Computational Analysis of Berberine–Inflammatory Protein Interactions Using Molecular Docking and Molecular Dynamics Simulations

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Abstract

Berberine is a bioactive alkaloid with potential therapeutic relevance. Computational approaches can help evaluate protein-binding interactions, structural stability, and predicted pharmacokinetic properties. To investigate berberine–protein interactions; assess complex stability; and predict its absorption, distribution, metabolism, and excretion (ADME) characteristics using in silico methods. Molecular docking was performed using AutoDock against five targets: peroxiredoxin-5 (1HD2), COX-1 (1EQH), COX-2 (1PXX), caspase-3 (3DEI), and TNF-α (2AZ5). Molecular dynamics simulations were conducted using GROMACS with the CHARMM force field for 100 ns. The RMSD, RMSF, radius of gyration (Rg), and solvent-accessible surface area (SASA) were analyzed. SwissADME was used to predict physicochemical and pharmacokinetic properties. Docking scores ranged from −5.7 to −8.9 kcal/mol, with the most favorable binding energy observed for caspase-3 (−8.9 kcal/mol), followed by TNF-α (−8.6 kcal/mol), COX-1 (−8.3 kcal/mol), COX-2 (−8.2 kcal/mol), and peroxiredoxin-5 (−5.7 kcal/mol). Hydrogen bonds were identified with Thr62 in caspase-3 and Lys126 in peroxiredoxin-5. MD simulations indicated a lower RMSD and RMSF, a stable Rg, and a lower SASA in the berberine-bound system. SwissADME predicted a molecular weight of 336.36 g/mol, a consensus LogP of 2.06, and a TPSA of 40.80 Å². Berberine satisfied major drug-likeness criteria, with predicted high gastrointestinal absorption and blood–brain barrier permeability. Limited solubility and potential CYP-mediated interactions warrant further investigation. The predicted binding of berberine, particularly that of caspase-3, was favorable, and the computational drug likeness characteristics were promising. Experimental validation is necessary to establish its biological activity and pharmacokinetic behavior.

Keywords: Berberine; Molecular docking; Molecular dynamics simulation; Caspase-3; Cyclooxygenase; SwissADME.

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INTRODUCTION

Inflammation is a complex biological response that protects the body against infection, tissue injury, and other harmful stimuli [1]. Although transient inflammation is essential for maintaining tissue homeostasis and initiating repair, persistent or dysregulated inflammatory responses can contribute to the development and progression of several chronic disorders, including neurodegenerative, cardiovascular, metabolic, autoimmune, and inflammatory diseases [2]. The inflammatory process is regulated through a network of signaling pathways involving numerous proteins, enzymes, receptors, and transcription factors [3]. Key molecular mediators such as cyclooxygenase-2 (COX-2), lipoxygenases, tumor necrosis factor-α (TNF-α), interleukins, nuclear factor-κB (NF-κB), and other inflammation-associated targets play important roles in initiating and sustaining inflammatory responses. Consequently, the modulation of these molecular targets represents an important strategy for identifying potential anti-inflammatory agents [4].

Berberine is a naturally occurring isoquinoline alkaloid widely distributed in several medicinal plants and has attracted considerable scientific interest because of its diverse pharmacological activities [5]. Previous experimental investigations have associated berberine with anti-inflammatory, antioxidant, antimicrobial, antidiabetic, cardioprotective, and neuroprotective effects. Its reported anti-inflammatory activity has been linked to the modulation of multiple cellular signaling pathways and inflammatory mediators rather than interactions with a single molecular target [6]. The precise molecular basis underlying its interaction with individual inflammatory proteins remains incompletely characterized. Understanding the structural interactions between berberine and inflammation-associated proteins may therefore provide valuable information regarding its possible molecular mechanism and therapeutic potential [7]. Computational drug discovery approaches provide efficient methods for investigating ligand–protein interactions at the molecular level [8]. Molecular docking is widely employed to predict the preferred binding orientation of a small molecule within the active or functional site of a target protein and to

estimate the relative strength of the interaction through docking scores. Analysis of the resulting complexes can further identify important noncovalent interactions, including hydrogen bonding, hydrophobic contacts, π–π interactions, and electrostatic interactions, which contribute to ligand recognition and stabilization. Such information can help identify potentially important amino acid residues involved in berberine binding and provide a structural rationale for its predicted biological activity. Molecular docking generally involves proteins and ligands in relatively static conformations and may not adequately reflect the dynamic nature of biological systems [9]. Molecular dynamics (MD) simulations can complement docking by evaluating the temporal stability and conformational behavior of protein–ligand complexes under simulated physiological conditions. Parameters such as the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, hydrogen bond formation, solvent-accessible surface area, and binding energy profiles can provide additional evidence regarding complex stability and molecular interactions [10]. Therefore, the present study was designed to computationally investigate the interactions of berberine with selected key inflammatory target proteins using molecular docking followed by molecular dynamics simulations.

1. Materials and Methods

1.1. Molecular Docking

Three-dimensional structures of the target proteins were retrieved from the Protein Data Bank (PDB) [11]. The protein structures used in this study included human peroxiredoxin-5 (1HD2), cyclooxygenase-1 (COX-1; 1EQH), cyclooxygenase-2 (COX-2; 1PXX), caspase-3 (3DEI), and tumor necrosis factor-alpha (TNF-α; 2AZ5). The active binding sites of all target proteins were identified using the PDB data server, and the key interacting residues reported by the sum of the PDBs are highlighted with an asterisk. The ligand berberine was downloaded from the PubChem database and prepared for molecular docking using Open Babel, where it was converted to a three-dimensional structure, protonated, and energy-minimized. Protein preparation, including the addition of polar hydrogens and assignment of Gasteiger charges, was carried out using AutoDock Tools. Molecular docking simulations were performed using AutoDock, which employs a grid box of dimensions 30 × 30 × 30 Å along the x, y, and z axes to encompass the predicted active site region [12, 13].

1.2. Molecular dynamics (MD) simulations

Molecular dynamics (MD) simulations were performed using the GROMACS simulation package to investigate the structural stability and dynamic behavior of the system. The CHARMM force field was employed. After appropriate system preparation and equilibration, a 100 ns production MD simulation was conducted under periodic boundary conditions. Trajectory analyses were carried out to evaluate the conformational stability and flexibility throughout the simulation period. The root mean square deviation (RMSD) was calculated to assess the overall structural stability of the system, while the root mean square fluctuation (RMSF) was used to analyze residue-level flexibility. The compactness of the system was examined using the radius of gyration (Rg), and the degree of solvent exposure was quantified by computing the solvent-accessible surface area (SASA). All analyses were performed using the built-in tools of GROMACS, and the resulting data were visualized and plotted with XMGrace [14].

1.3. ADME of Berberine

In silico pharmacokinetic and drug likeness assessments of the selected compounds were performed using the Swiss ADME web-based platform. The canonical SMILES structure of the compound was entered into the Swiss ADME interface, and the molecular structure was processed using the platform's integrated computational models. Physicochemical descriptors, including molecular weight, heavy atoms, hydrogen-bond acceptors and donors, rotatable bonds, TPSA, and molar refractivity, were recorded. Lipophilicity was predicted using iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT models. Water solubility, gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein interactions, cytochrome P450 inhibition, drug likeness, PAINS, Brenk alerts, lead likeness, bioavailability score, and synthetic accessibility were subsequently evaluated and documented [15].

2. Results and Discussion

2.1. Molecular Docking

Berberine docking against multiple target proteins revealed variable binding affinities and interaction profiles. For human peroxiredoxin-5 (1HD2), the docking grid was centered at x = 6.72, y = 43.975, and z = 28.783, and berberine exhibited a binding energy of −5.7 kcal/mol, resulting in the formation of a single hydrogen bond with Lys126. In cyclooxygenase-1 (COX-1; 1EQH), the grid center was defined at x = 26.156, y = 32.499, and z = 199.913, where berberine showed a stronger binding energy of −8.3 kcal/mol but did not form any hydrogen bond interactions. For cyclooxygenase-2 (COX-2; 1PXX), the grid was positioned at x = 25.111, y = 26.289, and z = 14.145, and berberine interacted with key active-site residues Ser530, Tyr355, and Arg120, yielding a binding energy of −8.2 kcal/mol. Docking against caspase-3 (3DEI) was performed using the grid center coordinates x = −47.177, y = 15.923, and z = −21.873, resulting in the most favorable binding energy of −8.9 kcal/mol, with a single hydrogen bond observed with Thr62. Finally, for tumor necrosis factor-α (TNF-α; 2AZ5), the grid box was centered at x = −13.687, y = 71.606, and z = 27.002, where berberine displayed a binding energy of −8.6 kcal/mol and no hydrogen

bond interactions were detected.

Table 1: Molecular docking results of berberine with selected target proteins showing binding energies and key hydrogen bond interactions at active sites.

Protein (PDB ID)	Ligand	Binding Energy (kcal/mol)	Interacting Hydrogen-Bond Residues
Human Peroxiredoxin-5 (1HD2)	Berberine	−5.7	Lys126
Cyclooxygenase-1 (1EQH)		−8.3	No interactions
Cyclooxygenase-2 (1PXX)		−8.2	Ser530, Arg120, Tyr355
Caspase-3 (3DEI)		−8.9	Thr62
Tumor Necrosis Factor-α (2AZ5)		−8.6	No interactions

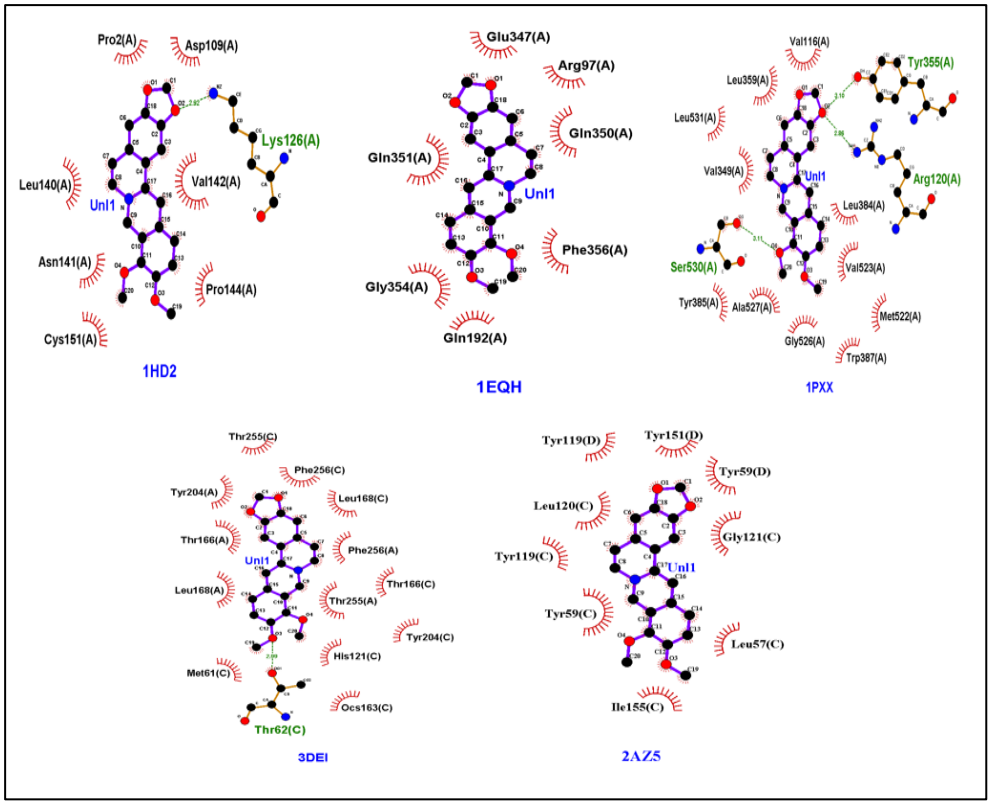


Figure 1: 2D ligand–protein interaction diagrams of berberine (Unl1) within the binding sites of different target proteins: 1HD2, 1EQH, 1PXX, 3DEI, and 2AZ5. The diagrams illustrate key interacting residues surrounding the ligand, with the hydrogen bonds shown as green dashed lines and the hydrophobic contacts represented by red arcs. Important interacting residues, such as Lys126, Arg120, Ser530, and Thr62, contribute to ligand stabilization through a combination of hydrogen bonding and hydrophobic interactions, highlighting the binding mode and affinity of berberine across multiple protein targets.

2.2. Molecular dynamics (MD) simulations

2.2.1. Root mean square deviation (RMSD)

The backbone RMSD profile reveals a pronounced difference between the apo and berberine-bound systems. The RMSD of the apo protein gradually increased after ~60 ns, reaching values up to ~1.5 nm, indicating significant conformational rearrangements and reduced structural stability. In contrast, the RMSD of the berberine-bound complex remained consistently low (~0.2–0.35 nm) throughout the simulation, suggesting that the binding of berberine stabilizes the protein backbone and prevents large structural deviations (Figure 2).

2.2.2. Root mean square fluctuation (RMSF)

Residuewise RMSF analysis further supported the stabilizing effect of berberine. The apo protein fluctuates more strongly, particularly in the N-terminal region and several loop regions, with RMSF values exceeding 2–3 nm for certain residues. Conversely, the berberine-bound complex shows markedly reduced fluctuations across the entire residue range, indicating restricted flexibility and enhanced rigidity upon ligand binding. This reduction in local mobility suggests strong ligand–

protein interactions that dampen residue motion (Figure 2).

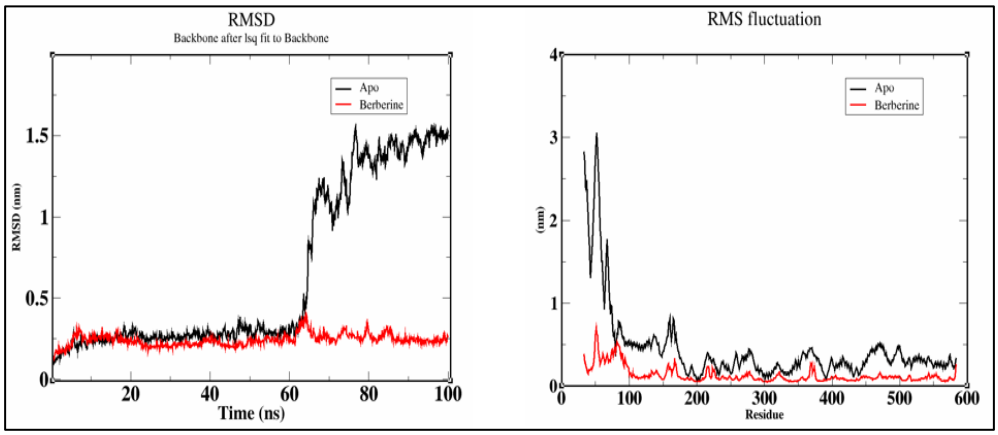


Figure 2: Backbone stability and flexibility analysis from molecular dynamics simulations. *(Left)* RMSD of the protein backbone over 100 ns showing greater structural deviation in the Apo form (black) than in the berberine-bound complex (red), indicating ligand-induced stabilization. *(Right)* RMSF per residue highlighting reduced fluctuations in the berberine-bound system, suggesting decreased flexibility upon ligand binding.

2.2.3. Radius of Gyration (Rg)

Analysis of the radius of gyration highlights changes in protein compactness. The apo system shows a noticeable increase in Rg after ~60 ns, reflecting partial unfolding or expansion of the protein structure. In contrast, the BBR-bound complex maintains a stable and lower Rg value throughout the simulation, indicating a more compact and structurally stable conformation. These findings suggest that the binding of berberine prevents protein expansion and preserves its folded state (Figure 3).

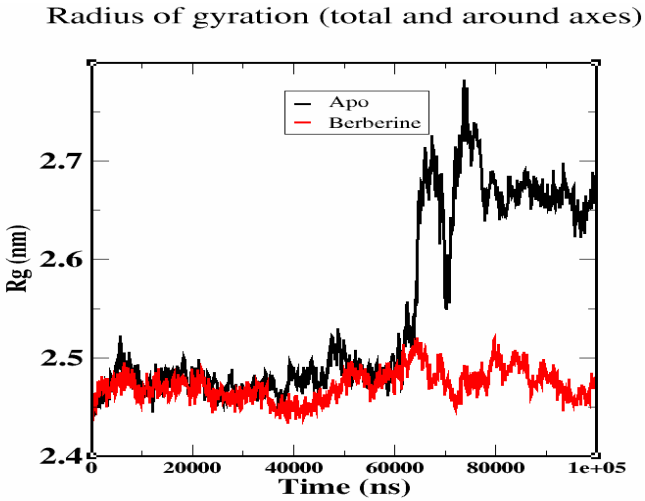


Figure 3: Radius of gyration (Rg) profile over the simulation time showing overall compactness of the protein in the Apo (black) and Berberine-bound (red) states. The Rg of the Apo system noticeably increased after ~60 ns, indicating structural expansion or reduced compactness, whereas that of the berberine-bound complex remained relatively stable and had a lower Rg, suggesting enhanced structural stability upon ligand binding.

2.2.4. Solvent-Accessible Surface Area (SASA)

SASA analysis revealed moderate fluctuations for both systems; however, the apo protein exhibited greater variability and a slight overall increase in solvent exposure over time. The berberine-bound complex displays relatively lower and more stable SASA values, implying reduced solvent exposure of the hydrophobic regions. This observation is consistent with the enhanced compactness and structural integrity induced by ligand binding (Figure 4).

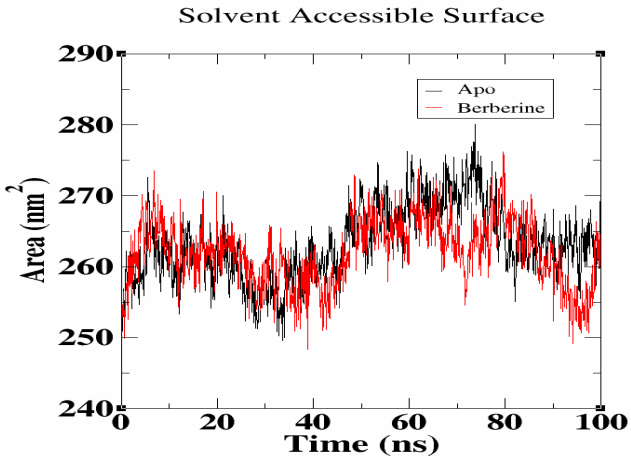


Figure 4: Time evolution of the solvent-accessible surface area (SASA) for the Apo (black) and berberine-bound (red) systems during the simulation. Both systems show moderate fluctuations; however, the SASA values of the berberine-bound complex are slightly lower and more stable overall, indicating a more compact structure and reduced solvent exposure upon ligand binding.

2.3. ADME Analysis

The Swiss ADME analysis of the investigated compound revealed a molecular formula of C20H18NO4+ with a molecular weight of 336.36 g/mol and 25 heavy atoms, including 16 aromatic heavy atoms, suggesting a structurally rigid and predominantly aromatic framework. The compound possessed two rotatable bonds, four hydrogen-bond acceptors, no hydrogen-bond donors, and a topological polar surface area (TPSA) of 40.80 Å², characteristics that may support favorable membrane permeability while maintaining adequate molecular polarity. The predicted lipophilicity values varied among the computational models, with a consensus LogP of 2.06, indicating moderate hydrophobicity and a potentially balanced affinity for aqueous and lipid environments. The estimated aqueous solubility values ranged from 4.00 × 10−4 to 2.30 × 10−2 mg/mL across the different prediction models, indicating limited-to-moderate water solubility, which may be associated with the aromatic character of the compound and relatively low hydrogen-bond donor capacity. Importantly, the compound was predicted to exhibit high gastrointestinal absorption and to permeate the blood–brain barrier, suggesting the potential for effective systemic absorption and central nervous system exposure. The predicted P-glycoprotein substrate status indicates that active efflux may influence its distribution and intracellular exposure. The compound was also predicted to inhibit CYP1A2, CYP2D6, and CYP3A4, whereas no inhibition was predicted for CYP2C19 or CYP2C9, highlighting the possibility of metabolic drug–drug interactions that would require experimental confirmation. Its skin permeation coefficient (log Kp = −5.78 cm/s) suggested relatively poor passive transdermal penetration. From a drug likeness perspective, the compound complied with Lipinski's rule of five without violations and satisfied the Ghose, Veber, Egan, and Muegge criteria, supporting favorable overall oral drug-like characteristics. The predicted bioavailability score of 0.55 further suggested a reasonable probability of oral bioavailability. No PAINS alerts were detected, reducing concern regarding common nonspecific assay-interference motifs; however, one Brenk alert associated with quaternary nitrogen functionality and one lead-likeness violation due to XLOGP3 > 3.5 were identified, indicating structural features that should be considered during lead optimization. The synthetic accessibility score of 3.14 suggested that the compound is reasonably accessible from a synthetic perspective. The in silico ADME profile indicates a promising pharmacokinetic and drug-like profile characterized by high gastrointestinal absorption, predicted BBB permeability, acceptable molecular polarity, moderate consensus lipophilicity, and compliance with major drug-likeness filters, although limited aqueous solubility, predicted P-glycoprotein-mediated efflux, potential CYP-mediated interactions, and the identified Brenk and lead-likeness alerts warrant further experimental investigation.

3. CONCLUSION

The results of the present computational investigation demonstrated that berberine preferentially interacts with several inflammation-associated target proteins, with the strongest binding affinity observed for caspase-3, followed by TNF-α, COX-1, and COX-2. Molecular dynamics simulations further indicated that compared with the apo system, the binding of berberine enhanced protein stability, reduced residue-level fluctuations, maintained structural compactness, and limited solvent exposure. Swiss ADME analysis additionally supported favorable drug-like characteristics, including high gastrointestinal absorption, predicted blood–brain barrier permeability, and compliance with major drug-likeness criteria. These findings support berberine as a promising multitarget anti-inflammatory candidate, warranting experimental validation of its predicted molecular interactions and pharmacological effects.

RESEARCH PAPER

Conflict of Interest: None

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