

IN SILICO MOLECULAR MODELING AND DOCKING STUDIES OF HERBAL COMPOUND MEDIATED INHIBITION OF $\kappa\beta$ KINASE β (INHIBITOR OF $\kappa\beta$ KINASE SUBUNIT β) FOR OSTEOARTHRITIS TREATMENT

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ABSTRACT

Objective: This study was intended to investigate and characterize the phytoconstituents of plant herbs and inhibitors, evaluate its anti-osteoarthritis (OA) and anti-inflammatory potential under *in silico* conditions.

Methods: Docking studies were performed to find out the maximum interaction between design ligands and selected five proteins using Schrodinger Software NY. Structures of selected proteins were downloaded from protein data bank.

Results: Morin, salubrinal, icariin, and chondroitin sulfate, and sesamol have higher binding energy than other compounds. Based on these properties out of 9 compounds we have selected 5 best-docked and compounds selected were molecular dynamics simulation with inhibitor of $\kappa\beta$ kinase subunit (IKK) β . Arg20, Leu21, Thr23, Lys44, Glu61, Glu97, Cys99, Lys106, Asp145, Asn150, and Asp166 were actively involved in H-bond interaction with ligands. Each compound has different binding modes, which reflects the difference of interacting residues with different functional groups. Morin has better interaction than other compounds. Either Cys99 or Lys44 found predominantly in most of the complex except in β -ecdysterone whereas both found interacting with morin.

Conclusion: The results revealed out that the herbal compounds can inhibit the IKK β protein. The virtual screening yielded five potential IKK β inhibitors: Morin, salubrinal, icariin, and chondroitin sulfate, and sesamol. This inhibitor shows good interaction, energy, and pharmacophoric activity and will be suitable for further experiments of an anti-OA target.

Keywords: Docking studies, Nuclear factor kappa β , Herbal medicine, Osteoarthritis, Inhibitor of $\kappa\beta$ kinase subunit β .

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INTRODUCTION

Osteoarthritis (OA), the most common of joint diseases, is characterized by a progressive loss of articular cartilage and inflammation of synovium. Chondrocytes, the resident cells in articular cartilage, play an important role in cartilage degradation in OA [1]. Previous studies showed that inflammatory cytokines were involved in the pathogenesis of OA. Stimulating of chondrocytes by interleukin (IL)-1 β lead to the production of matrix metalloproteinases (MMPs) and other inflammatory mediators such as nitric oxide and prostaglandin (PGE)2 [2,3]. Nuclear factor kappa β (NF $\kappa\beta$) in synovial lining cells, in fibroblast-like synoviocytes and also in chondrocytes of subjects affected by OA. NF $\kappa\beta$ is responsible for the induction of the transcription of genes encoding pro-inflammatory cytokines such as IL1 β and tumor necrosis factor (TNF) α , enzymes such as cyclo-oxygenase-2 that generate pro-analgesic mediators such as PGE2 as well as cartilage-degrading enzymes, e.g., the metalloproteinase (MMP-1, 3, and 13). These cytokines stimulate the production of degradative enzymes such as MMPs in cartilage, which is responsible for excessive cartilage matrix degradation in arthritis [4].

The nuclear transcription factor NF $\kappa\beta$ has a central role in the autoimmune, inflammatory, and destructive mechanisms that drive the progression of OA. Normally, NF $\kappa\beta$ is held in an inactive state in the cytoplasm by I κ B inhibitory proteins. In response to specific external stimuli, including TNF α and IL-1, the inhibitor of $\kappa\beta$ (I κ B) component of the complex is phosphorylated and degraded, resulting in the translocation of NF $\kappa\beta$ into the nucleus and induction of gene transcription. The signal-induced phosphorylation of I κ B involves two I κ B kinases, I κ B kinase subunit (IKK) α and IKK β . Various studies

indicate that IKK β plays the dominant role in the pro-inflammatory signal-induced phosphorylation of the I κ B protein. On the other hand, IKK α is dispensable for these functions but is essential for developing the epidermis and its derivatives. IKK β has been found to be responsible for some of the observed anti-inflammatory properties of marketed drugs such as aspirin and salicylates. Nowadays, the drugs used for the treatment of OA such as nonsteroidal anti-inflammatory drugs have numerous side effects. Therefore, it is urgently needed to seek safe and effective drugs to treat OA [5-7].

These target strategies only to affected cartilage and joints to avoid other undesirable systemic effects. In this paper, docking the IKK β has been described as a targeted by herbal compounds and inhibitors are Morin [8], Salubrinal [9], Icariin [10], chondroitin sulfate [11], sesamol [12], delphinidin [13], LBH589 [14], thymoquinone [15], and β -ecdysterone [16], which appeared well suitable to treat OA diseases.

METHODS

System configuration

All the research works were carried out on a high-performance workstation operated with Cent OS Version-6.5 Linux operating platform. Hardware specifications are high-performance computing workstation running with Intel Core i7 processor of 8 Cores and 16 GB RAM speed. Software specifications used are the commercial version of Schrödinger Software package, LLC, New York, NY 2015.

Protein and ligand preparation

The crystal structure of target protein Human I κ B kinase beta (IKK β), (protein data bank [PDB] ID: 4KIK) was retrieved from PDB [17]. The

typical structure file from the PDB is not suitable for immediate use in molecular modeling calculations, and so the crystal structure of IKK β was prepared through protein preparation wizard, implemented in Maestro 10.4 [18]. Missing residues were added using the prime loop modeling, which is also embedded in protein preparation wizard. First, the bond orders were assigned, hydrogen atoms were added, and all the crystallographic waters molecules were removed. The processing script optimizes the hydrogen-bonding network, rotating hydroxyl and thiol hydrogen, generating appropriate protonation and tautomerization states of His, and performing chi flips in Asn, Gln, and His residues. Optimized structure of IKK β was minimized using optimized potentials for liquid simulations (OPLS)-2005 force field until the average root-mean-square deviation (RMSD) of the non-hydrogen atoms reached 0.3Å.

Molecular docking

The docking protocol was carried out using glide [19-21]. It performs grid-based ligand docking with energetic and searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein. After ensuring that the protein and ligands were in the correct form for docking, the receptor-grid files were generated using a grid-receptor generation program. In this study, the grid was generated by using the predicted active site through the sitemap. Ligand docking panel was used to carry out the docking of all ligands of different charge. The receptor grid generated file was uploaded, and the precision extra precision (XP) was selected. Glide score was generated based on the following formula.

$$GScore = a * vdW + b * Coul + Lipo + Hbond + Metal + BuryP + RotB + Site. \quad (1)$$

Where, vdW=van der Waal energy, Coul=Coulomb energy, Lipo=lipophilic contact term, Hbond=hydrogen-bonding term, Metal=metal-binding term, BuryP=penalty for buried polar groups, RotB=penalty for freezing rotatable bonds, and Site=polar interactions at the active site.

Binding free energy calculation

The free energy of binding was calculated using prime MM-GB/SA approach [22]. In this approach, the docked poses were minimized using the local optimization feature in Prime, and the energies of the complex were calculated using the OPLS-AA (2005) force field and generalized-born/surface area (GB/SA) continuum solvent model. The free energy of binding, ΔG_{bind} is calculated as [23,24],

$$\Delta G_{bind} = \Delta E + \Delta G_{solv} + \Delta G_{SA} \quad (2)$$

$$\Delta E = E_{complex} - E_{protein} - E_{ligand} \quad (3)$$

Where, $E_{complex}$, $E_{protein}$, and E_{ligand} are the minimized energies of the protein-ligand complex, protein, and ligand, respectively. Prime uses a surface generalized born model employing a Gaussian surface instead of a van der Waals surface for better representation of the solvent-accessible SA.

$$\Delta G_{solv} = G_{solv}(complex) - G_{solv}(protein) - G_{solv}(ligand) \quad (4)$$

Where, $G_{solv}(complex)$, $G_{solv}(protein)$, and $G_{solv}(ligand)$ are the solvation free energies of the complex, protein, and ligand, respectively.

$$\Delta G_{SA} = G_{SA}(complex) - G_{SA}(protein) - G_{SA}(ligand) \quad (5)$$

Where, $G_{SA}(complex)$, $G_{SA}(protein)$, and $G_{SA}(ligand)$ are the SA energies for the complex, protein, and ligand, respectively. The rational criteria for selection of best compounds based on scoring and interaction parameters shown in XP docking with different charge model of ligands.

Molecular dynamics (MD) simulation

MD simulations were performed using Desmond with OPLS 2005 force field [25]. Prepared structures were imported in Desmond setup wizard, and they were solvated in the orthorhombic periodic box of TIP3P water molecules and neutralized using an appropriate

number of counter ions and 0.15M of salt concentration. A distance of 10 Å was set between the box wall and protein complex to avoid direct interaction with its own periodic image. Steepest descent method was used to minimize the energy of prepared systems with a maximum of 5000 steps until a gradient threshold (25 kcal/mol/Å) is reached. The systems were equilibrated using the default protocol provided in Desmond. The equilibrated systems were further carried to perform MD simulations for 20000 ps at a constant temperature of 300 K and the constant pressure of 1 atm with a time step of 2 fs.

RESULTS AND DISCUSSION

Molecular docking and binding free energy calculation

The prepared protein structure of IKK β was docked using glide with nine compounds taken for the study. The results of the docking studies were provided in Table 1, and it is revealed that Morin as better compound based on docking score. All docking results are monitored by scoring functions that predict how well the ligand binds in a particular docked pose. This scoring function will give the ranking of the ligands. In the present study, docking score was taken into consideration for the selection of best ligand. It is an empirical scoring function that approximates the ligand binding free energy. It includes various force field interactions such as electrostatic and van der Waals contributions which influence ligand binding [26-28]. Subsequently, the docked structures were carried for binding free energy calculation. The results of binding free energy calculation were provided in Table 2. It is found that binding energy values were well supporting the docking result. Morin, salubrinal, icariin, and chondroitin sulfate have higher binding energy than other compounds. All the other values contribute to the ΔG_{bind} , thereby reflects the total binding energy of the protein-ligand complex.

Binding pose analysis

The binding mode of the compounds with IKK β showed that H-bonds and salt bridges along with other non-bonded interactions were found to influence the interaction between the proteins and ligands. The two-dimensional diagram of protein-ligand interaction was shown in Fig. 1 whereas the three-dimensional view of the active site of protein-ligand interaction was shown in Fig. 2. It is found from Table 3 that Arg20, Leu21, Thr23, Lys44, Glu61, Glu97, Cys99, Lys106, Asp145, Asn150, and Asp166 were actively involved in H-bond interaction with ligands. Each compound has different binding modes, which reflects the difference of interacting residues with different functional groups. Morin has better interaction than other compounds. Either Cys99 or Lys44 found predominantly in most of the complex except in β -ecdysterone whereas both found interacting with Morin. The results obtained from the molecular docking reveals the key interacting residues required for IKK β inhibition.

MD simulation

MD simulations were performed to procure a better understanding of the dynamic behavior and stability of the protein-ligand complex. All the MD simulations were performed for 20000 ps. The change in the structural integrity was analyzed by calculating the RMSD and root

Table 1: Glide XP result of compounds under study

Compound CID	Compound name	Docking score	Glide emodel	Glide energy
5281670	Morin	-11.043	-65.769	-44.611
5717801	Salubrinal	-7.687	-75.464	-58.085
5318997	Icariin	-7.671	-81.167	-57.511
24766	Chondroitin sulfate	-7.531	-58.703	-47.349
68289	Sesamol	-7.171	-31.674	-24.597
68245	Delphinidin	-7.078	-60.993	-40.615
6918837	LBH589	-6.981	-65.362	-41.171
10281	Thymoquinone	-6.794	-31.482	-26.06
101778163	β -Ecdysterone	-6.387	-52.778	-40.423

CID: Compound identifier, XP: Extra precision

Table 2: Prime MM-GB/SA result of compounds under study

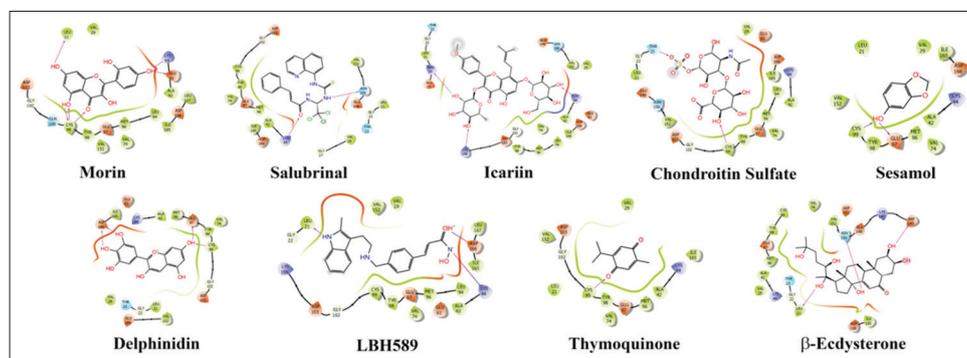
Compound CID	Compound name	ΔG_{bind}	ΔG_{bind} coulomb	ΔG_{bind} vdW	ΔG_{bind} Lipo	ΔG_{bind} Hbond	ΔG_{bind} Solv GB	ΔG_{bind} covalent
5281670	Morin	-67.041	-18.604	-34.288	-35.215	-1.755	22.864	-0.042
5717801	Salubrinal	-78.105	-12.153	-55.322	-40.684	-0.762	30.057	0.758
5318997	Icariin	-69.211	-20.536	-52.020	-44.319	-1.588	42.812	6.441
24766	Chondroitin sulfate	-30.044	30.800	-49.157	-32.415	-1.519	15.492	6.756
68289	Sesamol	-39.810	-11.872	-19.564	-16.385	-0.444	8.200	0.256
68245	Delphinidin	-57.261	79.699	-35.338	-27.333	-3.086	-73.844	2.641
6918837	LBH589	-60.215	100.386	-30.392	-52.303	-3.761	-80.093	5.948
10281	Thymoquinone	-60.783	-6.798	-24.210	-38.931	-0.247	7.151	2.252
101778163	B-Ecdysterone	-71.751	-18.480	-35.476	-57.727	-1.857	37.853	3.936

CID: Compound identifier, GB/SA: Generalized-born/surface area

Table 3: Hydrogen bonds in all complexes with their distances

Compound CID	Compound name	Interacting residues	H-bond acceptor	H-bond donor	Distance (Å)
5281670	Morin	Leu21	O (Pr)	H (L)	2.65
		Lys44	O (L)	HZ3(Pr)	1.98
		Glu61	OE1(Pr)	H (L)	2.58
		Cys99 (3)	O (Pr)	H (L)	1.84
			O (L)	H (Pr)	2.03
5717801	Salubrinal	Lys44 (2)	O (L)	H (Pr)	2.60
			O (L)	HZ3(Pr)	2.46
		Asn150	Cl (L)	HZ3(Pr)	2.98
			OD1(Pr)	H (L)	2.16
5318997	Icariin	Arg20	O (Pr)	H (L)	2.14
		Lys44	O (L)	HZ3(Pr)	2.05
		Lys106	O (L)	HZ3(Pr)	2.09
			O (L)	H (Pr)	1.89
24766	Chondroitin sulfate	Cys99	O (L)	H (Pr)	2.44
		Glu97	O (Pr)	H (L)	1.84
68289	Sesamol	Cys99	O (L)	H (Pr)	2.09
		GLU97	O (Pr)	H (L)	2.31
68245	Delphinidin	Cys99	O (L)	H (Pr)	2.06
			OD1(Pr)	H (L)	1.92
		Asp166 (2)	OD1(Pr)	H (L)	1.94
			O (Pr)	H (L)	2.00
6918837	LBH589	Lys44	N (Pr)	H (L)	2.56
		Cys99	O (L)	H (Pr)	2.10
10281	Thymoquinone	Leu21	O (Pr)	H (L)	2.51
		Asp145	OD1(Pr)	H (L)	1.72
		Asn150	OD1(Pr)	H (L)	2.07

CID: Compound identifier

Fig. 1: Two-dimensional interaction diagram of an inhibitor of $\kappa\beta$ kinase subunit β and its ligand complexes

mean square fluctuations (RMSF) over backbone atoms. Fig. 3 shows all the complexes have even deviations around 3Å and sometimes cross above 4Å. Fig. 4 shows RMSF of all residues in the protein on binding with five complexes. It shows salubrinal, and chondroitin sulfate fluctuates higher than the rest of the complexes, whereas Morin and

icariin fluctuate higher than sesamol. Highest fluctuation of 25Å was reached by the residues from 450 to 550 which were found to be the elongated chains. It was concluded from the trajectory analysis of all the protein-ligand complexes over 20000 ps time period of simulation that the ligand was stable in the binding pocket and did not dissociate

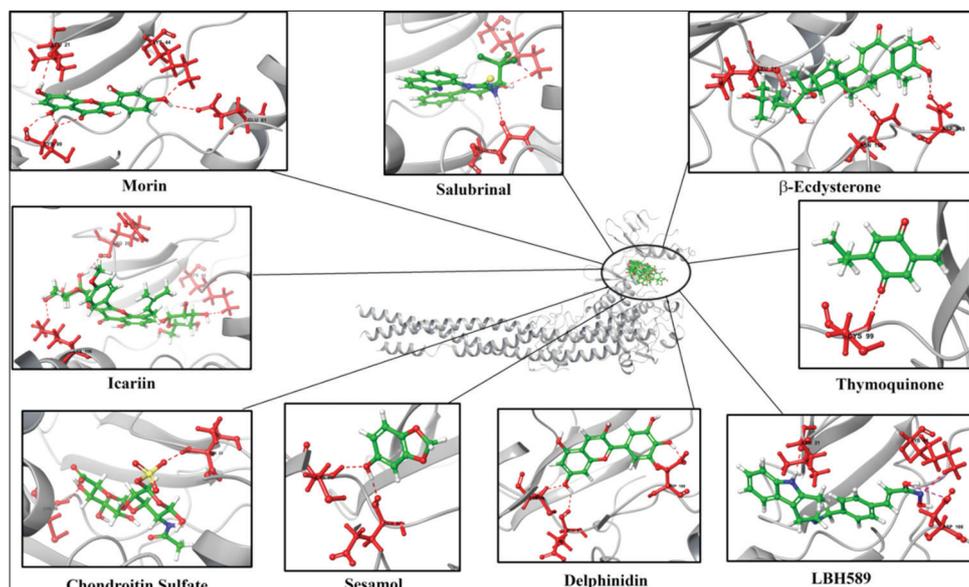


Fig. 2: Three-dimensional view of the active site of an inhibitor of $\kappa\beta$ kinase subunit β and its ligand complexes

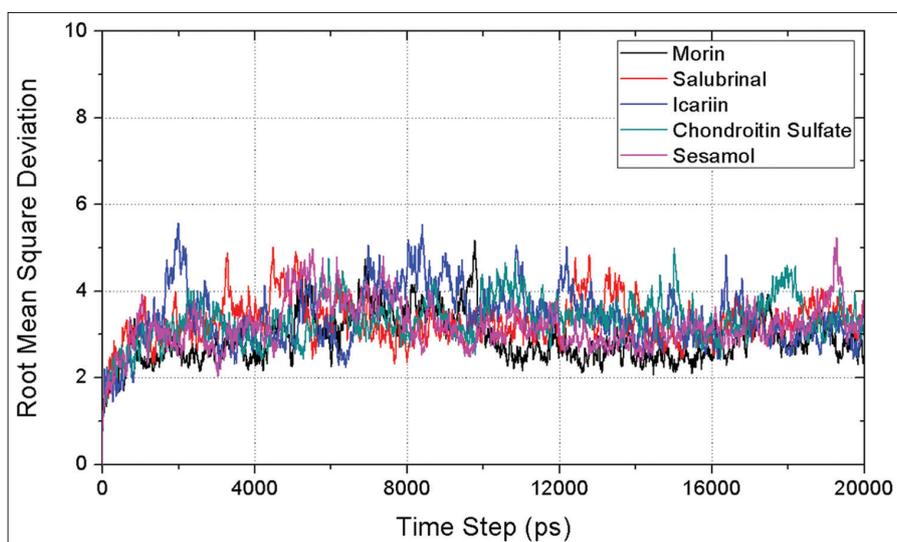


Fig. 3: Root mean square deviation of best five complexes over 20000 ps time period of simulation

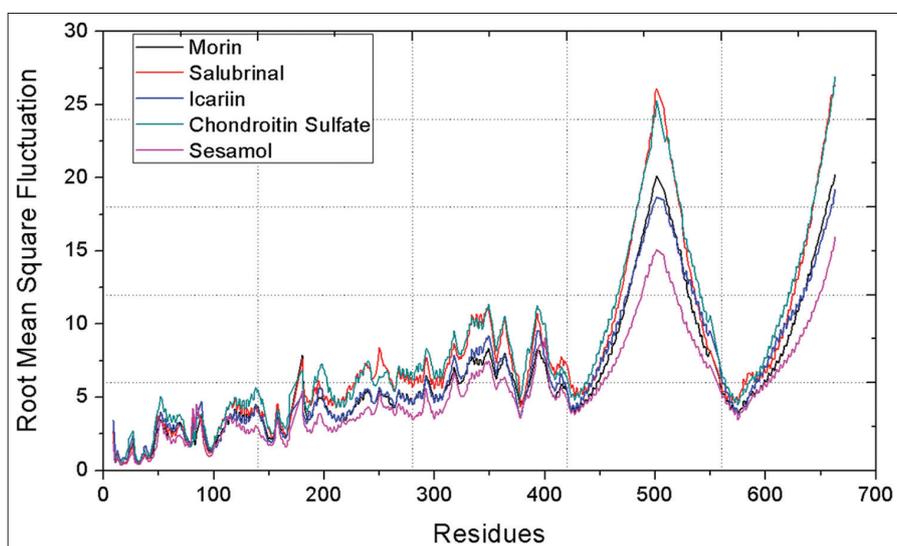


Fig. 4: Root mean square fluctuation of best five complexes over 20000 ps time period of simulation

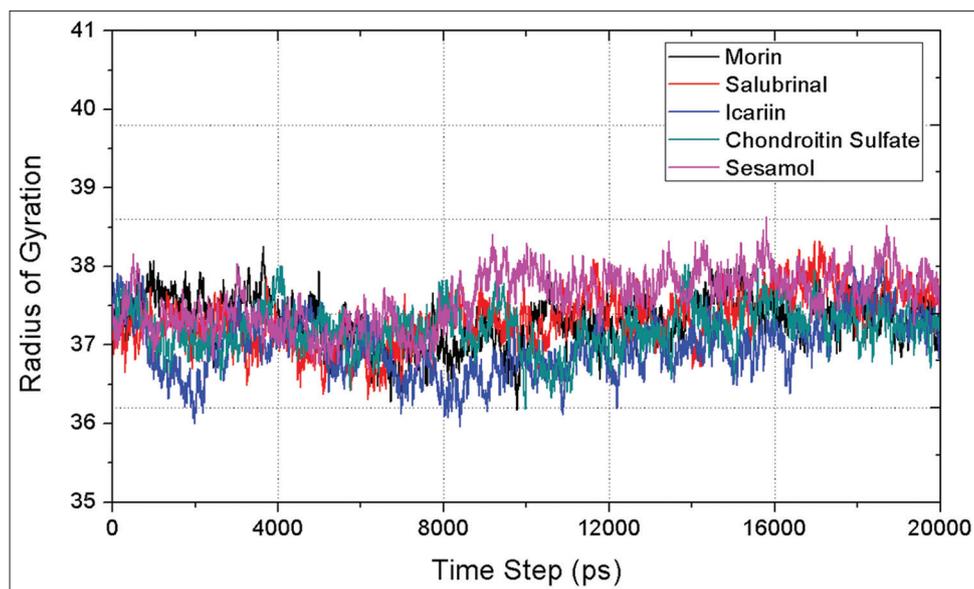


Fig. 5: Radius of gyration of best five complexes over 20000 ps time period of simulation

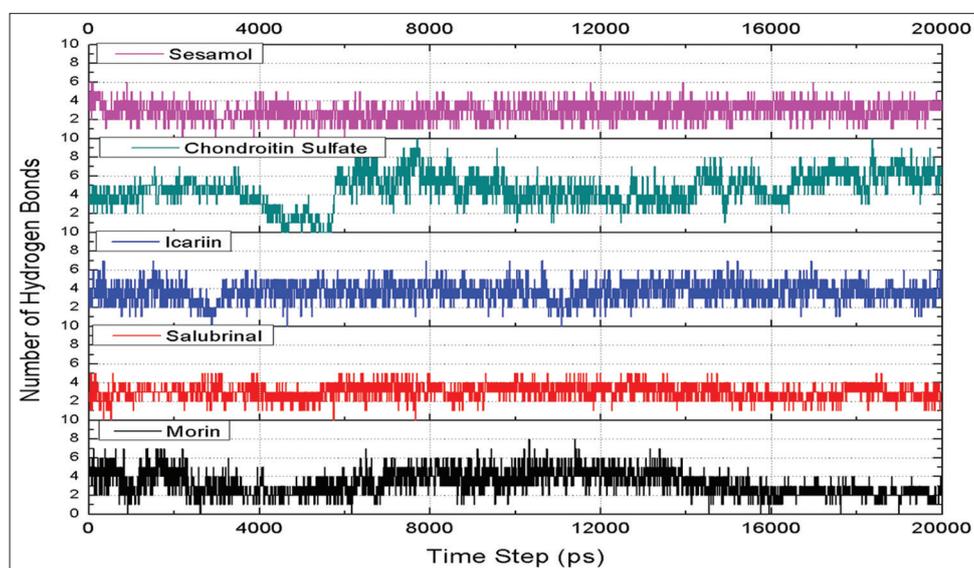


Fig. 6: Number of hydrogen bonds of best five complexes over 20000 ps time period of simulation

from the protein during the simulation. Further, the radius of gyration was calculated to predict the compactness of the protein-ligand complexes over the simulation time period, and the results were shown in Fig. 5. It was revealed that all the protein-ligand complexes did not lose compactness as the graph shows all the complexes were even with not many fluctuations. Fig. 6 shows the number of hydrogen bonds between the top 5 protein-ligand complexes which reveals that all the complexes have stable interaction except in the case of chondroitin sulfate where both loss and gain of more interaction happens during the simulation time period. Thus, the result obtained here confirms the structure and interaction stability of the protein-ligand complexes taken under study.

CONCLUSION

This *in silico* study revealed that $\text{NF}\kappa\beta$ is suppressing medicinal herbal phytochemicals by inhibiting the MMP-13 production in chondrocytes through downregulation of $\text{NF}\kappa\beta$ signaling pathways. In this study, five compounds were identified: Morin, salubrinal, icariin, and chondroitin sulfate, and sesamol. An *in vitro* study must be conducted

to explore the $\text{IKK}\beta$ inhibitory potential of these five compounds. In addition, five bioactive compounds have been mentioned that may serve as a promising therapeutic agent for the treatment of OA.

AUTHORS CONTRIBUTION

Since there is only one author for this manuscript, author contribution need not be mentioned.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

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