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Virtual screening of Ginsenosides for COX-2 inhibition: Insights from *in silico* docking studies

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Analgesic discovery has been a significant aspect to medical practice as they are critical tools in the management of pain and enhancement of procedures. Nonetheless, the side effects of these drugs, more so opioids and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), require constant research for safer analgesics. Herbal medicine, which dates back to historical cultures, has been considered as the reference source for modem pharmaceuticals. Of all the herbal components, ginseng has been noted for positive effects on the cardiovascular and immunological systems, pain relieving and anti-inflammatory effects. This work reports molecular docking methods that afford detailed information about the ginsenosides and Cyclooxygenase-2 (COX-2) binding, revealing aspects of drug- protein interactions. The communication processes involving amino acids which are arginine, valine, histidine, threonine and glycine were discussed and their importance highlighted especially in drug design. These ginsenosides include Rg1, Rd, and Rh2 were found to possess anti-inflammatory properties through the inhibition of COX-2, NF-κB, and cytokines. Threonine was identified as having a significant role in stabilizing certain inhibitors such as Ginsenoside Rh2 within the binding pocket of COX-2. Quantitative data supporting these observations revealed high degree of binding, with the Ginsenoside Rh2 having a binding energy value of -8.5 kcal/mol and thus it is considered as a good candidate for acting as a potent inhibitor. The findings presented in the article offer a better understanding of the potentially therapeutic value of compounds isolated from ginseng. As such, these findings are of significance for future drug development in the sphere of pain relief and anti-inflammatory therapy.

Keywords: Analgesics, Anti-inflammatory, Ginsenosides, Herbal medicine, Molecular docking

Pain and inflammation are integral components of the body's response to injury, infection, or other harmful stimuli[1]. These physiological processes serve as protective mechanisms, signalling that something is amiss and prompting the immune system to take action. Symptoms of pain include localized or widespread discomfort, ranging from mild to severe, and can be sharp, throbbing, or aching in nature[2]. Pain often accompanies injuries, diseases, or inflammatory conditions, hindering normal functioning and prompting individuals to seek medical attention. Inflammation, on the other hand, is characterized by redness, swelling, heat, and sometimes loss of function in the affected area. Various factors can contribute to pain and inflammation, such as infections, trauma, autoimmune disorders, or chronic diseases like arthritis[3]. Infections may trigger an immune response, leading to inflammation and pain as the body attempts to neutralize pathogens[4]. Trauma, including injuries or surgeries, can also induce inflammation as part of the healing process. Autoimmune disorders result from the immune system mistakenly attacking healthy tissues, causing chronic inflammation and pain. Arthritis, encompassing conditions like rheumatoid arthritis and osteoarthritis, involves inflammation of the joints, leading to pain and impaired mobility[5].

Cyclooxygenase-2 (COX-2) is a key enzyme involved in the molecular mechanism of pain. It plays a crucial role in the synthesis of prostaglandins, which are lipid compounds that contribute to inflammation, fever, and pain[6]. The activation of COX-2 occurs in response to various stimuli, such as tissue injury or inflammation, leading to the conversion of arachidonic acid into prostaglandin H2 (PGH2), a precursor of various prostaglandins. The molecular mechanism involves a complex cascade of events. First, arachidonic acid is released from cell membranes through the action of phospholipase A2. COX-2 then catalyzes the conversion of arachidonic acid to PGH2. PGH2 is subsequently converted by other enzymes into different prostaglandins, including those that contribute to the perception of pain. The amino acid residues in the COX-2 enzyme are crucial for its activity and function. The active site of COX-2

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contains amino acid residues that directly interact with arachidonic acid during catalysis[7]. The specific amino acid residues involved in the catalytic activity of COX-2 include serine, tyrosine, and arginine. These residues participate in the formation of a peroxidase active site, facilitating the conversion of arachidonic acid to PGH2.

Analgesics, while effective in managing pain, are associated with various adverse effects. Nonsteroidal anti-inflammatory drugs (NSAIDs) can cause gastrointestinal issues like ulcers and bleeding, as well as kidney problems. Opioid analgesics pose a risk of addiction, respiratory depression, and constipation. Long-term use may lead to tolerance and dependence. Acetaminophen, another common analgesic, can cause liver damage when taken in excessive amounts. NSAIDs and opioids may also contribute to cardiovascular complications[8]. Herbal medicine serves as a rich source of natural analgesics, offering alternatives to conventional pain relief. Plants like turmeric, willow bark, and ginger possess anti-inflammatory properties, reducing pain without the side effects associated with synthetic analgesics[9]. Kratom, derived from a Southeast Asian tree, has opioid-like effects and is used traditionally for pain relief[10]. Cannabis, containing cannabinoids like THC and CBD, exhibits analgesic properties and is increasingly explored for pain management[11]. Ginseng, a traditional medicinal herb, is increasingly recognized for its potential as a source of natural analgesics[12]. The active compounds in ginseng, known as ginsenosides, possess anti-inflammatory and pain-relieving properties. These compounds may modulate pain perception by interacting with receptors in the nervous system. Studies suggest that ginseng extracts exhibit analgesic effects in various pain models, making it a promising candidate for pain management[13]. The pharmacological studies of ginsenosides in relation to analgesics have been of great interest to researchers in view of the possible interaction between Ginsenosides and Cyclooxygenase-2 (COX-2). Ginsenosides are the bioactive metabolites of ginseng and they have shown potential antinociceptive and anti-inflammatory effects[14]. Recent molecular docking studies have provided detailed insights into the structural details of ginsenosides binding to COX-2, including a crucial role played by threonine in stabilising the interaction[15]. Especially, the anti-inflammatory effect of ginsenosides Rg1, Rd, and Rh2 has been implicated in the COX-2 suppression and modulation of NF-κB and cytokines[16]. Herbal medicines, like ginsenosides, offer potential advantages including fewer side effects, lower toxicity, and holistic benefits. Evidence supports their efficacy in modulating inflammation and pain pathways with fewer adverse effects compared to synthetic drugs. Studies show ginsenosides effectively inhibit COX-2, offering safer alternatives for pain management[17].

Herbal medicines such as ginsenosides therefore have benefits such as reduced side effects, less toxicity, and improved general health. It also proves that natural drugs are effective in the reduction of inflammation and pain signals with minimal side effects than synthetic drugs. Research further indicates that ginsenosides prevent the production of the enzyme COX-2 providing safer pain relief options[18].

Computer-aided drug design (CADD) has revolutionized the field of drug discovery by providing powerful tools for *in silico* analysis. Molecular docking, a crucial component of CADD, plays a pivotal role in identifying potential therapeutic agents. It involves the computational simulation of the interaction between small molecules and target proteins, predicting their binding affinity and orientation[19]. By simulating the molecular interactions at the atomic level, docking helps prioritize and optimize lead compounds, significantly expediting the drug development process. This *in silico* approach enhances the efficiency of hit identification, enabling researchers to focus on the most promising candidates for further experimental validation, ultimately accelerating the development of novel and effective therapeutic agents[20].

The research aims to conduct virtual screening of ginsenosides for COX-2 inhibition through in silico docking studies, elucidating the molecular interactions and identifying potential candidates for novel anti-inflammatory therapeutics.

Materials and Methods

Experimental setup

The software utilized for the study included Python 2.7, obtained from www.python.com, Molecular Graphics Laboratory (MGL) tools, and AutoDock 4.2 downloaded from www.scripps.edu. Discovery Studio Visualizer 4.1 was acquired from www.accelerys.com. The Python 2.7 language served as the foundation for the subsequent molecular docking experiments.

Docking studies

Molecular docking investigations were conducted on ginseng phytochemicals against Cyclooxygenase-2 (COX-2). The molecular structures of these phytochemicals were sourced from PubChem, while the COX-2 structure files (PBD ID: 4COX) were downloaded from the Protein Data Bank (PDB) at www.rcsb.org/pdb. Pre-processing steps involved editing the COX-2 structures, including the removal of heteroatoms and addition of C-terminal oxygen. GasteigerMarsili partial charges were assigned to ligands, non-polar hydrogen atoms were merged, and torsions were allowed during docking. Active pockets were identified using LigPlot, and the Computed Atlas of Surface Topography of Proteins server verified these pockets. LigPlot generates 2D diagrams illustrating protein-ligand interactions, displaying hydrogen bonds, hydrophobic contacts, and water-mediated bonds. Computed Atlas of Surface Topography of Proteins (CASTp) identifies and measures pockets on protein surfaces, aiding in the analysis of binding sites and ligand accessibilities.Docking employed the Lamarckian Genetic Algorithm for energy minimization with default parameters, and Discovery Studio was employed for result visualization.

Results

GinsenosideKch (Figure 1) engages in molecular interactions crucial for its binding, featuring hydrogen bonds with amino acids ASP 515, THR 94, and VAL 89. These hydrogen bonds facilitate specific and targeted connections between the compound and the protein or enzyme it interacts with. Additionally, GinsenosideKch forms van der Waals interactions with VAL 89, THR 94, ASP 515, ARG 513, and LYS 511. This array of van der Waals forces enhances the overall stability of the binding complex, contributing to the compound's effectiveness.

Ginsenoside Rd (Figure 2) exhibits a notable hydrogen bond with the amino acid HIS 356. This interaction with HIS 356 signifies a targeted and

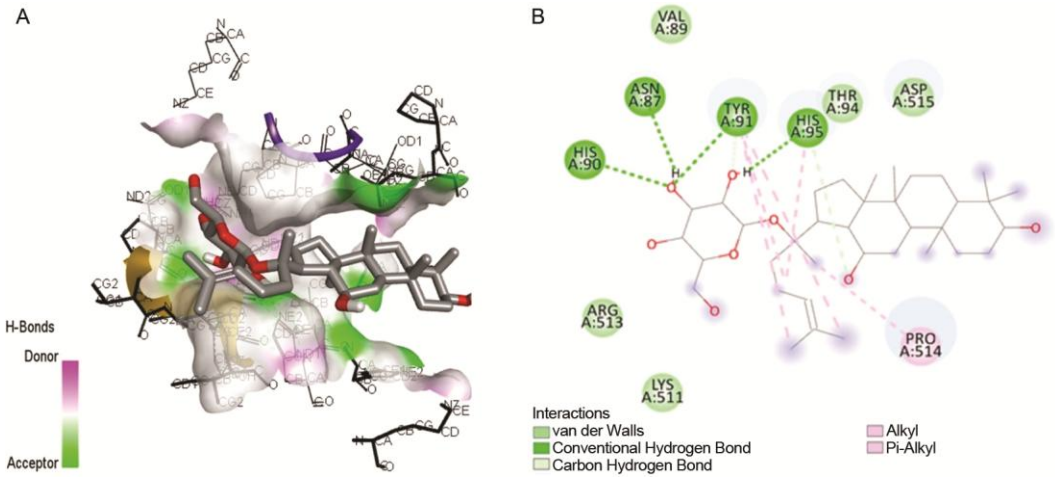

Fig. 1 — Docking interactions of GinsenosidesKch with COX-2 (A) 3D interactions; and (B) 2D interactions

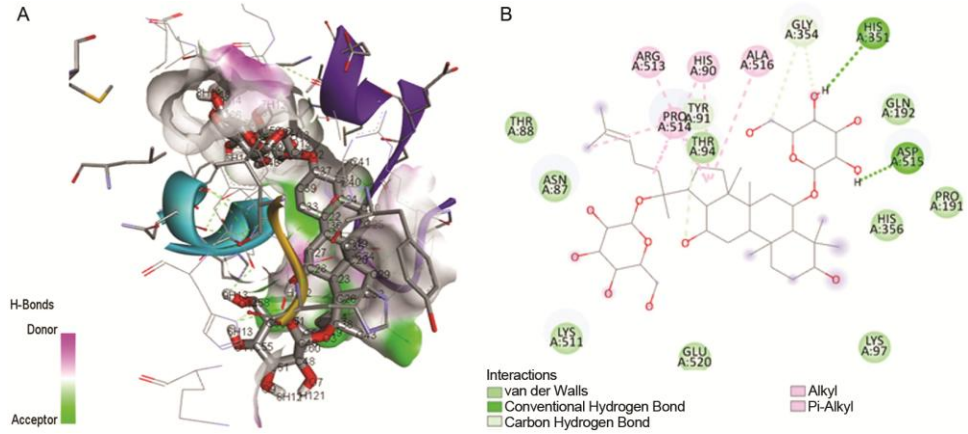

Fig. 2 — Docking interactions of Ginsenoside Rd with COX-2 (A) 3D interactions; and (B) 2D interactions

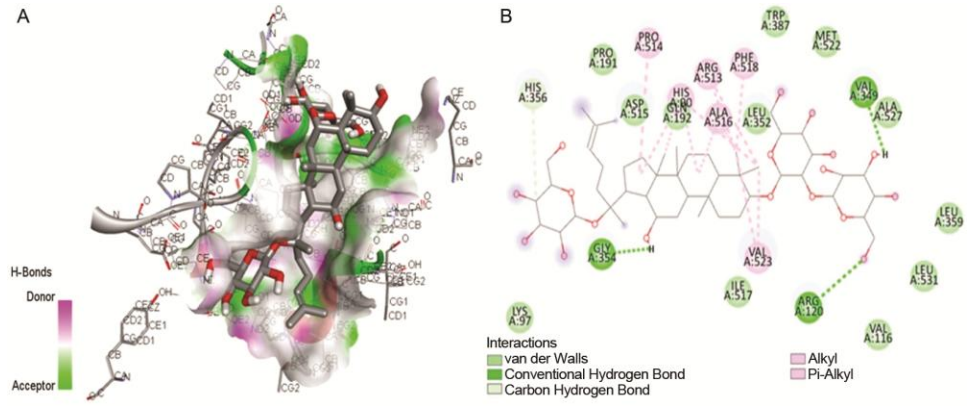

Fig. 3 — Docking interactions of Ginsenoside Rg1 with COX-2 (A) 3D interactions; and (B) 2D interactions

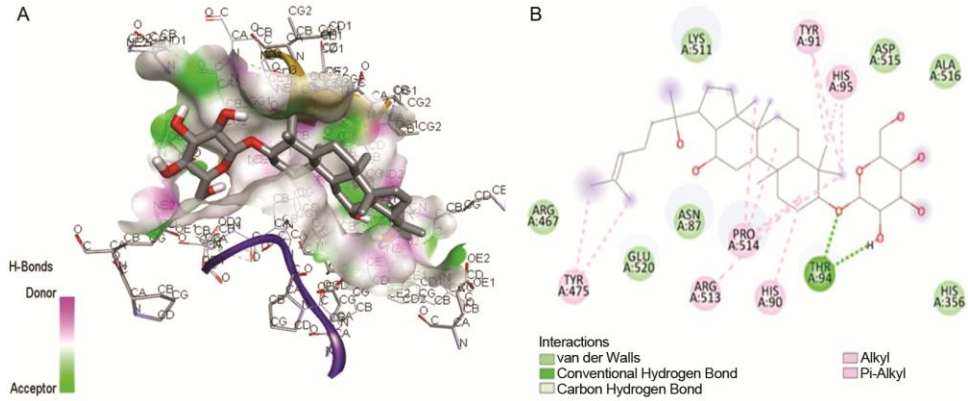

Fig. 4 — Docking interactions of Ginsenoside Rh2 with COX-2 (A) 3D interactions; and (B) 2D interactions

Table 1 — Molecular Docking studies of Ginsenosides with COX-2

Compound	Binding energy (kcal/mol)	Inhibition constant	H-bonds	van-der waals interactions
GinsenosideKch	-7.05	34.1 µM	Asp 515,Thr 94,Val 89	Val 89, Thr 94, Asp 515, Arg 513, Lys 511
GinsenosideRd	-7.25	21.34 µM	His 356	-
GinsenosideRg1	-8.01	5.74 µM	Tyr 91,Gly 354	-
GinsenosideRh2	-10.56	73.62 µM	Thr 94	Lys 511, Asp 515, Ala 516, His 356, Asn 87, Glu 520, Arg 467

selective binding mode, potentially influencing the biological activity of the compound. While details about van der Waals interactions are not provided, the presence of hydrogen bonds highlights the molecular specificity of Ginsenoside Rd, suggesting its potential role in modulating enzymatic or protein functions.

Ginsenoside Rg1 (Figure 3) establishes significant molecular interactions, forming hydrogen bonds with TYR 91 and GLY 354. The specific bonding with these amino acids suggests a targeted and intricate connection, potentially influencing the compound's biological activity. Hydrogen bonds play a crucial role in mediating selective interactions within biological systems, contributing to the overall stability of the binding complex. While details about van der Waals interactions are not provided, the presence of hydrogen bonds underscores the molecular precision of Ginsenoside Rg1.

Ginsenoside Rh2 (Figure 4) engages in a comprehensive network of van der Waals interactions with specific amino acids, including LYS 511, ASP 515, ALA 516, HIS 356, ASN 87, GLU 520, and ARG 467. This intricate molecular mechanism suggests a multifaceted interaction with its biological target, potentially a protein or enzyme. Van der Waals forces play a crucial role in stabilizing molecular complexes, contributing to the overall binding affinity. The involvement of diverse amino acids in these interactions indicates Ginsenoside Rh2's ability to establish a complex and specific binding pattern, influencing the target's structure or function (Table 1).

Discussion

The discovery of analgesics has indeed played a critical role in the development of new drugs and the field of medicine as a whole. Analgesics are substances that relieve pain, and they are a vital component of medical care[21]. The alleviation of pain is not only essential for improving the quality of life for individuals suffering from various conditions but also allows for better management of many medical procedures[22]. The development of analgesics has been a significant focus in pharmaceutical research, leading to the creation of various classes of drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and other pain-relieving medications. These drugs are used to manage pain associated with conditions ranging from chronic illnesses to post-operative recovery[23].

Indeed, while analgesics play a crucial role in managing pain, it is essential to acknowledge that some of these medications come with potential side effects. Addressing these side effects is imperative to enhance the safety and effectiveness of pain management strategies. Common side effects of analgesics, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), may include gastrointestinal issues, sedation, tolerance, dependence, and the risk of addiction[8]. Researchers and pharmaceutical companies are actively working to develop new analgesic compounds with improved safety profiles.

Herbal medicine has been a cornerstone of healthcare since ancient times, playing a pivotal role in healing mankind across diverse cultures and civilizations. Drawing from the vast reservoir of plant-derived compounds, herbal remedies have been integral to traditional medicine systems globally, offering therapeutic benefits for a myriad of ailments[24]. As scientific knowledge has advanced, herbal medicine has not only remained relevant but has also become a source of inspiration for the development of modern pharmaceuticals[25]. Many of the world's most potent and widely used drugs have their origins in plants. Aspirin, derived from willow bark[26], and the anti-malarial drug quinine from the cinchona tree are just a few examples[27]. The exploration of herbal medicine has led to the identification and isolation of active compounds, serving as prototypes for the synthesis of newer generation pharmaceuticals.

Ginseng finds its role in modulation of the body systems. Studies suggest that ginsenosides, the active compounds in ginseng, may have vasodilatory effects, promoting healthy blood circulation and potentially reducing the risk of cardiovasculardiseases[28]. The herb's adaptogenic properties are thought to help regulate blood pressure and cholesterol levels, contributing to overall cardiovascular well-being. Ginseng is also known for its immunomodulatory effects. It has been reported to enhance the activity of immune cells, supporting the body's defense mechanisms against infections and diseases. Research indicates that ginsenosides may have antioxidant and anti-inflammatory effects, potentially protecting nerve cells from damage and supporting cognitive function. These neuroprotective qualities make ginseng a subject of interest in studies related to neurodegenerative disorders and cognitive health[29,30].

The active compounds in ginseng, particularly ginsenosides, have been studied for their ability to alleviate pain and reduce inflammation. This analgesic property is particularly beneficial for individuals experiencing various forms of discomfort, providing a potential alternative or complementary approach to conventional pain management[31]. In addition to its analgesic effects, ginseng is recognized for its anti-inflammatory actions[32]. Studies have suggested that ginsenosides, the bioactive components of ginseng, may inhibit the activity of cyclooxygenase-2 (COX-2), an enzyme involved in the inflammatory response. By modulating COX-2, ginseng may help regulate the production of inflammatory mediators, contributing to the overall dampening of inflammation in the body. The prominent action of inhibiting COX-2 and modulating cytokine production positions ginseng as a promising candidate for managing inflammatory conditions and promoting overall immune health[33].

Molecular docking aids tounderstandhow a ligand, such as a drug or natural compound, interacts with a target protein at the atomic level. Such insights are crucial in drug discovery, allowing researchers to design and optimize compounds by precisely tailoring their interactions, ultimately advancing the development of effective pharmaceuticals with targeted and well-defined mechanisms of action[34,35]. In the current investigation, several Ginsenosides underwent molecular docking with the enzyme COX-2 to elucidate their interactions at the molecular level[36]. Through this computational approach, the preferred binding conformations and potential interactions between the Ginsenosides and specific amino acid residues within the COX-2 active site were explored.

Arginine, a positively charged amino acid, often assumes pivotal roles in protein functions. Its involvement in catalytic reactions, stabilizing intermediates, and interacting with substrates is well-documented[37]. Notably, Ginsenoside C-K demonstrates a significant interaction at Arginine 513 through van der Waals forces, a key active site within the hydrophobic cavity of COX-2.Valine, a nonpolar amino acid prominent in hydrophobic protein regions, contributes substantially to protein structure, stability, and specific interactions. This is exemplified by Valine 89, an active site component of COX-2, displaying docking interactions with Ginsenoside C-K. This result is in association with a study which investigated the anti-inflammatory and analgesic properties of ginsenoside metabolite compound K (CK) and its underlying mechanisms. In mouse and rat models, CK demonstrated dose-dependent alleviation of ear and paw edema, and analgesic effects against acetic acid-induced pain. The findings suggest that CK exerts its effects by down-regulating cyclooxygenase-2 (COX-2) expression, resulting in reduced prostaglandin E2 (PGE2) synthesis, highlighting the potential therapeutic significance of CK in modulating inflammation and pain through the regulation of COX-2 activity[16].

Histidine, with a unique pKa near physiological pH, serves as a pH sensor and participates in proton transfer reactions in proteins[38]. In COX-2, histidine residues are often crucial for catalysis, proton shuttling, and ligand interactions[39]. The observed hydrogen bonding of Ginsenoside Rd at His 356, adjacent to the Tyr 355 heme factor, underscores the intricate molecular interactions involved. One of the biological study signifies Ginsenoside Rd to exhibit anti-inflammatory effects which is associated with inhibition the expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS)-stimulated RAW264.7 cells and mouse liver. The observed reductions in nitric oxide, prostaglandin E2 (PGE2), and NF-κB activity suggest that ginsenoside Rd mitigates inflammation by down-regulating NF-κB and suppressing the expressions of iNOS and COX-2[40].

Threonine, known for its hydroxyl group, is implicated in phosphorylation events and contributes to protein structure and function[41]. In COX-2, Threonine residues, such as Thr 91 in the active site, reveal docking interactions with Ginsenoside RG1, indicating their role in substrate binding and catalysis.Glycine, the smallest amino acid, is esteemed for its flexibility, fitting into confined protein spaces. Glycine 354's interaction with Ginsenoside RG1, particularly in the vicinity of the active site's hydrophobic segment, exemplifies its role in accommodating ligands. Ginsenoside RG1 demonstrates significant potential in preventing and treating inflammatory diseases. Through its anti-inflammatory properties, Ginsenoside Rg1 has been shown to modulate immune responses and suppress inflammatory pathways[42].

Threonine, with diverse roles in proteins, including phosphorylation sites and catalytic activities, emerges as a stabilizer of inhibitors[43]. The interaction of Ginsenoside Rh2 with Thr 94, as evidenced in the study, underscores the potential significance of this interaction in stabilizing the inhibitor within the COX-2 binding pocket. A study affirms Ginsenoside Rh2 exhibited efficacy in down-regulating inflammatory genes, including COX-2, highlighting its impact on key inflammatory mediators such as COX-2 through the suppression of the NF-κB pathway

Conclusion

This study explored the interactions between ginsenosides and cyclooxygenase-2 (COX-2), a key enzyme in the inflammatory response. Using molecular docking simulations, we investigated how various ginsenosides (C-K, Rd, RG1, and Rh2) bind to specific amino acid residues within the COX-2 active site.The results revealed significant interactions between the ginsenosides and crucial amino acids like arginine, valine, histidine, threonine, and glycine. These interactions suggest that ginsenosides may inhibit COX-2 activity, potentially explaining their anti-inflammatory and analgesic effects observed in previous *in vivo* studies. Our findings support the potential of ginsenosides, particularly C-K, Rd, RG1, and Rh2, as therapeutic agents for managing inflammatory conditions and pain. Further research is warranted to validate these *in silico* results through *in vitro* and *in vivo* experiments. This study contributes to the scientific understanding of the mechanisms by which ginsenosides may exert their analgesic effects and paves the way for future investigations into their therapeutic applications.

Conflict of interest

All authors declare no conflict of interest.

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