperidin suggest the ability of hesperidin to bind to multiple targets involved in inflammation and immunomodulation. Hesperidin interacted with various chemokines and inflammatory mediators' *viz*. TNF-α, IL-1β, IL-6, and NOs. With each target, hesperidin demonstrated a noteworthy affinity for binding. Findings from the present study show that hesperidin may interact with several chemokines and inflammatory mediators. Further studies on hesperidin 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 immune-modulator.

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