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Research Article

BIOMOLECULES MEDIATED TARGETING OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN NEURONAL DYSFUNCTION: AN *IN SILICO* APPROACH

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ABSTRACT

Objective: Neurodegenerative diseases are a debilitating age-related disorder manifested by memory loss, impaired motor activity, and loss of muscle tone due to the accumulation of toxic metabolites in the brain. Despite the knowledge of factors causing neurodegenerative disorders, it remains irreversible and incurable. Growing evidence have currently advocated the physiological and pathological contribution of hypoxia-induced vascular endothelial growth factor (VEGF) in neuronal loss. The objective of this research report highlights biomolecules mediated targeting of VEGF activity based on *in silico* approaches that could establish a potential therapeutic window for the treatment of different abnormalities associated with impaired VEGF.

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

Results: Three-dimensional structure of VEGF was generated and Ramachandran plot obtained for quality assessment. RAMPAGE displayed 99.5% of residues in the most favored regions, 0.5% residues in additionally allowed, and no residues in disallowed regions in VEGF, showing that stereochemical quality of protein structure is good. Further, initial screenings of the molecules were done based on Lipinski's rule of five. Finally, we have found Naringenin to be most effective among three biomolecules in modulating VEGF activity 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 VEGF activity for the treatment of different abnormalities associated with impaired VEGF.

Keywords: Hypoxia, Vascular endothelial growth factor, Biomolecules, Active site prediction, Molecular docking.

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INTRODUCTION

Neurodegenerative diseases are pathological conditions that have an insidious onset and chronic progression. Different models have been established to study these diseases to understand their underlying mechanisms and to investigate new therapeutic strategies [1]. Several downstream signaling molecules are reported to trigger under hypoxia. Vascular endothelial growth factor (VEGF) is one of them, which is responsible for the formation of new blood vessels (angiogenesis) and lead to the supply of nutrients and oxygen for normal homeostasis [2]. Moreover, the crucial role of VEGF in the brain is not restricted only to controlling vessel growth: But it has direct effects on different types of neural cells including neural stem cells. Conversely, altered expression of this molecule has been implicated in virtually every type of angiogenic disorder, including those associated with cancer, ischemia, and inflammation [3]. Moreover, studies have also revealed the pathological implication of VEGF in the progression of neurodegenerative disorders (NDDs) including, Alzheimer's disease, Huntington disease, and amyotrophic lateral sclerosis. Recent genetic studies have revealed that reduced VEGF levels cause neurodegeneneration through impairing neural tissue perfusion [4]. Importantly, implementation of different biomolecules may helpful in regulating the altered levels of VEGF in cells. The growing evidence for an etiologic role of VEGF in neurodegeneration provides an underlying principle for considering the therapeutic potential of VEGF for NDDs, which are mostly not curable. In this framework, we have introduced different biomolecules (naringenin, quercetin, and sesamol) for targeting VEGF. These biomolecules have chemoprotective, anti-inflammatory, neuroprotective, and anti-aging property. For this purpose, we have performed *in silico* based structural and functional analysis of these molecules for revealing its therapeutic importance against neuronal loss through modulating the impaired expression of VEGF. The objective of this study is to explore neuroprotective action of these biomolecules in regulating the altered level of VEGF to attenuate the toxicity associated with toxic proteins in neuronal death.

METHODS

Visualization and quality assessment of three-dimensional (3D)-structure of VEGF

3D-structure of VEGF (ID: 1QTY) was generated using protein data bank (PDB), structural evaluation, and stereochemical analysis was 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 visualization of determined structures was performed using University of California, San Francisco Chimera.

Active site prediction

The active sites of VEGF were predicted using the Pock Drug tool (http://pockdrug.rpbs.univ-paris-diderot.fr/cgi-bin/index.py?page=home). The PDB structure of VEGF was uploaded and active sites were predicted using f-pocket estimation and setting ligand proximity threshold at 5.5.

Ligand optimization

Reported ligand molecules along with their physical and chemical properties were retrieved from PubChem Compound Database (http://

www.pubchem.ncbi.nlm.nih.gov/). PubChem is a composite database that is backed up by three primary databases, i.e., polycarbonate compounds (PC) substance, PC compund, 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 PC BioAssay provides information about Bioassays. Three 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/drugdesign/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 differentiate drug and non-drug such as properties of molecules. It is used to identify the possibility of success or failure due to drug-likeness for molecules fulfiling with two or more of the following rules: (a) Molecular mass should be <500 Da, (b) high lipophilicity (expressed as log p<5), (c) <5 hydrogen bond donors, (d) <10 hydrogen bond acceptors, and (e) molar refractivity should be between 40 and 130.

Preparation of protein and ligand molecules

Preparation of protein involves the addition of polar hydrogen atoms, neutralization 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 optimized 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

3D-structure visualization and quality assessment

3D-structure of VEGF was generated and visualized using UCSF Chimera (Fig. 1a). 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 VEGF. RAMPAGE checks the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structural geometry. Ramachandran plot was obtained for VEGF for quality assessment. RAMPAGE displayed 99.5% of residues in the most favored regions, 0.5% residues in additionally allowed, and no residues in disallowed regions in VEGF (Fig. 1b). Errat server was used to determine the accuracy of the model. Result of Errat showed 95.694% accurate structure for VEGF.

Active site prediction

Of top 10 pockets, VEGF had best pocket at P4 with a drug ability score of 0.95 and 0.01 standard deviation (Table 1). The volume of given pocket was 1338.57 cubic angstroms and fourteen residues were involved in interaction at this site.

Lipinski filter analysis of screened drugs

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. 2).

Molecular docking of VEGF with biomolecules

Biomolecules bound to VEGF at P4 pocket and same residues as predicted were involved in the interaction. The estimated free energy of binding for VEGF and naringenin was - 7.56 kcal/mol and total intermolecular energy was - 9.05 kcal/mol. Similarly, the estimated free energy of binding for VEGF and quercetin was - 7.10 kcal/mol and total intermolecular energy was - 8.89 kcal/mol. Likewise, the estimated free energy of binding for VEGF and sesamol was - 5.09 kcal/mol and total intermolecular energy was - 5.39 kcal/mol. Molecular docking pattern of VEGF with screened molecules (naringenin, quercetin, and sesamol) have been identified and depicted in Fig. 3. On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of VEGF with these molecules has been presented in Table 2.

Binding site of VEGF with selected compounds along with its reported Inhibitory active site

Binding site residues of VEGF interacting with naringenin, quercetin, and sesamol were found to be the same as the residues involved in their respective catalytic sites. Interacting residues of VEGF with naringenin, quercetin, and sesamol along with their identified catalytic sites have

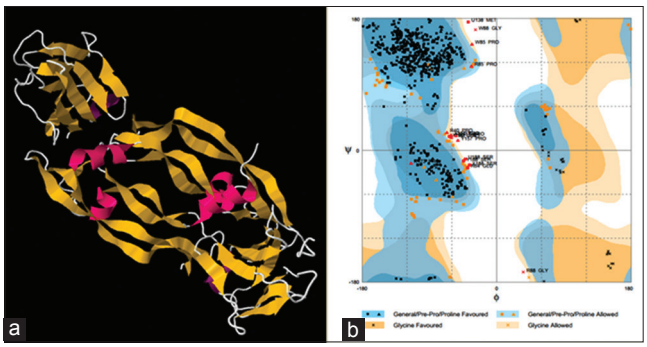

Fig. 1: (a) Three-dimensional-structure and (b) Ramachandran plot of vascular endothelial growth factor protein

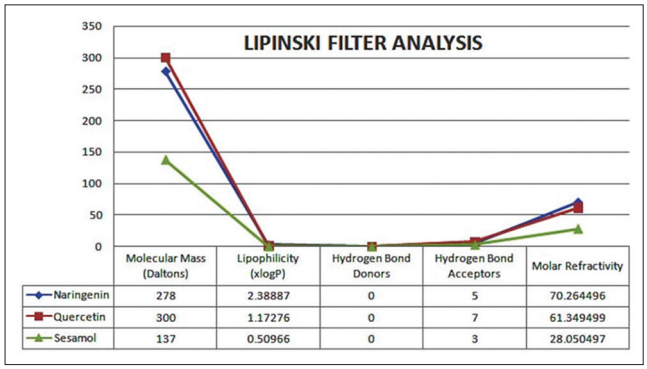

Fig. 2: Differentiation of drugs on the basis of Lipinski rule of five by Lipinski filter

Table 1: Active sites of vascular endothelial growth factor

Pockets	Vol. Hull*	Hydroph. Kyte*	Polar Res.*	Aromatic Res.*	Otyr atom	Nb. Res.*	Drugg Prob*	Standard Deviation
P 0	2205.45	-0.58	0.67	0.2	0.0	30.0	0.63	0.09
P 1	2500.5	-0.67	0.66	0.19	0.0	32.0	0.59	0.08
P 16	452.91	0.83	0.43	0.0	0.0	14.0	0.93	0.02
P 18	744.97	0.56	0.5	0.14	0.0	14.0	0.94	0.01
P 19	455.66	0.83	0.43	0.0	0.0	14.0	0.93	0.02
P 2	1622.2	-0.86	0.68	0.24	0.0	25.0	0.49	0.12
P 3	1819.24	-0.87	0.69	0.21	0.03	29.0	0.53	0.03
P 4	1338.57	0.34	0.62	0.19	0.04	21.0	0.95	0.01
P 5	1159.82	-0.3	0.67	0.24	0.04	21.0	0.83	0.02
P 6	1368.17	-1.2	0.75	0.06	0.0	16.0	0.14	0.02

been show in Table 3 and their two-dimensional and 3D pattern of interaction is presented in Fig. 4.

DISCUSSION

Neurodegeneration is an umbrella term for a range of conditions which primarily affect the neurons in the human brain [5]. Despite the knowledge of various factors which contribute in the occurrence and progression of NDDs, the exact cause and cure remains elusive. Abnormal expression of VEGF protein in terminally differentiated neurons is a recently known phenomenon which has been shown to drive neurodegeneration followed by apoptosis [6]. Free-radical injury of microvessels under hypoxia causes neuroinflammation and oligemia which thereafter leads to Aβ accumulation through vascular damage and the activation of proangiogenic factors including, hypoxia-inducible factor-1α, and VEGF-1 [7]. Further, invading macrophages and monocytes also causes neuronal damage through activation of VEGF-1 [8]. Similarly, low VEGF levels not only impair spinal cord perfusion and cause chronic ischemia of motoneurons but also deprive these cells of vital VEGF-dependent survival and neuroprotective signals.

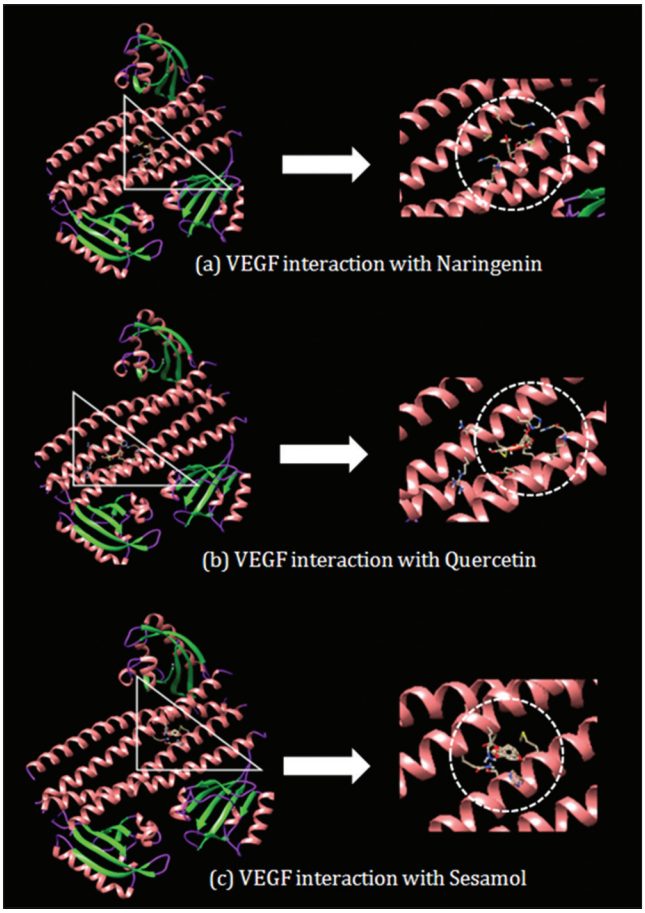

Fig. 3: Binding of vascular endothelial growth factor with selected compounds

Both phenomenons result in progressive degeneration of motoneurons, associated with muscle weakness, paralysis, and death [9]. Thus, it seems imperative to design therapeutic strategies aimed at attenuating the altered level of VEGF to inhibit the cascade of neurodegeneration.

Flavonoids have been advocated to exert human health benefits by antioxidant and anti-inflammatory mechanisms [10]. Naringenin reportedly prevent oxidative stress and nuclear factor kappa B (NF-κB)-mediated inflammatory brain damage in the rat model of focal cerebral injury. Further, prophylactic treatment with naringenin ameliorated functional outcomes and abrogated the ischemic brain injury by suppressing NF-κB-mediated neuroinflammation [11]. Similarly, a significant raise in neuronal survivability was observed with quercetin treatment in rats administered 6-OHDA. Both naringenin and quercetin also reversed the effect of hypobaric hypoxia and elicit neuroprotective response by reducing VEGF level in the murine model [12]. Further, sesamol pre-treatment restored oxidative defense possibly by its free radical scavenging lighted the neuroprotective effect of sesamol against 3-NP-induced neuronal damage [13]. Taken together, all these data provide convincing evidence of using VEGF interaction bioflavonoids such as naringenin and quercetin in attenuating the level of VEGF and in turn, inhibit the cascade of neuronal death.

RAMPAGE displayed 99.5% of residues in the most favored regions, 0.5% residues in additionally allowed and no residues in disallowed regions in VEGF, showing that stereochemical quality of protein structure is good. Result of Errat showed 95.694% accurate structure for VEGF. Lipinski filter analysis of all the compounds revealed that these compounds could act such as a drug and have drug-like property as these compounds meet the criteria of Lipinski Rule of five. Finally, molecular docking studies indicated that all these compounds can bind to and modulate the level of VEGF and possibly, halt or inhibit toxic proteins induced neuronal death in NDDs. Docking study revealed that all three compounds are interacting at the reported active binding site and binding atomic coordination was compared with the template complex coordination and found that docked drug coordination was similar with the known coordination. Amino acid residues of VEGF involved in interaction with naringenin, quercetin, and sesamol were found to be the same as the residues involved in binding with earlier used inhibitors. These observations clearly indicate that we can efficiently determine active site coordinates to investigate the effect of inhibitors on the functional active site of protein. In this result, the most effective compound was found to be naringenin as showing minimum inhibition constant, Ki, and lowest free energy of binding with maximum interacting surface area [14-17]. These findings can be further validated through *in vitro* and *in vivo* studies in neurodegeneration. Overall, although further work is required, these studies advocate the pivotal role of VEGF in NDDs and provide adequate grounds for estimating the potential therapeutic effectiveness of VEGF in their management.

CONCLUSION

The results of our study provide novel potential of biomolecules such as naringenin, quercetin, and sesamol in regulating VEGF expression in the brain, which has wider implications in the progression as well as protection against NDDs. Moreover, of these three biomolecules naringenin is showing better interaction with VEGF based on their minimum binding constant and highest negative free energy.

Table 2: Docking calculation of compounds with VEGF

Compound name	Estimated free energy of binding (kcal/mol)	Estimated binding constant (μM)	Estimated intermolecular energy (kcal/mol)	vdW+Hbond+desolv energy (kcal/mol)	Electrostatic energy (kcal/mol)	Estimated internal energy (kcal/mol)	Torsional free energy (kcal/mol)
Naringenin	−7.56	1.74	−9.05	−9.03	−0.02	+9.69	+1.19
Quercetin	−7.10	6.21	−8.89	−8.73	−0.16	+9.49	+1.79
Sesamol	−5.09	186.16	−5.39	−5.33	−0.06	+0.33	+0.30

VEGF: Vascular endothelial growth factor

Table 3: VEGF known inhibitory site and selected compounds interacting residues

Compounds	Interacting residues
Reported active site	PHE^{375}, VAL^{376}, ASP^{377}, HIS^{378}, ARG^{379}, VAL^{381}, ALA^{382}, GLY^{385}, GLN^{387}, PRO^{388}, GLN^{389}, GLU^{390}, LEU^{392}, LYS^{432} and ASN^{433} of chain A. GLN^{434}, MET^{605}, THR^{606}, GLU^{608}, GLN^{609}, LYS^{610}, LYS^{612}, GLU^{613}, and GLU^{616} of chain B. MET^{598}, HIS^{601}, SER^{602}, MET^{605}, THR^{606}, LEU^{607}, GLU^{608}, GNL^{609}, THR^{611}, LYS^{612}, GLU^{613}, ILE^{614}, ASP^{615} and GLU^{616} of chain C. ARG^{594}, MET^{598}, HIS^{601}, SER^{602}, MET^{605}, THR^{606}, GLN^{609}, LYS^{612} and GLU^{613} of chain F. SER^{241}, LYS^{242}, ASP^{259}, ARG^{260}, THR^{262}, GLU^{263}, LEU^{264}, ILE^{265}, GLY^{266}, HIS^{268}, PRO^{269}, GLU^{270}, ALA^{311}, LYS^{312}, HIS^{313}, GLY^{314}, GLY^{315}, TYR^{316}, VAL^{317}, TRP^{318}, VAL^{343}, and LEU^{344} of Chain G
Naringenin	MET^{605}, THR^{606}, GLU^{608}, and GLN^{609} residues of chain C and MET^{605}, THR^{606}, and GLN^{609} residues of chain F
Quercetin	GLN^{609}, LYS^{612}, GLU^{613}, and GLU^{616} residues of chain C and ARG^{594}, MET^{598}, HIS^{601}, and SER^{602} residues of chain F
Sesamol	MET^{598}, HIS^{601}, and SER^{602} residues of chain C and GLN^{609}, LYS^{612}, and GLU^{613} residues of chain F

VEGF: Vascular endothelial growth factor

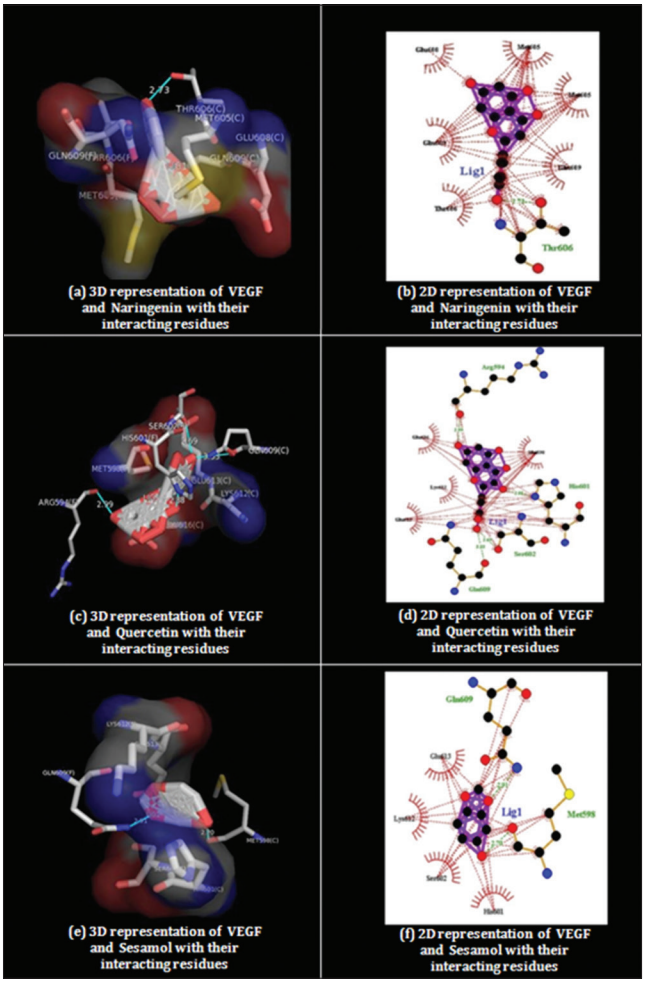

Fig. 4: Three-dimensional- and two-dimensional-representation of vascular endothelial growth factor and ligand interaction

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