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Note

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

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Plant, mineral, and animal products have been utilized as medications from the beginning of time to cure a variety of ailments. Use of medicinal herbs to modulate immune function has a rich history. Natural products serve as the foundation for contemporary pharmaceutical ingredients. Immunomodulation alters an individual's immune system by interfering with its normal processes. Immunomodulators derived from natural sources have been extensively studied in order to modify the immune system and prevent illness. Berberine is an alkaloid has been identified for its anti-inflammatory properties. In animal studies, Berberine was found to demonstrate analgesic properties. The current work is aimed to explore the *in silico* interactions of Berberine with various chemokines and inflammatory pathways. Berberine was docked with TNF-α, IL-1β, IL-6, and NOs in this investigation. Docking study demonstrated notable interactions with these targets. The present research provides insight into the development of new compounds for immunomodulation and the management of inflammatory illnesses. More research on Berberine and related flavonoids is necessary to assess its safety. As a result, Berberine can be regarded as a candidate for the advancement of an immunomodulatory agent.

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

Introduction

The immune system contains various types of cells are essential for pathogen elimination. Phagocytosis is among the mechanisms that destroys invading microorganisms *via* an enzyme-catalysed oxidative stress[1]. Various proinflammatory factors recruit and stimulate neutrophils and monocytes at the inflammatory sites during this process[2]. Excessive generation of oxygen species, on either hand, is proven to cause injury to the body's own structures and components, such as triglycerides, protein complexes, even nucleic acids, resulting in chronic aggravation[3]. Although acute inflammation aids in the fight against infections and the healing of tissue injury, persistent inflammation contributes to a variety of immunological illnesses[4]. Elevated expression of cytokines and growth factors is linked with a number of clinical conditions, including aberrant gene production control, undesired cell growth, as well as the development of chronic inflammatory response[5]. Inhibiting these factors, notably tumour necrosis factor-α (TNF-α) and Interleukin (IL-1β), has been shown to benefit a variety of clinical illnesses, notably inflammatory rheumatoid arthritis (RA) as well as other autoimmune conditions. TNF-α functions as a control trigger cytokine in a number of inflammatory events, such as the breakdown of chondrocytes[6] TNF-α concentrations in synovial fluid are associated with rheumatoid arthritis. The major cause of synovitis is TNF-α in conjunction with Interleukin-1β (IL-1β). In the rheumatoid arthritis (RA), it is thought that IL-1β acts before TNF-α[7]. The majority of articular injury in inflammatory arthritis is caused by IL-1β, which causes proteoglycan breakdown and inhibits proteoglycan biosynthesis. During the pro-inflammatory phase, IL-1β frequently collaborates with TNF-α to stimulate the synthesis of IL-6 and IL-8[8]. IL-6 also plays a majorin inflammation, mostly through inducing the creation of acute phase proteins by hepatocytes[8]. Cyclo-oxygenase (COX) is a key isoenzyme involved in production of thromboxane and prostaglandins. Prostaglandins are autacoids that hold significant roles in a variety of physiological and biochemical processes. COX-1 is expressed in numerous tissues, including the gut, kidneys, brain, airways, and spleen, whilst COX-2 is an inducible enzyme that is produced when cells are damaged[9]. Analgesic usage is coupled with renal and gastrointestinal toxicity (due to COX-1 suppression), limiting the use of effective analgesics amid pain and swelling[10].

Immune dysregulation leads to progressive harm in numerous organs, leading in discomfort, decreased quality of life, and premature mortality. The optimal

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medicine for treating immune-mediated inflammatory illnesses must establish prompt management of inflammation, minimizing tissue injury, and have an innocuous adverse impact pattern[11]. Presently available anti-inflammatory medications don't really match these criteria, typically exhibiting more side effects than is tolerable, less curative results than is wanted, or both[12].

Natural products have been regarded as a rich source of unique chemical structures that affect the immune system with little negative effects[13]. Phytochemicals are significant for food flavouring, insect resistance, and also as medications, including immunosuppressive compounds[14]. Furthermore, traditional herbal remedies as well as purified natural components could be used to drive the advent of new antiviral medications[15]. In other sense, more effective medications can be developed depending on the structure of naturally occurring molecules that display the intended functions. Plant alkaloids with their substantial therapeutic history can be particularly interesting candidates for the alleviation of autoimmune ailments[16]. Alkaloids are nitrogen-containing compounds with a small molecular weight which are found mostly in microbes, fungus, vegetation, and animals. They are secondary metabolites formed in plants in response to environmental modification and different biological stressors, endowing alkaloids with structural variety and substantial bioactivity. Because of such features, alkaloids are possible candidate molecules in future medication research, and they are gaining growing interest from researchers[17]. Berberine, an isoquinoline alkaloid derived primarily from the traditional Chinese remedies *Coptis chinensis* and *Phellodendron chinense*, has been shown to prevent acute colitis by modulating innate and adaptive immunity, intestinal barrier integrity, and intestinal micro flora[18–20]. There have been several accounts of analgesic as well as anti-inflammatory properties. Berberine was recently studied for its potential immunomodulatory properties, and it was shown to have protective impact on cellular immunity. Berberine was proven to be a potential immunomodulator since it lowered inflammatory response and diminished dendritic signalling pathways[20]. The current work attempts to investigate the fundamental mechanism of immunomodulation. The *in silico* interaction of berberine with several immunomodulatory cytokines was investigated in this study. In addition, the molecular basis of Berberine's interaction with COX-2 was identified.

Materials and Methods

Software

Python 2.7- language was downloaded from www.python.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, Discovery Studio visualizer 4.1 was downloaded from www.accelerys.com.

Docking protocol

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 COX-2 (PDB ID: 4COX).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 3Dchemical structure of Berberine 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 Berberine was carried out using the Autodock tools (ADT) v1.5.4 and autodock v 4.2 programs. Berberine 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

Molecular docking is a legitimate approach that assists in visualising the primary 'binding interactions' of the ligand with the recognized 'three-dimensional structure' of the protein. The research focused on interaction modalities that were required for major ‘structural interactions' and provided valuable information for the creation of inhibitors. Molecular docking is among the most well studied approaches for 'discovering' novel ligands for known targets. Most chemicals may be evaluated using a 'free energy binding' estimate. The value of free energy binding

represents the drug's 'affinity' for the targets. Likewise, the molecule with the least inhibition constant denotes the 'potential' compound[21].

In the present study, TNF-α (PDB ID: 2AZ5), IL-1β (PDB ID: 1ITB), IL 6 (PDB ID: 1P9M), and COX-2 (PDB ID: 4COX) were analysed for possible interaction with a Berberine.

The binding surface on the TNF-α dimer is made up of 16 contact residues, including 6 tyrosine residues. Leu 57, Tyr 59, Ser 60, Gln 61, Tyr 119, Leu 120, Gly 121, Gly 122, and Tyr 151 are the nine amino acids from chain A. The last seven residues (Leu 57', Tyro 59', Ser 60', Tyr 119', Leu 120', Gly 121', and Tyr 151') are a subset of those found in chain B[22]. In the present study, there was a notable hydrogen bonding of Berberine with Tyr151. Similarly, a notable interaction was seen at Tyr 119, Gln 61, Gly 121 (Fig. 1).

In case of IL-1β, the binding sites lie in the region of 11, 13-15, 20-22, 27, 29-36, 126-131, 147 and 149. Berberine demonstrated few major interactions as Tyr 127, Met 128 and Lys 112 (Fig. 2). Further, docking of Berberine with IL-6 revealed multiple interactions. With IL-6, Berberine demonstrated notable hydrogen bonding at Met 67. Wander waals interactions were also observed at Gly 154, Der 166, Phe 168, Gln 169, Gln 190 at C chain and Ala 56, Leu 165, Phe 173 (Fig. 3).

With respect to COX-2, berberine demonstrated Wander waals interaction at Gla 199, Phe 200, His

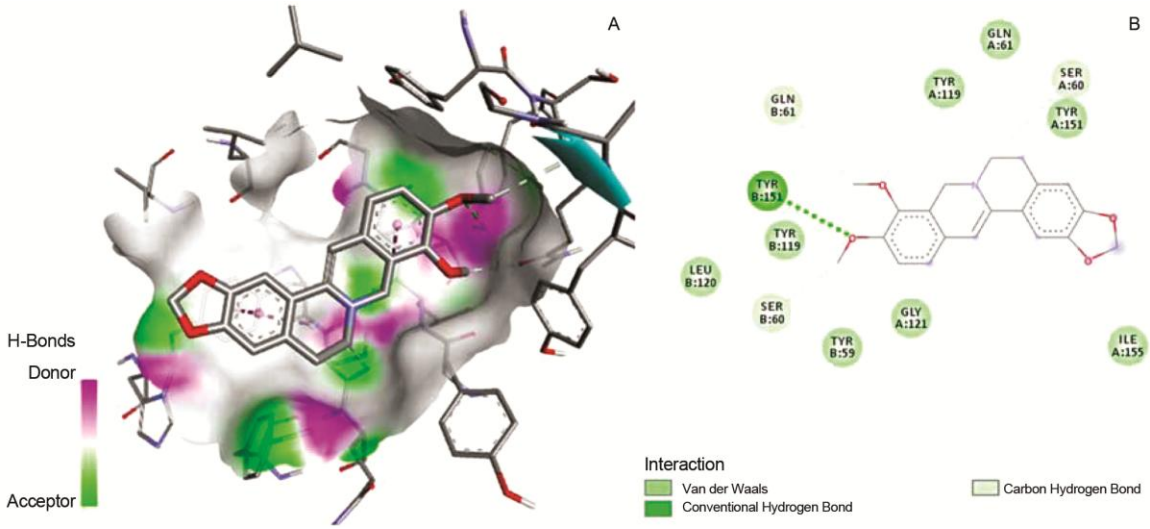

Fig. 1 — Docking interactions of Berberine with TNF-α (A) 3D interactions; and (B) 2D interactions

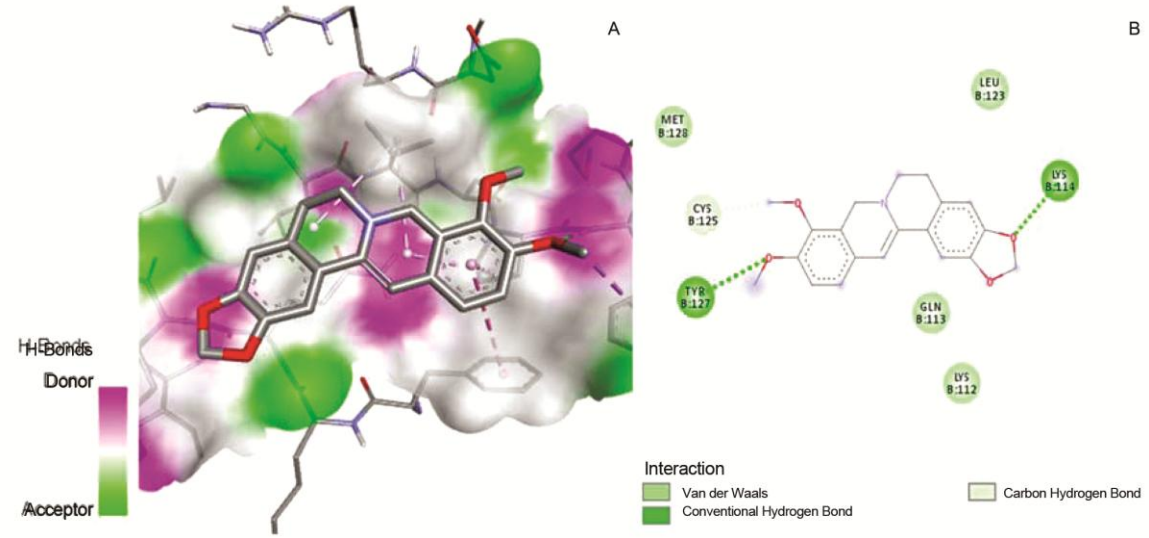

Fig. 2 — Docking interactions of Berberine with IL1-β (A) 3D interactions; and (B) 2D interactions

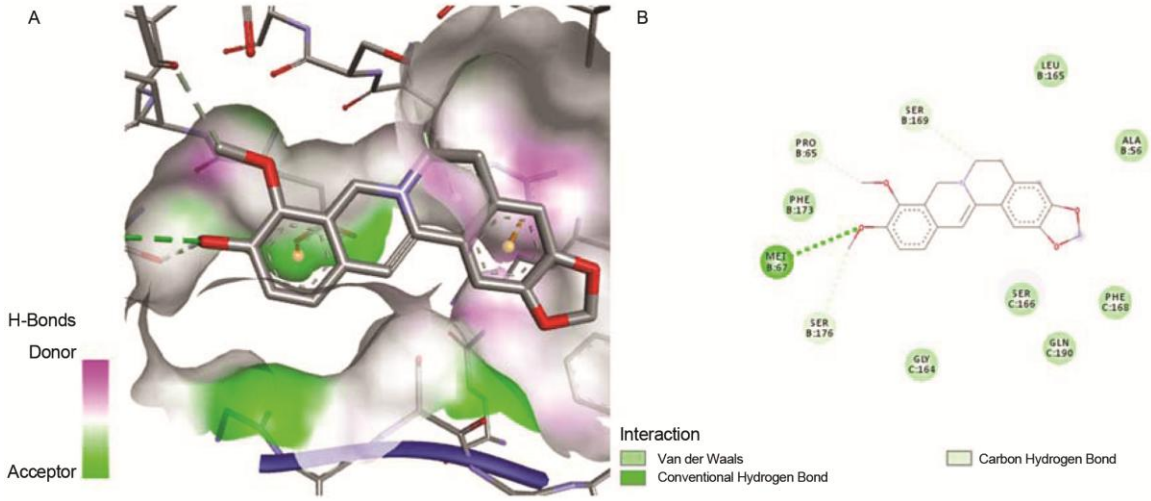

Fig. 3 — Docking interactions of Berberine with IL-6 (A) 3D interactions; and (B) 2D interactions

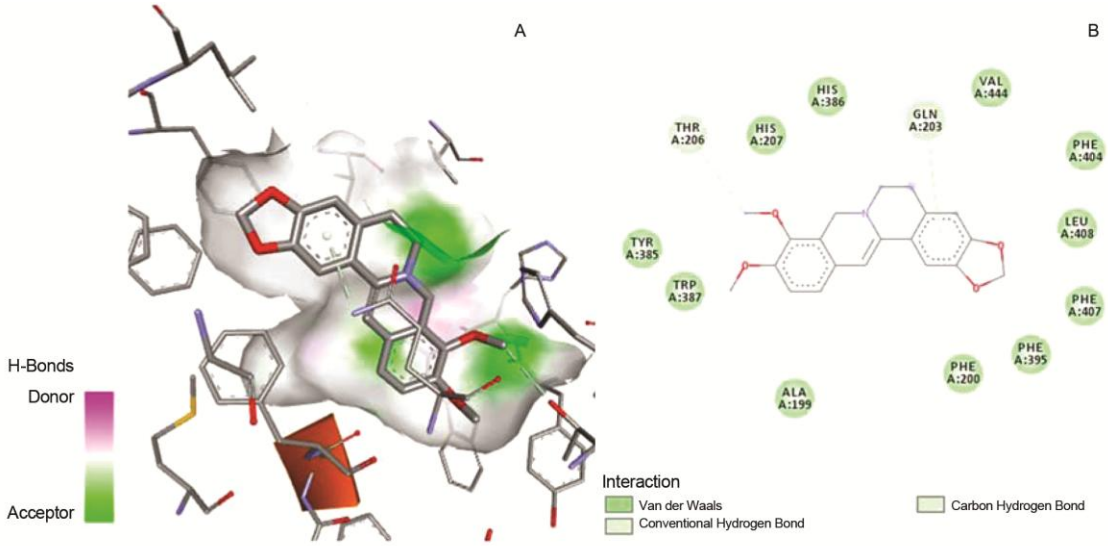

Fig. 4 — Docking interactions of Berberine with COX-2 (A) 3D interactions; and (B) 2D interactions

Table 1 — Molecular Docking studies of berberine on immunomodulatory targets

Targets	Binding Energy	Inhibition Constant
TNF-α	−7.03 kcal/mol	7.06 µM
IL-1β	−7.01 kcal/mol	7.22 µM
IL-6	−7.07 kcal/mol	6.57 µM
COX-2	−10.16 kcal/mol	39.78 nM

207, Try 385, His 386, Trp 387, Phe 395, Phe 404, Phe 407, Leu 408 and Val 444 at A chain. The interaction of berberine with Try 385 is crucial as it is a heam factor and also constitutes hydrophobic segment of pocket. The residues like Gla 199, Phe 200, His 207 lie within active site of COX-2 (Fig. 4).

Discussion

Cytokines play an important part in the development of inflammatory responses damage, which contributes to the rise of 'inflammatory disorders.' The two primary types of cytokines implicated in the development of 'hyperalgesia' are necrosis factors and interleukins. TNF-α, IL-1β, and IL-6 were selected for docking analysis in this work. Table 1 summarizes the docking results of the present study. TNF-α suppression by drug candidates has resulted in substantial breakthroughs in the treatment of rheumatoid arthritis and confirmed systemic suppression of such pro-inflammatory cytokine as a medical remedy. A molecule should attach to the

binding site of TNF-α to successfully suppress this cytokine. Berberine interacted with Tyr151, Tyr 119, Gln 61 and Gly 121 of TNF-α. The observations from the present study affirm the previous investigations where Berberine suppressed TNF-α-induced inflammatory component production and nuclear factor-B activation in vascular endothelial cells through stimulation of AMPK[23].

The IL-1 receptor is associated with transmitting cytokine-mediated inflammatory responses, while the IL-6 receptor is responsible for host cell proliferation and maturation, as well as immune system modulation during infections and autoimmune illnesses[24]. In the present study, docking interaction of berberine with IL-1β was determined. Seven out of ten postures were found at the active site or ligand binding site. IL-1 receptor interacts with its ligand through the amino acids Tyr 127, Val 16, Ala 109, Glu 11, Ile 110, Lys112, Leu 237, Asp 239, Ala 241, Tyr261, Asp 260 and Glu 252.A report signifies modulatory effect of berberine whereby berberine treatment resulted in suppression IL-1β induced inflammatory process in rat articular chondrocytes *via* down regulating MAPK[25]. It could be a possible reason for modulatory effect of berberine.

IL-6 is a four-helical bundled cytokine with normal up-up-down-down configuration of the helices and an extra, short -helix in the CD circuit. The IL-6R external section is made up with three areas: an N-terminal Ig-like motif and two Fn3 domains that together create the IL-6 binding CHR. The N-terminal Ig region has a deformed Ig-like fold and is not essential for cytokine binding or biological activity, while there is some indication that it is necessary for optimal receptor transportation. An appreciable hydrogen bonding interaction of berberine with at Met 67 of IL-6 could justify modulatory effect. Biological studies also signify suppression of IL-6 due to Berberine treatment[26,27].

COX-2 is an inducible enzyme. The COX-2 gene that encodes the cyclooxygenase-2 inducible isozyme is activated by particular inflammatory responses, implying that it is in charge of prostanoid production, that is implicated in inflammation and cytokine production. Pro-inflammatory factors are responsible for both the beginning and persistence of inflammation. As once initiating component is gone, the anti-inflammatory messengers involved in regulating inflammation level out the activity. Numerous causes, such as anxiety, chromosomal abnormalities, or environmental factors, disrupt this equilibrium and cause increased synthesis of prostaglandin E2 (PG E_2) produced from arachidonic acid *via* COX-2 up regulation, resulting in autoimmune illnesses. Increased PG E_2 levels cause a rise in the production of proinflammatory cytokines like IL-6, to which neutrophils and macrophage inflammatory responses activate. The intensification of inflammatory mediators signaling or the transition from an immediate to a systemic inflammatory response occurs as a consequence of IL-6 expression. One strategy for successfully treating inflammatory skin conditions is to reduce prostaglandin synthesis by reducing COX-2. The residues like Gla 199, Phe 200, His 207 lie within active site of COX-2. Previous reports also indicate suppression of COX-2 activity due to berberine[28,29]. These studies are in accordance to previous studies performed on natural products[30,31].

Conclusion

We used docking investigations on berberine to several inflammatory and immunomodulatory domains in order to explore and analyse the former's interaction with the latter *in silico*. Berberine's docking scores and interaction analysis reveal that it may bind to a variety of sites implicated in inflammation and immunomodulation. TNF-α, IL-1β, IL-6, and COX-2 were among the chemokines and inflammatory mediators that berberine interacted with. Berberine demonstrated a notable propensity for binding to each of the targets .According to the findings of this investigation, Berberine interacts with a variety of chemokines and inflammatory mediators. More research on Berberine and related flavonoids is needed to create and establish QSAR and QSPR investigations that could lead to the creation of innovative, effective, and safe immunomodulators.

Conflict of interest

All authors declare no conflict of interest.

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