Inhibition of p66Shc by Camellia sinensis Flavanols: A Potential Strategy Against Oxidative Stress in Alzheimer's Disease

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ABSTRACT

Reactive oxygen species (ROS) play essential roles in cellular processes, including immune defense and cell signaling. However, overproduction of the p66Shc protein leads to increased ROS levels and oxidative stress, greatly contributing to the progression of Alzheimer's disease (AD). This study investigated the inhibitory potential effects of flavanols from the *Camellia sinensis* plant on p66Shc to reduce ROS generation. These flavanols examined included catechin, epicatechin gallate (ECG), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epigallocatechin (EGC), epicatechin (EC), and gallocatechin (GC). A homology model of p66Shc was constructed, and molecular docking simulations were performed to explore the flavanols and p66Shc interactions. One-way analysis of variance testing showed significant differences in binding affinities, and GCG had the strongest interaction (−7.6 kcal/mol) via van der Waals interactions. ECG and EGCG followed (−6.9 kcal/mol) and formed additional pi–anion and pi–alkyl interactions. Catechin had a binding score of −6.4 kcal/mol, while the remaining flavanols had a score of −6.2 kcal/mol with varied interactions. These findings suggested that GCG, ECG, and EGCG could be explored further for their potential role in mitigating oxidative stress in AD.

Keywords: p66Shc, green tea flavanols, molecular docking, Alzheimer's disease, reactive oxygen species (ROS), *Camellia sinensis*

INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder characterized by the accumulation of amyloid-beta (Aß) plaques and tau tangles in the brain, leading to progressive declines in a person's memory and overall cognitive functioning. AD does not directly cause a person's death, but its onset can serve as an avenue for fatal conditions to afflict an individual. As AD worsens, its neuronal damage can spread to parts of the brain responsible for basic bodily functions such as mobility or communication, making a person weaker and more susceptible to accidents (Kumar et al., 2024). In 2020, estimates showed that over 50 million people worldwide were diagnosed with AD, with the cases projected to double every five years (Brejiyeh & Karaman, 2020). While no cure currently exists for AD, the onset of symptoms and their overall progression can be mitigated. Notably, reducing excess reactive oxygen species (ROS) in the brain may lower the chances of oxidative stress (OS), a key component in intensifying AD pathogenesis (Bhatt et al., 2021).

The p66Shc protein is a major ROS producer in mammalian vertebrates, and it is known to oversee both cell proliferation and apoptosis. However, previous research has also given prominence to the detrimental effects of overexpression of p66Shc on Aß toxicity in AD by increasing ROS production and reducing ROS-scavenging enzymes (Di Lisa et al., 2017; Kumar, 2019; Lone, 2023). In particular, phosphorylation of serine 36 (S36) in p66Shc's collagen homology 2 domain (CH2) is more likely in the presence of Aß. This causes the inhibition of the transcription of Forkhead (FOXO) proteins that play vital roles in producing ROS-scavenging enzymes (Lone et al., 2018).

Previously, flavanols derived from *Camellia sinensis* (*C. sinensis*), commonly known as green tea, have been found to inhibit ROS-producing enzymes like lipoxygenase, CYP2C9, and NAD(P)H, highlighting their use as effective and potent natural antioxidants (Rajak et al., 2023). *C. sinensis* is widely cultivated across South Asia, East Asia, Africa, and the Middle East (Tariq et al., 2010). Among the flavanols found in *C. sinensis* are catechin, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin (GC), and gallocatechin gallate (GCG), which have demonstrated neuroprotective and cardioprotective effects, highlighting its potential in therapeutic strategies (Gupta et al., 2007; Park et al., 2021). Our study intended to explore the interactions between *C. sinensis* flavanols and p66Shc and analyze the potential use of green tea as a natural intervention to mitigate the harmful effects of p66Shc with elevated levels.

This study explored the therapeutic potential of *C. sinensis* flavanols in mitigating the p66Shc protein through molecular docking, a computational technique that can predict how small molecules interact and bind with target proteins on an atomic level (Eberhardt et al., 2021; Trott & Olson, 2010). Now widely used in in silico studies, this technique simulates the binding process between a ligand and protein receptor, providing an estimated binding affinity that helps identify which ligands are most compatible to inhibit the target protein (Agu et al., 2023; Meng et al., 2011). In the context of AD, where p66Shc overexpression contributes to OS and worsens Aß toxicity (Kumar, 2019; Lone et al., 2018), molecular docking provides an approach to identify natural inhibitors that may regulate ROS production and neuronal damage. Specifically, it enabled the evaluation of *C. sinensis* flavanols by generating a visual simulation of their binding interaction with the p66Shc protein, allowing for the assessment of binding affinities and interaction patterns to predict how each flavanol, namely, ECG, EGCG, GCG, EGC, EC, GC, and catechin, may engage with the protein's active or regulatory sites. Through molecular docking, the study was able to efficiently identify those with the strongest inhibitory potential, offering structural

insights into their application as natural antioxidants for AD prevention and management (Gupta et al., 2007; Park et al., 2021; Rajak et al., 2023).

MATERIALS AND METHODS

Homology Modeling of p66Shc

Since the structure of the p66Shc protein was not readily available in the Protein Data Bank (PDB), a 3D model of the protein was created through homology modeling. To date, there has been no report of a previously developed homology model of p66Shc, making this the first attempt to characterize its structure. The protein sequence of p66Shc was obtained from the NCBI GenBank database (accession code: AAB49972.1) in FASTA format and used as the input for homology modeling. Homology modeling was performed using the SWISS-MODEL server (Schwede et al., 2003). The p66Shc protein sequence was uploaded into the web service to generate a model that contains sufficient query sequence coverage and sequence identity. Afterward, the most accurate structure was chosen based on the Global Model Quality Estimation (GMQE) values. As GMQE values range from 0 to 1, the higher values reflect greater confidence in the predicted molecular structure. Finally, the generated structure was saved in PDB format, prepared using MGLTools, and converted to PDBQT for molecular docking analysis.

Preparation of Ligands and Protein for Docking

3D structures of catechin (CID: 9064), EC (CID: 72276), ECG (CID: 107905), EGC (CID: 72277), EGCG (CID: 65064), GC (CID: 65084), and GCG (CID: 199472) were retrieved from PubChem in 3D conformer SDF format. These files were then converted into PDB format using the CACTUS Online SMILES Translator.

The PDB file of the 3D structure of p66Shc obtained from SWISS-MODEL was added to MGLTools for further preparation. Water molecules were removed, and polar hydrogens and Kollman charges were added to ensure better ligand bonding in the docking process. The prepared version of the model was then saved in PDBQT format. Ligands were also individually prepared and saved in the same format.

Statistical Postdocking Analysis

The binding energies obtained through the docking process were then analyzed using a one-way analysis of variance (ANOVA) test. Specifically, it was used to analyze the difference in binding energy values generated across the seven flavanol–protein interactions to determine whether there were significant differences between the means of the molecular docking scores under each flavanol and protein interaction. This test set a null hypothesis (H_0) equal to no significant difference in the effect of binding affinities across the seven flavanols, and an alternative hypothesis (H_1) was set equal to the presence of a significant difference. The study then used the *f*-distribution table, a table specialized in ANOVA testing, to prove the set hypothesis. A p-value of <0.05 was significantly different, and n was set to 9, representing the nine docking scores generated for each flavanol in AutoDock Vina.

After the one-way ANOVA test depicted significant differences between the means of the flavanols obtained in the molecular docking process, a post hoc analysis was conducted. Specifically, the Tukey's Honestly Significant Difference (HSD) test was utilized to compare all possible pairs of the sample means and determine which pairs are statistically significant. This ensured that the likelihood of a Type I error or a false positive remains within the desired significance level. Additionally, this allowed for a more comprehensive evaluation of pairwise differences to identify which flavanols

demonstrated significant results on the p66Shc protein.

The absolute difference between the group means was calculated and compared to the HSD value for each pairwise comparison. If the mean difference exceeded the HSD value, the null hypothesis for that pairwise comparison was rejected and consequently considered a statistically significant difference.

Postdocking Visualizations

A visualization of the bonds between the p66Shc protein and the seven different ligands was constructed using PyMOL. This was done by dragging the saved output file from AutoDock Vina and the protein PDBQT file into the program. The appearance of the protein was changed into the "surface" setting, while the ligands were changed into the "licorice" setting. Colors were randomly added to the ligands to make them easier to distinguish. The Draw function from the "Draw/Ray" feature was then used to save the visualization on screen without lowering the image quality.

2D diagrams showcasing the various types of bonds between the ligands and the amino acids of p66Shc were made using BIOVIA Discovery Studio Visualizer. First, the PDBQT file of the protein was inputted into the software. Then, the output ligand file created from AutoDock Vina was opened in a separate tab in the software. The model showcasing the best binding energy was copied and pasted onto the tab containing the protein. Afterward, bonds were identified and visualized using the "Show 2D Diagram" function. Lastly, the constructed diagram was saved in PNG format.

RESULTS AND DISCUSSION

p66Shc Model Selection and Molecular Docking

The FASTA sequence of p66Shc was submitted to SWISS-MODEL, where the model with the highest GMQE score of 0.63 was selected for further analysis. The final structural model is shown in Figure 1. Molecular docking simulations, as seen in Table 1, generated nine docking scores for each flavanol, revealing varying binding affinities between p66Shc and the seven C. sinensis flavanols. Among them, GCG exhibited the strongest interaction at −7.6 kcal/mol followed by ECG and EGCG displayed binding affinities equal to −6.9 kcal/mol. Catechin displayed a binding affinity of −6.4 kcal/mol, while EC, EGC, and GC each displayed affinities of −6.2 kcal/mol. It is worth noting that AutoDock Vina runs nine simulations of the ligand binding to the receptor, which produces similar but different numerical values for the binding affinity. The values of these tests were used in the one-way ANOVA test, but Table 1 and the succeeding PyMOL and BIOVIA visualizations simply reflected the best docking score produced.

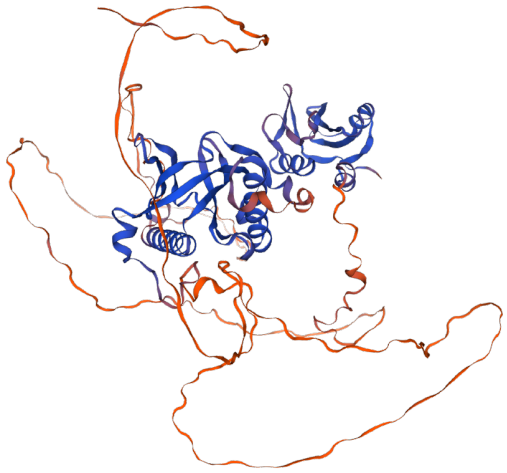

Figure 1. Homology model of p66Shc protein generated in SWISS-MODEL with a GMQE score of 0.63. Blue regions correspond to well-modeled structures, while orange regions indicate lower reliability.

Table 1. Binding Affinities of Green Tea Flavanols Against p66Shc Based on Molecular Docking Simulations.

Green Tea Flavanol	Binding Affinity (kcal/mol)
Catechin	−6.4
Epicatechin (EC)	−6.2
Epicatechin gallate (ECG)	−6.9
Epigallocatechin (EGC)	−6.2
Epigallocatechin gallate (EGCG)	−6.9
Gallocatechin (GC)	−6.2
Gallocatechin gallate (GCG)	−7.6

Visualizations and 2D Diagrams of Bonding Interactions

For the visualizations in PyMOL, Figure 2 shows the best binding position of the flavanols within the p66Shc protein. To facilitate better visualization, the binding site was enlarged to provide improved clarity and ensure a more detailed analysis.

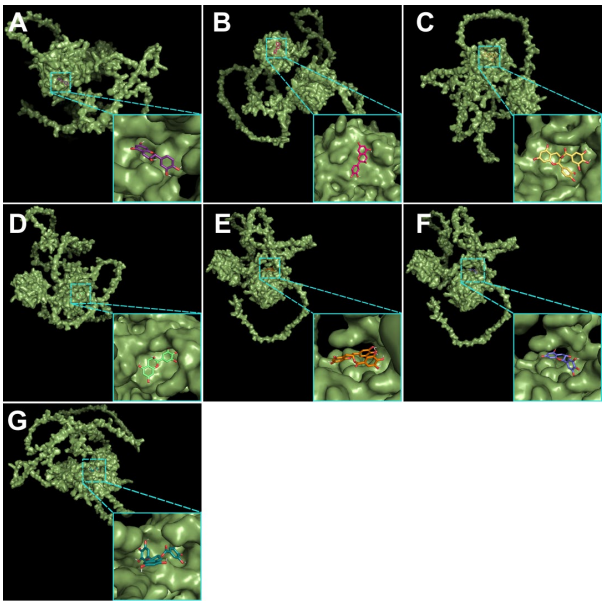

Figure 2. Enhanced ligand-pose visualizations of each flavanol–p66Shc interaction. The protein is shown in green; flavanols are depicted in distinct colors inside blue boxes. Panels depict the optimal binding conformation of (**A**) catechin, (**B**) epicatechin, (**C**) epicatechin gallate, (**D**) epigallocatechin, (**E**) epigallocatechin gallate, (**F**) gallocatechin, and (**G**) gallocatechin gallate when interacting with p66shc.

Afterwards, flavanol–protein interactions were then analyzed through BIOVIA Discovery Studio Visualizer, with the resulting 2D diagrams presented in Figure 3. These diagrams depict various molecular bonds present in each flavanol and p66Shc interaction. These are color coded in each diagram and included van Der Waals forces (green), pi–alkyl and alkyl (light pink), pi–cation and pi–anion (orange), carbon hydrogen (light green), and pi–pi stacked bonds (dark pink). Figure 3 also depicts p66Shc binding to catechin (Figure 3A), EC (Figure 3B), ECG (Figure 3C), EGC (Figure 3D), EGCG (Figure 3E), GC (Figure 3F), and GCG (Figure 3G).

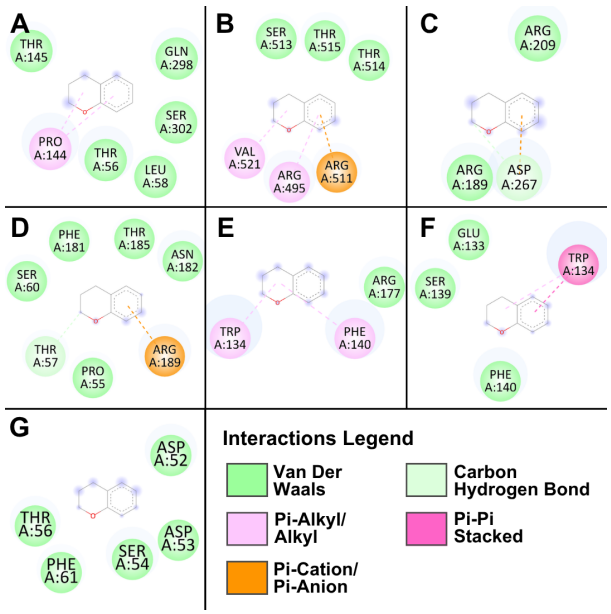

Figure 3. Generated 2D diagrams of each interaction between each flavanol and p66Shc. Blue halos indicate solvent-accessible surface area. (**A**) Catechin interacting with THR145, PRO144, THR56, LEU58, SER302, and GLN298. (**B**) Epicatechin interacting with VAL521, SER513, ARG495, THR515, ARG511, and THR514. (**C**) Epicatechin gallate interacting with ARG189, ASP267, and ARG209. (**D**) Epigallocatechin interacting with SER60, THR57, PHE181, PRO55, THR185, ARG189, and ASN182. (**E**) Epigallocatechin gallate interacting with TRP134, PHE140, and ARG177. (**F**) Gallocatechin interacting with SER139, GLU133, PHE140, and TRP134. (**G**) Gallocatechin gallate interacting with THR56, PHE61, SER54, ASP52, and ASP53.

One-Way ANOVA Test Results of Binding Affinities

A one-way ANOVA test was conducted to determine the statistical significance of these binding affinities. The sum of squares analysis indicated that the between-group variability, measured by the between sum of squares (BSS = 12.639), was significantly greater than the within-group variability, measured by the within sum of squares (WSS = 3.667), with computed mean square values of 2.105 and 0.065 for BSS and WSS, respectively. The resulting F-value of 32.149 ($p < 0.0001$) exceeded the f-critical threshold of 2.266, rejecting the null hypothesis and concluding that substantial differences were present between each *C. sinensis* flavanol tested.

Post Hoc Tukey's HSD Test Comparing *C. sinensis* Flavanols

The Tukey's HSD test, with an HSD critical value of 0.3689, identified significant pairwise comparisons that are shown in Table 2. In particular, these pairs had an absolute mean difference greater than the HSD value, and these pairs were catechin and EGCG (0.4), catechin and GCG (1.17), EC and ECG (0.61), EC and EGCG (0.73), EC and GCG (1.5), ECG and GCG (0.89), EGC and EGCG (0.43), EGC and GCG (1.2), EGCG and GC (0.41), EGCG and GCG (0.77), and GC and GCG (1.18), which were all found to be significant results that indicated meaningful differences among each group. Notably, these results showed that every pair that contained GCG yielded significant results, all of which were far greater than the HSD threshold, reaching values greater than 1.

Table 2. Significant Pairwise Comparisons of Green Tea Flavanols Based on Tukey's HSD Test Showing Absolute Mean Differences

Significant Pairs	Absolute Mean Difference
Catechin vs EGCG	0.4
Catechin vs GCG	1.16
EC vs ECG	0.61
EC vs EGCG	0.73
EC vs GCG	1.5
ECG vs GCG	0.89
EGC vs EGCG	0.43
EGC vs GCG	1.2
EGCG vs GC	0.41
EGCG vs GCG	0.77
GC vs GCG	1.18

Note. HSD = Honestly Significant Difference; EGCG = epigallocatechin gallate; GCG = gallocatechin gallate; EC = epicatechin; ECG = epicatechin gallate; EGC = epigallocatechin; GC = gallocatechin.

Analysis of Catechin and p66Shc Interaction

Catechin, with a docking score of −6.4 kcal/mol, ranked third in docking scores compared to the other flavanols in this study. The docking with this flavanol featured fewer diverse interactions compared to other stronger ligands. The flavanol's van der Waals forces with THR145, THR56, and LEU58, alongside a pi–alkyl bond with PRO144, indicated moderate binding stability. The hydrophobic pi–alkyl bond helped to improve the docking score due to its innate strength. However, catechin's lack of covalent or pi–anion interactions, which were seen in stronger ligands like ECG in the present study, may explain its lower binding affinity. Despite this, catechin has been proven to have beneficial effects in treating the symptoms of AD. A study by Okello and Mather (2020) observed that catechin had minor inhibitory effects on the acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes, leading to restored acetylcholine (ACh) synaptic levels. This is important as ACh levels are directly linked to the cholinergic neuron function and are responsible for cognitive functions such as memory. ACh levels are raised by inhibiting AChE and BuChE, and cognitive functions associated with cholinergic neurons are strengthened. This study recommends more studies on p66Shc and catechin interactions to further understand their effects on living systems and the mechanisms of their interactions.

Analysis of EC and p66Shc Interaction

With a binding affinity of −6.2 kcal/mol, EC ranked alongside GC and EGC in docking scores among the analyzed flavanols. EC formed van der Waals forces with amino acids SER453, THR514, and THR515, in addition to hydrophobic pi–alkyl bonds with VAL521 and ARG495. The pi–cation interaction with ARG511 provided additional stability, yet the absence of stronger covalent interactions, like those seen in ECG, likely accounts for EC's lower binding affinity. Regardless, EC has still been shown to play key roles in uplifting spatial memory conditions and regulating Aß

toxicity. A study by Diaz et al. (2019) found that rats with high amounts of Aß treated with EC showed lower latency times in finding an escape platform compared to their untreated counterparts. Furthermore, the study finds that EC successfully lowered ROS concentrations and prevented lipid peroxidation, subsequently blocking Aß toxicity. The study also suggested that the amelioration of the rats' spatial memory may be because Aß monomers adopt a new conformational form in the presence of EC, lowering the occurrences of fibril-prone Aß states that lead to further plaque development and the worsening of AD.

Analysis of ECG and p66Shc Interaction

ECG produced the second-best docking score of −6.9 kcal/mol along with EGCG. This may be attributed to the covalent carbon–hydrogen bond with amino acid ASP267, which is more stable than other possible interactions like van der Waals forces. An additional pi–anion interaction was observed, further strengthening the bond, but not to the same degree as the carbon–hydrogen. This high docking score followed a trend observed by Rajak et al. (2023), wherein ECG and EGCG had strong binding energies with ROS producers lipoxygenase, CYP2C9, and NAD(P)H oxidase. A study by Huang et al. (2007) showcased the inhibition of ECG towards ROS production in keratinocytes and lowered the overall chances of the occurrence of OS. Moreover, Chen et al. (2020) revealed that both ECG and EGC can inhibit the aggregation and production of Aß, with ECG being able to cross the blood-brain barrier to improve neural conditions and prevent the formation of plaques. This points to ECG as a prospective agent in preventing the further development of OS and AD by lowering Aß amounts and suppressing its toxicity. Additionally, the inhibitory abilities of ECG were also observed in a study by Zhu et al. (2021), where it inhibited uric acid production related to the overexpression of xanthine oxidase, subsequently highlighting its potential use in gout treatment.

Analysis of EGC and p66Shc Interaction

EGC scored the lowest-ranked binding affinity of -6.2 kcal/mol alongside EC and GC. Figure 3D illustrates that EGC formed Van der Waals forces with several amino acids, including SER60, THR57, PHE181, PRO55, THR185, and ASN182, contributing to the stabilization of the complex. Additionally, a Pi-Anion interaction with ARG189 further reinforced the binding, offering minor additional stabilization. The absence of stronger covalent or ionic interaction may explain the relatively lower binding affinity compared to its fellow flavanols. The reliance on weaker forces like Van der Waals interactions suggested that while EGC can form a stable complex with p66Shc, it does not exhibit stronger, more stable binding patterns in ligands with higher affinities. Although the score was relatively lower compared to the other flavanols in the present study, EGC still has the potential to inhibit ROS production. This was evident in a study by Ambigaipalan et al. (2020) which showcased the antioxidant capabilities of EGC, further revealing the increased potentness of its effects when lipophilized.

Analysis of EGCG and p66Shc Interaction

EGCG produced the second-best docking score of -6.9 kcal/mol, which is tied with the ECG score. Figure 3E shows that EGCG established van der Waals interactions with amino acid ARG177, helping with stability. Additionally, Pi-Alkyl bonds were formed with amino acids TRP134 and PHE140 to further stabilize the interaction by allowing hydrophobic packs to form between aromatic rings. This hydrophobic relationship provides notably good structural stability for the EGCG-p66Shc bind, thus resulting in a relatively good docking score. Of all the

flavanols discussed in the present study, EGCG is the most notorious for its many benefits to the body. It has been observed to have preventative effects on several cancer hallmarks like cell proliferation, and OS, which are known to accelerate tumor growth (Talib et al., 2024). Moreover, EGCG decreased the chances of kidney injury in mice (Chu et al., 2024). The results of the interaction between EGCG and p66Shc revealed the potential role of EGCG in AD prevention. A review by Lange et al. (2022) discussed EGCG's capabilities of lowering Aß deposition and occurrences of OS through free radical scavenging. EGCG's inhibition effect on the major adverse effects of p66Shc and its high docking score suggest its crucial part in preventing and de-escalating AD (Lone et al., 2018).

Analysis of GC and p66Shc Interaction

Similar to EC and EGC in this study, GC exhibited a binding affinity of -6.2 kcal/mol. GC formed van der Waals forces with SER139, GLU133, and PHE140 and featured a hydrophobic Pi-Alkyl bond with TRP134, which also involved Pi-Pi stacking. This stacking interaction contributes to its binding stability; however, the overall lack of additional stronger bonding types, such as covalent or Pi-Anion interactions, places it among the other lower-affinity ligands in the present study. This shows that GC can bind and inhibit p66Shc despite its relatively low docking score. The study invites experimentation between GC and Alzheimer's disease, as many mechanisms of its interactions are still unknown. Regardless, GC has been shown to have relevance in the hastening of delayed wound healing in mice with diabetes, which is a characteristic of AD (Vendidandala et al., 2021).

Analysis of GCG and p66Shc Interaction

GCG scored the highest-ranked binding affinity with a docking score of -7.6 kcal/mol in the present study. Bond analysis revealed five van der Waals interactions with the ligand, which can be seen in Figure 3G. Although van der Waals interactions are relatively weak compared to hydrogen bonds, which are hydrophobic interactions, the amount of interacting amino acids lowers the net Gibbs free energy in the overall system. Several studies in the past put a strong emphasis on GCG's easing of neurodegenerative conditions. A study on H22 cells in the brains of mice revealed that it helped inhibit glutamate's ROS production and the phosphorylation of mitogen-activated protein kinases (MAPK), subsequently lowering the chances of OS. It also revealed cell viability percentages similar to EGCG, a well-known compound in green tea for its potent effects (Park et al., 2021). Additionally, GCG has been known to revive the long-term potentiation (LTP) of neural pathways of older mice and even bring back synapse signal strengths similar to that of mice that were only 10 weeks old. As p66Shc has the potential to worsen AD and amplify Aß toxicity (Lone et al., 2018), the strong binding energy of GCG further sheds light on its role as a possible key player in preventing AD and ameliorating overall brain health.

Chromane Group in all interactions

The comprehensive examination of all bonding interactions revealed that the chromane group of each flavanol was the main interactor with p66Shc. Chromane is a compound that contains a fused pyran ring and phenyl ring, forming a heterocyclic chemical structure. In the past, derivatives of chromane, such as chromanol and chromanone, have been found to inhibit BuChE, a key contributor enzyme to AD pathology (Moutayakine et al., 2022). This further highlights the potential of green tea

flavanols in mitigating AD-related neurodegeneration and how it could play a significant supplementary role alongside other conventional AD treatments.

Limitations of the Study

The limitations of this study are rooted in the inherent nature of computational studies. The molecular docking simulations performed utilized static models of the target protein and the ligand molecules. Therefore, the results may not fully represent the dynamic and flexible nature of molecular interactions within biological systems. In reality, proteins and ligands undergo various conformational changes upon binding that are not fully accounted for by molecular docking simulations. As such, while AutoDock Vina provided valuable insights into possible binding affinities and interaction patterns of p66shc and the target ligand, the results must be considered preliminary and require further experimental testing before clinical applications.

Nevertheless, the results of this study have significant medicinal implications in the field of drug discovery and development. Specifically, the strong binding affinities observed between the seven *C. sinensis* flavanols and the p66Shc protein suggest their potential role in mitigating oxidative stress pathways associated with AD. Therefore, the findings of this study lay the groundwork for further *in vitro* and *in vivo* validation studies to be conducted by future researchers, which are critical to translating computational predictions into clinically viable treatment options for AD. In particular, the flavanols can be used as lead compounds in drug development to target p66Shc, which would consequently reduce the Aß plaques that accumulate in AD.

CONCLUSION

In this study, the binding affinities for *C. sinensis* flavanols revealed their potential to inhibit the production of p66Shc, which consequently aids in the mitigation of the progression of AD. While the one-way ANOVA test revealed that there were significant differences among the flavanols, a post hoc Tukey's HSD test revealed which specific pairs were statistically significant. Among these, GCG was found to be significantly different in all pairs, which showcased that GCG has the strongest potential to inhibit p66Shc. Moreover, GCG demonstrated the best docking score of −7.6 kcal/mol, indicating the strongest binding affinity due to the significant number of van der Waals interactions. In contrast, flavanols like EC, EGC, and GC shared the lowest scores (−6.2 kcal/mol), likely due to a lack of covalent or stronger pi–anion interactions, relying primarily on van der Waals forces and hydrophobic interactions for stabilization. Meanwhile, ECG and EGCG, with scores of −6.9 kcal/mol, showed strong binding through covalent carbon–hydrogen bonds, hydrophobic pi–alkyl bonds, and pi–anion interactions, suggesting they could be particularly effective in inhibiting p66Shc activity. Although catechin's docking score of −6.4 kcal/mol was moderate, its therapeutic potential in treating AD symptoms remains promising. The chromane group of all flavanols docked interacted with p66Shc, which supported its role in AD prevention due to its capacity to inhibit BuChE. Ultimately, the docking scores aligned with previously observed trends in ROS inhibition and treatment of AD, with this study demonstrating quantitative evidence of each flavanol's capacity to bind to the surface of p66Shc, triggering the activation of its beneficial effects.

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