**Ferulic acid as a potential drug for neurological disorder: An I*n silico* approach**

**ABSTRACT:**

Glycogen synthase kinase-3 (GSK-3) has been known to regulate various cellular and metabolic processes, including neuronal plasticity. Dysregulation of the GSK-3β isoform leads to the development of various neurological disorders such as Alzheimer’s, Parkinson’s, and Huntington’s disease. Thus, GSK-3β has gained major attention for therapeutic intervention in various neurological disorders. The present study aimed to explore natural derivatives of therapeutic value as potential inhibitors of GSK-3β for consideration in the treatment of neurological disorders. Using an *in silico* approach through virtual screening, molecular docking, and Molecular Dynamic (MD) simulation, we elucidate ferulic acid's binding interaction and affinity with GSK-3β residues. Our study revealed that ferulic acid (out of 545 phytochemicals from *Mangifera indica*) demonstrated effective inhibitory activity for GSK-3β. Interestingly, MD-simulation results showed that ferulic acid binds with three crucial amino acid residues of GSK-3β for modulating the activity. In addition, ferulic acid passed all Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/Tox) parameters, paving the way for drug design and development targeting neurological disorders. Our findings provide the basis for development of Ferulic acid as a potential GSK-3β inhibitor.

**Keywords:**  Alzheimer’s disease (AD), phytochemicals, bio-informatics, computational biology, ADME/Tox, molecular docking, molecular dynamic simulation.

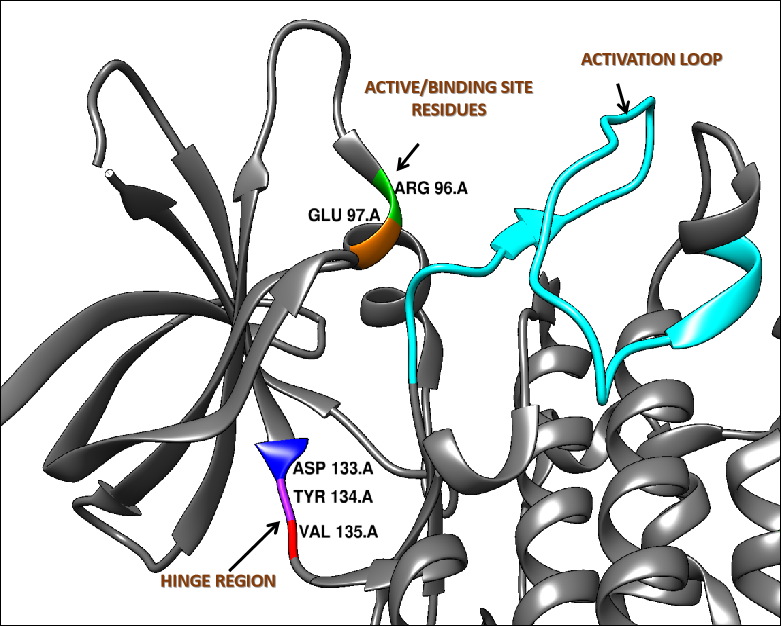
**1. INTRODUCTION**

Glycogen synthase kinase-3 (GSK-3) is a versatile enzyme involved in pathways regulating cellular differentiation, glial cell survival, neuronal morphology, neurogenesis, synaptic plasticity, learning, and memory (1, 2). Dysregulation of GSK-3 leads to the development of neurological disorders such as Alzheimer’s, Parkinson’s, Huntington’s disease, depression, schizophrenia, and amyotrophic lateral sclerosis (3). (Fig. 1summarizes the cellular mechanisms affected by GSK-3β in various neurological disorders)

**G:\GSK\Fig 1.tif**

**Fig. 1:** An overview of GSK-3β affecting various cellular mechanisms of neurons in the cortex and hippocampus brain regions of neurological disorders.

Structurally, GSK-3α and GSK-3β are two isoforms found in all cell types across the animal kingdom. The GSK-3β isoform consists of two lobes, namely the β-strand domain (N-terminal stretching from 25–138 amino acid residues) and an α-helical domain (C-terminal stretching from 139–343 amino acids). Located between these lobes is an interactive zone comprising an ATP-binding site, loop, and hinge region (Asp133-Val135) (4). The activation loop stretches from 200 to 226 amino acids residues. The β-strand domain is composed of seven antiparallel β-pleated sheets. Inside these sheets is an evolutionarily conserved loop of 4-5 short α- helices (spanning from 96 to 102 amino acids residues). In this loop, Arg96 and Glu97 play crucial roles in catalytic activity. Glu97 is located in the active site and forms a salt bridge with Lys85 (5, 6). The binding pocket residues Arg96 and Ala204 are unable to establish hydrogen binding when Glu97 fail to form a salt bridge with Lys85 (summarized in Fig 2). This leads to disruption of the phosphate-binding site, and the substrate-binding groove achieves a wider open conformation (7). We speculate that an interaction between a phytochemical (ligand) and specific residues in the enzyme can lead to GSK-3β inhibition. A GSK-3β inhibitor will be of pharmacological importance for the treatment of Alzheimer’s disease and other neurological disorders (8). Curative/therapeutic capabilities of plants around us provide an opportunity to search for a phytochemical with the potential to target key proteins in the pathways leading to neurological disorders. Such phytochemicals derived from common plants like *Mangifera indica* can be developed as drugs (secondary metabolites) against numerous ailments such as cancer, and neurological, metabolic, immunological, and genetic disorders (9) (10) (11).



**Fig 2:** A 3D model representation of the crucial sites like activation loop, hinge region, and active site occurring in GSK-3β.

Mango/ *M. indica* (Family Anacardiaceae)is an evergreen tree that mostly grows in tropical regions like southeast Asia (including India and Thailand). The hot and humid climateof southeast Asia favours the growth of *Mangifera indica*. The fruit and other parts of *M. indica* have been traditionally used for curative and therapeutic applications (12, 13). In the past two decades, numerous studies have highlighted the antibacterial, antitumour, and antioxidant potentials of Mango fruit peels (14). Whereas, few studies have noted that the water and ethanolic extract derived phytochemicals to confer antioxidant, antidiabetic, antimicrobial and immunomodulatory properties (13). The primary phytochemical Mangiferin derived from the ethanolic extract has demonstrated neuroprotective properties (15, 16). However, beside Mangiferin, there exist variety of phytochemicals whose therapeutic potential remain obscure due to differential extraction properties of these compounds (17, 18)**.** In the recent past computational biology and bioinformatics tools have paved the way for preliminary study and assessment of the therapeutic potential of various phytochemicals using an *in silico* approach. In the present study, using an *in silico* approach, we screened 545 phytocompounds found in *Mangifera indica.* We haveidentified the interaction between phytochemicals and the crucial residues of GSK-3β. Selected phytochemicals those passed the necessary *in silico* ADME/Tox parameters can be considered a potential inhibitor of the GSK-3β enzyme in the *in vitro* studies.

**2. MATERIALS AND METHODS**

**2.1 Phytochemical and ADME/Tox screening:**

Phytochemicals found in M. indica were screened using the Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) [https://cb.imsc.res.in/imppat/home](%20https://cb.imsc.res.in/imppat/home)) and Phytochemical and Drug Target DataBase (PDTDB): [https://pdt.biogem.org/search.php](%20https://pdt.biogem.org/search.php)) databases (19, 20). These phytochemicals were further screened for their drug-likeness and physicochemical properties using the web services ‘SwissADME’ (<http://www.swissadme.ch/>) and admetSAR (<http://lmmd.ecust.edu.cn/admetsar2/>) (21).

**2.3 Target protein selection and structural validation:**

The Research Collaboratory for Structural Bio-Informatics Protein Data Bank (RCSB-PDB) (<https://www.rcsb.org/>) was used to search for an X-ray crystallography structure of GSK-3β (22). The PDB ID 2JLD was selected as the structure had 2.35 Å resolution, 0.227 and 0.190 R-free and R-working values.

**2.4 Protein and Ligand Preparation:**

The GSK-3β structure, hereto referred to as 2JLD, was prepared using the protein preparation wizard suite of Schrodinger Maestro Suite version 2022-1. The default parameters include assigning bond orders, adding missing heavy atoms, assigning protonation states, and optimizing hydrogen bonds. Similarly, the shortlisted ligands were optimized using the ‘LigPrep’ module (23). The parameters, such as structure optimization and the addition of any missing atoms or partial charges using the force field OPLS-2005, were set for the ligands. After protein and ligand preparations, the protein grid box was created central to the active site of 2JLD. The active site of the enzyme was determined using the UNIPROT database version 2023-02 (<https://www.uniprot.org/help/uniprotkb>) (24).

**2.5 Molecular docking:**

The Molecular docking analysis was conducted using the Glide suite of Schrodinger Maestro Suite version 2022-1. Firstly, High-Throughput Virtual Screening (HTVS) was used to increase the hit rate and filter the shortlisted phytochemicals (25). Secondly, these phytochemicals were processed for molecular docking analysis using the Glide SP (standard precision) and XP (extra precision) suites (26) (27). Thirdly, the top five docking interactions were compared with a negative and positive control.

**2.6 Molecular Dynamic Simulation:** The phytochemical demonstrating the highest binding affinity with GSK-3β was subjected to Molecular Dynamic (MD) simulation using the ‘Desmond’ suite. The simulation was conducted using the isothermal-isobaric ensemble at 300 Kelvin and 1.013 bar pressure for 100 nanoseconds. The OPLS-2005 force field and a predefined TIP3P solvent model were utilized for running the MD simulation (28) Santos (29). The results of the MD simulation were analyzed using RMSD (Root Mean Square Deviation), RMSF (Root mean square fluctuation), and ligand-receptor interaction (ligand-atom points of contact over time, ligand-receptor point of contact plot at atomic scale).

**3. RESULTS AND DISCUSSION:**

**3.1. Phytochemical screening from *Mangifera indica:***

Certain comprehensive databases provide chemical structures, structure information, and chemical identifiers of phytochemicals. These databases provide chemical structures (ligands), which can be downloaded and used for *in silico* analysis. Previous studies have used IMPPAT and PDTDB databases to identify and potential inhibitors for enzyme targets like protein kinase C and phosphatidylinositol 4,5-bisphosphate 3-kinase in cancer-based studies (30, 31). In the present study we have utilized the IMPPAT and PDTDB databases, that revealed the presence of 545 phytochemicals in *Mangifera indica*. These phytochemicals were shortlisted based on their BBB (blood-brain barrier) crossing capabilities. Total 241 compounds filtered through on these parameters were subjected to molecular docking with GSK-3β (2JLD).

**3.2. Molecular docking analysis of phytochemicals with GSK-3β:**

The Schrodinger Glide module consists of three sub-modules, viz., HTVS, Glide SP, and Glide XP, designed for refined lead optimization. Few studies have utilized these sub-modules to shortlist inhibitor compounds against Monoamine Oxidase B, Chikungunya protease, and epidermal growth factor receptor (32-34). In the present study we used the sub-module HTVS to shortlist compounds based on their binding affinity with protein residues and drug-likeness properties, resulting in lead optimization. The phytochemicals (241) possessing BBB crossing capabilities were subjected to HTVS analysis. Out of 241 phytochemicals 161 compounds filtered by HTVS analysis were processed for lead optimization process,using the Glide SP and XP docking suites.

This exercise yielded only five phytochemicals having higher binding affinities to GSK-3β (2JLD) (Table 1). Among these phytochemicals, only ferulic acid (Table 1 and Fig. 3) demonstrated the highest binding affinity of −7.22 kcal/mol with 2JLD. A robust binding between a ligand-protein residue is established when there exists a low-energy orientations between them. It is also known that, hydrogen bond formation paired with a very low-energy orientations signify a strong interaction between the ligand and target protein residues. Using this criterion of filtering phytochemicals that have a binding affinity score below −1.00 kcal/mol would yield the best fit phytochemical for further *in silico* evaluation (35, 36).

The detailed molecular docking interactions of Ferulic acid using the 2D and 3D visualization analysis was conducted after confirming that there exist strong the binding affinities between the ligand and the protein (GSK-3β). The 2D and 3D interaction diagrams provided by Schrodinger Glide suite offers a visualization of the type of bonds along with the distance between ligand-protein.

Previous studies have classified the bond distances between a donor and acceptor for inhibition between 2.2 and 4.0 Å. The range between 2.2 and 2.5Å is classified as strong and covalent interactions, 2.5 to 3.5Å for moderate and electrostatic interactions, and 3.2 to 4.0Å is classified as weak and electrostatic interactions (37) (38).

Upon detailed analysis of the dock poses using the 2D interaction diagram **(**Fig. 4a**),** it was observedthat the OH groups of Ferulic acid formed hydrogen bonds with Lys85 and Val135 residues of GSK-3β (2JLD), thus establishing interaction between the Ferulic acid (ligand) and protein (GSK3B). Whereas, the 3D interaction diagram displayed the hydrogen bond distance between Ferulic acid and amino acid residues of GSK-3β (measured using the ‘Measure’ tool of Maestro Suite). The results shown in Fig. 4b display the bond distance between the OH (hydroxyl) group of Ferulic acid with Lys85 to be 2.88Å. While OH and carboxylic group of Ferulic acid, each formed one hydrogen bonds with Val135, having a bond distance of 2.19Å and 2.15Å respectively. As per earlier studies on GSK-3β structure,-, the amino acid residues Lys85 and Val135 play a crucial role in GSK-3β function. Lys85 is vital for salt-bridge formation with Glu97 (an active site residue) (7), whereas Val135 is part of the (hinge region) interactive zone—an important residue in the GSK-3β (4). Therefore, it can be deduced that the formation of a hydrogen bond between Ferulic acid and Lys85 might restrict GSK-3β chain region movements. Similarly, the hydrogen bond formed between Ferulic acid and Val135 may restrict GSK-3β hinge movement. Both these stearic hinderances posed by Ferulic acid to the GSK-3β, can lead to the inhibition of the enzyme activity. The *in silico*, experimental procedures require validation of the results based on the comparison with a positive and negative control (39, 40). Therefore, we compared the Ferulic acid - GSK-3β docking results (interactions and bond distances) of with a positive and negative control.

A known GSK-3β inhibitor, SAR502250 [(S)-2-(2-(4-flurophenyl) morpholine)-1-methyl-[4,4’-bipyrimidin]-6 (1H)-one] was used as a positive control (41). It was observed that SAR502250 interact with GSK-3β with binding energy of −4.40 kcal/mol and formed hydrogen bonds with Val 101 and Asp166 (Fig. 4c) of the enzyme. Both these amino acids are non-crucial residue of GSK-3β. Additionally, the bond distances between SAR502250 and Val101 and Asp166 of the enzyme were 2.32 Å and 4.60 Å (Fig. 4d) respectively. The interaction of SAR502250 with non-crucial GSK-3β residues and its low binding affinity as compared to Ferulic acid indicate the suitability of Ferulic acid over SAR502250 as potent GSK-3β inhibitor.

As negative control, we docked Ferulic acid with hexokinase (a kinase family member involved in glycolysis) (42). The docked results demonstrated a binding energy of −6.3 kcal/mol between Ferulic acid with hexokinase. Ferulic acid formed hydrogen bond with Asp73 with a bond distance of 3.19Å (Fig. 4e and 4f), which again is non crucial residue of GSK-3β. These results, indicate that ferulic acid demonstrate specificity and higher affinity in interaction with GSK3B in comparison to other enzyme/protein. Such interactions are characteristic of established inhibitors. From these results we speculate that Ferulic acid can counteract GSK-3β activity without obstructing function of other cellular enzymes in vivo.. .

The assessment of ligand-protein interaction using molecular docking methods is limited by the absence of entropic and solvation effects occurring in the cellular milieu. This limitation can be overcome by MD simulation methods that provide an atomic spectrum of dynamic interaction between target ligand and protein(43).

**3.3 Molecular dynamic simulation of Ferulic acid GSK-3β:**

The MD simulation provides a detailed visualization of ligand-protein interactions on an atomic scale, considering a few physiological parameters such as pressure, temperature, pH, water, ions, and other molecules in the system. These parameters mimic the cellular environment by placing a solvation layer along with a force field around the target ligand protein. Thus, MD simulation approaches are used to assess the overall stability of ligand-protein complexes (28) (29) (44). We conducted the MD simulation between Ferulic acid and GSK-3β for 100 ns. The acceptable RMSD values for ligand-protein are usually between 0-3.0Å in a MD simulation with stable and normal conditions (45). MD simulation report generated the RMSD (Fig. 5a) values between 1.5 Å and 2.1 Å for GSK-3β and Ferulic acid (as shown in Fig. 5b). The RMSF value obtained were within the allowed range of 0.6 to 1.8 Å, thereby indicating a normal fluctuation in the RMSF range.

The timeline representation of ligand-protein interaction (Fig. 6a top panel)demonstrated total number of specific contacts GSK-3β residues make with OH and carboxylic groups of Ferulic acid over the 100 ns simulation. The total points of contact fluctuated between 2 and 9, with an average of 5, characteristic of the fluid nature between GSK-3β and ferulic acid. The bottom panel of Fig. 6adisplays which GSK-3β amino acid residues made contact with the OH and carboxylic groups of ferulic acid during the trajectory course. Moreover, the residues that have made more than one specific contact with Ferulic acid are represented by a dark orange hue. Our results demonstrate Ferulic acid to have persistent interactions with Val135 and Asp200. The darker orange intensity emphasizes that Val135 had more interactions with Ferulic acid (more than 90 ns) as compared to Asp200. Fig. 6bshows hydrogen bond (green bar) formation between Ferulic acid and GSK-3β residues during the interactions, along with the significant interaction fractions with residues Val135 and Asp200. The interaction fraction value above 1.0 was due to the multiple contacts made by GSK-3β residues made with ferulic acid. The percent interaction diagram demonstrates the interactions occurring more than 30.0% during the 100 ns simulation trajectory, as shown in Fig. 6c.Our findings suggest a constant interaction of 91% with the carboxylic group and 98% with the OH group of Ferulic acid with the crucial GSK-3β residue Val135. Additionally, 76% of the interaction was observed with another crucial GSK-3β residue, Asp200, during the simulation course.

It is known that the hinge motion in an enzyme structure provides flexibility and allows substrate binding during the reaction. In the case of GSK-3β, the hinge region is found between the β-strand domain and α-helical domain; thereby, obstruction in this region might inhibit the enzyme hinge motion (46). While the activation loop in an enzyme restricts substrate access, it controls catalysis (47). MD simulation for 100 ns results demonstrated interactions with Val135 (hinge region residue) and Asp200 (activation loop residue) with the OH and carboxylic groups of Ferulic acid. Such an interaction might inhibit the hinge motion along with preventing substrate access and catalysis of GSK-3β. Additionally, \results display hydrogen bond formation for 90 ns with the crucial GSK-3β residues. Overall, these actions are predicted to inhibit GSK-3β,

In order for a phytochemical or drug to qualify for *in vitro* or *in situ* assessment, certain pharmacokinetic and Drug-likeness properties must be tested. Once qualified these testing using an ADME/Tox assessment, the compounds can be used for *in vitro* analysis (48, 49). Therefore, we subjected Ferulic acid to the standard ADME/Tox analysis.

**3.4. ADME/Tox Analysis of Ferulic Acid:**

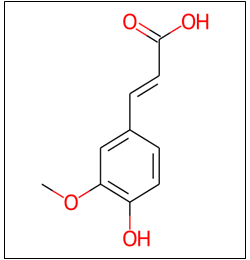
Bioinformatics-based web services aid in the generation of drug-likeness, pharmacokinetics, and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/Tox) capability/profiles of any given compound. Such properties aid in drug discovery, drug development, and preventing drug rejection during *in vivo* studies (50) Daina (21). In the present study, we utilized SwissADME and admetSAR web-services that provide drug-likeness, compound physicochemical and medical chemistry properties of any given compound. Further, these web services were used to explore the drug properties of Ferulic acid. It was found that ferulic acid having molecular weight of 194.19 g/mol showed bioavailability score of 0.85. It is established that, a bioavailability score of ~0.56 is a preferred value if the compound passes the Lipinski's rule of five. While a score below 0.17 bioavailability score signifies that the compounds fails to qualifies physicochemical and oral bioavailability prediction (48, 51, 52). Furthermore, Ferulic acid demonstrated ability pass through Blood-Brain- Barrier and gastrointestinal (GI) tract.

The Drug-likeness characteristics provide the structural, physiochemical and pharmacokinetic properties of a drug for early stages of drug discovery. These pharmacokinetic properties calculate the number of rotatable bonds, Topological polar surface area, molecular weight, number of H-bond donors and acceptors, and octanol-water partition coefficient, and number of aromatic rings of a compound drug. After qualifying these rules, a compound/drug is deemed fit for further *in vivo* orclinical trials (48, 52, 53). We found that, except for the Pfizer 3/75 rule, Ferulic acid passed other drug-likeness properties such as Lipinski’s rule of 5, Ghose rule, Veber rule, Egan rule, and GSK 4/400 rule (Table 2).

The BOILED EGG model graphically represents TPSA and WLOGP (lipophilicity) values, along with BBB or GI,and thepossibility of a compound being a P-glycoprotein (P-gp) substrate. Additionally, in this model, the red dot indicates the compound to be a P-gp non-substrate, while the blue dot indicates the compound to be a P-gp substrate (53-55). The BOILED EGG model of Ferulic acid (Fig. 7a) shows that this compound has a TPSA value of 66.76 Å² and a WLOGP value of 1.39. Additionally, the position of the red dot indicates the compound to be both BBB and GI tract permeable and a P-gp non-substrate. Many therapeutic agents are P-glycoprotein substrates and carry the risk of being ejected from the cells. The *in silico* P-gp inhibition prediction parameter states the possibility of a molecule/drug to be a P-gp substrate or not (50).

The bioavailability radar represents physicochemical and oral bioavailability properties such as flexibility (0< Number of rotatable bonds< 9), instauration (0.25< Fraction Csp3< 1), insolubility (-6< Log S[ESOL]< 0), lipophilicity (-0.7< XLOGP3< +5.0), polarity (20Å2< TPSA< 130Å2), and size (150g/mol< MW< 500 g/mol) of compounds (53, 55, 56). In the case of Ferulic acid (Fig. 7b and Table 3), the number of rotatable bonds was three: instauration of 0.10, LogS [ESOL] value of -2.11, TPSA value of 66.76 Å², and molecular weight of 194.18 g/mol. Therefore, Ferulic acid qualified the parameters of physiochemical and oral bioavailability. The toxicity assessment of Ferulic acid revealed negative probabilities for the Ames mutagenesis test, carcinogenicity, hepatotoxicity, and nephrotoxicity. Simultaneously, it is biodegradable. In addition, Ferulic acid qualified the acute oral toxicity under class IV (non-toxic and non-irritant) (55, 57) (Table 4).

According to the ADME/Tox criterion, an ideal therapeutic drug must be able to cross the gastrointestinal and blood-brain barrier, apart from having a low polar surface area, reduced molecular flexibility, and passing other drug-likeness parameters (48, 52, 55, 58). Our ADME/Tox analysis reveals that ferulic acid is able to pass these criteria. Thereby suggesting ferulic acid as a possible GSK-3β inhibitor.



**Fig. 3:** Ferulic acid (2D chemical structure) has two exposed OH groups that allow hydrogen bond formation with the ‘R’ group of crucial GSK-3β amino acids.

G:\GSK\Fig 3.tif

**Fig. 4: Comparative 2D and 3D Molecular docking results showing interaction along with the bond distances of Ferulic acid, positive control and negative control with GSK-3β: a.** The 2D interaction diagram shows two OH and carboxylic groups of ferulic acid forming hydrogen bonds (violet arrows) with crucial GSK-3β residues Lys85 and Val135. **b.** The bond distance between the OH and carboxylic groups of ferulic acid with crucial GSK-3β residues Lys85 (2.88Å) and Val135 (2.19Å and 2.15Å) signifies the strong binding affinity required for inhibition. **c.** The positive control SAR502250 interacts with two non-crucial GSK-3β residues, Val 101 (-NH group) and Asp166 (-OH group). **d.** While the positive control SAR502250 and GSK-3β residues Val101 and Asp166 demonstrate a bond distance of 2.32Å and 4.60Å, respectively **e.** The negative control, Ferulic acid, interacts with the Asp73 (OH group) residue of hexokinase. These results indicate effective *in silico* GSK-3β inhibition. **f.** The negative control Ferulic acid and hexokinase residue Asp73 have a bond distance of 3.19Å. Altogether, the bond distance of ferulic acid with SAR502250 and hexokinase shows weak interactions with GSK-3β residues. As opposed to these results, our finding is that ferulic acid was able to form strong hydrogen bindings with crucial GSK-3β residues.

G:\GSK\Fig 4.tif

**Fig. 5: RMSD and RMSF graphs denoting the similarity and fluctuations between Ferulic acid-GSK-3β coordinates in MD simulation:** **a.** The RMSD graph represents the quantitative measure of ferulic acid and GSK-3β for 100 ns. This graph also demonstrates a difference of 1.5 Å to 2.1 Å between ferulic acid and GSK-3β **b.** The RMSF plot represents the average deviation of ferulic acid and GSK-3β residues for 100 ns. The plot shows a fluctuation between ferulic acid and GSK-3β between 0.6 and 1.8 Å.

G:\GSK\Fig 5.tif

**Fig. 6: Detailed MD simulation representation of Ferulic acid interactive groups with crucial GSK-3β residues:** Additional MD simulation results further affirm the RMSD and RMSF results. **a.** The top panel of the diagram represents the points of contact for GSK-3β made with ferulic acid. The bottom panel shows residue interactions of GSK-3β with ferulic acid. GSK-3β residues Val135 (dark orange) and Asp200 (orange) bonded for 90 ns with ferulic acid during the 100 ns MD simulation. **b.** The interaction diagram shows Ferulic acid OH and carboxylic groups interaction fractions of above 1.0 with Val 135 and Asp200 (GSK-3β residues), respectively, during the 100 ns simulation. **c.** A detailed schematic diagram of ligand-atom interactions with the GSK-3β residues describes that the two OH and carboxylic groups of ferulic acid formed 91% and 98% of hydrogen bonds with Val135 and 76% with Asp200, respectively, during the 100 ns simulation run.

G:\GSK\Fig 6.tif

**Fig. 7: Pharmacokinetic and drug-likeness profiles of ferulic acid:** The ADME/Tox properties, drug-likeness, and medical chemistry of ferulic acid were checked using SwissADME and AdmeSAR web services before proceeding with molecular docking and MD simulation. **a.** The BOILED EGG model of ferulic acid (red dot) demonstrates that ferulic acid can cross the blood-brain barrier (yellow area) and is a potential non-P-GP substrate. **b.** Similarly, the bioavailability radar of ferulic acid plots drug-likeness properties shows LIPO (lipophilicity), SIZE (molecular weight), POLAR (polarity), INSOLU (insolubility), INSATU (instauration), and FLEX (flexibility). It was observed that ferulic acid abides by all the bioavailability radar parameters, except instauration. Overall, these ADME/Tox properties qualify ferulic acid as a possible GSK-3β inhibitor.

**Table 1:** The top five binding affinity scores of phytochemicals after docking with GSK-3β (PDB ID: 2JLD) demonstrating Ferulic acid to have the highest binding affinity as compared to the other phytochemicals present in *Mangifera indica*.

|  |  |
| --- | --- |
| **Phytochemical** | **Docking score (kcal/mol)** |
| Ferulic acid | −7.22 |
| Benzoic acid | −6.79 |
| Carvacrol | −6.74 |
| Thymol | −5.98 |
| (+)-delta-Cadinene | −5.43 |

**Table 2:** Different parameters ofFerulic acid. ADME Profile and Drug-likeness Properties.

|  |  |
| --- | --- |
| **Parameters of Ferulic acid** | **Score** |
| Bioavailability score | 0.85 |
| Solubility class [ESOL] | Soluble |
| Solubility class [Silicos-IT] | Soluble |
| Blood Blood-brain barrier permeation | Yes |
| Gastrointestinal absorption | High |
| Log Kp (Skin permeation, cm/s) | -6.41 |
| Number of PAINS structural alerts | 0.0 |
| Number of Brenk structural alerts | 1.0 |
| CYP1A2 inhibitor | No |
| CYP2C19 inhibitor | No |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| P-glycoprotein substrate | No |
| Number of Lipinski’s rule of 5 violations | 0 |
| Lipinski’s rule of 5 | Passed |
| Number of Ghose rule violations | 0 |
| Ghose rule | Passed |
| Veber rule | Good |
| Egan rule | Good |
| GSK 4/400 rule | Good |
| Pfizer 3/75 rule | Bad |
| Weighted quantitative estimate of drug-likeness (QEDw) score | 0.72 |
| Molecular weight (g/mol) | 194.19 |

**Table 3:** Bioavailability radar showing lipophilicity (XLOGP3), size (MW: molecular weight), polarity (TPSA), insolubility (LogS [ESOL]), insaturation (Fraction Csp3) and flexibility (Number of rotatable bonds) the respective value range and values occurring in Ferulic acid.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Value range** | **Score** |
| Lipophilicity | -0.7< XLOGP3< +5.0 | 1.51 |
| Size | 150g/mol< MW< 500 g/mol | 194.18 g/mol |
| Polar | 20Å2< TPSA< 130Å2 | 66.76 Å² |
| Insolubility | -6< Log S[ESOL]< 0 | -2.11 |
| Insaturation | 0.25< Fraction Csp3< 1 | 0.10 |
| Flexibility | 0< Number of rotatable bonds< 9 | 3 |

**Table 4:** A Few Toxicity properties such as Ames mutagenesis, acute oral toxicity, biodegradation, carcinogenicity, hepatotoxicity and nephrotoxicity of Ferulic acid.

|  |  |
| --- | --- |
| **Property** | **Ferulic acid** |
| Ames mutagenesis | – |
| Acute oral Toxicity (c) | Class IV |
| Biodegradation | – |
| Carcinogenicity (binary) | – |
| Insaturation | – |
| Flexibility | – |

**CONCLUSION**

The progression of neurological disorders is the most prevalent non-communicable disease, with the highest burden among all diseases. Several studies convincingly proved that GSK-3β has emerged as potential target for the treatment of neurological disorders. Present *in silico* study revealed that Ferulic acid, a phytochemical from *M. indica* has numerous therapeutic potentials to be developed as inhibitor drug against GSK-3β. The bioinformatics- based virtual screening and molecular docking methods revealed that, Ferulic acid demonstrated strong specific interactions with GSK-3β characteristic of inhibitory potential. Ferulic acid could form hydrogen bond with crucial GSK-3β residues Lys85 and Val135. Further, the MD simulation displayed a strong association between Ferulic acid with GSK-3β residues Val135 and Asp200 for more than 90 and 70 ns simulation time, respectively. Ferulic acid also qualified all the ADME/Tox parameters of Drug-likeness, pharmacokinetics, bioavailability, and toxicity predictions. These ADME/Tox renders Ferulic acid as an optimum choice as therapeutic against GSK-3β in neurological disorders.

The MD simulation, ADME/Tox profile, and binding affinity values predicted that Ferulic acid can be a choice of drug over other compounds. A possible explanation for the preference of Ferulic acid over SAR502250 might be due to certain pharmacokinetic properties. For decades, GSK-3 inhibitors such as AZD1080, LY2090314, lithium salt, SB-216763, and tideglusib have been chemically synthesized and used for treatment various neurological disorders. These drugs demonstrate toxicity, lethal side effects, and safety issues during *in vivo* and clinical trials (59). However, poor pharmacokinetics and safety issues related to synthetic compounds have been the leading causes of drug rejection in clinical trials (60). These issues could be overcome by using phytochemicals, owing to their diverse and favorable chemical scaffolds, beneficial ADME/Tox profiles; along with high degree of binding affinity towards target proteins. The phytochemicals are naturally optimized toward their cellular targets and have proven broad safety profiles and efficacy over synthetic compounds (61) (62) (63) (64).

The results of present study provide compelling evidence that, the interaction between the phytochemical Ferulic acid and crucial GSK-3β residues can be inhibitory to the enzymatic activity of GSK-3β. This study paved the way for harnessing the potential of the natural derivative Ferulic acid for further *in vitro* and *in vivo* experimental validation and exploration as a drug candidate for therapeutic intervention in neurological disorders.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

We hereby declare that, No generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

**References:**

1. Hagen T, Di Daniel E, Culbert AA, Reith AD. Expression and characterization of GSK-3 mutants and their effect on beta-catenin phosphorylation in intact cells. J Biol Chem. 2002;277(26):23330-5.

2. Horgusluoglu E, Nudelman K, Nho K, Saykin AJJAJoMGPBNG. Adult neurogenesis and neurodegenerative diseases: a systems biology perspective. 2017;174(1):93-112.

3. Eldar-Finkelman H, Martinez A. GSK-3 Inhibitors: Preclinical and Clinical Focus on CNS. Front Mol Neurosci. 2011;4:32.

4. Emmerich TD, Hayes JM. In Silico-Motivated Discovery of Novel Potent Glycogen Synthase-3 Inhibitors: 1-(Alkyl/arylamino)-3H-naphtho[1,2,3-de]quinoline-2,7-dione Identified as a Scaffold for Kinase Inhibitor Development. Pharmaceuticals (Basel). 2023;16(5).

5. Balasubramaniam M, Mainali N, Bowroju SK, Atluri P, Penthala NR, Ayyadevera S, et al. Structural modeling of GSK3beta implicates the inactive (DFG-out) conformation as the target bound by TDZD analogs. Sci Rep. 2020;10(1):18326.

6. ter Haar E, Coll JT, Austen DA, Hsiao H-M, Swenson L, Jain JJNsb. Structure of GSK3β reveals a primed phosphorylation mechanism. 2001;8(7):593-6.

7. Sun H, Jiang YJ, Yu QS, Luo CC, Zou JW. Effect of mutation K85R on GSK-3beta: Molecular dynamics simulation. Biochem Biophys Res Commun. 2008;377(3):962-5.

8. Eskandarzadeh M, Kordestani-Moghadam P, Pourmand S, Khalili Fard J, Almassian B, Gharaghani S. Inhibition of GSK\_3beta by Iridoid Glycosides of Snowberry (Symphoricarpos albus) Effective in the Treatment of Alzheimer's Disease Using Computational Drug Design Methods. Front Chem. 2021;9:709932.

9. Vaishnav P, Demain AL. Unexpected applications of secondary metabolites. Biotechnology Advances. 2011;29(2):223-9.

10. Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines. 2015;2(3):251-86.

11. Shukla S, Habbu P, Kulkarni V, Jagadish K, Pandey A, Sutariya V. Endophytic microbes: a novel source for biologically/pharmacologically active secondary metabolites. Asian J Pharmacol Toxicol. 2014;2(3):1-6.

12. Yap KM, Sekar M, Seow LJ, Gan SH, Bonam SR, Mat Rani NNI, et al. Mangifera indica (Mango): A Promising Medicinal Plant for Breast Cancer Therapy and Understanding Its Potential Mechanisms of Action. Breast Cancer (Dove Med Press). 2021;13:471-503.

13. Kumar M, Saurabh V, Tomar M, Hasan M, Changan S, Sasi M, et al. Mango (Mangifera indica L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. Antioxidants (Basel). 2021;10(2).

14. Jahurul MH, Zaidul IS, Ghafoor K, Al-Juhaimi FY, Nyam KL, Norulaini NA, et al. Mango (Mangifera indica L.) by-products and their valuable components: a review. Food Chem. 2015;183:173-80.

15. Feng ST, Wang ZZ, Yuan YH, Sun HM, Chen NH, Zhang Y. Mangiferin: A multipotent natural product preventing neurodegeneration in Alzheimer's and Parkinson's disease models. Pharmacol Res. 2019;146:104336.

16. Temviriyanukul P, Kittibunchakul S, Trisonthi P, Kunkeaw T, Inthachat W, Siriwan D, et al. Mangifera indica 'Namdokmai' Prevents Neuronal Cells from Amyloid Peptide Toxicity and Inhibits BACE-1 Activities in a Drosophila Model of Alzheimer's Amyloidosis. Pharmaceuticals (Basel). 2022;15(5).

17. Phan MAT, Paterson J, Bucknall M, Arcot J. Interactions between phytochemicals from fruits and vegetables: Effects on bioactivities and bioavailability. Critical reviews in food science nutrition. 2018;58(8):1310-29.

18. Rallabandi HR, Mekapogu M, Natesan K, Saindane M, Dhupal M, Swamy MK, et al. Computational methods used in phytocompound-based drug discovery. Plant-derived Bioactives: Chemistry

Mode of Action. 2020:549-73.

19. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, et al. IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. Sci Rep. 2018;8(1):4329.

20. Rajagopal B. Pdtdb an integrative structural database and prediction server for plant metabolites and therapeutic drug targets.

21. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717.

22. Rose PW, Bi C, Bluhm WF, Christie CH, Dimitropoulos D, Dutta S, et al. The RCSB Protein Data Bank: new resources for research and education. Nucleic Acids Res. 2013;41(Database issue):D475-82.

23. Iwaloye O, Elekofehinti OO, Oluwarotimi EA, Kikiowo BI, Fadipe TM. Insight into glycogen synthase kinase-3beta inhibitory activity of phyto-constituents from Melissa officinalis: in silico studies. In Silico Pharmacol. 2020;8(1):2.

24. Khare N, Maheshwari SK, Rizvi SMD, Albadrani HM, Alsagaby SA, Alturaiki W, et al. Homology Modelling, Molecular Docking and Molecular Dynamics Simulation Studies of CALMH1 against Secondary Metabolites of Bauhinia variegata to Treat Alzheimer's Disease. Brain Sci. 2022;12(6).

25. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. 2004;47(7):1739-49.

26. Mishra V, Pathak CJIjobm. Human Toll-Like Receptor 4 (hTLR4): Structural and functional dynamics in cancer. 2019;122:425-51.

27. Singh A, Mishra A. Leucoefdin a potential inhibitor against SARS CoV-2 Mpro. Journal of Biomolecular Structure and Dynamics. 2021;39(12):4427-32.

28. Hollingsworth SA, Dror RO. Molecular Dynamics Simulation for All. Neuron. 2018;99(6):1129-43.

29. Santos LHS, Ferreira RS, Caffarena ER. Integrating Molecular Docking and Molecular Dynamics Simulations. Methods Mol Biol. 2019;2053:13-34.

30. Alshehri SA, Wahab S, Almoyad MAA. In silico identification of potential protein kinase C alpha inhibitors from phytochemicals from IMPPAT database for anticancer therapeutics: a virtual screening approach. Journal of Biomolecular Structure Dynamics. 2024;42(18):9463-74.

31. Mohammad T, Hussain A, Alajmi MF, Hasan S, Yadav DK, Hassan MI. Identifying potential inhibitors of phosphatidylinositol 4, 5-bisphosphate 3-kinase: Molecular dynamic insights into the interaction and inhibitory mechanism. Chemical Physics Impact. 2024;8:100458.

32. Mateev E, Georgieva M, Zlatkov A. Improved Molecular Docking of MAO-B Inhibitors with Glide. Biointerface Res App Chem. 2022;13:159.

33. Singh Jadav S, Nayan Sinha B, Pastorino B, De Lamballerie X, Hilgenfeld R, Jayaprakash V. Identification of pyrazole derivative as an antiviral agent against Chikungunya through HTVS. Letters in Drug Design Discovery. 2015;12(4):292-301.

34. Abdelmalek D, Smaoui F, Frikha F, ben Marzoug R, Msalbi D, Souissi A, et al. Computational identification of new TKI as potential noncovalent reversible EGFRL858R/T790M inhibitors: VHTS, molecular docking, DFT study and molecular dynamic simulation. Journal of Biomolecular Structure Dynamics. 2024;42(9):4870-87.

35. Naik SR, Bharadwaj P, Dingelstad N, Kalyaanamoorthy S, Mandal SC, Ganesan A, et al. Structure-based virtual screening, molecular dynamics and binding affinity calculations of some potential phytocompounds against SARS-CoV-2. Journal of Biomolecular Structure Dynamics. 2022;40(15):6921-38.

36. Vora J, Patel S, Athar M, Sinha S, Chhabria MT, Jha PC, et al. Pharmacophore modeling, molecular docking and molecular dynamics simulation for screening and identifying anti-dengue phytocompounds. J Biomol Struct Dyn. 2020;38(6):1726-40.

37. Panigrahi SK, Desiraju GR. Strong and weak hydrogen bonds in the protein-ligand interface. Proteins. 2007;67(1):128-41.

38. Huang BJIoLBS, Area P-PI. Identification of pockets on protein surface to predict protein–ligand binding sites. 2013:25-39.

39. Parvez MK, Rehman MT, Alam P, Al-Dosari MS, Alqasoumi SI, Alajmi MF. Plant-derived antiviral drugs as novel hepatitis B virus inhibitors: Cell culture and molecular docking study. Saudi Pharmaceutical Journal. 2019;27(3):389-400.

40. Ghalloo BA, Khan K-u-R, Ahmad S, Aati HY, Al-Qahtani JH, Ali B, et al. Phytochemical profiling, in vitro biological activities, and in silico molecular docking studies of Dracaena reflexa. Molecules. 2022;27(3):913.

41. Griebel G, Stemmelin J, Lopez-Grancha M, Boulay D, Boquet G, Slowinski F, et al. The selective GSK3 inhibitor, SAR502250, displays neuroprotective activity and attenuates behavioral impairments in models of neuropsychiatric symptoms of Alzheimer's disease in rodents. Sci Rep. 2019;9(1):18045.

42. Zheng M, Wu C, Yang K, Yang Y, Liu Y, Gao S, et al. Novel selective hexokinase 2 inhibitor Benitrobenrazide blocks cancer cells growth by targeting glycolysis. 2021;164:105367.

43. Gioia D, Bertazzo M, Recanatini M, Masetti M, Cavalli AJM. Dynamic docking: a paradigm shift in computational drug discovery. 2017;22(11):2029.

44. Singh S, Singh VKJFips, function,, dynamics. Molecular dynamics simulation: methods and application. 2020:213-38.

45. Sukanya S, Choudhary BS, Mehta P, Filipek S, Malik R. Structure-based virtual screening, biological assessment, and MD simulation studies of novel CNS compatible GSK-3β inhibitors as potential Alzheimer’s disease therapeutics. 2022.

46. Arfeen M, Bhagat S, Patel R, Prasad S, Roy I, Chakraborti AK, et al. Design, synthesis and biological evaluation of 5-benzylidene-2-iminothiazolidin-4-ones as selective GSK-3beta inhibitors. Eur J Med Chem. 2016;121:727-36.

47. Adams JAJB. Activation loop phosphorylation and catalysis in protein kinases: is there functional evidence for the autoinhibitor model? 2003;42(3):601-7.

48. Awadelkareem AM, Al-Shammari E, Elkhalifa AEO, Adnan M, Siddiqui AJ, Snoussi M, et al. Phytochemical and in silico ADME/Tox analysis of Eruca sativa extract with antioxidant, antibacterial and anticancer potential against Caco-2 and HCT-116 colorectal carcinoma cell lines. Molecules. 2022;27(4):1409.

49. Batool A, Parveen S, Shafiq N, Rashid M, Salamatullah AM, Ibenmoussa S, et al. Computational study of ADME-Tox prediction of selected phytochemicals from Punica granatum peels. Open Chemistry. 2024;22(1):20230188.

50. van de Waterbeemd H, Gifford E. ADMET in silico modelling: towards prediction paradise? Nat Rev Drug Discov. 2003;2(3):192-204.

51. Martin YC. A Bioavailability Score. J Med Chem. 2005;48:3164-70.

52. Supandi S, Wulandari MS, Samsul E, Azminah A, Purwoko RY, Herman H, et al. Dipeptidyl peptidase IV inhibition of phytocompounds from Artocarpus champeden (Lour.) Stokes: In silico: molecular docking study and ADME-Tox prediction approach. Journal of Advanced Pharmaceutical Technology Research. 2022;13(3):207-15.

53. Wekesa EN, Kimani NM, Kituyi SN, Omosa LK, Santos CB. Therapeutic potential of the genus Zanthoxylum phytochemicals: A theoretical ADME/Tox analysis. South African Journal of Botany. 2023;162:129-41.

54. Daina A, Zoete V. A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. ChemMedChem. 2016;11(11):1117-21.

55. Abdurrahman S, Ruslin R, Hasanah AN, Mustarichie R. Molecular docking studies and ADME-Tox prediction of phytocompounds from Merremia peltata as a potential anti-alopecia treatment. Journal of Advanced Pharmaceutical Technology

Research. 2021;12(2):132-9.

56. Ritchie TJ, Ertl P, Lewis R. The graphical representation of ADME-related molecule properties for medicinal chemists. Drug Discov Today. 2011;16(1-2):65-72.

57. Cronin MT, Madden J, Enoch S, Roberts D. Chemical toxicity prediction: category formation and read-across: Royal Society of Chemistry; 2013.

58. Ahsan MJ, Samy JG, Khalilullah H, Nomani MS, Saraswat P, Gaur R, et al. Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. Bioorg Med Chem Lett. 2011;21(24):7246-50.

59. Arciniegas Ruiz SM, Eldar-Finkelman H. Glycogen Synthase Kinase-3 Inhibitors: Preclinical and Clinical Focus on CNS-A Decade Onward. Front Mol Neurosci. 2021;14:792364.

60. Priyadarshi K, Shirsath K, Waghela NB, Sharma A, Kumar A, Pathak CJJoBS, et al. Surface modified PAMAM dendrimers with gallic acid inhibit, cell proliferation, cell migration and inflammatory response to augment apoptotic cell death in human colon carcinoma cells. 2021;39(18):6853-69.

61. Bhatnagar M. Novel leads from herbal drugs for neurodegenerative diseases. Herbal drugs: ethnomedicine to modern medicine. 2009:221-38.

62. Ahmad S, Ullah F, Sadiq A, Ayaz M, Imran M, Ali I, et al. Chemical composition, antioxidant and anticholinesterase potentials of essential oil of Rumex hastatus D. Don collected from the North West of Pakistan. BMC Complement Altern Med. 2016;16:29.

63. Mir NT, Saleem U, Anwar F, Ahmad B, Ullah I, Hira S, et al. Lawsonia Inermis Markedly Improves Cognitive Functions in Animal Models and Modulate Oxidative Stress Markers in the Brain. Medicina (Kaunas). 2019;55(5).

64. Ayaz M, Ullah F, Sadiq A, Kim MO, Ali T. Editorial: Natural Products-Based Drugs: Potential Therapeutics Against Alzheimer's Disease and Other Neurological Disorders. Front Pharmacol. 2019;10:1417.