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Molecular Docking, Drug-Likeness and Toxicity Prediction to Determine the Role of the Taxifolin on Neurological Diseases

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Abstract

Through an in-silico approach, the effects of taxifolin on tau, alpha 2 macroglobulin (A2M) and alpha 1 anti-chymotrypsin (ACT), proteins are investigated in relation to neurological disorders. In order to facilitate protein interaction, the ligand taxifolin (extracted from the PubChem Database) and protein receptor molecules (extracted from the PDB) are prepared, converted to PDBQT format, and uploaded in an auto dock. The effects of taxifolin on tau protein, ACT, and A2M are still being studied, but the preliminary findings are promising. These interactions suggest that taxifolin may have a complex role in controlling neuroinflammation, proteostasis, and neurodegeneration in neurological illnesses due to their substantial binding affinity for tau, ACT, and A2M protein. Taxifolin shows promise as a therapy for neurological disorders by targeting tau protein, ACT, and A2M. Lipinski's Rule states that Taxifolin administered orally should not violate more than one condition. Taxifolin examined was in category IV, which is under the dosage of 300 < LD50 = 2000 mg/kg. A significant root mean square value was 0.000, with a docking score of -7.2 for alpha 1 antichymotrypsin and taxifolin. Interactions in 2D and 3D include conventional hydrogen bonds, carbon-hydrogen bonds, and unfavourable donor-donor interactions. The chosen root mean square value was associated with a significant docking score of -10.2 for alpha2 macroglobulin and taxifolin. The docking score for alpha 2 macroglobulin in a two-dimensional structure, emphasising pi-donor bonds, unfavourable donor-donor interactions, and conventional carbon-hydrogen connections. According to these findings, taxifolin has a significant affinity for alpha 2 macroglobulin. Tau had a high root mean square value and a docking score of -7.5 with tau protein. Tau's 2D and 3D structures contain pi-alkyl contacts, pi-stacking interactions, unfavourable donor-donor interactions, and carbon-hydrogen bonds. Its ability to raise A2M activity, decrease ACT expression, and stop tau protein aggregation suggests a variety of potential neuroprotective benefits.

Keywords: *ACT, A2M, Neurological Disorders, Tau, Taxifolin.*

Introduction

Plant cell vacuoles contain water-soluble glycosides, or flavonoids, which are phenolic compounds and secondary plant metabolites [1]. In addition to being constitutive agents, flavonoids are also present in plant tissues where they are produced in reaction to microbial infection. These are the most important plant pigments for several uses, including colouring flowers, turning them yellow, and colouring petals to attract pollinating animals [2]. Its antimicrobial and antioxidant properties are widely recognised. Conifers like *Pinus roxburghii*, *Larix sibirica*, and *Cedrus deodara* are common places to find it. Numerous investigations have demonstrated

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the broad spectrum of pharmacological effects exhibited by flavonoids, which include neuroprotective, anti-inflammatory, antioxidant, and anti-Alzheimer's disease characteristics [3]. The degree of polymerization and hydroxylation structural class, various substitutions and conjugations, and metal chelation activity of flavonoids are the main causes of these actions [4]. In addition to being referred to as Tax (2,3-dihydroquercetin or 3,5,7,3′,4′-pentahydroxy-flavanone), the flavonoid taxifolin is frequently found in onions, grapes, olive oil and citrus fruits [5]. Tax is a frequently occurring bioactive ingredient in foods and herbs, exhibiting a wide range of pharmacological and biochemical properties, including anti-inflammatory, anti-tumor, hepatoprotective, anti-diabetic, neuroprotective and cardioprotective benefits. It also aids in the prevention of AD [5]. In vitro studies revealed that taxifolin prevented the 42-residue amyloid-β protein (amyloid-β1–42) from aggregating, which is crucial for the onset of AD [6,7]. This shows that taxifolin might help treat or prevent AD. However, taxifolin is believed to be insufficient to prevent intracerebral amyloid-β aggregation and has a limited ability to traverse the blood-brain barrier (BBB) [8]. The aberrant build-up of tau protein, which results in neurofibrillary tangles and neuronal dysfunction, is a defining Alzheimer's disease (AD) and other tauopathies. It has been shown that taxifolin can prevent tau protein aggregation and facilitate its removal, which may slow the course of the disease and slow cognitive deterioration. Moreover, taxifolin has demonstrated interactions with ACT and A2M, two essential proteins implicated in the control of inflammation and neurodegeneration. In Alzheimer's disease, the inflammatory biomarker ACT is increased, which exacerbates neuroinflammation and neuronal destruction. ACT is classified as a serine protease inhibitor (serpin) within the protein family. It is particularly renowned for its association with Alzheimer's disease, where it can potentially contribute to the formation and buildup of beta-amyloid plaques, which are another hallmark of the disorder. In AD patients, there is increased concentrations of ACT have been observed in the brain [9,10], cerebrospinal fluid (CSF) and serum. Elevated concentrations of ACT in the bloodstream are linked to cognitive decline in elderly individuals. This indicates that ACT has the potential to be used as a biomarker for the early identification of illness. Research conducted using transgenic mouse models of Alzheimer's disease (AD) has demonstrated the involvement of ACT in the development of AD. The results indicate that the expression of a human ACT gene promotes the formation of amyloid plaque and leads to cognitive decline [11].

Excess calcium, excitotoxicity, and mitochondrial dysfunction are the main causes of the many neurodegenerative illnesses [3] that emerge; these factors all play a part in oxidative stress-mediated neurodegeneration [12]. Disorders that are primarily dependent on the tau protein for their aetiology, such as Alzheimer's disease and other illnesses known as tauopathies, are particularly noteworthy. In healthy neurons, tau works to maintain the stability of microtubules, which are vital components of the cell's structural framework. However due to peculiar changes in these illnesses, tau becomes hyperphosphorylated and aggregates form neurofibrillary tangles, an indication of pathogenic anomalies [13].

The integrity of microtubules is impacted by these tangles, which impedes axonal transport, malfunctions neurons, and eventually leads to neurodegeneration. Neuropathology explains that Alzheimer's disease (AD) is characterised by the formation of neurofibrillary lesions inside neurons, which are made up of a protein called tau that is connected with microtubules. Additionally, there is a significant presence of neuritic plaques outside neurons, which are composed of the β-amyloid peptide [14,15]. An important component of the innate immune

system, A2M has been connected to neuronal damage indicators in preclinical AD's cerebrospinal fluid (CSF) and to the likelihood of incident AD in factors that predict cognitive impairment in healthy individuals. The levels of A2M in the blood show a close correlation with the concentrations of tau and phosphorylated tau, which are markers of neuronal damage in the cerebrospinal fluid. Additionally, higher baseline levels of A2M in the serum are associated with a threefold increased likelihood of developing clinical indications of Alzheimer's disease in men [16].

Proteases that aid in the cleavage of tau proteins are among the proteases that can have their activity altered by the protease inhibitor A2M. It has been demonstrated that taxifolin increases A2M expression and activity, which may reduce the pathogenic consequences of tau protein and lessen its proteolytic cleavage [16]. In order to evaluate the impact of taxifolin on neurological illnesses, this study uses an in-silico method to examine how taxifolin affects neurodegenerative proteins like tau, alpha 1 antichymotrypsin, and alpha 2 macroglobulin.

Materials and Methods

Drug-likeness Prediction

The drug-likeness of pharmaceuticals relates to their eligibility for ingestion, which can be determined using Lipinski's Rule of Five. The substance is projected to be incorporated or infused if the calculated logP (ClogP) value is higher than 5.37, the molecular mass (MW) is larger than 500 g/mol, there are over 10 acceptors, and there are over five H-bond donors. Taxifolin can be chosen based on their drug score. Bioactive compounds that have greater drug assessment scores have been considered better drug prospects. The Swiss ADME predictor was used for screening bioactive compounds in this investigation. This reveals details regarding the unique properties of bioactive compounds, including the number of hydrogen acceptors, rotatable bonds and hydrogen donors. The substances were screened using Lipinski's Rule of Five, and it was found that they could proceed to the molecular docking process without any violations.

Oral Toxicity Prediction

taxifolin toxicity was predicted by utilising the PRO TOX-II online tool (https://tox.charite.de/protox3/). This site calculated the LD50 of taxifolin via input files in Canonical Smiles format.

Protein Target Preparation

The PDB format was utilised to obtain the protein structures of tau (tiqp), alpha-2-macroglobulin (1bv8), and alpha-1-antichymotrypsin (1qmn) from the Protein Data Bank (PDB). Autodoc software was used to process the structures so that additional analysis could be performed. Hydrogen atoms, which were absent in the protein structures, were introduced subsequent to the removal of water molecules and hydrogen atoms. In order to enhance the distribution of electric charge inside the structures, Kollman Charges were incorporated into the proteins using the Autodoc application. The protein structures were prepared for the subsequent round of molecular docking investigations thanks to this procedure.

Preparation of the Ligand

The taxifolin (439533) ligand was acquired and stored in the Structure-Data File (SDF) format. The ligand underwent ligand synthesis techniques utilising the PubChem database. Energy reduction techniques were employed to optimise the ligand's structure and decrease its energy level, making it ready for subsequent molecular docking investigations.

Molecular Docking

Molecular docking studies [17] were conducted with the support of the Discovery Studio initiative. The protein structures (ACT, A2M, and Tau) were used in the docking simulations with the ligand Taxifolin. The

docking programme utilised algorithms to forecast the binding orientations and affinities of the ligand within the binding sites of the target proteins [18]. The docking calculations took into account many aspects such as shape complementarity, electrostatic potentials, and protein-ligand interactions in order to determine probable binding positions. The docking programme produced potential binding positions and arranged them according to their binding energy or scores. These scores provide an indication of the anticipated intensity of the interaction between the protein target and the ligand. The docking results were evaluated and analysed to determine probable binding processes and interactions [19]. It was necessary to comprehend the results of the molecular docking studies in order to determine the likely interactions and binding affinities of Taxifolin with the target proteins [20]. This comprehension also illuminates potential methods of action by providing insights into the molecular mechanisms underlying Taxifolin's impact on the chosen protein targets. In summary, to generate the protein target, modifications to the protein structures were required, including the introduction of hydrogen atoms and Kollman Charges, as well as the elimination of water molecules. Energy-efficient methods were employed in the ligand synthesis process through the utilisation of PubChem. The Discovery Studio programme was employed to conduct molecular docking tests, which forecasted the binding affinities and mechanisms of Taxifolin to the target proteins. This thorough approach allowed for the exploration of possible correlations and yielded valuable new knowledge about the molecular mechanisms that drive the action.

Results

Predicting Drug-likeness

The Swiss ADME website is utilised for predicting the physiochemical properties of Taxifolin, as found in Table 1. Lipinski's Rule of Five was utilized ligand filter and screen, leading to the finding of Taxifolin, a bioactive molecule that remained unchanged. Lipinski's Rule states that a Taxifolin administered orally should not violate greater than one condition.

Table 1. Lipinski's Rule for Taxifolin Produced by Using the Swiss ADME Server

S. No	Compound Name	Lipinski's Rule of Five					
		iLogP < 5	**Molecular Weight <500**	**Hydrogen Acceptor < 10**	**Hydrogen Donor < 5**	**Drug-likeness Lipinski's rule follows**	**Violation**
1	Taxifolin	1.30	304.25	7	5	Yes	0

Toxicity Analysis

Toxicity prediction of a chemical is an important step in the creation of new medications. In silico toxicity, assessment is a quicker and more affordable method than in vivo toxicity screening on animals. It can significantly reduce the number of animals needed for experimental studies. Attempts are now being made to study toxic effects, mainly through the use of in-silico models. There are several online programs accessible that can calculate the acute toxic effects, median lethal dose, probability of neurotoxicity or cardiotoxicity, and other relevant variables of substances. The ProTox tool, which predicts the LD50 in rodents, was also used. Chemical compounds are classified into six classes by LD50 values (Table 2).

1.Category I: LD50 = 5 mg/kg.
2. Category II: 5 < LD50 = 50 mg/kg.
3. Category III: 50 < LD50 = 300 mg/kg.
4. Category IV: 300 < LD50 = 2000 mg/kg.
5. Category V: 2000 < LD50 = 5000 mg/kg; and
6. Category VI: LD50 > 5000 mg/kg.

Taxifolin examined was in category IV.

Table 2. ProTox Web Server's Toxicity Prediction for Taxifolin

Compound Name	LD_{50} (mg/kg)	Toxicity Class		Hepatotoxicity	Neurotoxicity	Cardiotoxicity	Cytotoxicity
Taxifolin	2000	4		Inactive	Inactive	Inactive	Inactive

Molecular Docking

Table 3 displays the root mean square values and the log value indicating affinity for alpha 1 anti-chymotrypsin with Taxifolin from the docking analysis. Although the affinity value is more than -4.5, the RMSD is 0.000. A significant root mean square value was also chosen, in addition to a docking score of -7.2.

Table 3. Log table Displaying the Affintiy and Root Mean Square Deviation (RMSD) Value of Alpha 1 Antichymotrypsin and Taxifolin

Mode	Affinity (Kcal / mol)	Dist from rmsd l.b	Best Mode rmsd u. b
1	-7.2	0.000	0.000
2	-7.1	16.101	19.147
3	-7.1	18.366	21.012
4	-7.1	32.942	35.133
5	-6.9	34.878	36.266
6	-6.8	30.712	33.940
7	-6.7	1.566	6.921
8	-6.7	31.584	34.559
9	-6.7	17.643	19.841

Table 4 in our study shows the docking study results, along with the root mean square values of alpha 2 macroglobulin with Taxifolin and the log value indicating affinity. The affinity value is more than -4.5 and the RMSD value is 0.000. The selected root mean square value was accompanied with a significant docking score of -10.2.

Table 4. Log table Displaying the Affintiy and Root Mean Square Deviation (RMSD) Value of Alpha 2 Macroglobulin with Taxifolin

Mode	Affinity (Kcal / mol)	Dist from rmsd l.b	Best Mode rmsd u. b
1	-10.2	0.000	0.000
2	-10.0	2.297	3.409
3	-9.9	1.786	3.313
4	-9.8	1.627	6.424
5	-9.7	1.290	1.812
6	-9.4	2.306	3.669

7	-9.3	2.922	5.476
8	-9.0	4.132	6.271
9	-8.8	33.395	35.563

The docking investigation's findings are displayed in Table 5, along with the tau protein's root mean square values with taxifolin and an affinity log value. The affinity value is more than -4.5 and the RMSD value is 0.000. Tau shown a strong root mean square value together with a docking score of -7.5.

Table 5. Log table Displaying the Affintiy and Root Mean Square Deviation (RMSD) Value of Tau Protein with Taxifolin

Mode	Affinity (Kcal / mol)	Dist from rmsd l.b	Best Mode rmsd u. b
1	-7.5	0.000	0.000
2	-7.1	2.770	4.063
3	-7.1	4.182	8.228
4	-7.0	18.862	23.300
5	-6.9	5.905	7.903
6	-6.8	4.394	6.733
7	-6.8	3.183	6.030
8	-6.7	3.115	6.294
9	-6.7	21.689	24.039

The docking score and visual representation of the 2D and 3D structures of Alpha 1 antichymotrypsin are displayed in Figures 1 and 2, respectively. The two-dimensional structure contains interactions such as normal hydrogen bonds, carbon-hydrogen bonds, and unfavourable donor-donor interactions. This indicates that there is a binding affinity between taxifolin and AACT, which leads to alternating expression of AACT and neurodegenerative disorders.

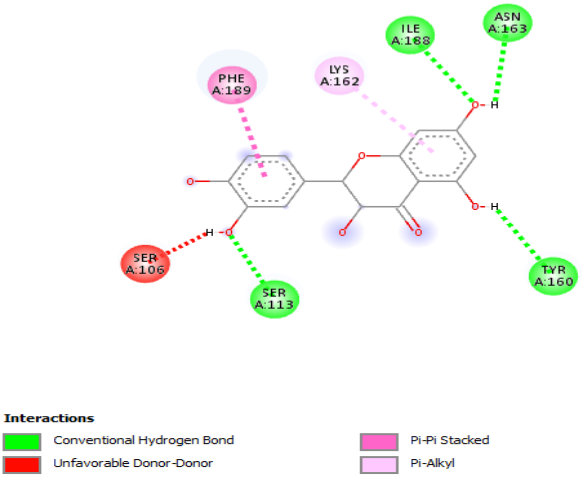

Figure 1. Shows Alpha 1 anti-chymotrypsin Interacts in 2D Structure with Taxifolin

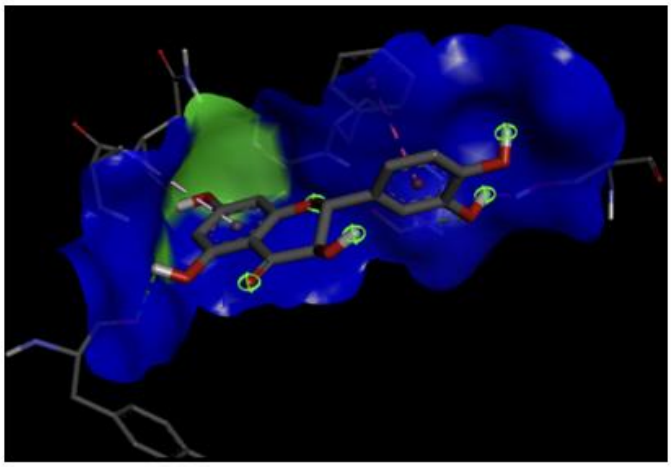

Figure 2. Alpha1 Antichymotrypsin Interacts in 3D Structure with Taxifolin

The docking score visualisation is presented for both 2D and 3D picture structures, as seen in Figures 3 and 4. These figures depict the docking score for alpha 2 macroglobulin in a 2D structure, highlighting pi-donor bonds, unfavourable donor-donor contacts, and standard carbon-hydrogen connections. According to these results, taxifolin has a strong affinity for alpha 2 macroglobulin and can therefore be effective in treating neurological conditions.

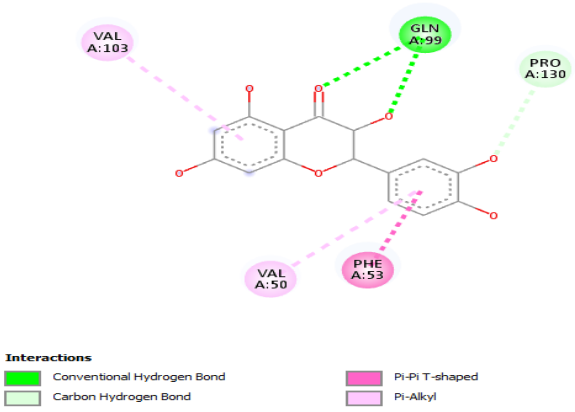

Figure 3. Shows the Interaction between Alpha 2 Macroglobulin and Taxifolin in a 2 D Structure

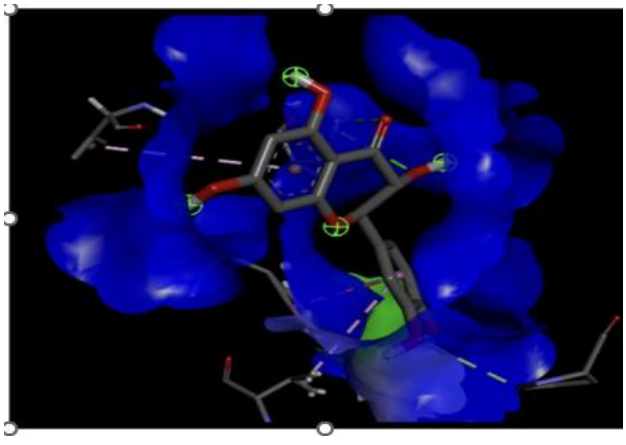

Figure 4. Alpha 2 Macroglobulin Interacts in3D Structure with Taxifolin

The docking score is graphically depicted by the 2D and 3D images shown in Figures 5 and 6. The two-dimensional structure of tau, which includes pi-alkyl contacts, pi-stacking interactions, unfavourable donor-donor interactions, and carbon-hydrogen bonds, is depicted in Figure 5. Consequently, the impact of taxifolin is mediated by its binding to tau protein, which prevents the aggregation of neurological disorders.

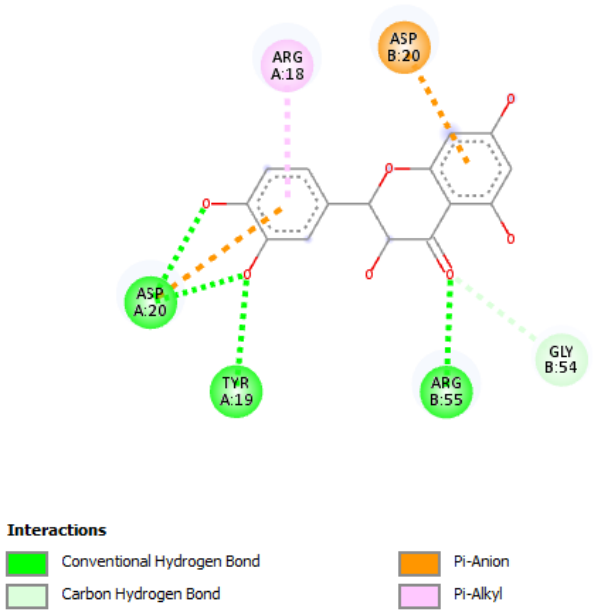

Figure 5. Shows the Interaction between Tau and Taxifolin in a 2D Framework.

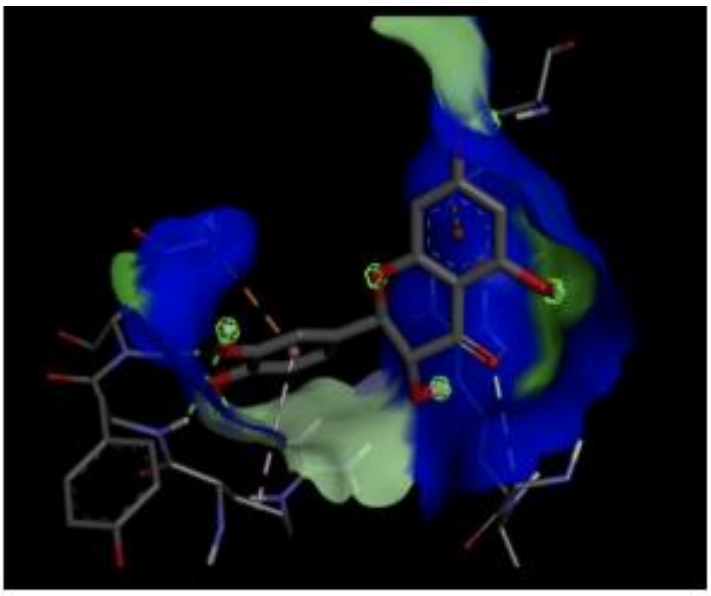

Figure 6. Tau Protein Interacts in 3D Structure with Taxifolin

The high binding properties of the proteins (tau protein, alpha 2 macroglobulin, and alpha 1 anti chymotrypsin) with the taxifolin have been represented in table 6.

Table 6. Comparison of Molecular Docking Results of Taxifolin on Tau, ACT, A2M Family Members

Result analysis	Visualization software	Protein	Ligand	Docking score	Amino acid residue
Auto dock 1.5.7	Discovery software	Alpha-1- antichy motrypsi n (1qmn)	Taxifolin CID: 439533	-7.2	Conventional Hydrogen bond: SER A:113, ILE A:188, ASN A:163, TYR A:160 Unfavourable donor-donor: SER A:106 Pi-pi stacked: PHE A:189 Pi-Alkyl: LYS A:162
		Alpha-2-macroglobulin (1bv8)		-10.2	Conventional Hydrogen Bond: GLN A:99 Carbon Hydrogen Bond: PRO A:130 Pi-Pi T-shaped: PHE A: 53 Pi-Alkyl: VAL A: 50, VL A:103
		Tau (5iqp)		-7.5	Conventional Hydrogen Bond: ASP A:20, TYR A:19, ARGBB:55 Carbon Hydrogen Bond: GLY B:54 Pi-Anion: ASP B:20 Pi-Alkyl: ARG A:18

Discussion

Dihydroquercetin, or taxifolin, is a flavonoid that can be obtained from a variety of plant sources. In structural biology, docking studies are a computer technique used to study the interactions between a tiny chemical, such as taxifolin, and a target protein or receptor. Many ridges and grooves are present in the protein structure, according to a recent study that used docking techniques to examine the interaction between TAX and haemoglobin. The finding that TAX attaches to the cavity encircled by the α-1 and α-2 sub-units confirms that hydrophobic interactions and hydrogen bonding play a major role in the binding process [21,22].

AACT controls the NF-κB signalling pathway, which results in the release of cytokines linked to disorders of the nervous system, including TNF-α and IL-6. AACT binds to IL-6 genes to cause inflammation and apoptosis, which in turn causes neurodegenerative diseases, according to a study that employed STRING to examine the proteins that interacted with AACT [23].

A2M is known to bind and remove amyloid-beta (Aβ) peptides, which collect and form plaques in the brains of AD patients. A2M Amyloid-beta (Aβ) peptides are known to assemble and form plaques in the brains of AD patients. A2M is known to bind and remove these peptides. Aβ accumulation and plaque development can result from A2M dysfunction or genetic variations that interfere with the clearance process. dysfunction or genetic variants may disrupt the clearance pathway, leading to Aβ buildup and plaque formation.

According to research findings, patients with AD had increased levels of A2M in their cerebral fluid and plasma, which may be an indication that the protein is being elevated in reaction to the illness. According to experimental models, A2M can change inflammation and neuronal survival, two factors that are crucial for the advancement of neurodegenerative diseases.

When tau protein builds up inappropriately in neurodegenerative circumstances, it creates neurofibrillary tangles, which change function and the structure of neurons and lead to their death. This is the first step towards the onset and progression of neurological illnesses. Post-translational changes that may impact tau protein hydrophilicity, stability, and spatial conformation are associated with tau protein aggregation in neurodegenerative diseases, which in turn promotes the formation of neurofibrillary tangles and tau protein aggregation [24]. Therefore, changes in tau protein may prevent the disease from aggregating.

Taxifolin binds to these neurodegenerative proteins and changes the impact of the neurological illnesses caused by these proteins.

Conclusion

The results of this study show that taxifolin protects against neurological illnesses and modifies neurological proteins such as tau, alpha 2 macroglobulin and alpha 1 antichymotrypsin (as shown in fig. 7). For this material to regulate these three genes and function as a therapeutic agent, we will need to figure out which neurological illnesses can be investigated both in vivo (using animal models) and in vitro (using neural cell lines).

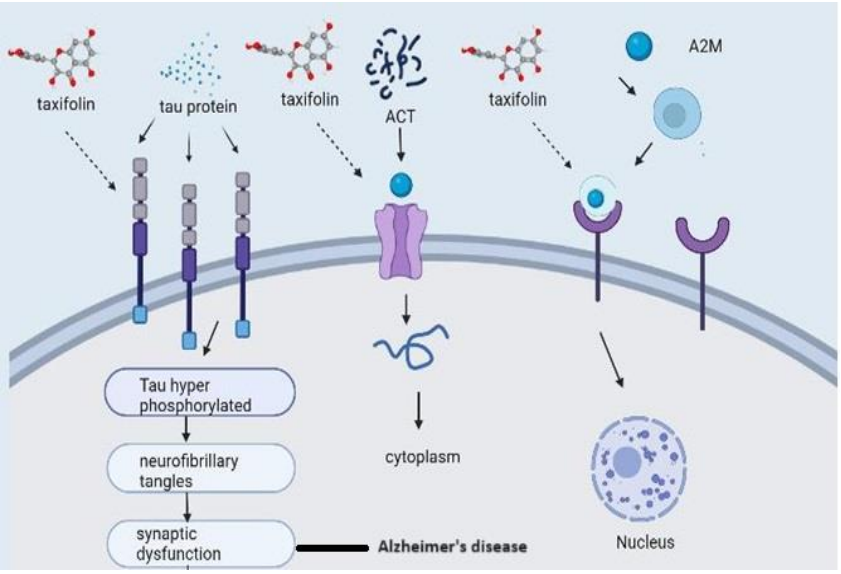

Figure 7. Represents the Interaction of Taxifolin with Alpha 1 Antichymotrypsin, Alpha 2 Macroglobulin, Tau Protein and Genes Alteration Protects from Neurodegenerative Disorders

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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