

## Computational Insights into the interaction of Piperine with Immune Regulatory Proteins: A Docking Study

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The natural products, such as plant, mineral, and animal compounds, have been used for medicinal purposes for centuries. The use of medicinal herbs to modulate immune function has a rich history, and natural products serve as the foundation for contemporary pharmaceutical ingredients. Immunomodulation is the process of modifying 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. One such natural product that has been studied for its immunomodulatory properties is Piperine. Piperine is a chemical compound found in black pepper and other spices, and it has been found to have anti-inflammatory and analgesic properties in animal studies. In this context, present study is based on *insilico* docking studies to investigate the interaction of Piperine with various chemokines and inflammatory pathways. The results of these docking studies demonstrated that Piperine exhibited an excellent interaction with targets such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and COX-2. This research provides insight into the development of new compounds for immunomodulation and the management of inflammatory illnesses. However, further research on Piperine and related flavonoids is necessary to assess their safety as a potential immunomodulatory agent.

**Keywords:** Alkaloids, COX-2, Cytokines, Piperine

The immune system comprises a variety of cells that are necessary for pathogen clearance. Phagocytosis is one of the methods that uses enzyme-catalyzed oxidative stress to kill invading bacteria. During this process, many proinflammatory agents attract and activate neutrophils and monocytes at the inflammatory sites<sup>1</sup>. Excessive production of oxygen species, on the other hand, has been shown to harm the body's own structures and components, such as triglycerides, protein complexes, and even nucleic acids, leading in chronic exacerbation. While acute inflammation benefits in the fight against infections and tissue repair, chronic inflammation leads to a number of immunological disorders<sup>2</sup>. Increased cytokine and growth factor expression has been associated to a variety of clinical disorders, including abnormal gene production control, unfavourable cell proliferation, and cancer. Increased cytokine and growth factor expression has been associated to a

variety of clinical problems, including abnormal gene production control, unwanted cell proliferation, and the establishment of a persistent inflammatory response. Hindering these factors, particularly TNF- $\alpha$  and IL-1 $\beta$ , has been proven to help a range of clinical disorders, including inflammatory arthritis (RA) and other autoimmune ailments. Tumor necrosis factor acts as a control trigger cytokine in a variety of inflammatory processes, including chondrocyte disintegration. TNF- $\alpha$  levels in synovial fluid are linked to rheumatoid arthritis. TNF- $\alpha$ , in concert with IL-1 $\beta$ , is the most common cause of synovitis<sup>3</sup>.

It is considered that IL-1 $\beta$  acts before TNF- $\alpha$  in the RA. The bulk of inflammatory arthritis articular damage is produced by IL-1 $\beta$ , which induces proteoglycan degradation and limits proteoglycan production. IL-1 $\beta$  commonly combines with TNF- $\alpha$  during the pro-inflammatory phase. Both interleukin-1 (IL-1 $\beta$ ) and microbial substances promote the production of interleukin-6 and interleukin-8. IL-6 also has a role in inflammation, mostly via promoting the production of proteins during the acute phase by hepatocytes<sup>4</sup>. COX is an important isoenzyme in the

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synthesis of thromboxane as well as prostaglandins. Prostaglandins are autacoids with important functions in many physiological and metabolic processes. COX-1 is found in many organs, including the stomach, kidneys, brain, lungs, and spleen, whereas COX-2 is an upregulation enzyme that is activated when cells are injured. Because of COX 1 inhibition, analgesic use is associated with renal and gastrointestinal toxicity, limiting the use of beneficial analgesics in the face of discomfort and swelling<sup>5</sup>.

Immune dysregulation causes gradual organ damage, resulting in pain, a worse life expectancy, and early death. The best treatment for managing immune-mediated autoimmune disorders must provide fast inflammation control, minimize tissue harm, and have a low unfavorable effect pattern. Anti-inflammatory drugs now accessible do not meet these criteria, generally displaying more adverse effects than is bearable, fewer curative outcomes than is desired, or both. Natural goods have long been thought to be a rich source of distinct chemical patterns that have no detrimental impact on the immune system<sup>6</sup>. Phytoconstituents have an important role in food flavoring, insect resistance, and treatment, including immunosuppressive substances. Additionally, traditional herbal medicines and purified natural components may be employed to accelerate the development of novel antiviral drugs. In other words, by studying the structure of naturally occurring molecules which perform the desired activities, more effective drugs can be designed<sup>7</sup>. Plant alkaloids, with their extensive medicinal history, are particularly intriguing possibilities for the treatment of autoimmune diseases. Alkaloids are a sort of nitrogen-containing chemical molecule formed from amino acids that has a low molecular weight and is found mostly in bacteria, fungi, plants, and mammals<sup>8</sup>.

For centuries, medicinal plants have been utilized as a source of nutrition, fragrances, and, in herbal medicine, as a treatment for a variety of ailments. *Piper nigrum*, a member of the Piperaceae family, is among the most extensively used herbs and spices worldwide. The inclusion of the phytochemical piperine gives it a characteristic harsh taste. Besides from being used as a flavor, *Piper nigrum*, is also widely employed in medicine, preservation, and fragrance<sup>9</sup>. Piperine concentration varies with the pepper plant and ranges from 2 to 7.4% in black pepper. Piperine has been shown in several *in vitro* and *in vivo* experimental studies to have antiproliferative,

anticancer, antiangiogenesis, antioxidant, antidiabetic, anti-obesity, antihyperlipidemic, antibacterial, antiaging, and immunomodulatory properties. Piperine has also been shown to have hepatoprotective, anti-allergic, anti-inflammatory, and neuroprotective effects<sup>10</sup>.

The current study aims to elucidate the basic mechanism of immunomodulation. In this work, the *In silico* interaction of piperine with different immunomodulatory cytokines was examined. Moreover, the chemical underpinning of Piperine's interaction with COX-2 was discovered.

## Materials and Methods

### Software

Python 2.7- language was downloaded from [www.python.com](http://www.python.com), Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from [www.scripps.edu](http://www.scripps.edu), Discovery Studio visualizer 4.1 was downloaded from [www.accelerys.com](http://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- $\alpha$  (PDB ID: 2AZ5), IL 1 $\beta$  (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 3D chemical structure of Piperine was retrieved (<http://pubchem.ncbi.nlm.nih.gov/>). These .sdf and .mol files obtained from PubChem were converted into .pdb files using Marvin 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 Piperine was carried out using the Autodock tools (ADT) v1.5.4 and autodock v 4.2 programs. Piperine 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<sup>11</sup>.

## Results

Molecular docking is a legitimate approach that assists in visualizing 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<sup>12</sup>. Likewise, the molecule with the least inhibition constant denotes the 'potential' compound. TNF- $\alpha$  (PDB ID: 2AZ5), IL 1 $\beta$  (PDB ID: 1ITB), IL-6 (PDB ID: 1P9M), and COX-2 (PDB ID: 4COX) were explored for probable interactions with Piperine in this investigation (Fig. 1).

In case of TNF- $\alpha$ , Piperine demonstrated van der Waals interaction at residues Ile A 155, Ser A 60, Gly A 121, Tyr B 59, Tyr B 119, Tyr A 151, hydrogen bonding at Tyr B 151, carbon hydrogen bond at Ser B 60,  $\pi$ -amide bond at Leu A 120 and  $\pi$ -alkyl bond at Tyr A 59. Piperine interacted with six amino acid residues through van der Waals interactions. These residues are Ile A 155, Ser A 60, Gly A 121, Tyr B 59, Tyr B 119, and Tyr A 151. It is possible that some or all of these residues are located in or near the active site of TNF- $\alpha$ , and that Piperine is interacting with them to stabilize the complex. Piperine formed a hydrogen bond with the Tyr B 151 residue. This residue is located near the active site of TNF- $\alpha$ , and the hydrogen bond may be important for stabilizing the complex. Piperine formed a carbon-hydrogen bond with the Ser B 60 residue. This interaction may

also help to stabilize the complex. Piperine formed a  $\pi$ -amide bond with the LeuA 120 residue. This type of interaction involves the stacking of an aromatic ring (in this case, the piperine molecule) with the planar peptide bond of an amino acid residue (in this case, LeuA 120). This interaction may also help to stabilize the complex. Piperine formed a  $\pi$ -alkyl bond with the Tyr A 59 residue. This type of interaction involves the stacking of an aromatic ring (in this case, the piperine molecule) with an alkyl group on an amino acid residue (in this case, the side chain of Tyr A 59). This interaction may help to stabilize the complex by providing additional hydrophobic interactions between the two molecules. Overall, these interactions suggest that Piperine is able to bind to the active site of TNF- $\alpha$  (2AZ5) and stabilize the complex through a variety of noncovalent interactions. These interactions may modulate the activity of TNF- $\alpha$  by altering its interactions with other proteins or signaling molecules.

In case of IL-1 $\beta$ , vanderwaal bond at Glu A 105, Ile A 106 Lys A 109, Phe 146, Thr 147, Phe 150, hydrogen bonding at Arg A 11, Gln A 149, carbon hydrogen bonding at Met A 148, amide  $\pi$ -stacked bonding at Ans A 108 and  $\pi$ -alkyl bonding at Leu A 110 was seen. Van der Waals bonding involves weak interactions between non-polar atoms, such as those found in hydrophobic amino acids. In this case, Piperine interacted with several amino acids in IL-1 $\beta$  through van der Waals interactions, including Glu A105, Ile A106, Lys A109, Phe 146, Thr 147, and Phe 150. These interactions contribute to the stability of the complex by increasing the contact area between

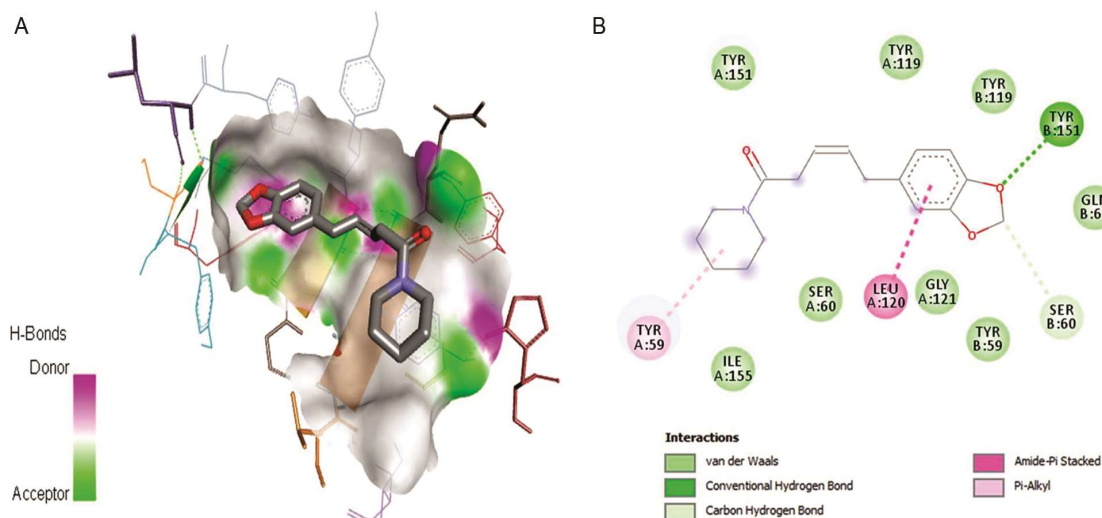


Fig. 1 — Docking interactions of Piperine with TNF- $\alpha$  (A) 3D interactions; and (B) 2D interactions

piperine and IL-1 $\beta$ . Hydrogen bonding is a strong interaction between a hydrogen atom and an electronegative atom such as oxygen or nitrogen. Piperine forms two hydrogen bonds with IL-1 $\beta$ : one with Arg A11 and another with Gln A149. These interactions are important for maintaining the specificity and affinity of the complex. Carbon-hydrogen bonding involves weak interactions between carbon and hydrogen atoms in hydrophobic amino acids. In the piperine-IL-1 $\beta$  complex, a carbon-hydrogen bond is observed between Piperine and Met A148, which contributes to the hydrophobic interactions between the two molecules. Amide  $\pi$ -stacking bonding involves interactions between  $\pi$ -electrons in an aromatic ring and the electron cloud of an amide group. In the piperine-IL-1 $\beta$  complex, Piperine forms an amide  $\pi$ -stacking bond with Asn A108, which is thought to contribute to the stability of the complex.  $\pi$ -alkyl bonding involves interactions between an aromatic ring and an alkyl group. Piperine forms a  $\pi$ -alkyl bond with Leu A110 in IL-1 $\beta$ , which helps to anchor Piperine in the binding site. Overall, the various types of non-covalent interactions observed between Piperine and IL-1 $\beta$  in the 2NVH complex play an important role in stabilizing the complex and providing specificity for Piperine's anti-inflammatory effects (Fig. 2).

Piperine interacted with several amino acids in the active site of IL-6 through Vander Waals interactions, including Ala B 58, Leu B 165, Asn B 61, Asn B 60, Thr B 162, and Gln B 154. Vander Waals interactions occur due to the fluctuating electron density in atoms and molecules, and are important for stabilizing protein-ligand complexes. Piperine formed a carbon

hydrogen bond with Leu B 64. This type of bond involves a carbon atom in the ligand (piperine) interacting with a hydrogen atom in the protein (IL-6). Piperine formed a  $\pi$ -cation bond with Lys B 66. This type of bond occurs between a positively charged atom (in this case, the epsilon amino group of lysine) and the  $\pi$ -electron density of an aromatic system (in this case, the piperine molecule). Piperine forms a  $\pi$  donor hydrogen bond with Asn B 63. This type of bond occurs between a  $\pi$  electron donor (in this case, the Piperine molecule) and a hydrogen atom attached to a polar atom (in this case, the amide nitrogen of asparagine). Piperine interacted with the lone pair of electrons on the nitrogen atom of Leu B 62 through a  $\pi$  interaction. This type of bond occurs between an electron-rich system (in this case, the nitrogen lone pair) and an electron-deficient system (in this case, the piperine molecule). Piperine formed an alkyl bond with Met B 161. This type of bond involves a carbon atom in the ligand (Piperine) interacting with a carbon atom in the protein (methionine). Piperine formed a  $\pi$ -alkyl bond with Leu B 158. This type of bond occurs between an alkyl group (in this case, the isopropyl group of piperine) and an aromatic ring (in this case, the phenyl ring of Leu B 158). Overall, the interactions between piperine and IL-6 in the active site involve a combination of hydrophobic and aromatic interactions, as well as various types of non-covalent bonds. These interactions help to stabilize the protein-ligand complex, and contribute to the ability of piperine to inhibit IL-6 activity (Fig. 3).

Piperine interacted with the COX-2 protein through Vander Waals interactions with the following amino

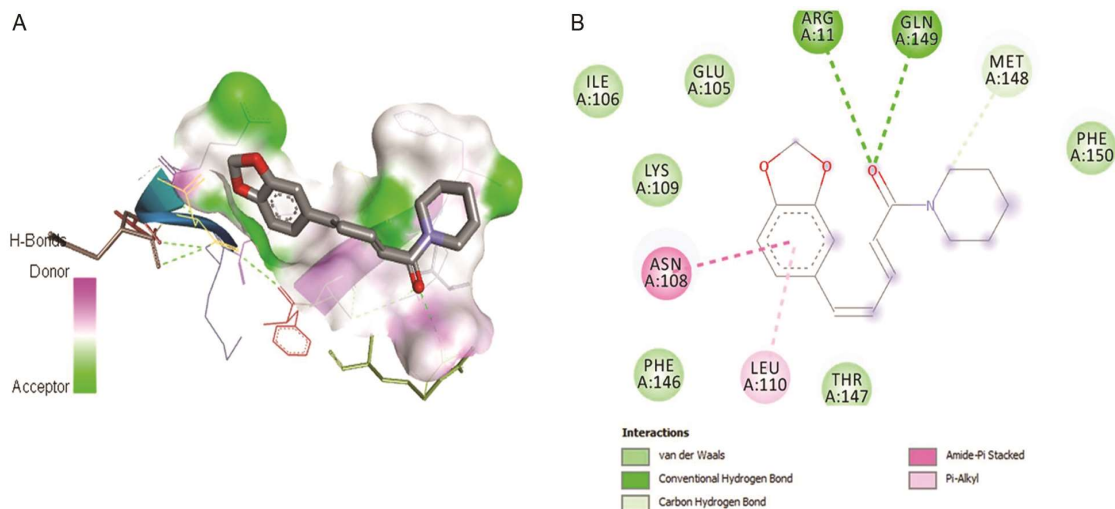


Fig. 2 — Docking interactions of Piperine with IL-1 $\beta$  (A) 3D interactions; and (B) 2D interactions

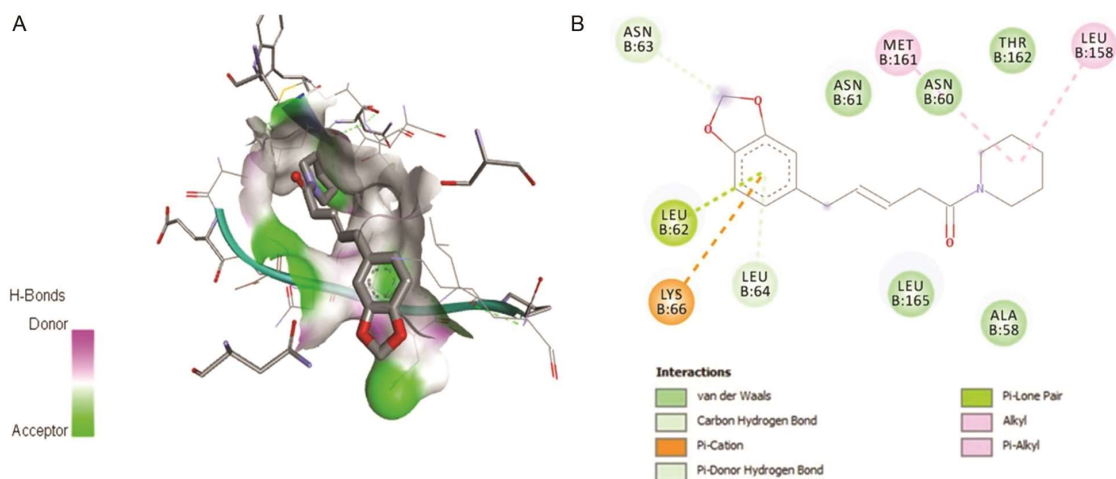


Fig. 3 — Docking interactions of Piperine with IL-6 (A) 3D interactions; and (B) 2D interactions

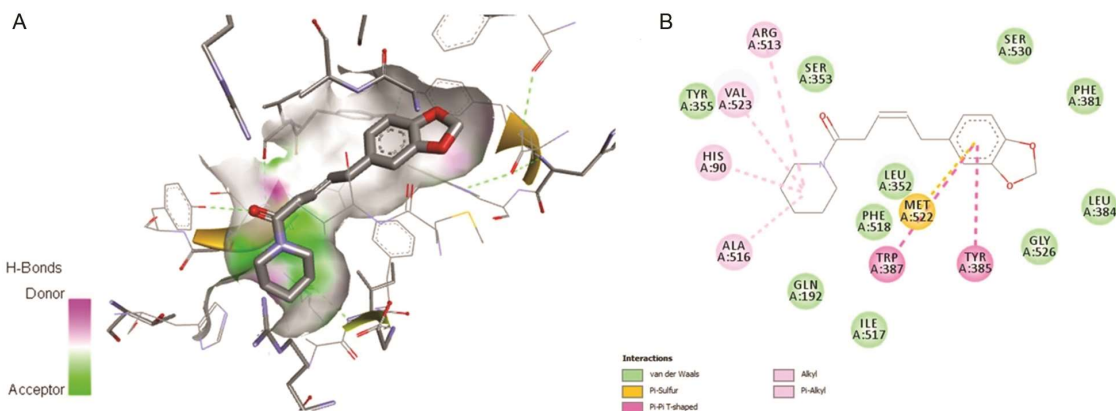


Fig. 4 — Docking interactions of Piperine with COX-2 (A) 3D interactions; and (B) 2D interactions

acid residues: GlnA 192, Phe A 518, Leu A 352, Gly A 526, Leu A 384, Phe A 381, Ser A 530, and Ser A 353. Vander Waals interactions involve the attraction between two atoms or molecules due to fluctuations in their electron densities. Piperine formed a covalent bond with the sulfur atom of Met A 522 in COX-2. This type of interaction is known as  $\pi$  sulfur bonding. Piperine also formed  $\pi$ - $\pi$  T shaped bonding interactions with the aromatic rings of TrpA 384 and Tyr A 385 in COX-2. These interactions involve the stacking of two aromatic rings in a T-shaped orientation. Piperine formed an alkyl bond with the side chains of Ala A 516 and His A 90 in COX-2. An alkyl bond involves the sharing of a pair of electrons between two atoms to form a covalent bond. The binding of piperine with COX-2 involves interactions with amino acid residues that are also involved in the binding of arachidonic acid. For example, piperine forms Vander Waals interactions with LeuA 384, which is also involved in the binding of arachidonic

acid. Additionally, piperine forms  $\pi$ - $\pi$  T shaped bonding interactions with the aromatic rings of TrpA 384 and Tyr A 385, which are also involved in the binding of arachidonic acid. These similarities in the binding interactions suggest that piperine may compete with arachidonic acid for binding to COX-2. Moreover, piperine forms a covalent bond with the sulfur atom of Met A 522, which is not directly involved in the binding of arachidonic acid. This suggests that piperine may have a unique mechanism of action compared to arachidonic acid and other non-steroidal anti-inflammatory drugs (NSAIDs) that target COX-2 (Fig. 4).

## Discussion

The active site of TNF- $\alpha$  in the 2AZ5 crystal structure is a groove formed by the association of two TNF- $\alpha$  monomers. The groove is lined with amino acid residues that interact with ligands or other proteins, and it includes a zinc ion that is coordinated

by three histidine residues (His 95, His 105, and His 106). The active site is located at the interface between the two TNF- $\alpha$  monomers and is involved in the binding of ligands and receptors that regulate TNF- $\alpha$  signaling. It appears that Piperine is interacting with amino acid residues located near the active site of TNF- $\alpha$ , but it is not clear whether Piperine is directly interacting with the zinc ion or other key residues within the active site. The van der Waals interaction of Piperine with Ser A 60 and Gly A 121 may be close to the active site, and the hydrogen bonding with Tyr B 151 may also be important for ligand binding in the active site<sup>13</sup>.

IL-1 $\beta$  is an important mediator of inflammation, and its activity is tightly regulated to prevent excessive inflammation, which can lead to tissue damage and disease. By binding to the protein and modulating its activity, Piperine has the potential to act as an anti-inflammatory agent. The 2NVH structure shows that Piperine binds to a pocket located near the surface of IL-1 $\beta$ . This pocket is known to be important for the protein's activity, as it is involved in the binding of IL-1 $\beta$  to its receptor and subsequent signaling events. The binding of Piperine to this pocket likely alters the conformation of IL-1 $\beta$  and interferes with its ability to interact with its receptor, thereby inhibiting its activity. Additionally, the specific interactions between Piperine and amino acids in IL-1 $\beta$  at the atomic level suggest that Piperine binds to the protein with high specificity and affinity. This binding may stabilize the protein and prevent its degradation, thereby enhancing its activity. Moreover, the binding of Piperine to IL-1 $\beta$  may alter the protein's interactions with other proteins and molecules in the cell, leading to downstream effects on inflammation and immune responses. There is only one active site of Interleukin 1 $\beta$  (IL-1 $\beta$ ), which is located near the surface of the protein and is known to be important for the protein's activity. The active site of IL-1 $\beta$  is involved in the binding of the protein to its receptor and subsequent signaling events. In the case of the PDB ID 2NVH complex, the pocket that piperine binds to is located within this active site. This binding pocket is surrounded by residues that are critical for IL-1 $\beta$  activity, such as Glu 109, Phe 146, and Thr 147, which form important interactions with IL-1 $\beta$ 's receptor. It's worth noting that while there may be other regions on the IL-1 $\beta$  protein that interact with Piperine, the active site is likely the most important site of interaction, as it is directly involved in the protein's activity and function<sup>4</sup>.

The docked crystal structure of the piperine-IL-6 complex (PDB ID: 1P9M) shows that Piperine binds to the active site of IL-6, which is a hydrophobic pocket located at the interface between the two subunits of the IL-6 homodimer. Piperine interacted with several key amino acid residues in the IL-6 active site through various types of non-covalent interactions, including hydrophobic,  $\pi$ -cation,  $\pi$ -alkyl,  $\pi$  donor hydrogen bonding, carbon hydrogen bonding, and  $\pi$ -lone pair interactions. These interactions contribute to the ability of Piperine to inhibit IL-6 activity, which may have potential therapeutic applications in various inflammatory diseases. Piperine interacts with several key amino acid residues in the IL-6 active site, including Ala B 58, Leu B 62, Leu B 64, Asn B 63, Leu B 158, Met B 161, Thr B 162, Gln B 154, and Lys B 66. These amino acid residues are located within the hydrophobic pocket of IL-6, and they are involved in various types of non-covalent interactions with Piperine, including hydrophobic interactions,  $\pi$ -cation interactions,  $\pi$ -alkyl interactions,  $\pi$  donor hydrogen bonding, carbon hydrogen bonding, and  $\pi$ -lone pair interactions. Overall, the major location in IL-6 where piperine is demonstrating interactions is the hydrophobic pocket at the interface between the two subunits of the IL-6 homodimer, which is the active site of IL-6. Piperine interacts with specific amino acid residues in this active site through a combination of various types of non-covalent interactions, which contribute to its ability to inhibit IL-6 activity. Therefore, it can be concluded that piperine is indeed showing interaction with IL-6, and this interaction is mediated through a combination of non-covalent interactions with specific amino acid residues in the IL-6 active site<sup>14</sup>.

The atomic-level details of the binding of arachidonic acid and piperine with COX-2 (PDB ID 4COX) involve interactions with specific amino acid residues and regions of the protein. Arachidonic acid binds to the active site of COX-2, which is a deep hydrophobic pocket formed by the interaction of several amino acid residues. The binding of arachidonic acid involves several interactions with the amino acid residues within this pocket. For example, the carboxylate group of arachidonic acid forms salt bridges with ArgA 120 and Arg A 513, while the hydrophobic tail of arachidonic acid interacts with several hydrophobic residues, including Leu A 352, Ile A 503, Val A 509, and Leu A 531. The binding of arachidonic acid also induces a conformational change in COX-2

that allows for the subsequent chemical reactions to produce prostaglandins. Piperine binds to COX-2 in a similar hydrophobic pocket near the active site, but its interactions with specific amino acid residues are different from those of arachidonic acid. Piperine forms Vander Waals interactions with several amino acid residues in COX-2, including GlnA 192, Phe A 518, Leu A 352, Gly A 526, Leu A 384, Phe A 381, Ser A 530, and Ser A 353. Piperine also forms a covalent bond with the sulfur atom of Met A 522 and  $\pi$ - $\pi$  T shaped bonding interactions with the aromatic rings of Trp A 384 and Tyr A 385. The binding interactions of arachidonic acid and piperine with COX-2 involve specific amino acid residues and regions of the protein, which contribute to their distinct mechanisms of action. While both compounds bind to the same hydrophobic pocket in COX-2, the differences in their binding interactions may contribute to the different effects they have on COX-2 activity and downstream signaling pathways<sup>15-17</sup>.

### Conclusion

Piperine was docked to different inflammatory and immunomodulatory domains to explore and analyse the former's interplay with the latter *In silico*. The docking scores and interaction analyses of piperine show that it can bind to a number of locations involved in inflammation including immunomodulation. Piperine interacted with chemokines as well as inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and COX-2. Piperine displayed a strong affinity with all of the targets. Piperine interacts with a number of and inflammatory mediators, based on the findings of this study. Further study on Piperine but instead related flavonoids is required to develop and construct QSAR and QSPR studies that might lead to the development of novel, efficacious, and affordable immunomodulators.

### Conflict of interest

All authors declare no conflict of interest.

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