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Original Research Article

Molecular docking and molecular dynamics simulation studies on the effect of baicalein on breast and ovarian cancers

Xiaoci Cao*, Guohong Xin, Baoshuan Fang
Department of Medical Oncology, Hebei General Hospital, No. 348 Heping Road, Shijiazhuang City, Hebei Province, China

For correspondence:* ***Email:* *hbcaoxiaoci@aliyun.com*

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Abstract

Purpose: *To carry out molecular docking and molecular dynamics simulation analysis on the effect of baicalein and its derivatives on breast cancer and ovarian cancer.*
Methods: *The potential ligand binding site of cyclooxygenase-2 (COX-2) was predicted, and virtual screening was carried out on baicalein, a plant-based herbal compound, and its natural derivatives. Furthermore, molecular dynamics (MD) simulation was used to confirm the validity and stability of the docking protocol.*
Results: *Molecular docking showed strong molecular interaction of baicalein and its derivatives with COX-2. The results of docking showed that baicalein, baicalein 6-O-glucoside, and baicalein monohydrate had total scores of -528.26, -505.65 and -496.83 kj/mol, respectively, relative to scores of -481.29 kj/mol for ibuprofen (NSAID), and -466.80 kj/mol for the anticancer agent, olaparib ($p < 0.05$). There were strong hydrogen bonding and electrostatic interactions at the active of the enzyme when compared to market-approved drugs such as COX-2 inhibitors (NSAIDs) and other anti-cancer drugs. Baicalein and its derivatives, as plant-based compounds, had lower probabilities of health effects such as heart attacks and strokes, than synthetic drugs.*
Conclusion: *Baicalein and its derivatives have promising potential as effective anti-breast cancer and anti-ovarian cancer agents through inhibition of COX-2 but these findings require validation via in vivo studies*

Keywords: *Cyclooxygenase-2, Ovarian cancer, Breast cancer, Baicalein, Olaparib, Molecular docking*

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INTRODUCTION

Breast cancer and ovarian cancer account for most of the mortalities associated with gynecological malignancies [1]. Several reports have indicated that COX-2 expression is linked with breast cancer and ovarian cancer [2,3]. Cyclooxygenase-2 (COX-2; EC 1.14.99.1) is linked to the production of inflammatory mediators such as prostaglandins, thromboxane, prostacyclin, and oncogenic factors [4]. The gene that encodes COX-2 is *ptgs2* [4]. The enzyme (COX-2) is a homodimer; each monomer has about 70 kD and three structural domains [5]. There are also reports from various animal studies and clinical experiments indicating the

ubiquitous nature of COX-2 and its overexpression as a signal for mammary carcinogenesis [3,6]. Therefore, the inhibition of COX-2 has become a strong strategy for preventing and treating ovarian and breast cancers [7]. It has also been reported that COX-2 is abundantly expressed in the tissues and cells of both ovarian and breast cancer, thereby promoting cancer cell proliferation and invasion [8]. Moreover, COX-2 is associated with regulation of genes in the carcinogenic pathways of breast and ovarian cancers [9]. Animal studies on tumors in mice and rats have also reported the association of high levels of COX-2 expression with tumorigenesis which was a very responsive treatment after the inhibition of COX-2 [10]. In fact, overexpression of COX-2 has been reported in different cancers whose progression was determined by the regulation of different signaling factors and pathways [10]. There is evidence that agents which inhibit COX-2 reduce the risk of breast cancer and ovarian cancer in women without disease, and reduce recurrence risk and mortality in women with breast cancer [11]. Furthermore, COX-2 overexpression has been observed in tumors of breast, ovary, lung, and liver [12,13]. However, cancer patients usually have to deal with relapse and continuous therapy because of resistance to chemotherapy. Chemotherapy drugs are usually synthetic agents which produce health problems and toxicity signs. Moreover, they are toxic not only to the cancerous cells but also to normal healthy cells [13]. Therefore, the search for natural plant-based phytochemicals and compounds for cancer treatment is very important. There are also recent reports which suggest the beneficial effects of phytochemicals that are cytotoxic to cancerous cells but minimally toxic to normal cells [14]. Thus, phytochemicals may serve as alternatives for cancer treatment and therapy. The present investigation was focused on the inhibition of COX-2 with the phytochemical compound baicalein (5,6,7-trihydroxy flavone) and its natural derivatives present in roots of *Scutellaria baicalensis* and *Scutellaria lateriflora* (Family: Lamiaceae). The study was based on computational insights into the molecular interactions of baicalein and its natural derivatives as COX-2 inhibitors, using molecular docking studies, MD simulation, and *in silico* ADME toxicity analysis.

METHODS

Protein preparation and sequence alignment

Cycloxenase-2 from *Homo sapiens* (NCBI Accession no.: AAA58433) does not have a 3D structure available in the Protein Data Bank (http://www.rcsb.org/). Therefore, the 3D structure of cycloxenase-2 (PDB ID: 6COX) from *Mus musculus* (mouse) was retrieved from the databank. The two sequences share a sequence homology of about 90 %, with a conserved catalytic domain and a conserved active site. Hence, it was considered for the molecular docking study. It had a resolution of 2.80 Å and it was complexed with a HEM cofactor.

For molecular docking purposes, chain A of the crystal structure and protoporphyrin IX containing Fe (HEM) were loaded and prepared according to MVD 7.0 protocol. The amino acid sequence of cyclooxygenase-2 (COX-2) of *Homo sapiens* (NCBI Accession No. AAA58433) and that of *Mus musculus* (NCBI Accession No. AAA39918) were retrieved from the UniProt database (http://www.uniprot.org/). In order to determine the homologous and similar regions, their amino acid sequences were aligned using ClustalW in CLC Main Workbench (Qiagen, Aarhus N, Denmark). Additionally, secondary structure regions of COX-2 of *Homo sapiens* and *Mus musculus* were predicted in order to determine the homologous regions (helix and strands regions). Furthermore, the amino acid sequences of the two species were analyzed using hydropathy plot and protein charge plot, so as to understand the similarities between the two enzymes.

Chemical dataset

The 2D structure of baicalein (CID_5281605) and its natural derivatives viz. baicalein monohydrate (CID_5281605), baicalein 6-O-glucoside (CID_5321896), and baicalein (1-) (CID_86289522) were downloaded from PubChem [15]. Additionally, for validation of the docking protocol, two approved drugs (NSAIDS) in the market, i.e., ibuprofen and melphalan, and two approved anti-cancer drugs olaparib and doxorubicin were retrieved from PuBChem database. The energies of these compounds were optimized using MM2 force field methods [16], and saved as sybyl mol2 (3D format) file format using ChemOffice 2010.

Molecular docking simulation

Docking was performed using Molexus MVD (MVD 7.0, Molexus, Denmark). Initially, the cavity detection program of MVD was applied and the potential ligand binding site of COX-2 enzyme was predicted. The cavity had a volume of 115.71 $Å^3$ and a surface area of 332.8 $Å^2$. The binding site was set inside a restriction sphere of radius 15 Å (X: 25.92, Y: 39.46, Z: 29.48) with

0.30 Å grid resolution. The bonds were also set at flexible mode with the amino acid side chains which were placed inside the restriction sphere of the cavity. Furthermore, the RMSD threshold was set at 2.00 Å for 100.00 energy penalties. At least 20 docking engine runs were executed, and the finest orientation from the 20 runs was considered for in-depth analysis.

Molecular dynamics simulation

Molecular dynamics simulations for the protein and the protein-ligand complexes were performed using GROMACS 5.0. The complexes and systems were first stabilized and globally minimized using Protein Preparation Tool of MVD 7.1. The protonation states of all the complexes were also corrected prior to simulation. Hydrogen-bonding inconsistencies and mis-protonation were also checked. The topology profile of COX-2 (PDB ID: 6COX) was operated with OPLS-AA/L force field, while those of the protein-ligand docked complexes were operated with GROMOS96 43a1 force field. The coordinates and topologies of the ligands were generated and solvation was accomplished through configuration using SPC solvent model. The system ensemble was equilibrated with NPT (300 K) and progressed with NVT (1 ns). Molecular dynamics simulation run was carried out for 10 nanoseconds (ns) and the trajectories were examined for their stability based on the deviations and shifts of the root mean square deviation (RMSD).

RESULTS

Sequence alignment

The amino acid sequence alignment of cyclooxygenase-2 (COX-2) of *Homo sapiens* (NCBI Accession no. AAA58433) and *Mus musculus* (NCBI Accession no. AAA39918) aligned using ClustalW in CLC workbench are shown in Figure 1. The alignment depicts the regions of similarities between the amino acid sequences as well as the secondary structures. From Figure 1, it is very evident that both sequences share a common secondary structure (helix and strand regions). Moreover, Figure 1 displays the conserved regions as well as the consensus regions between the two amino acid sequences. The comparative analyses of the hydropathy plot and protein charge plot are presented in Figures 2 and 3, respectively. It is evident from Figures 2 and 3 that there is no significant difference at the protein level between COX-2 enzymes of *Homo sapiens* and *Mus musculus*.

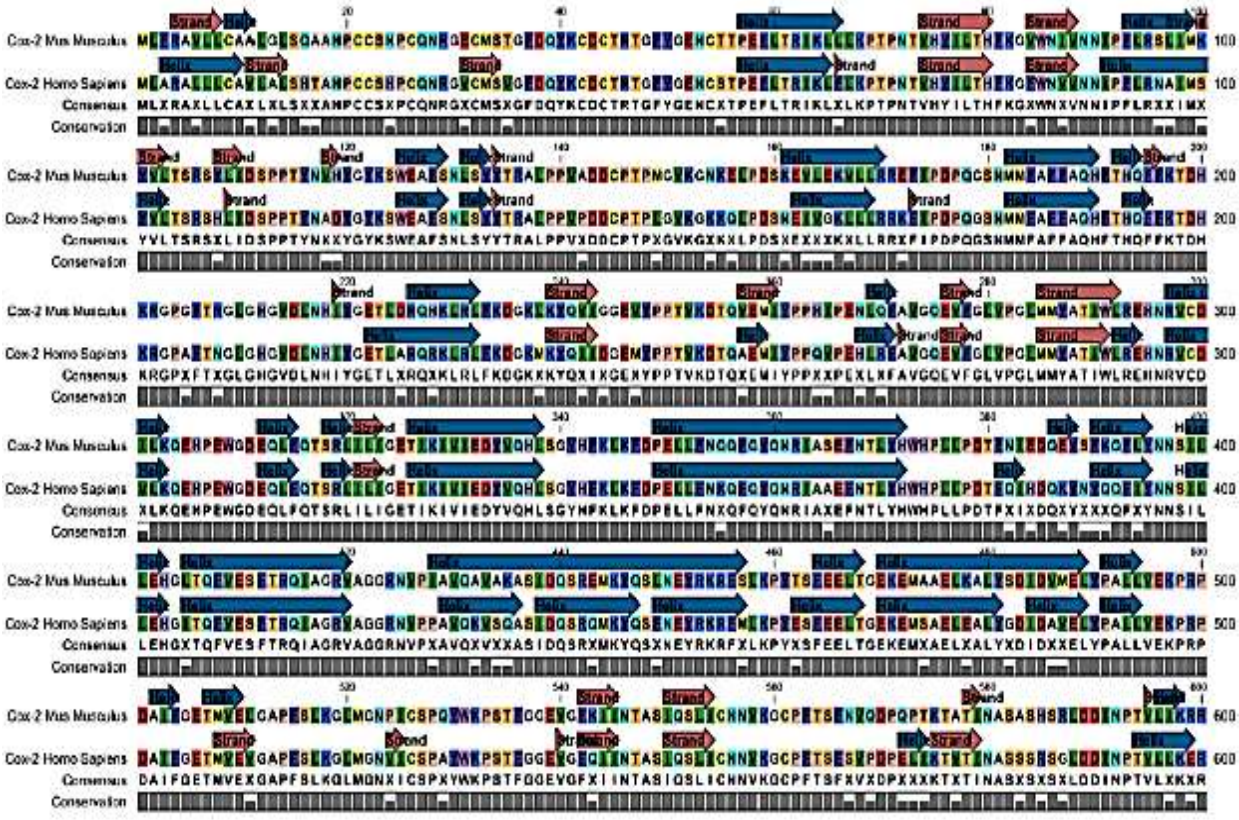


Figure 1: Amino acid sequence alignment of *Homo sapiens* and *Mus musculus* COX-2

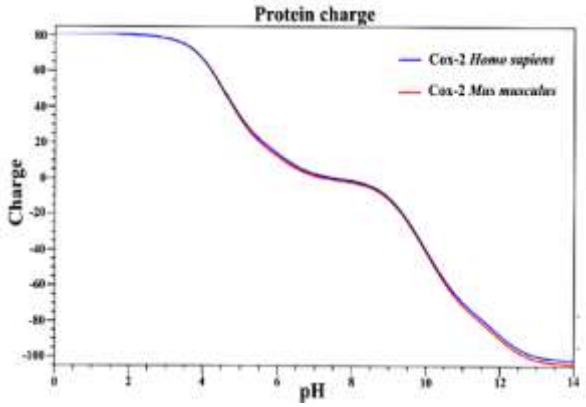


Figure 2: Protein charge plot of *Homo sapiens* and *Mus musculus* COX-2

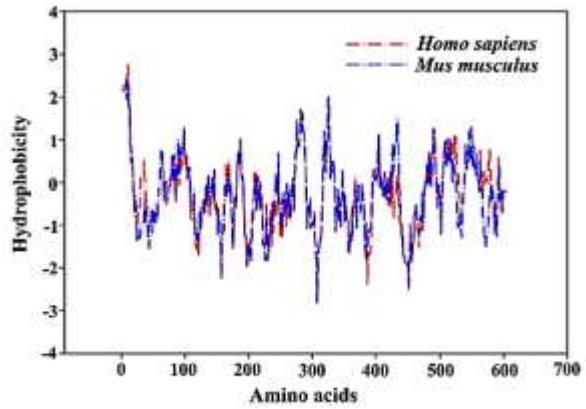


Figure 3: Hydropathy plot of *Homo sapiens* and *Mus musculus* COX-2

Molecular docking scores

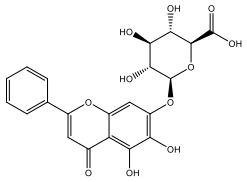
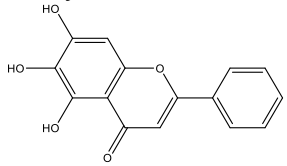
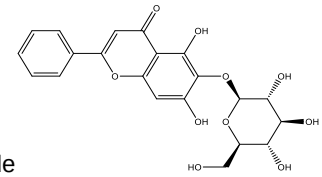
The docking scores, interaction energy values and hydrogen bonding energy values are shown in Table 1. The MolDock scores ranged from -131.83 kJ/mol (baicalein) to -75.89 kJ/mol (baicalein (1-), while the total docking scores ranged from-528.26 kJ/mol (baicalein) to -343.50 kJ/mol (baicalein (1-)). The interaction energy values ranged from -179.97 kJ/mol (baicalein) to -106.32 kJ/mol (melphalan). It was observed that baicalein, baicalein monohydrate and baicalein 6-O-glucoside had higher MolDock scores, rerank scores, interaction energies (in terms of negative energy) and overall total docking scores, than the control and the reference drugs (ibuprofen, olaparib, doxorubicin and melphalan). This indicated that the herbal compounds had more favorable molecular interactions at the binding site of COX-2 than the reference compounds. Additionally, baicalein, baicalein 6-o-glucoside and baicalein monohydrate were investigated with respect to details of the ligand-protein interactions such as the interacting residues, interaction distances, and their energy values, and the results are presented in Table 2.

From molecular interaction analysis, it was revealed that baicalein exhibited molecular interactions with Arg120(NH1), Arg120(NH2), Gly526, Ser530 and Tyr 355, while baicalein 6-O-glucoside exhibited molecular interactions with His90, Thr94, Gln192(OE1), Gln192(NE2), Leu352, Tyr355, Ile517 and Phe518. In contrast, baicalein monohydrate interacted with His90, Gln192, Ser353, Tyr355 and Phe518. The snapshots of the binding poses and energy maps of baicalein, baicalein 6-O-glucoside and baicalein monohydrate are shown in Figure 4, Figure 5 and Figure 6, respectively. The energy maps depicted in Figure 4 b, Figure 5 b and Figure 6 b revealed that these compounds docked in the region which was most favorable for steric interaction, electrostatic interaction, as well as hydrogen bond acceptor- and donor-favorable interactions, resulting in molecular interactions necessary for inhibiting the enzyme.

Table 1: Total docking score of the investigated compounds against COX-2

Ligand	MolDock Score (kj/mol)	Rerank Score (kj/mol)	Interaction (kj/mol)	Protein (kj/mol)	HBond (kj/mol)	LE1 (kj/mol)	LE3 (kj/mol)	Total (kj/mol)
Baicalein	-131.83	-24.71	-179.97	-179.97	-6.90	-4.12	-0.77	-528.26
Baicalein 6-O-glucoside	-113.79	-60.56	-160.43	-160.43	-4.82	-3.67	-1.95	-505.65
Baicalein monohydrate	-118.77	-94.44	-133.38	-133.38	-5.64	-6.25	-4.97	-496.83
Ibuprofen	-124.18	-19.38	-165.55	-165.55	-2.13	-3.88	-0.61	-481.29
Olaparib	-100.60	-87.24	-133.01	-133.01	-3.55	-5.03	-4.36	-466.80
Doxorubicin	-100.03	-86.48	-133.36	-133.36	-3.83	-5.00	-4.32	-466.39
Melphalan	-92.79	-80.37	-106.32	-106.32	-5.21	-6.19	-5.36	-402.55
Baicalein(1-)	-75.89	59.71	-158.83	-158.83	-9.25	-1.95	1.53	-343.50

Table 2: Molecular Interaction analysis of the top 3 docking hits

Compound	Amino acids	Distance (Å)	Energy (kj/mol)
Baicalein 	Tyr 355	3.07	-2.5
	Arg120(NH1)	3.30	-1.13
	Arg120(NH2)	2.77	-0.5
	Gly526	2.91	-2.5
	Ser530	2.73	-2.5
Baicalein monohydrate 	Phe518	3.38	-1.10
	Gln192	3.09	-2.5
	Ser353	3.05	-2.5
	Ser353	3.26	-1.69
	His90	2.67	-2.5
	Tyr355	3.51	-0.44
Baicalein 6-O-glucoside 	Leu352	3.20	-1.98

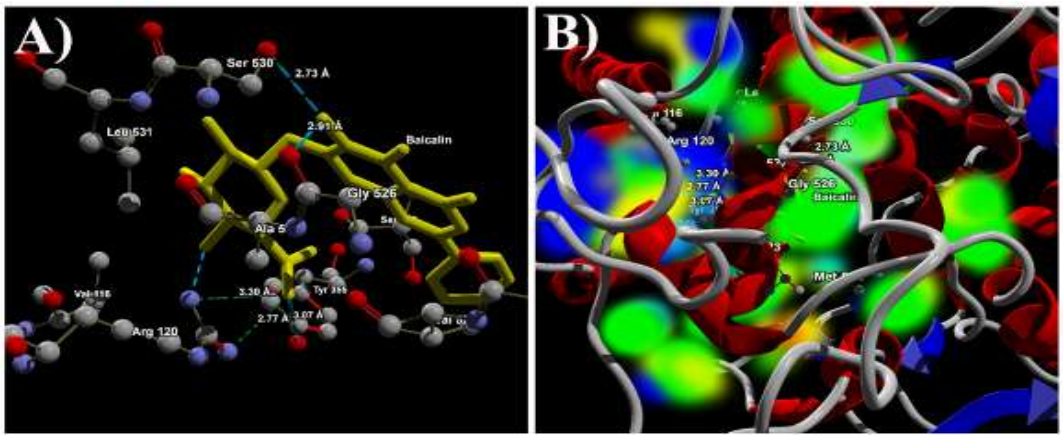


Figure 4: (A) Molecular interactions between baicalein with the active site amino acid residues and (B) Energy map indication of baicalein

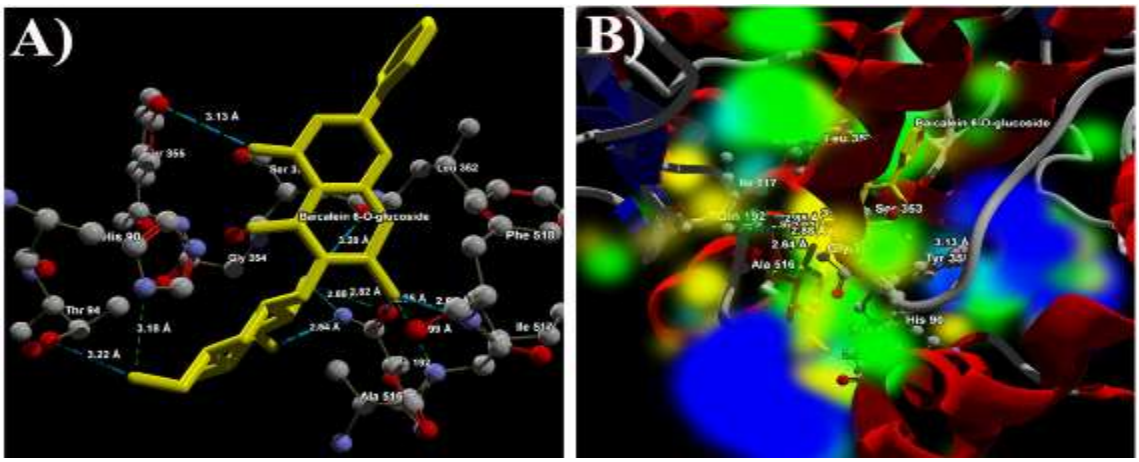


Figure 5: (A) Molecular interactions between baicalein 6-O-glucoside with the active site amino acid residues and (B) Energy map indication of baicalein 6-O-glucoside

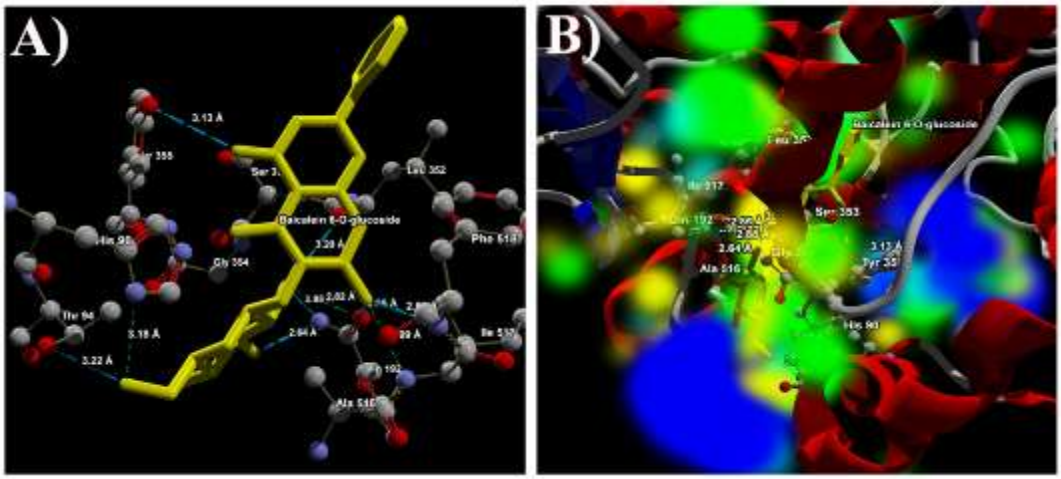


Figure 6: (A) Molecular interactions between baicalein monohydrate with the active site amino acid residues and (B) Energy map indication of baicalein monohydrate

Molecular dynamics (MD) simulation

The molecular dynamics simulation analysis revealed the stability of trajectory during the 10 ns MD simulation, as evidenced from the root mean squared deviation (RMSD) analysis shown in Figure 7. Overall, the results of RMSD analysis indicated relative stability.

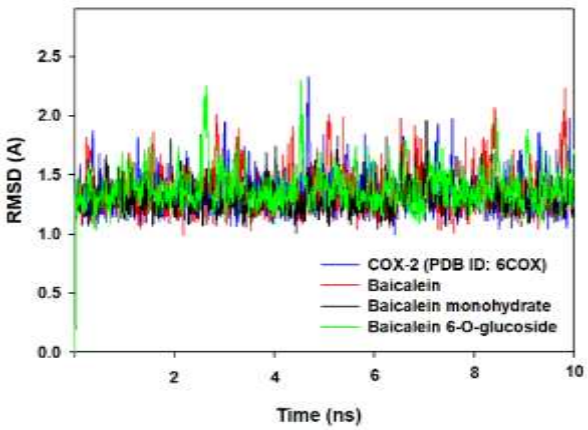


Figure 7: Molecular dynamics simulation of COX-2 and COX-2-baicalein complex, COX-2-baicalein 6-O-glucoside complex and COX-2-baicalein monohydrate complex

DISCUSSION

In the present study, baicalein and its natural derivatives showed strong inhibitory potential against COX-2 enzyme from *Mus musculus* through favorable molecular interactions at its active site. The amino acid sequences of COX-2 from *Homo sapiens* and COX-2 from *Mus musculus* share a sequence similarity of approximately 95%. Thus, since the 3D structure of COX-2 of *Homo sapiens* was not available in the Protein Data Bank, it is reasonably valid to assume that inhibition of the COX-2 from *Mus musculus* should give the same result as inhibition of the COX-2 from *Homo sapiens* [17]. Moreover, a homology model of COX-2 might lead to a negative impact since there are only few amino acid differences (10 amino acids) between the two sequences. Additionally, the hydropathy plot of the hydrophobic amino indicated that the two sequences had equally distributed and similar frequencies of hydrophobic amino acids which might be involved in ligand binding and molecular interactions.

Ovarian cancer and breast cancer are ranked among gynecological cancers with highest fatality rates in China [18]. The docking study demonstrated the molecular interactions of the ligands with the protein of interest (COX-2). The scoring function was based on derived PLP defined in terms of E_{inter} and E_{intra} [19]. This technique is widely used in the drug discovery process as a powerful tool for identifying potential lead compounds [19]. In these investigations, baicalein and its natural derivatives showed better evaluation scores than the marketed approved drugs such as ibuprofen (COX-2 inhibitor) and anti-cancer drugs (olaparib, doxorubicin, and melphalan) which are administered for treating ovarian and breast cancers.

The use of these drugs served as validation of the docking protocol in this study. Although the targeted enzyme COX-2 is linked to inflammatory responses, its overexpression is a signal for various types of mammary carcinogenesis, including breast and ovarian cancer [20]. Moreover, COX-2 is thought to play major role in the regulation of the apoptotic pathway than in inflammation, and numerous studies have investigated its effect on cancer progression [21]. Apoptosis is generally conditioned by genetic factors and physiological processes, and its pathways are mostly regulated by pro-apoptotic and anti-apoptotic proteins [15]. However, overexpression of COX-2 may influence apoptosis. Based on the results of the present study, baicalein, and its derivatives showed favorable molecular interactions with COX-2, based on strong evidence from the molecular docking scores and MD simulation analysis. There are also reports from investigations of cancer progression indicating that certain flavonoids showed good docking scores with COX-2 [22]. In fact, the binding interactions at the active site of COX-2 cause inhibitory effects, and might induce cell apoptosis through the mitochondrial signaling pathway [22].

The presence of certain energy interactions such as electrostatic, hydrophobic, steric, and hydrogen bonding interactions also contributed to the higher binding affinities of the docked compounds. Although water molecules were removed during the docking process, hydrophobic effect played a major role by forcing the water molecules to aggregate and disrupt the hydrogen bonds in protein–ligand docked complexes [23]. This increased the binding affinity between the protein and the ligand because of the strong inter- and intra-molecular interactions (electrostatic, hydrophobic, steric, and hydrogen bonding interactions). The overexpression and involvement of COX-2 in various types of cancer tissues including breast and ovarian cancer indicate the need for inhibition of COX-2 for effective chemo-preventive treatment of these cancers [24]. The present investigation provides a detailed analysis of the interaction between COX-2 molecular target and the docked compounds, as well as a discussion of how these compounds are involved in anticancer effects.

CONCLUSION

Molecular docking simulation studies reveal that baicalein and its natural derivatives exerted strong inhibitory effect on COX-2. Since baicalein and its derivatives are plant-based, there are less chances of adverse health effects such as heart attacks and strokes. Additionally, the energy map reveals that baicalein and its natural derivatives are bound to the region of the enzyme which favors steric, electrostatic, hydrogen donor, and hydrogen acceptor interactions. Moreover, from amino acid sequence alignment, it has been revealed that COX-2 from *Mus musculus* and *Homo sapiens* shares about 90 % sequence homology, with similar secondary structure regions and the same distribution of hydrophobic amino acids. Therefore, baicalein and its derivatives may serve as good anti-cancer agents, but *in vivo* studies are required to buttress these findings.

DECLARATIONS

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Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaoci Cao, Guohong Xin, and Baoshuan Fang designed the study and the methodology, performed computational investigation, and wrote the entire manuscript.

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