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Original Article

MOLECULAR DOCKING STUDIES FOR THE COMPARATIVE ANALYSIS OF DIFFERENT BIOMOLECULES TO TARGET HYPOXIA INDUCIBLE FACTOR-1α

NIRAJ KUMAR JHA[1], PRAVIR KUMAR[1†]

[1]Molecular Neuroscience and Functional Genomics Laboratory, Delhi Technological University (Formerly DCE), Delhi 110042
Email: pravirkumar@dce.edu

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ABSTRACT

Objective: Hypoxia plays a significant role in governing many vital signalling molecules in the central nervous system (CNS). Hypoxic exposure has also been depicted as a stimulus for oxidative stress, increase in lipid peroxidation, DNA damage, blood-brain dysfunction, impaired calcium (Ca^{2+}) homoeostasis and agglomeration of oxidized biomolecules in neurons, which act as a novel signature in diverse neurodegenerative and oncogenic processes. On the contrary, the presence of abnormally impaired expression of HIF-1α under hypoxic insult could serve as an indication of the existence of tumors and neuronal dysfunction as well. For instance, under hypoxic stress, amyloid-β protein precursor (AβPP) cleavage is triggered due to the higher expression of HIF-1α and thus leads to synaptic loss. The objective of this research is to perform comparative studies of biomolecules in regulating HIF-1α activity based on *in silico* approaches that could establish a potential therapeutic window for the treatment of different abnormalities associated with impaired HIF-1α.

Methods: We employed various *in silico* methods such as drug-likeness parameters namely Lipinski filter analysis, Muscle tool, SWISS-MODEL, active site prediction, Auto Dock 4.2.1 and LigPlot1.4.5for molecular docking studies.

Results: 3D structure of HIF-1α was generated and Ramachandran plot obtained for quality assessment. RAMPAGE displayed 99.5% of residues in the most favoured regions. 0% residues in additionally allowed and 0.5% disallowed regions of the HIF-1α protein. Further, initial screenings of the molecules were done based on Lipinski's rule of five. Cast P server used to predict the ligand binding site suggests that this protein can be utilised as a potential drug target. Finally, we have found *Naringenin* to be most effective amongst three biomolecules in modulating HIF-1α based on minimum inhibition constant, Ki and highest negative free energy of binding with the maximum interacting surface area during docking studies.

Conclusion: The present study outlines the novel potential of Biomolecules in regulating HIF-1α activity for the treatment of different abnormalities associated with impaired HIF-1α.

Keywords: Hypoxia-inducible factor-1α (HIF-1α), Biomolecules, Active site prediction, Molecular docking

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INTRODUCTION

Hypoxia plays a decisive role in controlling many important signalling molecules in the central nervous system (CNS) [1]. At the higher altitude, low barometric pressure causes accelerated expression of HIF gene. Hypoxia inhibits prolyl hydroxylation of HIF-1α leading to aggregation of a functional heterodimeric transcription factor (both HIF1 α and HIF1 β subunits) [2]. Hypoxia-inducible factor-1 (HIF-1) is a key transcriptional factor which is amenable for cellular adaption to low oxygen tension. It is a heterodimer comprising of an oxygen-regulated α-subunit and a constitutively expressed β-subunit that regulates a series of genes associated with iron metabolism, angiogenesis, cell proliferation/survival and glucose metabolism [3].

The activity of HIF-1 is regulated by post-translational modifications on different amino acid residues of its subunits, mainly the α-subunit. It has been reported that under hypoxic insult, the activity of HIF-1 and expression of its associated downstream genes, such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO) are altered in a range of neurodegenerative diseases (NDDs) including Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS) [4]. It has also been reported that hypoxia causes reduced synaptic transmission associated with neuronal death by causing neuronal dysfunction [5]. Thus, elucidation of pathophysiological mechanisms caused due to hypoxic insults on the cerebral nervous system (CNS); their therapeutic regulation awaits much importance. Further, experimental and clinical evidence has revealed that regulating the expression of HIF-1α might improve the cellular and tissue damage in the NDDs. This regulatory role on the expression of HIF-1α can be accompanied by employing different biomolecules.

Naringenin (5, 7, 4-trihydroxyflavanone, NGEN) is a flavanone, mainly found in the citrus fruits and tomato. It is known to be act as a multi-functional agent. For example, it acts as a powerful anti-oxidant, anti-depressant, anti-inflammatory and neuroprotective compound [6]. Similarly, *Sesamol* is the major constituent of sesame seed oil (*Sesamum indicum*) possessing powerful antioxidant property. Further, it acts as chemoprotective, anti-inflammatory, neuroprotective, hepatoprotective, and anti-aging biomolecule [7]. Another, well-known biomolecules, *Quercetin* (2-(3,4-dihydroxyphenyl)-3,5, 7-tryhydroxy-4H-chromen-4-one, QUR) possess anti-oxidant, anti-inflammatory and in some cases anti-cancerous activities [8]. These biomolecules are having neuroprotective properties as proposed to be potent therapeutic agents in many diseases, including cognitive impairment associated with neuronal damage. Although, regulating the function of HIF-1α are crucial to be investigated that have protective effects on the pathological ambiances resulting from hypoxic insults in the brain. Therefore, in this research report, we have done *in silico* based comparative study of three different biomolecules (*Naringenin, Sesamol* and *Quercetin*) in order to regulate the impaired functions of HIF-1α. These molecules have been identified and selected for this study through Lipinski rule of five. However, several *in vitro* studies have explored its neuroprotective activities in different neuronal cell cultures and animal models [9, 10]. But a comparative study of these biomolecules on the expression of HIF-1α by using *in silico* tools has not been explored so far, which we are going to explore in this article. In the present study, we reported the Phylogenetic and Physico-chemical properties of HIF-1α gene, since we have selected this gene as a target protein for docking study. Further, Homology modelling visualization and quality assessment of 3D-structure of HIF-1α has been addressed. Additionally, active site prediction and ligand optimisation for both the biomolecules and protein has been done in order to perform molecular docking. Further, we found and docking study revealed that all three compounds were interacting at the reported active and binding site. Finally, in this result the most effective compound was found to be *Naringenin* as showing

minimum Inhibition Constant, Ki and highest negative free energy of binding with maximum interacting surface area suggesting that *Naringenin* could be effective as HIF-1α regulators and therefore act as potential therapeutic molecules for treating patients suffering from hypoxic insults.

MATERIALS AND METHODS

Retrieval of hypoxia inducible factor protein and its function recognition

The amino acid sequence of hypoxia inducible factor protein HIF-1α with accession number Q16665.1 of *Homo sapiens* was retrieved from NCBI database and was used for homology search using Basic Local Alignment Search Tool (BLAST). Protein functional elucidation was done using Interproscan server (https://www.ebi.ac.uk/interpro/search/sequence-search).

Phylogenetic relationship and physicochemical properties

For multiple sequence analysis Muscle tool (http://www.ebi.ac.uk/Tools/msa/muscle/) was used and a phylogenetic tree was constructed using Muscle tool based on NJ (Neighbor-joining) plot without distance correction. ProtParam (http://web.expasy.org/protparam/) was used to predict physicochemical properties. The parameters computed by ProtParam included the molecular weight, theoretical PI, aliphatic index and grand average of hydropathicity (GRAVY).

Homology modelling, visualisation and quality assessment of 3D-structure of hypoxia-inducible factor

Homology modeling was used to determine the 3D-structure of HIF-1αisoforms. A BLASTP search with default parameters was performed against the Brookhaven Protein Data Bank (PDB) to find suitable templates for homology modelling. Template with PDB ID: 4H6J was retrieved for HIF-1αprotein from PDB. The Protein Structure Prediction Server SWISS-MODEL (http://swissmodel.expasy.org/) was used for homology model construction. Once the 3D-structure of proteins was generated, structural evaluation and stereochemical analysis were performed using RAMPAGE (http://www.mordred.bioc.cam.ac.uk/~rapper/rampage.php). Errat server was used to find the accuracy of the structure and visualisation of determined structures was performed using UCSF Chimera.

Active site prediction

Castp Server (http://www.sts.bioe.uic.edu/castp/) was used to predict the active sites of the protein. Castp could also be used to measure area, the circumference of mouth openings of each binding site insolvent and molecular accessible surface. PDB file of protein was uploaded in the server and it showed the ligand binding sites present in protein and the site with maximum surface area and maximum surface volume was selected and all the amino acid residues involved in binding with ligands were retrieved.

Ligand optimization

Reported ligand molecules along with their physical and chemical properties were retrieved from Pub chem compound database (http://www.pubchem.ncbi.nlm.nih.gov/). Pubchem is a composite database that is backed up by three primary databases, i.e. PC substance, PC compound, and PC BioAssay. Pubchem provides biological activity and chemical information of small molecules. PC substance contains information about the substances; PC compound contains information about chemical compounds, and PCBio assay provides information about Bioassays. Four compounds (*Naringenin, Quercetin,* and *Sesamol*) were selected. SDF files of Ligands were converted in PDB file with the help of Open Babel tool that could be used for docking study. Visualization of Molecular Structure of compounds was done using Pymol.

Lipinski filter analysis of screened drugs

An online tool Lipinski Filter (http://www.scfbio-iitd.res.in/software/drug design/lipinski.jsp) was used to retrieve the information about drug-likeness properties of Biomolecules with the help of Lipinski rule of five. Lipinski rule helps to differentiate drug and non-drug like properties of molecules. It is used to identify the possibility of success or failure due to drug-likeness for molecules fulfilling with two or more of the following rules: (a) Molecular Mass should be less than 500 Dalton, (b) High Lipophilicity (expressed as logP less than 5), (c) Less than 5 hydrogen bond donors, (d) Less than 10 hydrogen bond acceptors and (e) Molar refractivity should be between 40-130.

Preparation of protein and ligand molecules

Preparation of protein involves the addition of polar hydrogen atoms, neutralisation of charge and removal of any miscellaneous structures from the protein molecule by Autodock 4.2.1 whereas ligand preparation involves the neutralization of charge.

Molecular docking studies

Prepared and optimised structures of ligands and protein were ultimately used for molecular docking using Autodock 4.2.1 for predicting the possible protein–ligand interactions and the results that include the understanding of the association that involves H-bonding and hydrophobic interactions were analyzed using LigPlot1.4.5, a program to generate schematic diagrams of protein-ligand interactions.

RESULTS

Retrieval of hypoxia inducible factor protein and its functional elucidation

Based on functional domain sequence of well-characterized gene/protein, a homology search was done using BLAST. We have successfully hunted 5 isoforms (table 1) of protein HIF-1α on the basis of families and domains identified from Interproscan results. Interproscan study revealed that all homologues proteins for HIF-1α were belonging to Hypoxia-inducible factor, α-subunit family (IPR021537), Hypoxia-inducible factor-1αfamily (IPR001321), Myc-type, basic helix-loop-helix domain (IPR011598), PAS domain (IPR000014), PAS fold (IPR013767), PAS fold 3 (IPR013655), HIF-1α, transactivation domain, C-terminal (IPR014887) and a repeat of PAC motif (IPR001610)respectively (fig. 1).

Table 1: Hunted HIF-1α related proteins

S. No.	Accession No.	Protein	Score	Identity	E Value
1	NP_001521.1	hypoxia-inducible factor 1-alpha isoform 1	1721	100%	0
2	AKI70676.1	HIF1A	1719	99%	0
3	AAC68568.1	hypoxia-inducible factor 1 alpha subunit	1718	99%	0
4	NP_001230013.1	hypoxia-inducible factor 1-alpha isoform 3	1696	99%	0
5	Q9XTA5.1	Hypoxia-inducible factor 1-alpha	1624	95%	0

Phylogenetic relationship and physicochemical properties

For multiple sequence analysis, Muscle tool was used and found that amino acid residues were conserved in most of the isoforms of the protein HIF-1α (fig. 2a). A phylogenetic study of HIF-1αhunted proteins revealed that HIF-1α and HIF1A were in the same cluster as they share the same homology and HIF-1α isoform 1 was in another cluster while HIF-1α isoform 3 and HIF-1α subunit were differed from others (fig. 2b). ProtParam showed that Mol. wt. of HIF-1α was 92670.4 Daltons. An isoelectric point for HIF-1αwas 5.17 which indicates that protein was negatively charged. The GRAVY index of-0.573 for HIF-1α is indicative of hydrophilic and soluble protein (table 2).

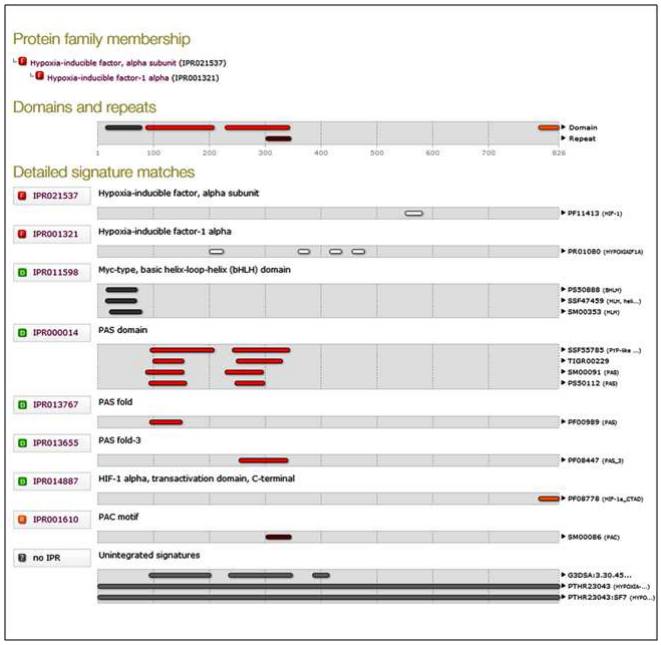


Fig. 1: Interproscan result for HIF-1-αfamily and their domain identification

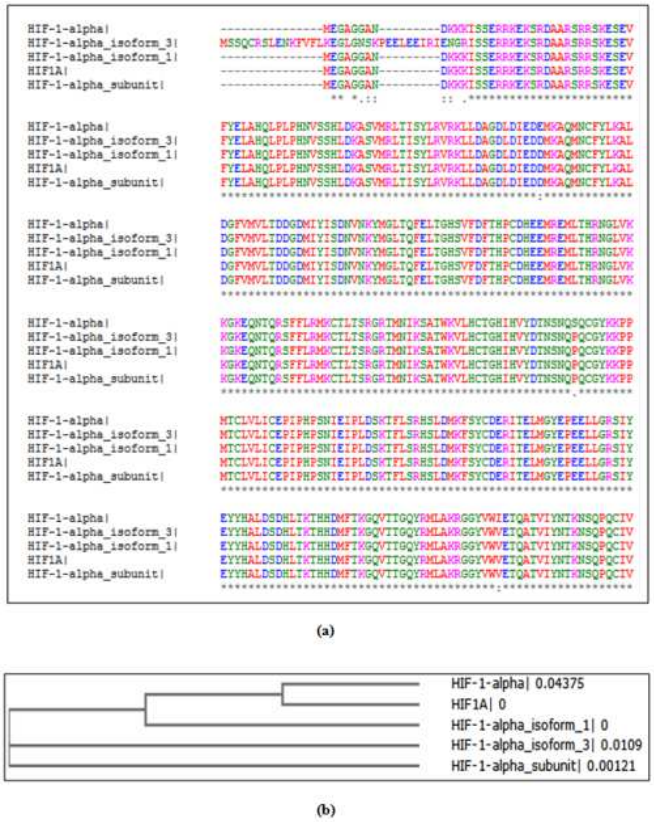


Fig. 2: (a) Multiple sequence alignment and (b) phylogenetic analysis of all HIF-1αisoforms

Table 2: Physico-chemical properties of HIF-1α

Properties	HIF-1α
Molecular Formula	$C_{4027}H_{6410}N_{1108}O_{1309}S_{43}$
Molecular Weight (Daltons)	92670.4
Theoretical PI	5.17
Aliphatic Index	74.96
Grand Average of Hydropathicity (GRAVY)	-0.573

Homology modelling

Prediction of 3D-structure of proteins provides us precise functional information of how proteins interact and localize in their stable conformation. Homology modelling is a most common structure prediction method in structural genomics and proteomics. The best matching template was selected for the target protein on the basis of sequence homology using PDB Advance Blast. The template is experimentally determined 3D-structure of protein that shares sequence similarity with the target sequence. Template showed a sequence identity of 99.07% for HIF-1αisoforms. 3D-structure of HIF-1αwas generated using Swiss-Model Server. The Z-score is indicative of overall model quality and is used to check whether the input structure is within the range of scores typically found for native proteins of similar size. Z-score of the template and query model was obtained by SWISS-MODEL. Z-score for HIF-1α was-0.81 suggesting a good structure (table 3).

Table 3: Swiss model server result showing template structure used in homology modelling, sequence identity and quality score of the model generated

Gene name	Modelled residue range	Based on template	Sequence identity	QMEAN Z-score
HIF-1α	139-811	4H6J	99.07%	-0.81

3D-structure visualization and quality assessment

3D-structure of HIF-1α transcription factor was generated and visualized using UCSF Chimera (fig. 3a). Even though there were no steric clashes in the structure generated, it was assessed for geometric and energy aspects. Ramachandran plot was used to check the reliability of predicted 3D-structure of hypoxia inducible factor protein HIF-1α. RAMPAGE checks the stereochemical quality of a protein structure by analysing residue-by-residue geometry and overall structural geometry. Ramachandran plot was obtained for HIF-1αfor quality assessment. RAMPAGE displayed 99.5% of residues in the most favoured regions, 0.5% residues in additionally allowed and no residues in disallowed regions in HIF-1αProtein (fig. 3b). Errat server was used to determine the accuracy of the model. The result of Errat showed 95.694% accurate structure for HIF-1α protein.

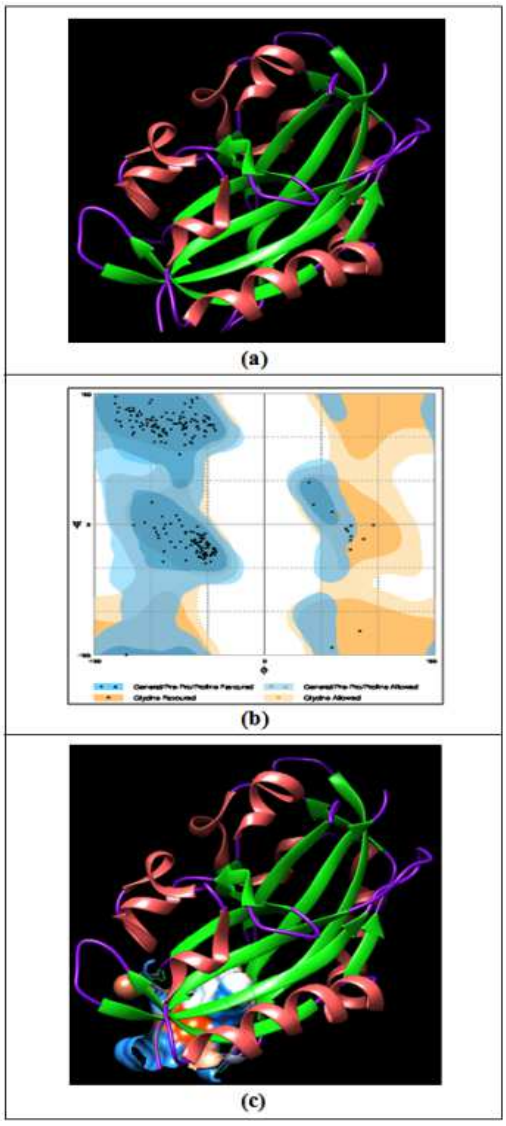
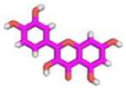
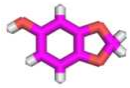


Fig. 3: (a) 3D-Structure, (b) Ramachandran Plot and (c) Active site of generated HIF-1α model

Active site prediction

CastP server was used to predict the ligand binding sites in the generated 3D-structure of HIF-1α. This server calculates the possible active sites from the 3D atomic coordinates of the protein (fig. 3c). Among the twenty-nine binding sites obtained from CastP for HIF-1α, site 29 was highly conserved within the active site of the protein. The Predicted site 29 consisted 415.4 Cubic angstroms site volume out of the 1661.1 Cubic Angstroms of protein volume. The residues in site twenty-nine are shown in (table 6).

Table 4: Physico-chemical properties of natural compounds used for docking study

Characteristics	Naringenin	Quercetin	Sesamol
Molecular weight	272.25278 g/mol	302.2357 g/mol	138.12074 g/mol
Molecular Formula	$C_{15}H_{12}O_5$	$C_{15}H_{10}O_7$	$C_7H_6O_3$
Molecular Structure			
IUPAC Name	5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	1,3-benzodioxol-5-ol
Rotatable Bond Count	1	1	0
Topological Polar Surface Area	$87A^2$	$127A^2$	$38.7A^2$
Heavy Atom Count	20	22	10
Complexity	363	488	126

Further, the screening of ligand molecules was done on the basis of Lipinski's rule of five. Lipinski filter analysis revealed that all the compounds selected possessed drug likeness and can be used for docking purposes (fig. 4).

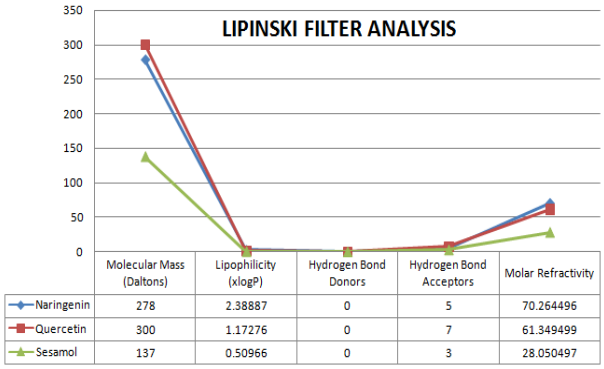


Fig. 4: Differentiation of drugs on the basis of lipinski rule of five by lipinski filter

Docking calculation of compounds with HIF-1A

HIF-1α interaction with *Naringenin*

Free energy of binding with *Naringenin*was-8.48 kcal/mol and Est. Inhibition Constant, Ki was found to be 605.40nM. Intermolecular Energy was found to be-9.68 kcal/mol. VdW+Hbond+desolv Energy and Electrostatic Energy was-9.62 kcal/mol and-0.06 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 9.70 kcal/mol and 1.19 kcal/mol.

HIF-1α interaction with *Quercetin*

Free energy of binding with *Quercetin*was-8.22 kcal/mol and Est. Inhibition Constant, Ki was found to be 945.18 nM. Intermolecular Energy was found to be-10.01 kcal/mol. VdW+Hbond+desolv Energy and Electrostatic Energy was-9.81 kcal/mol and-0.20 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 9.51 kcal/mol and 1.79 kcal/mol.

HIF-1α interaction with *Sesamol*

Free energy of binding with *Sesamol* was-5.13 kcal/mol and Est. Inhibition Constant, Ki was found to be 174.23 µM. Intermolecular Energy was found to be-5.43 kcal/mol. VdW+Hbond+desolv Energy and Electrostatic Energy was-5.34 kcal/mol and-0.08 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 0.32 kcal/mol and 0.30 kcal/mol. Docked energy estimation of HIF-1α is shown in table 5 and interaction of HIF-1α with ligands is shown in (fig. 5).

Binding site of HIF-1α with selected compounds along with its reported Inhibitory active site

Binding site residues of HIF-1α interacting with *Naringenin, Quercetin* and *Sesamol* were found to be the same as the residues involved in their respective catalytic sites. Interacting residues of HIF-1α with *Naringenin, Quercetin* and *Sesamol* along with their identified catalytic sites have been shown in (table **6**) and their 2D and 3D pattern of interaction is presented in (fig. 6).

Table 5: Docking calculation of compounds with HIF-1α

Compound name	Est. free energy of binding	Est. binding constant	Est. intermolecular energy	vdW+Hbond+desolv energy	Electrostatic energy	Est. internal energy	Torsional free energy
Naringenin	-8.48 (kcal/mol)	605.40 nM	-9.68 (kcal/mol)	-9.62 (kcal/mol)	-0.06 (kcal/mol)	+9.70 (kcal/mol)	+1.19 (kcal/mol)
Quercetin	-8.22 (kcal/mol)	945.18 nM	-10.01(kcal/mol)	-9.81 (kcal/mol)	-0.20 (kcal/mol)	+9.51 (kcal/mol)	+1.79 (kcal/mol)
Sesamol	-5.13 (kcal/mol)	174.23 µM	-5.43 (kcal/mol)	-5.34 (kcal/mol)	-0.08 (kcal/mol)	+0.32 (kcal/mol)	+0.30 (kcal/mol)

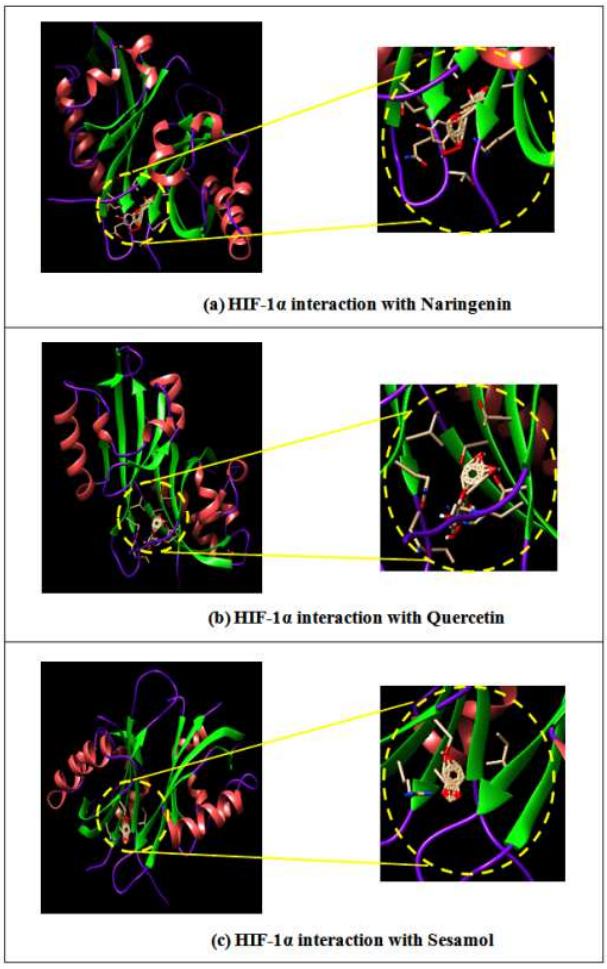


Fig. 5: Binding of HIF-1α with selected compounds

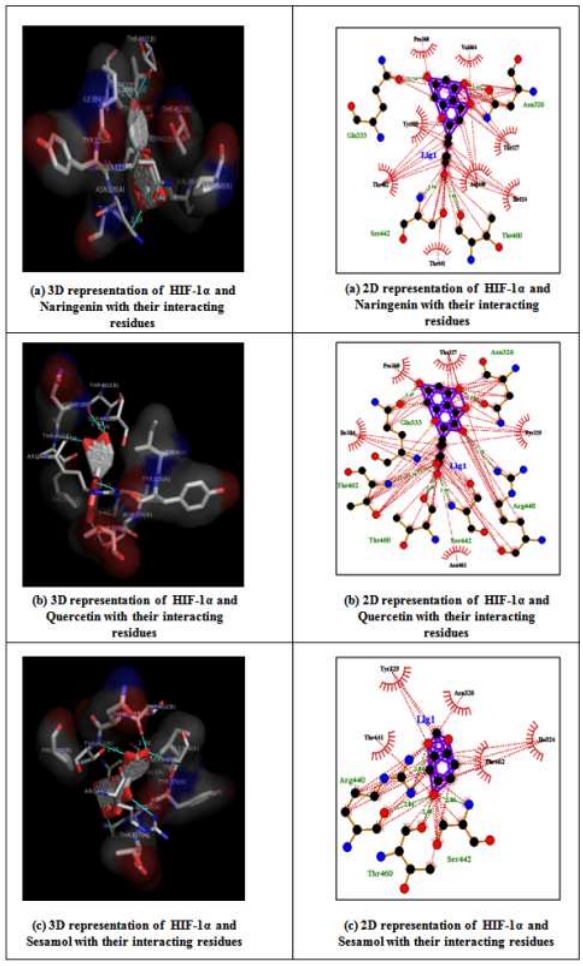


Fig. 6: 3D-and 2D-representation of HIF-1α and ligand interactio

Table 6: HIF-1α known inhibitory site and selected compounds interacting residues

Compounds	Interacting residues
Reported Active Site	ILE^{324}, TYR^{325}, ASN^{326}, THR^{327}, LYS^{328}, GLN^{333} and CYS^{334} of chain A and CYS^{358}, GLN^{359}, PRO^{360}, ARG^{362}, MET^{426}, ARG^{440}, THR^{441}, SER^{442}, THR^{460}, ASN^{461}, THR^{462}, ASN^{463}, VAL^{464} and LYS^{465} of chain B.
Naringenin	ILE^{324}, ASN^{326}, TYR^{325}, THR^{327} and GLN^{333} residues of chain A and PRO^{360}, ARG^{440}, THR^{441}, SER^{442}, THR^{460}, THR^{462} and VAL^{464} residues of chain B.
Quercetin	ILE^{324}, TYR^{325}, ASN^{326}, THR^{327} and GLN^{333} residues of chain A and PRO^{360}, ARG^{440}, SER^{442}, THR^{460}, ASN^{461} and THR^{462} residues of chain B.
Sesamol	ILE^{324}, TYR^{325} and ASN^{326} residues of chain A and ARG^{440}, THR^{441} SER^{442}, THR^{460} and THR^{462} residues of chain B.

DISCUSSION

Further, recent therapeutics advancement in hypoxia-mediated aberrations reveals the promising role of natural compounds as potent neuroprotective agents. By this *in the silico* investigation, we have successfully hunted 5 unique hits using BLAST [11] based on functional domain sequence and optimized the full-length genes of HIF-1α on the basis of families and domains identified from Interproscan results. These isoforms belong to Hypoxia-inducible factor, alpha subunit family (IPR021537), Hypoxia-inducible factor-1 alpha family (IPR001321), Myc-type, basic helix-loop-helix domain (IPR011598), PAS domain (IPR000014), PAS fold (IPR013767), PAS fold 3 (IPR013655), HIF-1 alpha, transactivation domain, C-terminal (IPR014887) and a repeat of PAC motif (IPR001610) and catalyse functions based on its activity to regulate the transcriptional activity in areas of vascularization and angiogenesis, energy metabolism, cell survival and tumour invasion. Further, the Phylogenetic study of HIF-1αrevealed that hypoxia-inducible factor 1α and HIF1A were in same cluster as they share the same homology and hypoxia-inducible factor 1αisoform 1 was in another cluster while Hypoxia-inducible factor 1-alpha isoform 3 and hypoxia-inducible factor 1αsubunit were differed from others [12]. ProtParam results showed that isoelectric point was 5.17 which indicates that protein was negatively charged thus it could be better for docking analysis. TheGRAVYindexis-0.573 for HIF-1α is indicative of hydrophilic and soluble protein. Template showed 99.07% sequence identity for HIF-1αprotein which is another important property for proper ligand interaction. 3D-structure of HIF-1αwas generated by using SWISS MODEL Server [13] and visualized using UCSF Chimera [14]. Z score for HIF-1αwas 0.81 respectively suggesting that input structure is within the range of scores typically found for native proteins of similar size. RAMPAGE displayed 99.5% of residues in the most favoured regions, 0.5% residues in additionally allowed and no residues in disallowed regions in HIF-1αProtein, showing that stereochemical quality of protein structure is good and which is important for proper docking. The result of Errat showed 95.694% accurate structure for HIF-1αprotein. Among the twenty-nine binding sites obtained from CastP Server for HIF-1α, site 29 was highly conserved within all the binding sites of HIF-1αprotein [15]. Further, active site prediction was performed since it is useful to

determine potential sites for ligand binding in molecular docking. Three compounds (*Naringenin, Quercetin* and *Sesamol*) obtained from different medicinal plants were selected for molecular docking study at *in silico* level. Lipinski Filter Analysis of all the compounds revealed that these compounds could act like a drug and have drug-like property as these compounds meet the criteria of Lipinski Rule of five [16]. Docking study revealed that all four compounds are interacting at the reported active and binding site [17, 18]. Inhibition Constant, Ki of *Naringenin, Quercetin* and *Sesamol* for HIF-1α was found to be 605.40 nM, 945.18 nM and 174.23 μM respectively suggesting that all the selected compounds are effective as HIF-1α inhibitors. Investigation of active and binding sites within HIF-1α protein and gives a better idea for a valuable drug target site and drug interaction with the highest affinity. In this result, the most effective compound was found to be *Naringenin* as showing minimum Inhibition Constant, Ki and highest negative free energy of binding with maximum interacting surface area [19-22].

CONCLUSION

In the light of the above analysis, we found that modulating the HIF-1α activity could be helpful for curing many disease progressions. Since, altered expression of this important transcription factor directs many abnormalities, including neuronal dysfunction and cancers. Further, *in silico* studies revealed that biomolecules might have a role in the inhibition of HIF-1α and in the prevention of hypoxia-mediated cellular dysfunction. All the biomolecules which have been selected for docking study are having drug-like property and may act as potential biomolecules for inhibiting or targeting the altered expression of HIF-1α. The *in silico* molecular docking study results revealed that all the biomolecules are having minimum binding energy and have good affinity toward the active pocket, thus, they may be supposed as good inhibitor of HIF-1α. Inhibition Constant, Ki of *Naringenin, Quercetin* and *Sesamol* for HIF-1α was found to be 605.40 nM, 945.18 nM and 174.23 μM respectively, advocating that all the selected compounds are effective as HIF-1α inhibitors. Additionally, investigation of active and binding sites within HIF-1α protein gives a better idea for a valuable drug target site and drug interaction with the highest affinity. Finally, in this result the most effective compound was found to be *Naringenin* as showing minimum inhibition constant, Ki and highest negative free energy of binding with the maximum interacting surface area. Thus, the role of natural compound *Naringenin* with HIF-1α provides a novel remedial approach among all three biomolecules based on docking studies and provide a potential curative biomarker for the treatment of patients suffering from hypoxic injuries.

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CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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