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Daidzein attenuates inflammation and pain via TRPV1/ERK/COX-2 pathway modulation: insights from computational and in vivo studies

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Mehreen Afridi[1], Ashraf Ullah Khan*[2], Abbas Iqbal[1], Muhammad Zubair[1], Danyal Khan[1], Haroon Afridi[1], Amir Zeb[1]

[1]Department of Pharmacy, Faculty of Pharmaceutical Sciences, Abasyn University Peshawar, 25000 Peshawar, Pakistan.
[2]Department of Pharmacy, City University of Science and Information Technology, Peshawar, Pakistan.
Drmehreenafridii@gmail.com; ashrafwazir6@gmail.com; abbasiqbal976@gmail.com; janzubair84@gmail.com; danyalkhan11536@gmail.com; haroon.hk8229@gmail.com; amir.zeb@abasyn.edu.pkmanahilf198@gmail.com.
*Correspondence: aukhan@bs.qau.edu.pk

ABSTRACT: The animal and computational studies were conducted to elucidate the anti-inflammatory and analgesic potential of the Daidzein (isoflavone in nature). The molecular docking of the Daidzein was commenced against the inflammatory and analgesic targets i.e., COX-2 (Cyclooxygenase-2), ERK (Extracellular receptor kinase), and TRPV1 (Transient receptor Potential Vanilloid 1) protein. The molecular docking was followed by the molecular dynamic (MD) simulation assess the dynamic stability of the complexes over time. Following MD simulation, the binding free energy calculations were conducted to determine the thermodynamic binding affinity. After the computational studies, the results were validated using the acetic acid-induced writhing and formalin-induced models. The molecular docking of the Daidzein showed multiple hydrophilic and hydrophobic interactions. The MD simulation analysis showed that the Daidzein_COX-2, Diadizein_ERK, and Daidzein_TRPV1 complex showed that complexes remain stable using RMSD (Root mean square deviation), RMSF (Root mean square fluctuations), RoG (Radius of Gyration), SASA (Solvent accessible surface area) and hydrogen bond analysis. The binding free energy calculations using MM-PBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) and MM-GBSA Molecular Mechanics Generalized-Born Surface Area) revealed favorable binding free energy and the total energy of the system remains negative. Furthermore, the Daidzein showed marked reduction in the writhing movement and increased the pain threshold. Similarly, the Daidzein also evidently reduced the Formalin-induced biphasic pain response in animals when the results were compared with the Disease control. In conclusion, the Daidzein portrayed promising

anti-inflammatory and analgesic activities using computational and animal studies, however, additional studies will be required to employ it clinically.
Keywords: Daidzein; Inflammation; Analgesic; MD simulation; MM-PBSA; MM-GBSA

1. Introduction

The inflammatory process plays a key role in the pathogenesis of various diseases including arthritis, infections, neurological disorders, and cancer (L. Chen et al., 2018). The inflammatory process is considered as a protective response against the offending agent with the intention to limit the spread of the harmful onslaught by cordoning off the area and prevent the systemic dissemination of the inflammation (Ronchetti, Migliorati, & Delfino, 2017). The inflammatory process can be broadly classified into two types based on the duration i.e., acute inflammation and chronic inflammation. The acute inflammation lasts from minutes to hours, while the chronic inflammatory process lasts from days to months. Acute inflammation if not treated appropriately may worse and lead to chronic inflammation (Chatterjee, 2016; Stelzer, Rosen, et al., 2016). The inflammatory process is associated with the activation of the immune cells to the site of injury, release of the pro-inflammatory mediators such as cytokines, chemokines, and oxidative stress markers (Carrasquillo & Gereau, 2008). These inflammatory mediators not only induce the infiltration of the immune cells but also induce the activation of the nearby cells. In order to deal with the inflammatory conditions both chronic and acute form, we take support of non-steroidal anti-inflammatory drugs (NSAIDs) drugs (Carrasquillo & Gereau, 2008). The various NSAIDs which are in clinical practice to deal with inflammatory conditions includes Aspirin, Diclofenac, and Ibuprofen (L. Chen et al., 2018). The various side effects includes GIT problems, vomiting, headache, bleeding, cardiovascular problems and stroke (L. Chen et al., 2018). The *Pueraria lobata* plant (a traditional Chinese medicine also known as Kudzu) have been reported for various traditional uses including relieving body heat (as an anti-pyretic), high blood pressure associated headache etc. Similarly, the Dadizein have also been reported for numerous pre-clinical biological activities anti-inflammatory, analgesic, antioxidants and improving brain functions (Stelzer, Rosen, et al., 2016). Extracts and secondary metabolites from *Pueraria lobata* including Dadizein have shown potential pharmacological effects, such as an anti-diabetic effect, an anti-inflammatory effect, estrogen-like activity, and anti-cancer property (Stelzer, Plaschkes, et al., 2016). Computational approaches have recently been focused for the drug development and discovery process because of their rapid screening of large number of compounds and their reliability (Chatterjee, 2016; Wu et al., 2020). The molecular docking, network pharmacology and poly-pharmacology shifted the trend of one drug/one target/one disease concept to development of multitarget drugs (Wu et al., 2020). The molecular docking, network pharmacology and poly-pharmacology shifted the trend of one drug/one target/one disease concept to development of multitarget drugs (Wu et al., 2020).

In the present study, the active constituent i.e., Daidzein commonly found in the Pueraria lobata plant was evaluated for anti-inflammatory and analgesic potential using computational and in vivo approaches. The Daidzein have been previously reported for various biological activities including the antioxidants, anti-inflammatory, anti-cancer etc. based on the previously reported studies it was anticipated that the Daidzein will possess anti-inflammatory and analgesic activities by tagretin the COX-2, ERK, and TRPV1 receptor.

2. METHODOLOGY

2.1. Chemical reagents

The chemical and reagents used in the study includes Formalin (for the induction of the biphasic pain in animals), Acetic acid (for the induction of the writhing movement), normal saline and Daidzein. Daidzein was provided by the Prof. Yeong Shik Kim (Seoul National University, Republic of South Korea, South Korea). All the chemical and reagents used in the current study were of research grade.

2.2. Molecular docking

The virtual screening of the Daidzein was conducted against the COX-2 (PDB ID: 5F19), ERK (PDB ID: 5NHJ), and TRPV1 (PDB ID: 3J5Q) using the AutoDock vina. The target protein for the molecular docking involved in the pain and inflammation were downloaded from the RCSB-PDB website as PDB file (Kushwaha et al., 2021). The protein were prepared using the UCSF_Chimera version_16. The co-crystalized ligands were removed, water molecules were deleted, desired chain was selected, and missing residues were added using the Swiss-Model software. Furthermore, the Dock prep function was commenced to prepared the protein and

saved as PDB file (Jose, Varughese, Parvez, & Mathew, 2024; Sharma et al., 2022). Similarly, the Daidzein molecule was downloaded from the PubChem database as Structural data file and prepared by adding polar hydrogen and minimized using UCSF_Chimera version_16. Following, the ligand and protein preparation,

2.3. Ligand Prepration

The Daidzein was downloaded from the PubChem database as SDF file. The ligand was prepared before the molecular docking by adding polar hydrogen bonds, energy was minimized and converted to PDB file format as reported previously (Ghasemlou, Uskoković, & Sefidbakht, 2023; Schneidman-Duhovny, Dror, Inbar, Nussinov, & Wolfson, 2008).

2.4. Protein preparation

The target proteins such as COX-2 (PDB ID: 5F19), ERK (PDB ID: 5NHJ), and TRPV1 (PDB ID: 3J5Q) were downloaded from the PDB website as PDB files. The missing residues were added using the SWISS-MODEL online plateform and the best model was selelcted and downloaded as PDB file. The proteins were further processed by removing the extra chain, adding polar hydrogens, co-crytalized ligands and water molecules were removed using the dock prep function of the UCSF-Chimera version 16 software and saved as PDB file as reported previously (Botelho et al., 2020; Rafi et al., 2022; Schneidman-Duhovny et al., 2008).

2.5. Molecular dynamic simulation

The molecular docking analysis was followed by the molecular dynamic simulation to assess the ligand-protein stability using GROMACS_24.1 software. The ligand-protein complexes after the molecular dynamic simulation were separated into the LIG.mol2, and REC.pdb and the topologies of the system were prepared using the SwissParam software. The TIP3P water model and the CHARMM27 all atom force field was employed for the system. In order to reduce the steric clashes, the system was minimized using the steepest descent model and the system was minimized for total of 50000 steps. The system equilibration was conducted in two steps for 100 ps i.e., NVT and NPT. Finally, the production run was conducted for 50 ns to obtain the RMSD, RMSF, RoG and SASA (Ahmad, Abbasi, Shahid, Gul, & Abbasi, 2021; S. Srivastava, Pandey, Kumar, Siddiqui, & Dubey, 2025).

2.6. Binding free energy calculations

Following the MD simulation for 50 ns using GROMACS software, the binding free energies of all complexes were commenced using gmx_mmpbsa_py software compatible with the GROMACS. The various favorable binding energies were conducted for all three complexes using both MM-PBSA and MM-GBSA. A total of 500 trajectories frames were analyzed starting from 1 to 500 and the interval of 1 was kept (Karpun et al., 2023; Zare, Solhjoo, Sadeghpour, Sakhteman, & Dehshahri, 2023).

2.7. Toxicokientics, pharmacokinetics, and phsyico-chemical studies of Daidzein

The toxicity of any compound is key factor in implementing the drug for the clinical practice. The lower the toxicity of the compound, the higher is the acceptability following potency of the compounds. The toxicity of the Daidzein was determined using the pkCSM software as reported previously. Similarly, the pharamcokinetic parameters of the Dadizein was determined including the absorption, distribution, metabolism, and excretion using SwissADME software as previously reported. Furthermore, the physico-chemical paramters of the Daidzein were determined using the pkCSM and SwissADME software (Elkarhat, Charoute, Elkhattabi, Barakat, & Rouba, 2022; Sundar, Thangamani, Manivel, Kumar, & Piramanayagam, 2019).

2.8. Animals

The mice (male albino BALB/c) were procured from the animal facility of National Institute of Health (NIH), Islamabad, Pakistan. The age of the animal was between 6-7 weeks and the average weight of the animal was 28-33 gram. Before the commencement of the animal's activities, the Animals Ethical Committee approved the studies and assign the approval number of **IERC-AUP 2024-007**. In the present study, it was tried level best to avoid any unnecessary harm to the animals and number of animals were kept as minimum as possible. After the completion of the experiments, the animals were killed using cervical dislocation by keeping the animal ethical committee regulations.

2.9. *In vivo* inflammatory model

The in vivo anti-inflammatory activities were performed to confirm the computational study results. Briefly, after the molecular docking analysis the best compounds based on the negative binding energy was selected and further investigated for the in vivo anti-inflammatory

activity in mice as reported previously. The *in vivo* studies were performed using acetic acid-induced writhing movement and formalin-induced biphasic pain responses. During the formalin and acetic acid-induced model the animals were divided into various groups as discussed below.

Normal control/Vehicle control (received only normal saline). Disease/Negative control (received only acetic acid or formalin). Positive control (received inducer and Ketoprofen 5 mg/kg). Treatment control 1 (received inducer and Daidzein 1 mg/kg). Treatment control 2 (received inducer and Daidzein 5 mg/kg). Treatment control 1 (received inducer and Daidzein 10 mg/kg).

2.10. Statistical analysis

The result of the study was presented using mean ± S.D (n=5). The data was analyzed using one-way analysis of variance (ANOVA) followed by post hoc test to determine the statistical significance amongst various groups. The criteria of $p < 0.05$ is chosen for statistical significance. The GraphPad Prism software version_5 was used plot the data.

3. RESULTS

3.1. Molecular docking analysis

The molecular docking analysis was performed to assess the binding interaction and binding affinity between the ligand and protein. The molecular docking analysis showed that the Daidzein interacted with the target proteins i.e., COX-2, ERK, and TRPV1 via multiple hydrogen bonds and hydrophobic bonds. The size of COX-2_Daidzein complex was (x= 25.0, y= 23.2797, and z= 50.1339), while the dimension of the COX-2_Daidzein complex was (x= 4.5342, y= -12.7319038015, and z= 21.9330047503). The size of ERK_Daidzein complex was (x= 25.0, y= 25.0, and z= 48.76511), while the dimension of the ERK_Daidzein complex was (x= -3.95, y= 47.5962, and z= 15.550734532). The size of TRPV1_Daidzein complex was (x= 33.515969, y= 25.0, and z= 50.36461), while the dimension of the TRPV1_Daidzein complex was (x= 182.8882, y= 175.708, and z= 120.58813). The green line showed the hydrogen bonds, while blue colors show the hydrophobic bonds between the ligand and protein. The binding energy of the COX-2_Daidzein was -7.1 kcal/mol, the binding energy of the ERK_Daidzein complex was -6.7 kcal/mol, and the binding energy between the TRPV1_Daidzein complex was -6.2 kcal/mol. The interaction between the Daidzein and the protein targets were visualized using Discovery studio software in 3-Dimemsional and 2-Dimensional view as shown in the **Figure 1**.

3.2. Molecular dynamic simulation

The molecular dynamic simulation was employed for all three complexes using GROMACS software version 2024.1 The dynamic stability was assessed for all three complexes i.e., COX-2_Daidzein complex, ERK_Daidzein complex and TRPV1_Daidzein complex for 50 ns using RMSD analysis. The RMSD analysis showed that the ERK_Daidzein complex and TRPV1_Daidzein complex remain stable, however, the COX-2_Diadzein complex showed higher RMSD value compared to other complexes **Figure 2**. Similarly, the RMSF, SASA, Rg and hydrogen bond analysis was performed for all three complexes **Figure 3, 4, 5,** and **6**.

3.3. Binding free energy calculations

The MD simulation was followed by the energetics calculations using Poisson-Boltzmen Surface Area and Genenralized-Born Surface Area by employing the gmx_mmpbsa_py software compatible with the GROMACS software. During these calculations, total of 500 trajectroies frames were assessed by keeping interval of 1, starting from 1-500 frames. The results of the COX-2_Daidzein complex using MM-PBSA showed negative total binding energy and favorable interactions **Figure 7**. The higher the negative energies of the complex, the more favroble binding interactions. Similarly, the MM-GBSA analysis of the COX-2_Daidzein complex also showed favorable interaction and negative total binding energies **Figure 8**. The MM-PBSA is more reliable than then the MM-GBSA, however, the MM-PBSA requires more time and computational resources as compared to the MM-GBSA. Similarly, the MM-PBSA and MM-GBSA analysis of the ERK_Daidzein complex and TRPV1_Daidzein complex showed favorable binding enenrgies and negative total binding energies as shown in the **Figure 10, 11, 13**, and **14**.

The per-residue decomposition analysis of all complexes i.e., COX-2_Daidzein complex, ERK_Daidzein complex, and TRPV1_Daidzein complex was conducted to reveal the interacting amino acid within the binding pocket and their binding enenrgies as shown in the **Figure 9, 12,** and **15.**

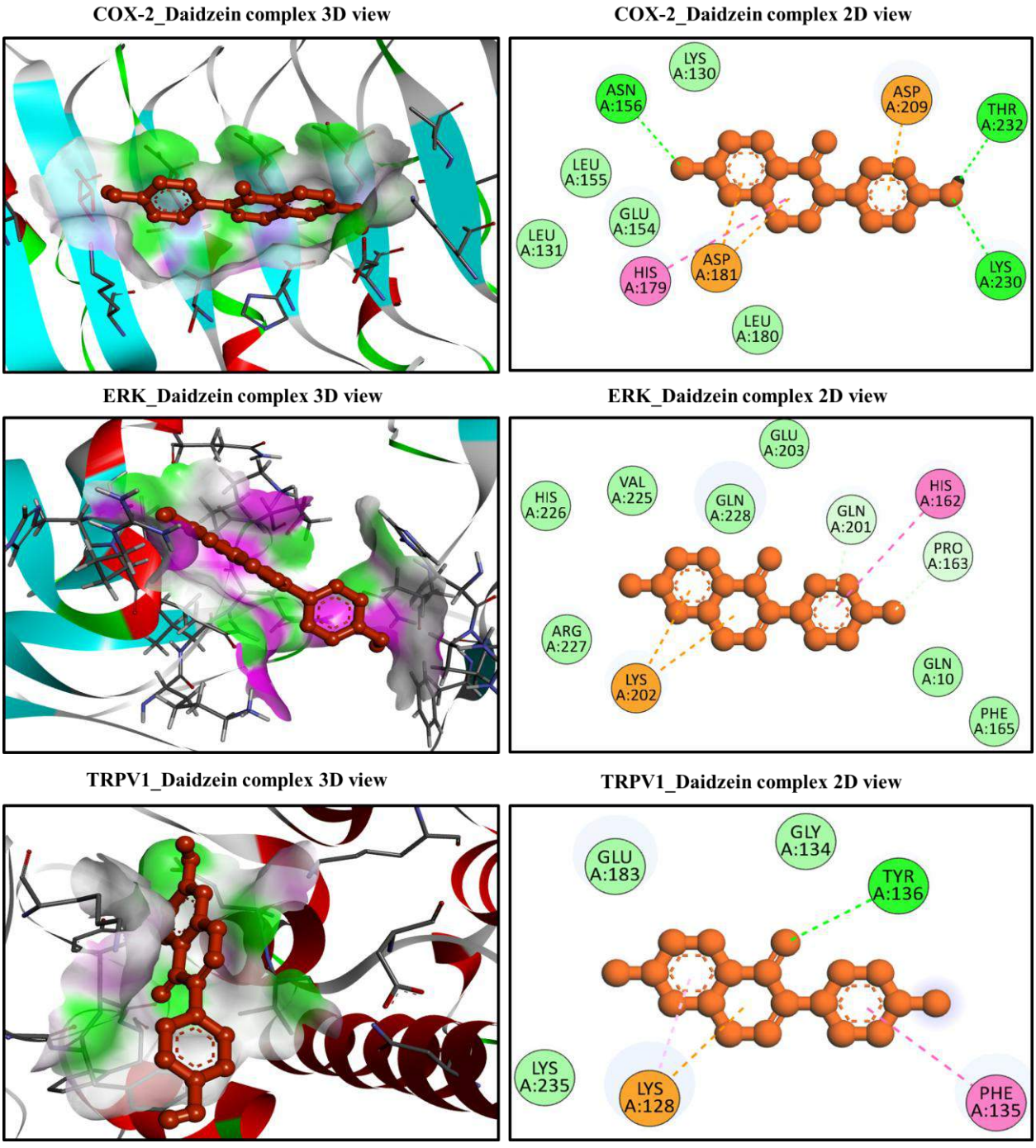

Figure 1. The molecular docking analysis was performed using Daidzein as ligand COX-2, ERK, and TRPV1 as protein target. The molecular docking analysis showed that Daidzein interacted with the 5F19

(COX-2), 5NHJ (ERK), and TRPV1 protein via multiple hydrogen and hydrophobic bonds. The results of interaction were expressed in 3-Dimensional and 2-Dimensional view.

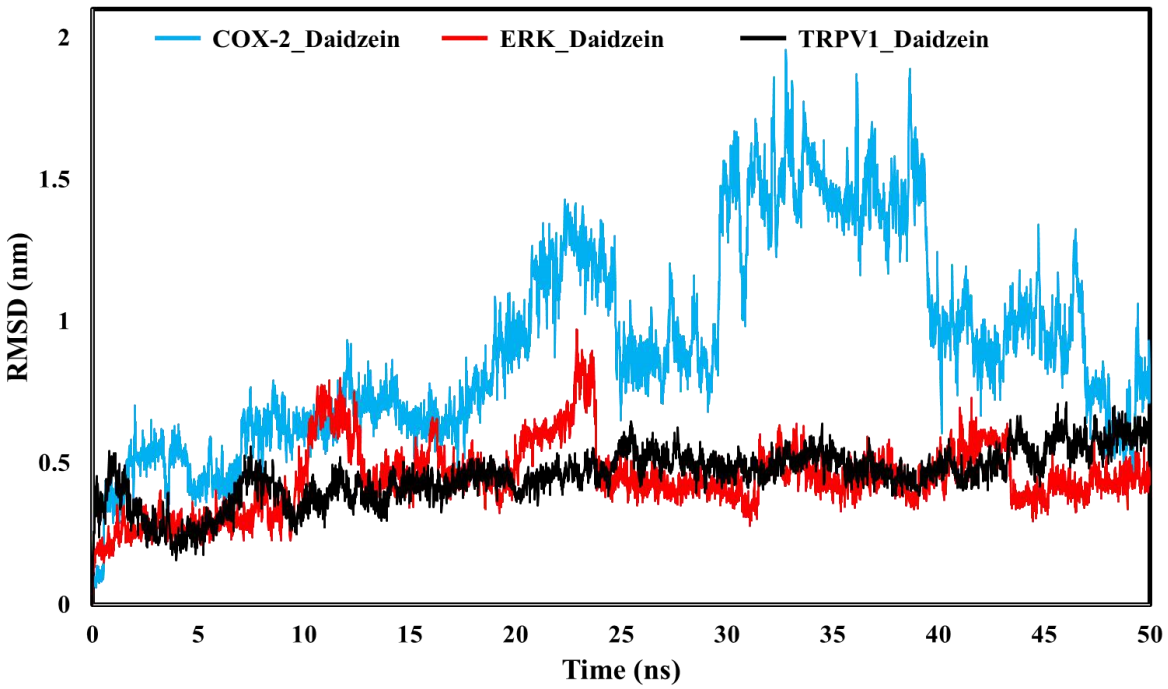

Figure 2. The RMSD analysis of all three complexes for 50 ns i.e., COX-2_Daidzein complex, ERK_Daidzein complex, and TRPV1_Daidzein complex protein

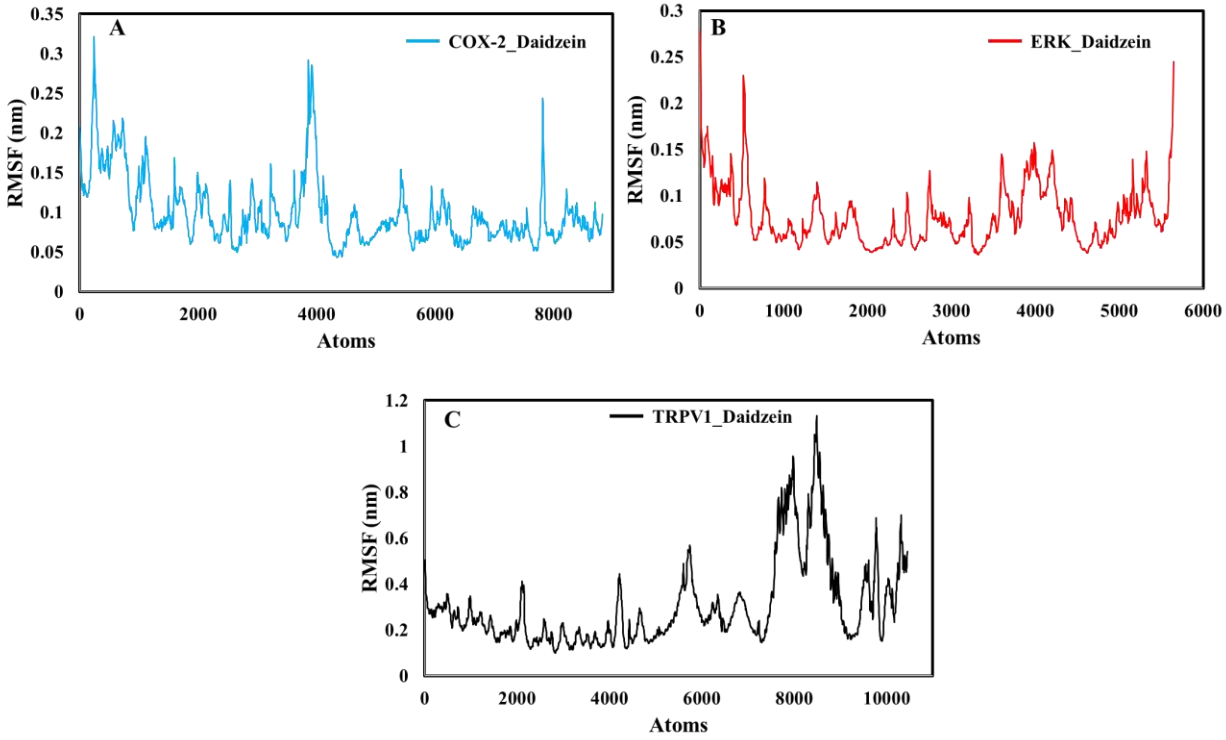

Figure 3. The RMSF analysis of all three complexes for 50 ns i.e., COX-2_Daidzein complex, ERK_Daidzein complex, and TRPV1_Daidzein complex protein

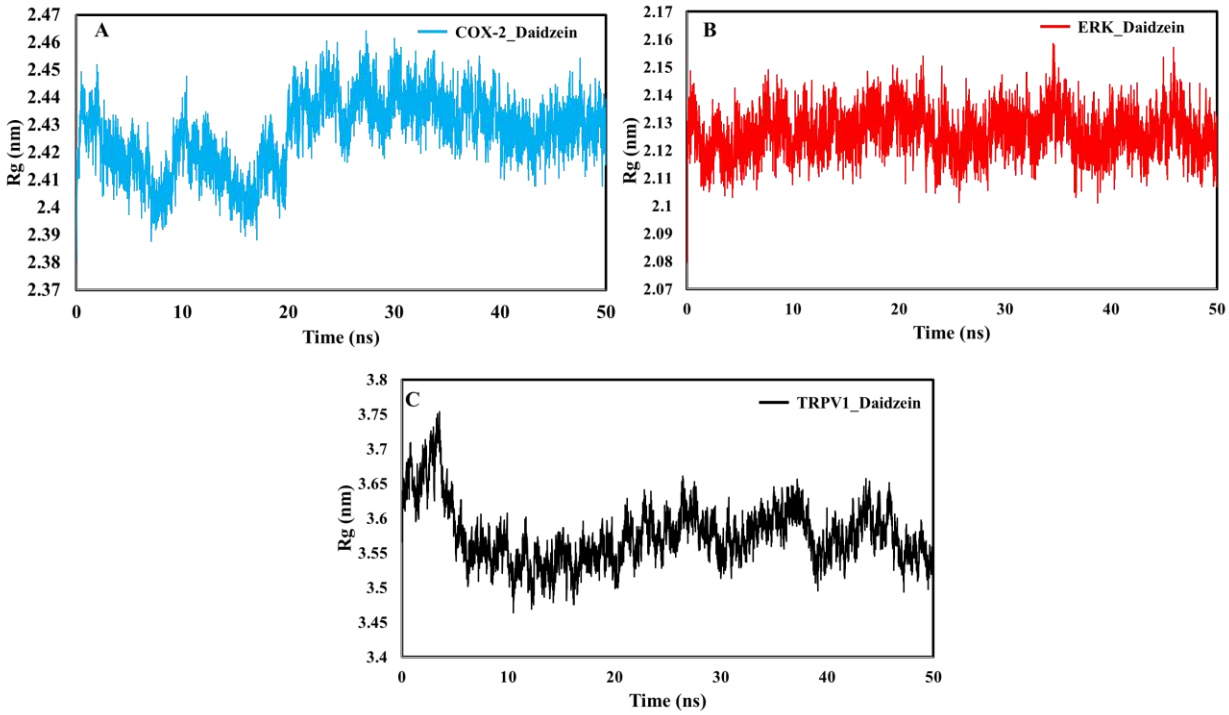

Figure 4. The Rg analysis of all three complexes for 50 ns i.e., COX-2_Daidzein complex, ERK_Daidzein complex, and TRPV1_Daidzein complex protein

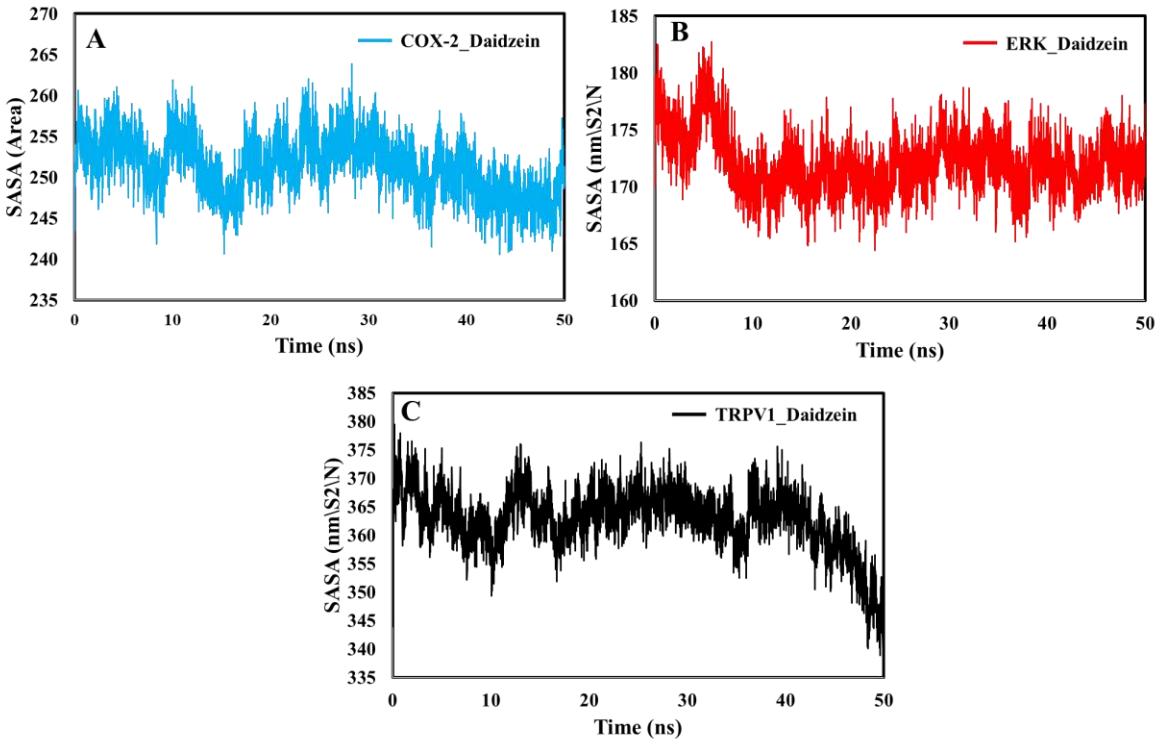

Figure 5. The SASA analysis of all three complexes for 50 ns i.e., COX-2_Daidzein complex, ERK_ Daidzein complex, and TRPV1_Daidzein complex protein

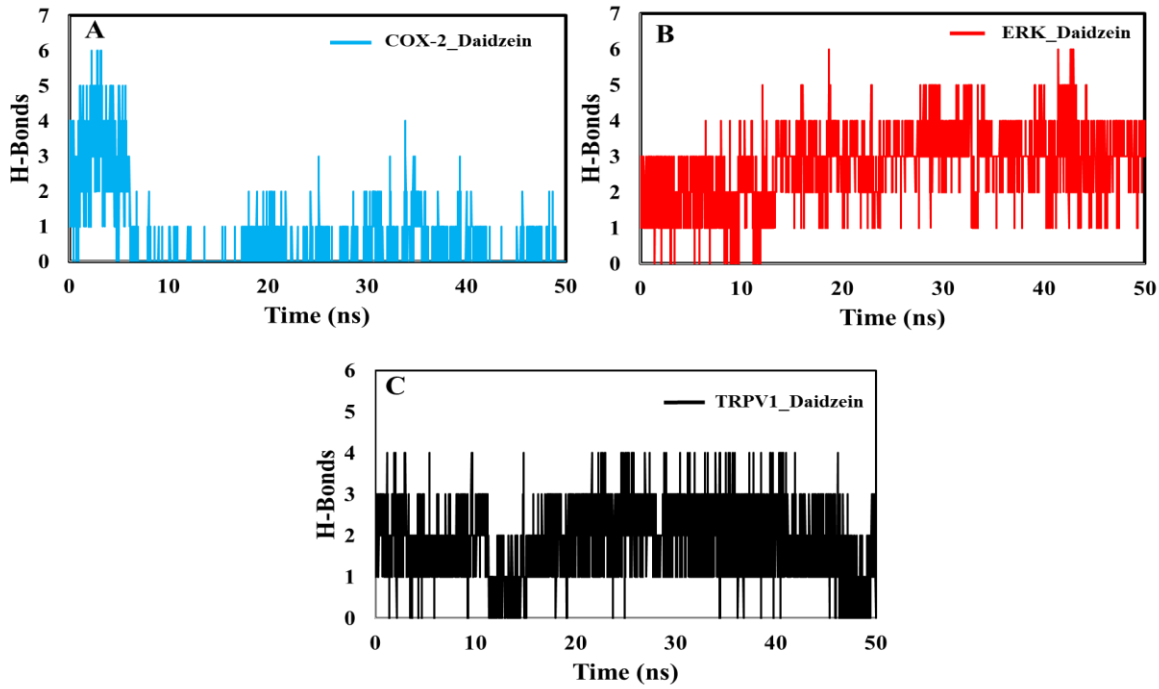

Figure 6. The H-Bonds analysis of all three complexes for 50 ns i.e., COX-2_Daidzein complex, ERK_Daidzein complex, and TRPV1_Daidzein complex protein.

3.4. The toxicokinetic analysis of the Pueraria lobata compounds

The toxicity of profile of the Daidzein was determined against various organs such as liver, heart, skin, mutagenicity, and carcinogenicity, maximum and minimum oral toxicity. The toxicokinetic profile assessment of the Dadizein showed no significant toxicity against the vital organs as shown in **Table 1**.

Table 1. The toxicokinetic analysis of the Daidzein against various vital organs using computational analysis.

S. No	Ames toxicity	herG I inhibitor	herG II inhibitor	Hepatotoxicity	Skin Sensitization	T.Pyriformis toxicity	Minnow toxicity	Max. tolerated dose (human)	Oral Rat Acute Toxicity (LD50)	Oral Rat Chronic Toxicity (LOAEL)
Daidzein	NO	NO	NO	NO	No	0.693	1.035	0.187	2.164	1.187

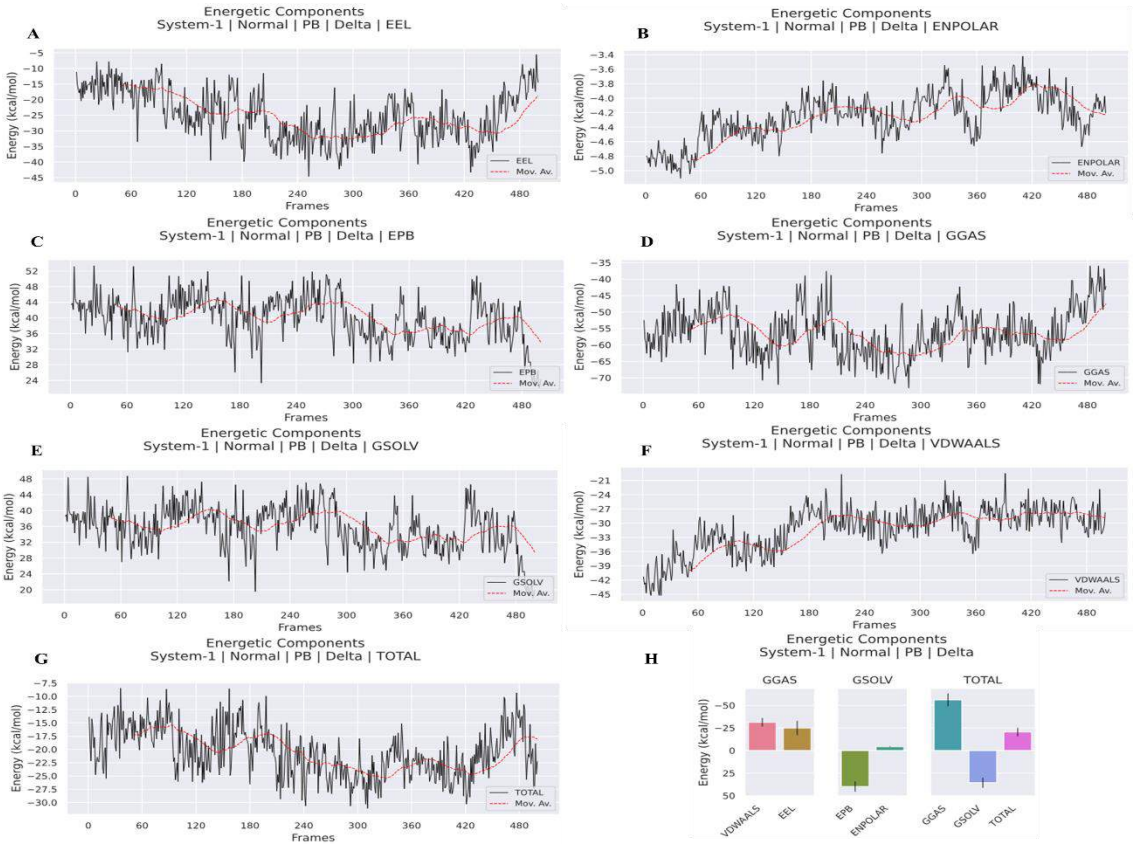

Figure 7. The MM-PBSA analysis of the COX-2_Daidzein complex using gmx_mmpbsa_py software and various parameters were assessed such as EEL, ENPOLAR, EPB, GGAS, GSOLV, VDWAALS, and toal energy.

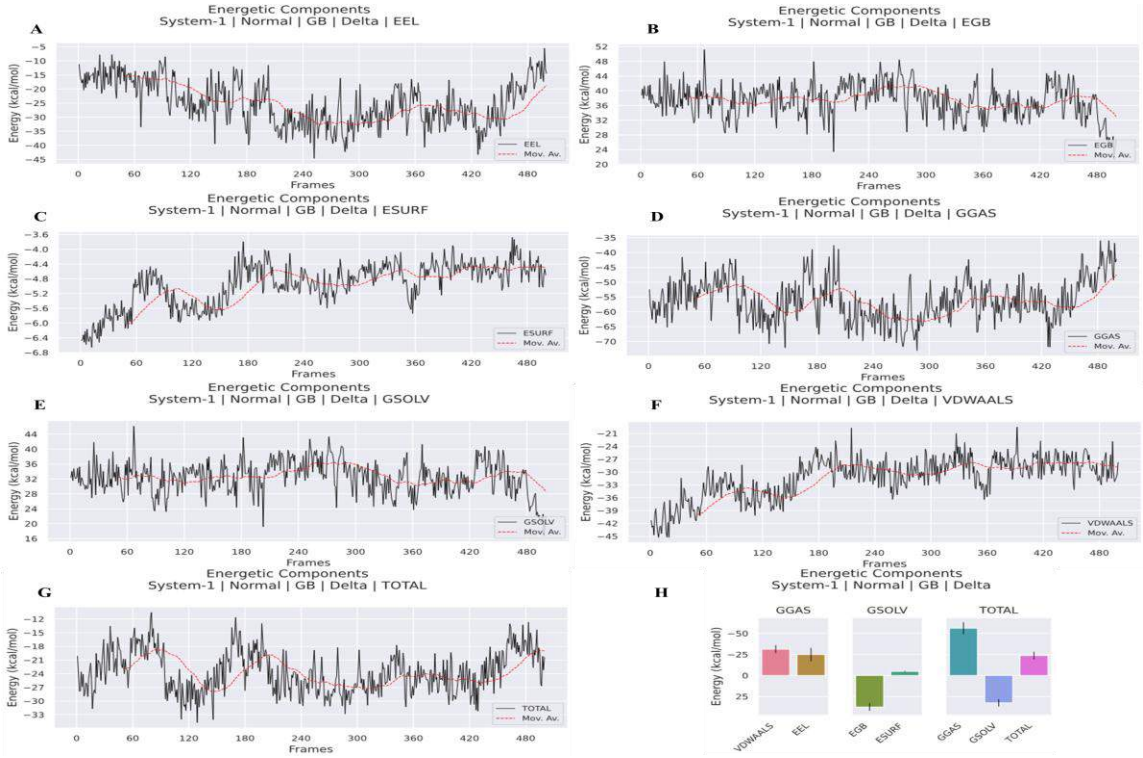

Figure 8. The MM-PBSA analysis of the COX-2_Daidzein complex using gmx_mmpbsa_py software and various parameters were assessed such as EEL, ESURF, EGB, GGAS, GSOLV, VDWAALS, and toal energy.

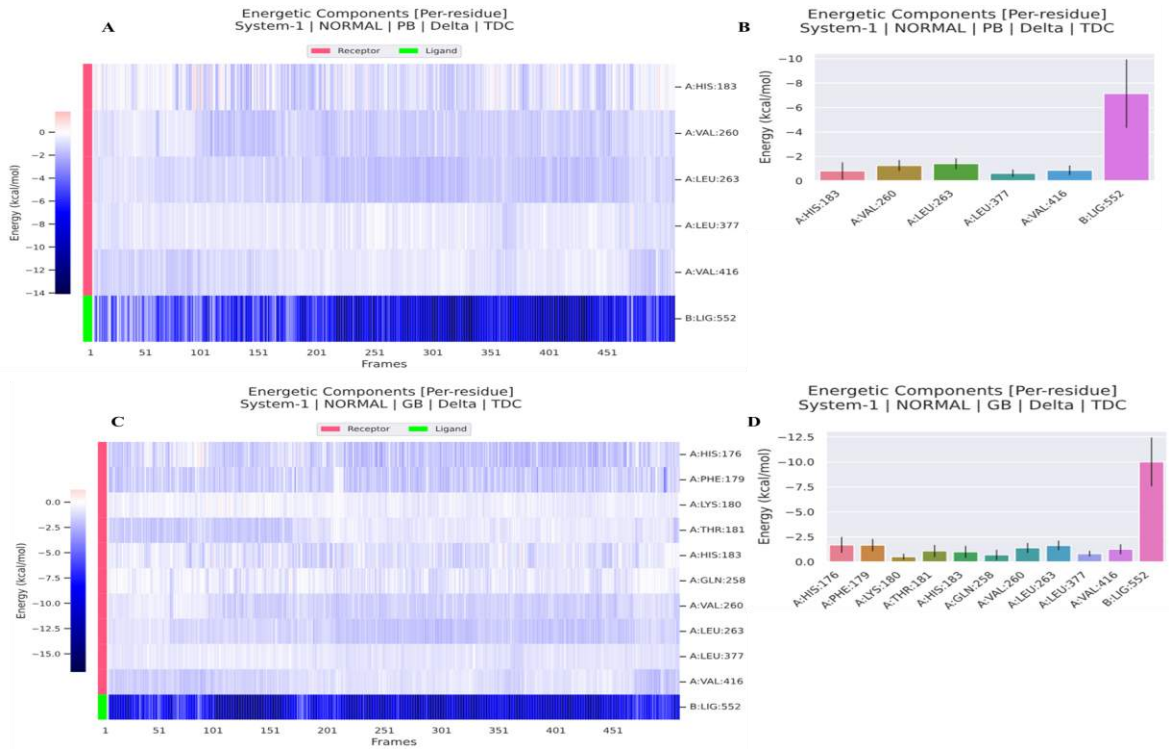

Figure 9. The per-residue decomposition studies of the COX-2_Daidzein complex using both PB and GB analysis.

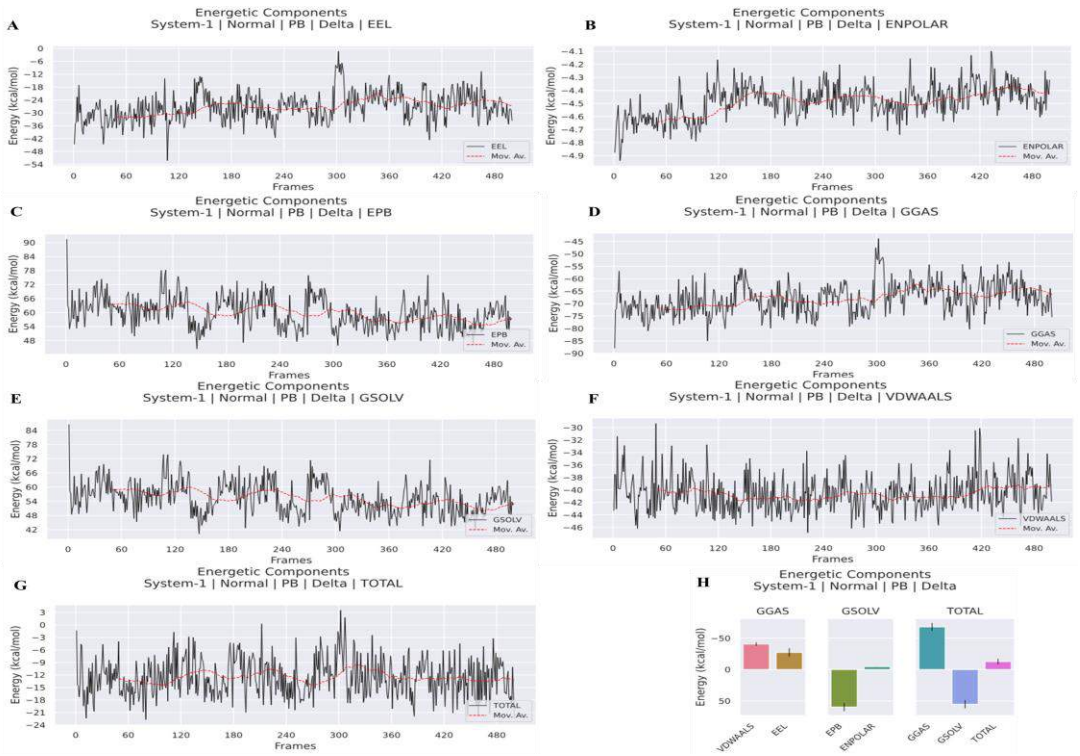

Figure 10. The MM-PBSA analysis of the ERK_Daidzein complex using gmx_mmpbsa_py software and various parameters were assessed such as EEL, ENPOLAR, EPB, GGAS, GSOLV, VDWAALS, and toal energy.

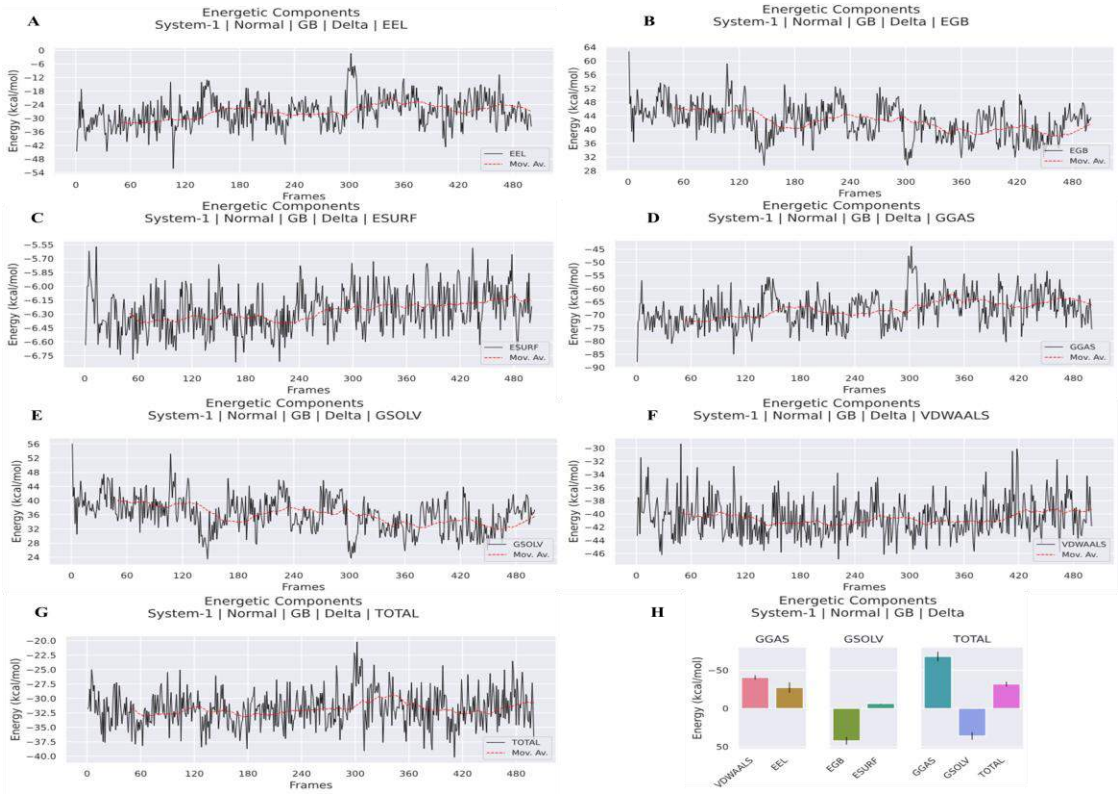

Figure 11. The MM-PBSA analysis of the ERK_Daidzein complex using gmx_mmpbsa_py software and various parameters were assessed such as EEL, ESURF, EGB, GGAS, GSOLV, VDWAALS, and total energy.

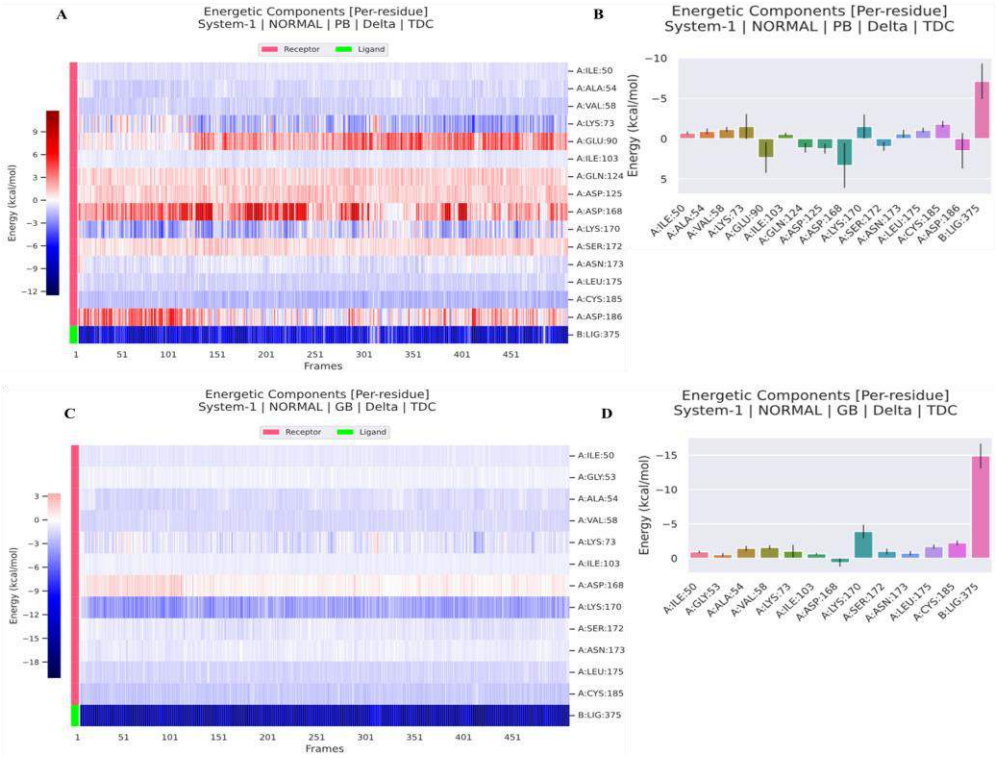

Figure 12. The per-residue decomposition studies of the ERK_Daidzein complex using both PB and GB analysis.

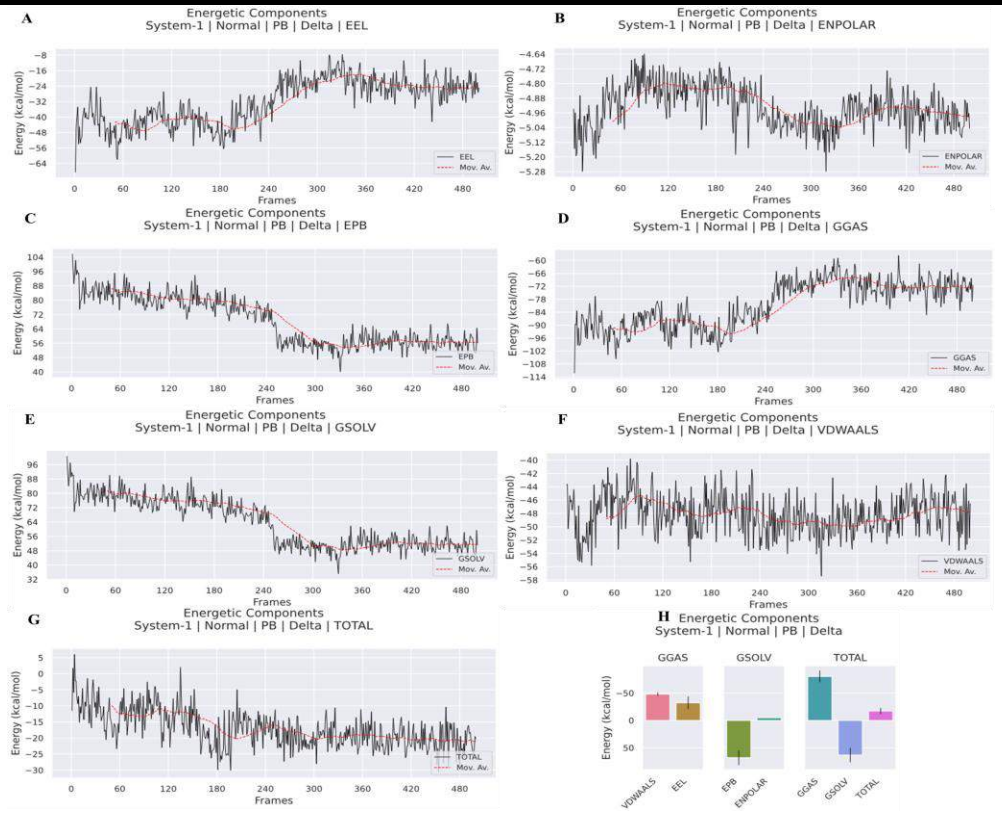

Figure 13. The MM-PBSA analysis of the TRPV1_Daidzein complex using gmx_mmpbsa_py software and various parameters were assessed such as EEL, ENPOLAR, EPB, GGAS, GSOLV, VDWAALS, and toal energy.

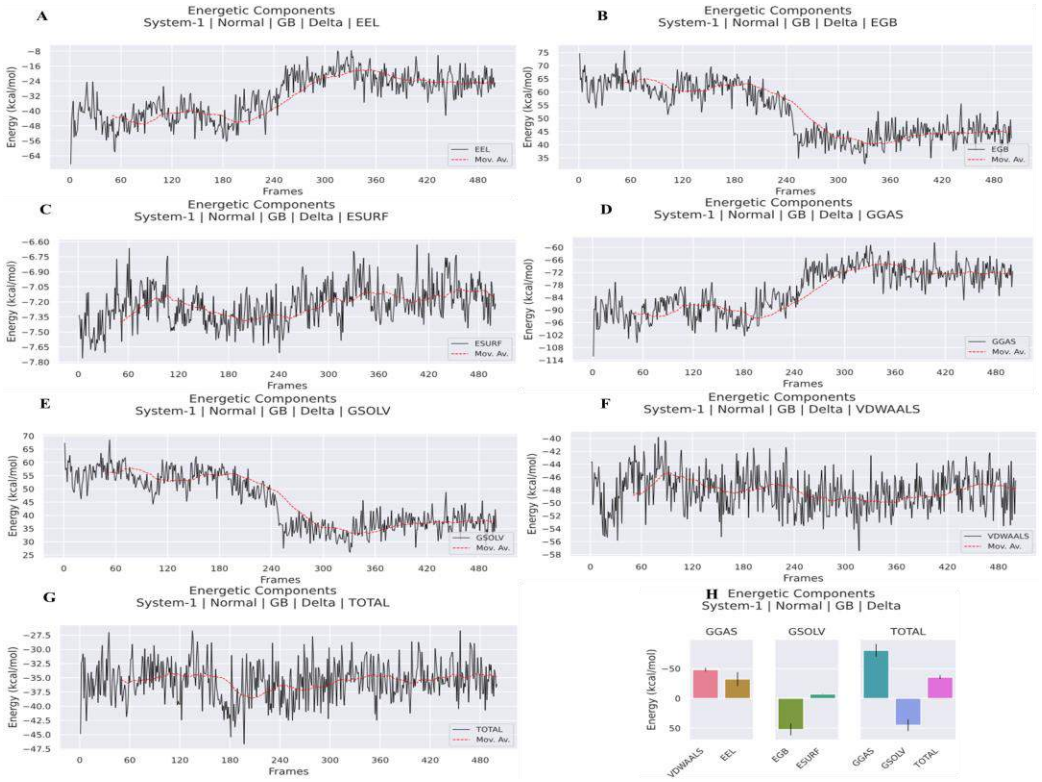

Figure 14. The MM-PBSA analysis of the TRPV1_Daidzein complex using gmx_mmpbsa_py software and various parameters were assessed such as EEL, ESURF, EGB, GGAS, GSOLV, VDWAALS, and toal energy.

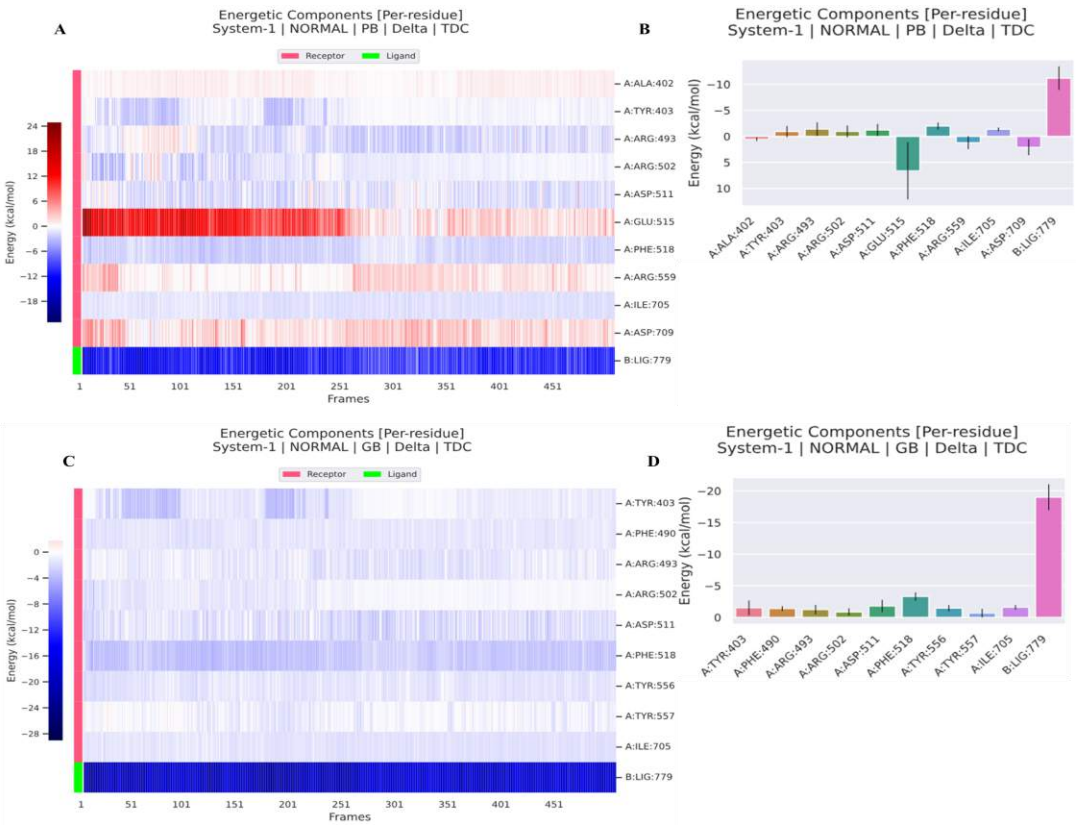

Figure 15. The per-residue decomposition studies of the TRPV1_Daidzein complex using both PB and GB analysis.

3.5. The pharmacokinetic parameter assessment

he pharmacokinetic profile of the Daidzein was assessed using various parameters such as Absorption, distribution, metabolism, and excretion using SwissADME software (A. Srivastava et al., 2022). The pharmacokinetic parameters play crucial role in the understanding of the pharmacodynamic profile of the drugs and its behavior within the living system. The SwissADME software was used to assess the pharmacokinetic parameters such ADME, Drug likeness, physcio-chemical behavior, and medicinal chemistry aspects. The results also show radar analysis which indicates whether the compound follows the drug like behavior or not (A. Srivastava et al., 2022). The results showed that Daidzein follows most of the medicinal chemistry rules and no significant violation of the rule was observed. The Daidzein also showed moderate water solubility and high absorption was noticed from the GIT. The compound also followed most of the drug likeness behavior such as Lipinski rule and the bioavailability 0.55 was observed. The Daidzein also showed no P-gp substrate which indicates that drug will have high absorption fraction. The results of the study are shown in **Figure 16**. The various parameters that were assessed include percent GIT absorption, BBB permeability, Caco-2 permeability, P-glycoprotein substrate, and cytochrome inhibitors as reported in **Table 2.**

Table 2. The pharmacokinetic parameters assessment of the selected compounds Using a computational approach.

Compounds	GI absorption	BBB Permeation	Caco-2 permeability	P-glycoprotein substrate	P-glycoprotein inhibitor	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor
Daidzein	High (94.83)	Yes	0.903	Yes	NO	Yes	Yes	Yes	NO

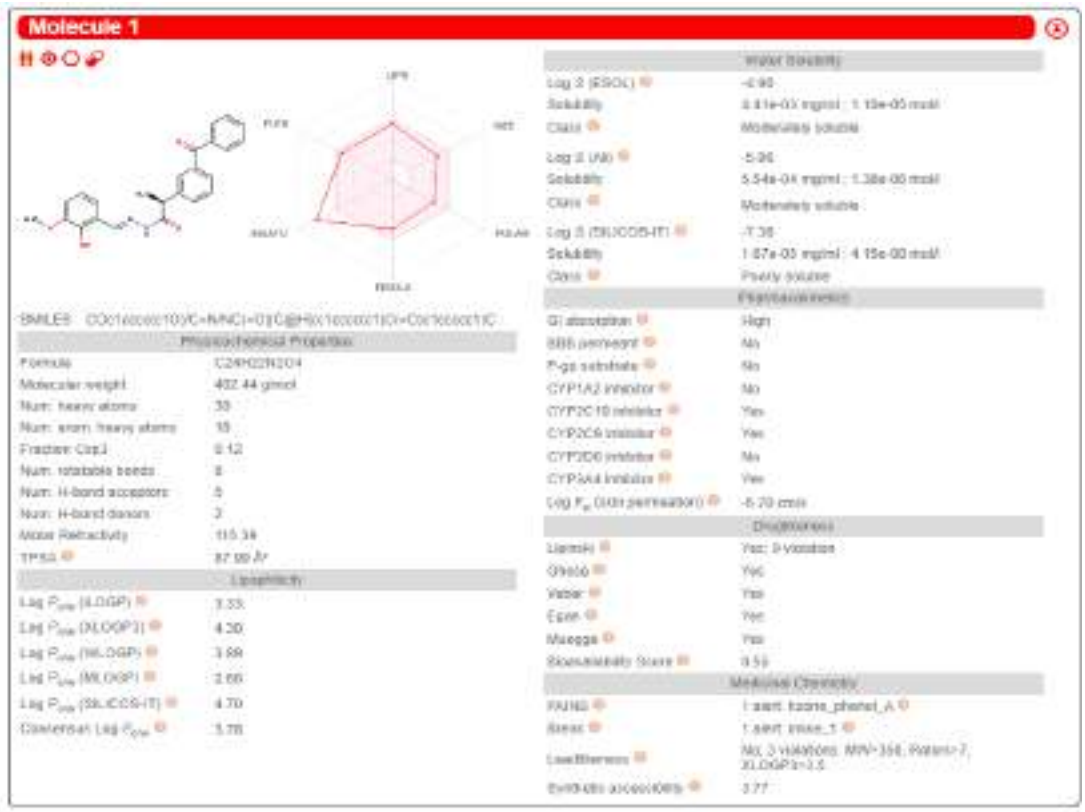

Figure 16. The pharmacokinetic analysis of the Daidzein using SwissADME online server. The results indicate various parameters such as radar analysis, physico-chemical properties, lipophilicity, water solubility etc.

3.6. Phsycio-chemical parameters assessment

The physico-chemical parameters assessment of the Daidzein was performed using in silico approaches. The various parameters that were assessed include molecular weight, LogP, number of rotatable bonds, acceptors, donors, and surface area of all the ligands as shown in **Table 3**.

Table 3. The physico-chemical parameters assessment of the Dadizein using in silico approaches.

S. No	Molecular weight	LogP	Rotatable bonds	Acceptors	Donors	Surface area
Daidzein	254.241	2.8712	1	4	2	107.725

3.7. Effect of Daidzein on the Acetic acid-induced writhing movement

The in vivo activity was performed using acetic acid-induced writhing movement and the inhibitory effect of the Daidzein was studied. The acetic acid-induced writhing pain is commonly employed to study the visceral pain in animals and evaluate the effect of the new chemical moiety (Oh et al., 2015). The acetic acid was administered intraperitoneally (I.P) into the mice of all the recruited groups. The acetic acid administration showed marked increase in the writhing movement in the disease control mice. The animal treated with the Daidzein showed dose dependent reduction in the writhing movement following acetic acid. Similarly, the positive control intervention with the Ketoprofen showed marked reduction in the writhing movement as compared to the disease control as shown in **Figure 17**.

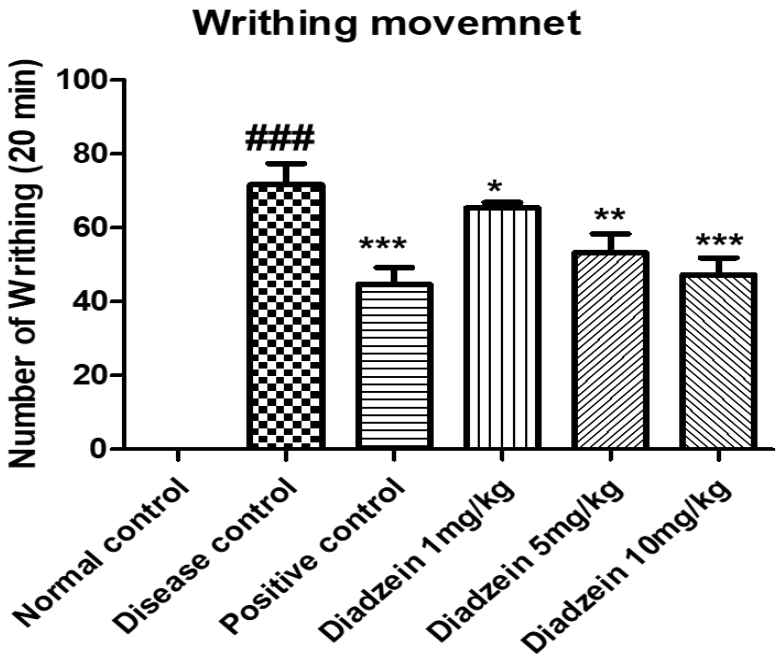

Figure 17. The effect of the Daidzein treatment on the acetic acid-induced writhing movement. The Daidzein treatment showed marked reduction in the writhing movement as compared to the disease control. Similarly, the Positive control treatment also showed marked reduction in the writhing movement. The results were analyzed using One-Way ANOVA followed by the post hoc Dunnet test. The P value less than 0.05 was chosen as criteria for statistical significance.

3.8. Effect of Daidzein in the Formalin-induced biphasic pain response

The formalin-induced biphasic pain model is frequently used pre-clinically to evaluate the anti-inflammatory and analgesic potential of new compounds. The formalin-induced pain model is divided into two phases i.e., Early phase and late phase. The early phase involves the activation of the neurons and is called neurogenic phase, while the late phase involves inflammatory reaction is called inflammatory phase. The early phase lasts for 10 min and the late phase lasts for 10-20 minutes (Lee, Jang, Jung, Kim, & Cho, 2012). The formalin was administered to the plantar surface and before the administration of formalin, animals were pre-treated with different compounds. The formalin administration showed marked nociceptive behavior, however, the Daidzein administration showed marked attenuation in the painful behavior in both phases. Similar results were seen with the Dexamethasone used as positive control as shown in **Figure 18**.

4. DISCUSSION

The natural products are cheap and important source of new drug development and natural product can be exploited for new drug development by structural modification (X. Chen et al., 2021). The natural products have been extensively used for the treatment of various diseases including inflammation, pain, anti-microbial, anti-cancer, hepatoprotective etc (Naidu, Tripathi, Nagar, Mishra, & Poluri, 2024). The natural product provides not only an important source of new molecules but also pave the ways of structural modification to produce

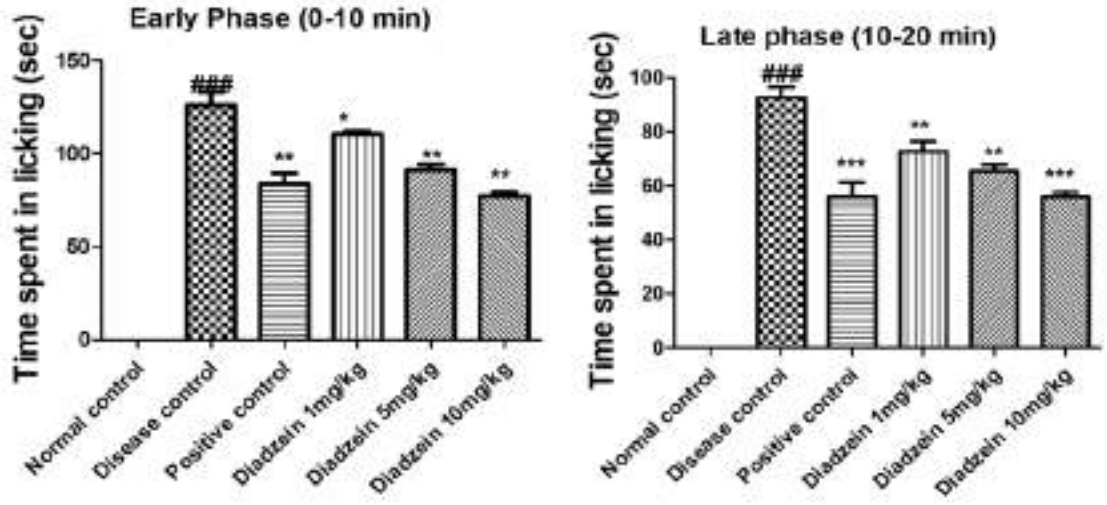

Figure 18. The effect of the Daidzein treatment on the Formalin-induced biphasic pain responses. The Daidzein treatment showed marked reduction in the painful behavior such as paw licking, paw biting, and jumping compared to the disease control. Similarly, the Positive control treatment also showed marked reduction in the painful behavior. The results were analyzed using One-Way ANOVA followed by the post hoc Dunnet test. The P value less than 0.05 was chosen as criteria for statistical significance.

drug with increase pharmacokinetic and pharmacodynamic properties (Botelho et al., 2020; X. Chen et al., 2021). The natural products are cheap and important source of new drug development and natural product can be exploited for new drug development by structural modification (X. Chen et al., 2021). The natural products have been extensively used for the treatment of various diseases including inflammation, pain, anti-microbial, anti-cancer, hepatoprotective etc (Naidu, Tripathi, Nagar, Mishra, & Poluri, 2024). The natural product provides not only an important source of new molecules but also pave the way for structural modification to produce drug with increase pharmacokinetic and pharmacodynamic properties (Botelho et al., 2020; X. Chen et al., 2021). The drugs from the natural source as analgesic has a rich history and various drugs have been reported from natural source as analgesic such as morphine, aspirin etc. Similarly, natural analgesics have been modified structurally to improve their activity and reduce the toxicity associated with the original moiety (Xi et al., 2023). Inflammation is a key event in numerous diseases, and the inflammation

process is an evolutionary alert system in response to the actual or potential tissue damaging response (Xi et al., 2023). The inflammatory process consists of several cardinal features such as pain, fever, redness, swelling and function Lesia. These features of inflammation with all good intention tend to protect the body from the harmful onslaught, however, this exaggerated response makes the patient discomfort and needs pharmacological intervention to relive the discomfort (H. K. Srivastava & Sastry, 2012; Xi et al., 2023). The pain is induced by the activation of the local nerves by the inflammatory cytokines, edematous pressure and direct activation of the brain somatosensory cortex and associated parts such as cingulate and insular cortex (Xi et al., 2023). During inflammation, the inflammatory cytokines not only sensitize the nearby nerves but also trigger the direct damage to nearby tissues by activating the COX signaling. This signaling pathway induces the inflammatory microenvironment and causes the necrosis of the cells, which further increases the pain (Xi et al., 2023).

The pain sensation travels from the site of injury through the first order neurons into the spinal cord and from spinal cord second order neurons to the thalamus (Zhang & An, 2007). The third order neurons from the thalamus reaches to the somatosensory cortex and the pain is perceived to generate an appropriate motor response (Zhang & An, 2007). The pain is chief complain associated with the inflammatory conditions and compel the patient to visit the physician. The pain during transmission process undergoes modulation at several level with the spinal cord (Sommer & Kress, 2004). The first important pain modulation takes place at the level of the first order and second order neurons by the endogenous neurotransmitter such as enkephalin, endorphins and encephalin which are considered as endogenous opioid (Sommer & Kress, 2004). The neurotransmitter at this level is substance-P (for slow pain sensation) and glutamate (for fast pain sensation) and modulated by the GABA interneurons followed by activation of touch receptor (Sommer & Kress, 2004). This pain modulation is called the Gate theory of pain control. Similarly, the pain is also modulated by the descending pain modulation by the DAG and PAG, from which the endogenous opioid neurons descend to the spinal cord at the level of the first order neurons (Sommer & Kress, 2004). These endogenous pain modulation neurotransmitters are activated by the second neurons reaching the thalamus and give innervation to these nuclei to increase the activity of the endogenous opioids (Milligan et al., 2005). Furthermore, the Raphe nuclei and caeruleus or locus caeruleus also release serotonin and nor-epinephrine which activates the opioid neurons to induce the release of additional endogenous opioids and reduce the pain intensity. Most of the NSAIDs work on the peripheral pain mechanism to attenuate the pain sensation, while the opioid derivatives act on the central pain modulation mechanism (Milligan et al., 2005). The NSAIDs act on the COX-2 enzyme responsible for the prostaglandins synthesis which triggers the inflammation. The inflammatory process consists of cellular and vascular events which are orchestrated by a variety of inflammatory mediators (Milligan et al., 2005). The cellular events include the extravasation and diapedesis of the immune cells to the site of injury such as RBCs, WBCs and even platelets, while the vascular events include the blood vessels dilation and contraction of the endothelial cells to make the way for the passage of transudate and exudate (Milligan et al., 2005).

In the present study the effect of the Daidzein was evaluated against the inflammation and nociception using in vivo and computational methods. The computational analysis was performed using the Daidzein compound against the anti-inflammatory pain induced by the Formalin and Carrageen. The computational results showed that Daidzein significantly higher binding energy against various target such as COX-2, ERK, and TRPV1. Similarly, the Daidzein interacted with these protein targets via multiple Hydrogen bonds and hydrophobic bonds. The results of the present study also confirmed by the previously reported studies that inhibition of the COX-2, ERK, and TRPV1 protein is associated with the reduction in inflammation and nociception (Mao et al., 2023). Furthermore, the computational studies reported previously also showed that inhibition of the ligand interaction with the COX-2, ERK, and TRPV1 also exhibited marked anti-inflammatory and analgesic activity (Mao et al., 2023).

The in-silico toxicity and pharmacokinetic analysis was performed for all the constituents from the Pueraria lobata. The results of the study showed that Daidzein exhibited no promising toxicokinetic and pharmacokinetic profile was in an acceptable range compared to the other constituents. Based on the computational analysis, the results were extended towards the in vivo activities. The in vivo activity was performed to validate the computational results

against the inflammation and nociception. The in vivo results of the Daidzein showed significant reduction in the inflammation and nociception in both acetic acid-induced visceral pain and formalin-induced biphasic pain response. The acetic acid-induced visceral pain model has been used commonly for the assessment of new analgesic compound and inhibition of the acetic acid-induced nociceptive behavior have been reported as promising analgesic (Pavao-de-Souza et al., 2012). Similarly, formalin-induced biphasic pain responses model has been commonly used method for the evaluation of new analgesic compounds. The results of the present study were consistent with the previously reported results that inhibition of the formalin-induced nociception is associated with significant analgesic potential (Gwon et al., 2021). In conclusion, the Daidzein showed significant binding interaction with the inflammatory and analgesic targets via multiple hydrogen and hydrophobic bonds. Furthermore, the results of the in vivo analysis showed marked analgesic and anti-inflammatory potential of the Daidzein. Overall, the Daidzein exhibited marked anti-inflammatory and analgesic potential, however, to employ it clinically further molecular investigation such as western blot and Immunohistochemistry is required.

5. CONCLUSIONS

The present study investigated the anti-inflammatory activities of Daidzein using computational and in vivo activities. The molecular docking analysis of the compounds were performed against the various inflammatory target such as COX-2, ERK, and TRPV1 protein. Following molecular docking analysis, the Daidzein compound was subjected to further analysis such as MD simulation, and binding free energy calculations. The Daidzein anti-inflammatory activities against the acetic acid-induced visceral pain and formalin-induced biphasic pain responses were studied. The results of the present study revealed that Daidzein treatment significantly reduced the writhing movement as compared to the negative control or disease control. Similarly, the formalin-induced biphasic pain responses are commonly used to evaluate the analgesic and anti-inflammatory potential of new compound pre-clinically. The formalin-induced biphasic pain can be divided into the two phases i.e., early phase which is called as neurogenic phase and late phase which is called as inflammatory phase. The early last for 10 min, while the late phase lasts from 10-20 min. The Daidzein treatment significantly reduced the painful behavior in both neurogenic and inflammatory phase compared to the disease control. In conclusion, the Daidzein showed marked anti-inflammatory and analgesic potential using computational and in vivo approaches, however, to utilize it clinically additional studies will be required.

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