Pharmacokinetics and Molecular Docking for Identification of Potential Drug Targets in Tangeretin's Neuro-Protection

Sundararajan Balasubramani (✉ sundarpbt87@gmail.com)

Southwest University

Arulkumar Rasu

Bharathidasan University

Ponnambalam Venuvanalingam

Bharathidasan University

Zhiqin Zhou (✉ zhouzhiqin@swu.edu.cn)

Southwest University

Research Article

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Abstract

Background

Tangeretin obtained from *Citrus limonum* L. represents the key secondary metabolite amongst other citrus derived polymethoxyflavones eliciting a comprehensive bioactive potential establishing versatility in abating neurodegenerative disorders.

Objective

The present study was aimed to decipher additional targets and interacting mechanisms for tangeretin in elucidating the neuro-inflammatory profiles through computer aided drug designing and deciphering potential molecular mechanisms.

Methods

Pharmacokinetic evaluation was will be performed for ADMET properties comprising Liver Toxicity, Metabolism, blood brain barrier permeability, cardiotoxicity, mitochondrial toxicity, mutagenicity by vNN web server and SWISS-ADME for drug likeliness deriving medicinal properties of tangeretin, comparatively. Polar surface area-based drug assimilatory profiles were catalogued using Molinspiration toolkit for confirming the tangeretin's bioavailability and solubility. Extra-precision flexible molecular docking studies was preceded using Glide XP incorporated in Schrödinger, LLC, New York, NY, 2021 for protein (5-Beta-reductase, PLA2, IRAK4, COX-1, COX-2, 5-LOX, NIK) – ligand (tangeretin) interaction assessment. Phylogenetic analysis of the selected protein targets in docking experiments were assessed for closely relatedness by maximum likelihood iterations using MEGA 10.2.6. Further tangeretin-gene targets-interaction assessment was cross-verified in STITCH and SWISSTARGET prediction data for affirming putative drug targets.

Results

Pharmaokinetic assessment revealed the tangeretin's role in safety, efficacy and druglike properties based on chemi-informatic profiles. Further selected proteins utilized for flexible docking corresponds to 3KK6, 1CX2 (Cyclooxygenases family), 2B03 (Phospholipase A2), 4IDV (NF-KB-inducing kinase), 6O9D (Interleukin-1 receptor-associated kinase domain) and 3BV7 (steroid 5beta-reductase) that were assessed for binding affinity patterns revealed the efficacy in the order of 5-Beta-reductase > PLA2 > IRAK4 > COX-1 > COX-2 > 5-LOX > NIK. Phylogenetic relatedness proved that 3BV7 posed the ancestral node for rest of the selected proteins emphasizing cascade mechanisms in absorption potentiating additional protein-protein interaction and crosstalk. Comparative gene target analysis revealed two putative potential targets namely AKT1 and CYP1A1.

Conclusion

Pharmacokinetic based docking assessment and network pharmacology studies pertaining neuro-inflammatory protection are coerced with confirmation of putative targets aiding the safety, efficacy, and efficiency of tangeretin.

1. Introduction

A plethora of Research has been accounted for the polymethoxylated flavone, tangeretin against various neurodegenerative disorders in establishment of anti-inflammatory and neuroprotection, a comprehensive network remains unraveled. Molecular mechanisms in elucidating the lipopolysaccharide induction and subsequent anti-inflammatory cascades have been adequately reported comprising proteins like matrix metalloproteinases and inflammatory cytokines[1]. With the advent of systems pharmacology approaches, computer aided drug designing perspectives have been advocated for high throughput screening and interaction pathway analysis corresponding drug targets for resultant positive clinical outcomes [2] Neuroprotection mediated by natural products are also attributed for lipopolysaccharide induction, microglial activation and differential regulation regulating the neuro-inflammatory protective signal cascade pathways ranging from NF- κ B pathway to PI3K/Akt pathway[3]. Thus a explicit attention towards traditional Chinese medicine and other related therapeutics were utilized for high-throughput virtual screening for envisaging safety, efficacy of low molecular weight medicinal compounds for broad spectrum bioactivity [4, 5]. The prominent application aspects of tangeretin has been recently proved efficient against Zika fever and Corona viruses through virtual screening for pharmacokinetics and pharmacodynamics revealed more assimilatory safety and less toxicity [6]. Nevertheless network pharmacology and docking alone couldn't affirm the ADMET properties and blood brain permeability of significant phenolic compounds bioactive potentials [7]. The scenario of neuroprotection and anti-inflammatory properties of cheminformatics and systems biology of tangeretin requires abridging the pitfalls rendering ease of commercial applications. Based on the limitations to address the issues for affirming tangeretin's efficacy, safety, drug likeliness, assimilatory patterns and pursuit for putative gene/protein targets were preceded. Hence the present analysis incorporates pharmacokinetic analysis for drug safety profiles, molecular docking with non-steroidal inflammatory drug target proteins and comparative mining for putative gene targets for tangeretin.

2. Materials And Methods

2.1 Preparation of tangeretin

Pubchem database (<http://pubchem.ncbi.nlm.nih.gov/>) was utilized to retrieve the basic structure for tangeretin in SMILES format. OPENBABEL 3.1.1[8] corresponds to the toolbox utilized for file format conversion. The two dimensional structures were obtained in PDB format and converted to three dimensional format employing Arguslab 4.0.1 through clean geometry optimization [9]. Root-mean-square deviation (RMSD) based *in vacuo* optimization and energy minimization for deriving a rough

structure was preceded with hydrogen addition, gasteiger charges for fixing unusual bonding patterns using CHARMM force field and final three dimensional structure was generated in Arguslab[10].

2.2 Pharmacokinetic evaluation for Druggability profiles for tangeretin

Comparative pharmacokinetic evaluation was performed vNN web server [11] and SWISS-ADME [12]. Chemoinformatic assessment in physicochemical properties encompassing the specific descriptors including molecular weight, molecular refractive potentials, polar surface topology for calculating absorption, digestion, metabolism and excretion. The drug likeliness was characterized using molinspiration tool (<https://www.molinspiration.com/cgi-bin/properties>) to derive the bioactivity scores and druggability values of tangeretin. Bioavailability radar in SWISS-ADME was utilized to evaluate the pharmacokinetics and friendliness application of tangeretin in the medicinal chemistry realms. Further lipophilicity values based on $\log P_{o/w}$ estimation, water solubility using linear correlation coefficient R^2 , blood-brain barrier permeability, Human Liver Microsomal Stability, mitochondrial membrane potential (MMP) Mitochondrial Toxicity, Cytotoxicity (HepG2) Cytotoxicity, Drug-Induced Liver Injury (DILI), Cytochrome P450 Inhibition (Drug-Drug Interaction), human ether-à-go-go-related gene (hERG) hERG Blockers, mutagenicity, P-glycoprotein (Pgp) dependent cancer inhibition, maximum recommended therapeutic dose and drug-drug interactions were affirmed.

2.3 Target proteins and protein preparation for molecular dockin

Proteins chosen for the docking studies comprise of the pivotal targets for non-steroidal anti-inflammatory drugs similar to tangeretin. The respective proteins with PDB Id's are 3KK6, 1CX2 (Cyclooxygenases family), 2B03 (Phospholipase A2), 4IDV (NF-KB-inducing kinase), 6O9D (Interleukin-1 receptor-associated kinase domain) and 3BV7 (steroid 5beta-reductase). The three dimensional structures were retrieved from the RCSB PDB database (<https://www.rcsb.org/>). Protein preparation wizard available in the Schrödinger suite (Schrödinger, LLC, New York, NY, 2021) was utilized for determining the protonation of histidine residues, hydrogen bond optimization, and restrained minimization for arriving at the rough optimized geometrical bonds and angles [13]. Analysis of cleft pockets and solvent accessible surface area were calculated using CASTp server (<http://sts.bioe.uic.edu/castp/index.html?1yys>) and GETAREA (<http://curie.utmb.edu/getarea.html>), respectively. Atomic solvent accessible surface area corresponds to area/energy/residue using 1.4 Å⁰ radius of water probe was used. Poisson–Boltzmann method was preceded to compute the electrostatic potentials with 80.0 dielectric constant values for 20 cycles of iteration in the Swiss-PdbViewer, DeepView v4.1[14].

2.4 Phylogenetic analysis of protein targets

The protein targets were assessed for phylogenetic information to decipher the closeness and evolutionary information of the protein drug targets to derive additional information of the proteins

chosen. A phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) version 10.1 program (<https://www.megasoftware.net/>) using the maximum likelihood method[15].

2.5 Docking Studies

Docking studies were carried out using LigPrep, Protein Preparation Wizard and Glide modules integrated in maestro and available in Schrodinger package (Maestro, Schrödinger, LLC, New York, NY, 2015). 3D conformer of the studied Tangeretian ligand is freely available in Pubchem database (ID: 68077). This pdb structure is taken for initial structure for Ligprep module in maestro. Energy minimization of the Tangeretian carried out using OPLS_2005 force field and possible states has been produced by Epik (pH 7.0) and conformational searches performed. Final structure has been used for docking studies. Docking studies of Tangeretian with COX-1 were performed using PDB ID: 3KK6 which have similar A and B chain. Therefore, chain A has been taken for molecular docking studies. Docking studies of Tangeretian with COX-2 were performed using PDB ID: ICX2 which have identical A, B, C and D chains. Therefore, chain A has been chosen for molecular docking studies. Docking studies of Tangeretian with 5-LOX were performed using PDB ID: 3V99 which is composed of different A and B chains. Therefore, both chains A and B has been considered for the docking studies. Docking studies of Tangeretian with PLA2 were performed using PDB ID: 2B03 which is made up of only one chain A. Docking studies of Tangeretian with NIK were performed using PDB ID: 4IDV which have identical four chains A, B, C and D. Therefore, only chain A has been considered for docking study. Docking studies of Tangeretian with IRAK4 were performed using PDB ID: 6O9D which have identical four chains A, B, C and D. Therefore, only chain A has been considered for docking study. PDB ID: 3BV7 has been considered for 5-beta-reductase. 3BV7 have similar A, B chains and chain A has been taken for the further molecular docking studies. During the preparation of above proteins in Protein Preparation Wizard, missing Hydrogen atoms, side chains and loops were added using prime. The highest ranked grids were used to identify the active site of Tangeretian ligand with the above seven proteins. Extra precision(XP) level of accuracy is used in order to generated Glide score and Flexible 10,000 poses of Tangeretian were calculated with each of the above proteins. Top ranked pose of each of the above proteins and binding sites are analyzed using maestro software.

2.6 Comparative mining for putative targets of tangeretin

Previously, the ligand molecule tangeretin was retrieved for potential bioactivity using STITCH database (<http://stitch.embl.de/>). Further comparative mining for detecting putative gene targets based on Gene Ontology and target prediction using SWISSTARGET[16]. Comparative verification among tangeretin-target- interaction pathway deciphering putative gene targets were rigorously examined to derive additional and putative targets.

3. Experimental Procedures Related

Optimization of polymethoxy flavones separation from *Citrus tangerina* Tanaka were optimized in our laboratory employing macroporous resins for adsorption coupled with preparatory HPLC [17]. Earlier,

network pharmacology and the corresponding signal cascade were elucidated for establishing the neuro-protective anti-inflammatory pathways mediated by tangeretin (Sundararajan et al., 2021, communicated). NOS2, PTGS2, NOX4, HMOX1, IL2, ADORA2A, ADORA1, and ADORA3 genes were identified as bioactive gene targets and a cumulative involvement of 14 pathways involved in blood-brain barrier compatibility were affirmed. Moreover, network pharmacology prediction along with transcriptomic analysis revealed insulin receptor (InsR) and insulin-like growth factor-I (IGF-1) receptor as potential additional targets. With this confirmatory experimental background, the present assessment is put forth in establishing additional targets that confirm the anti-inflammatory neuro-protection of tangeretin.

4. Results

4.1 Druggability, drug likeliness and assimilatory profiles

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions are enlisted based on comparative predictions for holistic bioactive potentials emancipated by tangeretin. Table 1 shows the appropriate druggability and drug assimilatory potentials. The canonical SMILES format for tangeretin was retrieved from the Pubchem compound database

(COC1=CC=C(C=C1)C2=CC(=O)C3=C(O2)C(=C(C(=C3OC)OC)OC)OC) and was assessed for their intrinsic bioactivity. Molecular formula for tangeretin is $C_{20}H_{20}O_7$ and the pubchem CID corresponds to 68077.

The protective efficacy of tangeretin is evident from the tabulation. Drug-induced liver injury (DILI), cytotoxicity against hepG2 cell line parameters, mitochondrial toxicity combating matrix metalloproteinases disruption, drug interaction comprising cytochrome p450 inhibition, human ether-à-go-go-related gene (hERG) blocking indicating cardio-protectiveness, P-glycoprotein (Pgp) substrates and inhibitors showing carcinogenicity profiles, human liver microsomal stability for hepatotoxicity, mutagenicity and maximum recommended therapeutic dose were assessed. The results establish the positive benefits of tangeretin through specificity and sensitivity depicting optimal values and applicability for drug likeliness. Figure 2 illustrates the two dimensional structure and three dimensional structure of tangeretin, along with drug likeliness score of 0.29 vitalizing the equality in mode of action compared to the available bioactive drugs. The SWISSADME predictions showing the bioavailability radar for physicochemical region depicting the total polar surface area of $76.36 \text{ \AA}^2$, lipophilicity with consensus $\log P_0/w$ of 3.02. Further the size, polarity, poorly soluble in water, insaturation, flexibility, $\log K_p$ of -6.41 m/s reveal the bioavailability score 0.55 and synthetically accessible values affirm the drug leadlike characteristic of tangeretin (Supplementary table 1). pKa value of -3.04 and blood brain score of 4.25 with zero stereo centers confirms the prospective of drug availability and effectuating benefits rendered by tangeretin (Table 2).

4.2 Target proteins, Phylogeny and intricate docking analysis

The target proteins chosen were appropriately analyzed for protein-ligand interaction assessment owing to the fact that they are primarily involved in assimilation and pose similarity for non-steroidal anti-

inflammatory drug targets. The present study is the first report of the combinatorial pharmacological bioactivity together with elucidation of signal cascades in neuro-protective anti-inflammatory nature of tangeretin. The target proteins utilized in the molecular docking experiments correspond to 3KK6, 1CX2, 2B03, 4IDV, 6O9D and 3BV7. The binding affinity (in kcal/mol) patterns corroborates to the sequential binding ranging with 5-Beta-reductase (-9.017) > PLA2 (-7.010) > IRAK4 (-4.938) > COX-1 (-3.879) > COX-2 (-3.651) > 5-LOX (-3.635) > NIK (-3.080). The target protein 3BV7 nomenclature for 3-oxo-5-beta-steroid 4-dehydroxygenase revealed the molecular evolutionary pattern and statistical significance shows the basis of the protein with regard to molecular phylogeny (Figure 3). These results clearly depict the choice of target proteins and necessary binding patterns in confirming the potential neuro-protection of tangeretin based on molecular docking. Based on the active site residues, cleft region residues and comparative analysis of binding residues show occupancy of solvent accessible surface area with the energy value (81857.60 total area/energy). The interacting residues were shown in the Figure 4. Among which Ser 225 and Lys 273 shows Hydrogen bonding with backbone, TRP 230 interact via Pi-Pi stacking. The interacting residues by and large comprise of serine, threonine and tyrosine residues at the interacting interface showing the involvement of serine-threonine-tyrosine kinase signaling pathway. Hence further comparative proteomics, multi-omics approaches together with systems biology in elucidating the potential targets will shed insights into in vivo assessment and randomized controlled trials. Docked poses and interacting key residues of other proteins are given in Figure 5 and 6 respectively.

4.3 Detection of putative targets based comparative mining

Comparative mining of STITCH database and SWISSTARGET prediction showed gene target analysis of two putative targets namely AKT1 and CYP1A1. Although they have been revealed in the involvement of anti-inflammatory neuroprotection, a rational pathway in the tangeretin's anti-inflammatory neurological protection requires further studies. The molecular docking results also affirm the involvement of AKT1 gene products. Furthermore, the functional roles emancipated by tangeretin are attributed to G-protein coupled receptors binding patterns showing the large-scale receptor-based affinities showing the tangeretin's role in signal cascades for anti-inflammatory properties (Figure 7). The present cheminformatics assessment the involvement of protein kinases involved in activating kinase phosphorylation signaling pathway (Activated protein kinase B). Thus, the putative protein targets identified after rigorous mining for additional candidates established 3BV7 (5-Beta-reductase), AKT1 and C1PYA1 as putative targets for tangeretin's anti-inflammatory neuroprotective efficacy.

5. Discussion

ADMET prediction studies are preferred by scientists worldwide, as they aid in drug discovery showing cost-effectiveness and minimization of *in vivo* experiments [18]. Similar studies in evaluating the beneficial potentials of tangeretin against Zika fever and COVID-19 were proved through cheminformatics and molecular studies [6]. Ample reports show anti-inflammatory mechanisms of tangeretin, however neuroprotection through lipopolysaccharide induction, microglial activation and blood

brain barrier permeability effects have not been reported, authoritatively [15]. The proteins and signaling pathways involved in neuroprotection have been proved through immunoinformatics assessment suggesting immunization against neurodegeneration proteins like TRAF6 and SQSTM1 was hypothesized for positive effects[19]. Although the present preliminary assessment reveals positive and optimal clinical outcomes in the future, appropriate clinical trials will show beneficial prospects. In a similar *in silico* study, Phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway has been proved effective in neuroprotection, antioxidant and anti-inflammatory bioactivities of fucosterol through network pharmacology analysis[20]. An extensive review addressing the assessment of structure-activity relationship in determining the bioactivity of flavonoids for biomedical applications stresses the pharmacokinetics as an essential study [21]. *In silico* studies for docking perspectives in studying the citrus peel extract combined effect on retarding metastasis of breast cancer was addressed to matrix metalloproteinases-9 downregulation [22]. A similar effect was observed in mitigating the mitochondrial toxicity through pharmacokinetic studies of tangeretin in the present *in silico* study. Combinatorial docking for curcumin and tangeretin in binding affinities in the NFkB signaling pathway showed upstream regulation indicating proteosomal cleavage[23]. Similarly the docking of tangeretin showed the molecular dissection for activated protein kinase signaling through serine threonine kinase regulation. Additional research through transcriptomic profiling for assessing upregulation/downregulation of specific genes involved through quantitative studies will have more implications.

Enzyme kinetics, spectroscopy analysis and dynamics simulation studies showed precise structure-activity relationship and effective inhibition against pancreatic lipase[24], showing determinative ADMET profiles of citrus tangeretin. An *in silico* tangeretin estimation for abatement of hepatocellular carcinoma showed the involvement of Cox-2 enzymes [25] depicting the hepatoprotective efficacy similar to the pharmacokinetic results revealed from the present study. Tangeretin targeting putative targets revealed from the assessment shows 3BV7 (5-Beta-reductase), AKT1 and C1PYA1 for anti-inflammatory properties against neurodegenerative diseases. Metastasis was inhibited by PI3K/Akt signaling cascade in breast cancer therapeutics. However, the dissection of interaction between tangeretin and 5-Beta-reductase with AKT1 suggests an analogous mechanism depicting anti-inflammatory neuroprotection. Further hepatoprotectivity for tangeretin enhancing drug assimilatory levels and bioavailability of the small molecule is affirmative along with hindering mitochondrial toxicity with matrix metalloproteinases-9. Moreover, expression profiling for mRNA levels confirms the comparative data mining approach adopted in the present study for proving tangeretin's effectuating medicinal roles by PTGS2 (Supplementary table 1,2).

Inhibition of lipase of tangerine pith at 40.67% and *in silico* validation through molecular docking proved the cost-effectiveness of *Citrus* flavonoids[26]. *Citrus* peel flavonoids were found to have antagonistic activities against collagenase and elastase with skin protection[27]. Similar results were obtained for tangeretin in the physiochemical and pharmacokinetic evaluation indicating the protective efficacies for neuroprotection, consequently. The results of the present study can be compared to the neuroprotective signaling cascades enumerated against Alzheimer's disease and Parkinsonism through a comprehensive review[28]. Cardioprotectiveness of tangeretin was affirmed for the interplay between PTEN/AKT/mTOR pathways *in vivo* [29]. The putative targets obtained from the study will have pivotal significance in the

future for microglial activation strategy and blood brain barrier compatibility. A recent review has pointed out for the significant encapsulation methods for enhancing the prominent application of phenolics, viz. tangeretin for enhanced availability and accessibility for potent bioactivity and protection[30]. Hence the explicit advancements for tangeretin applications with friendly approach without toxicity can foresee immense research prospects. On the contrary, tangeretin and atorvastatin have utilized nanocarriers for targeted delivery for colon cancer treatment [31]. Henceforth the scientific therapeutics and nutraceutical potentials of tangeretin research have a long path of excellence in biomedical applications. Nevertheless the commercialization, patent scenario and largescale randomized controlled trials will shed future applications of tangeretin, significantly.

6. Conclusion

Putative targets were obtained based on pharmacokinetic evaluation of tangeretin. Chemo informatics, molecular docking, and comparative mining for detecting novel gene targets and protein targets was preceded and 3BV7 (5-Beta-reductase), AKT1 and C1PYA1 were derived putative and unique. The anti-inflammatory neuroprotection mechanisms are summarized. However, the future research gaps are addressed for prospective applications.

Declarations

Author Contributions

[#]B. S and A.R. contributed equally to the work. Finally, all authors P.V, Z.Z, X.G and Q.C discussed the results and contributed to the final manuscript.

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Competing Interests

The authors declare no competing interests.

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Supplementary Tables

Supplementary Tables 1 and 2 are not available with this version.

Figures

Figure 1

Figure 1. a) basic chemical structure of tangeretin, b) three dimensional structure of tangeretin, c) Drug likeliness of the ligand, d) Bioavailability radar of the compound.

Figure 2

Figure 2. Interacting protein network partners with tangeretin in the STITCH database.

Figure 3

Figure 3. Molecular phylogeny of the selected proteins belonging to the non-steroidal inflammatory drug targets.

Figure 4

Figure 4. Docked pose of Tangeretin with 5-beta-reductase and key interacting residues.

Figure 5

Figure 5. Docked pose of Tangeretin with selected proteins (a) COX-1 (b) COX-2 (c) 5-LOX (d) PLA2 (e) NIK and (f) IRAK4

Figure 7

Figure 7. Distribution chart depicting the major protein targets as revealed by SWISSTARGET showing pattern variations.