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Competitive Inhibition and Synergistic Effects of Nutraceutical and Metabolite Molecules on Anti-Acetylcholinesterase Activity

Munteha Girgin[1], Shirin Tarbiat[1], Sevim Isik[2,3], Nigar Kantarci-Carsibasi[1*]

[1]Department of Chemical Engineering, Uskudar University, Uskudar, Istanbul, Turkey.
[2]Department of Molecular Biology and Genetics, Uskudar University, Uskudar, Istanbul, Turkey
[3]Uskudar University, Stem Cell Research and Application Center (USKOKMER), Istanbul, Turkey.

Abstract: The rapidly increasing prevalence of Alzheimer's disease (AD) poses a significant global public health threat. While medications such as Donepezil, Galantamine, and Rivastigmine are used, their serious side effects and limited healing fail to provide a definite cure. Consequently, combination therapies are being explored to enhance the efficacy of existing drugs. This study aims to evaluate the anti-acetylcholinesterase activities of previously identified nutraceutical and metabolite compounds, namely Queuine, Etoperidone, and Thiamine. Combined use of Queuine with Donepezil, Etoperidone, and Thiamine on acetylcholinesterase enzyme inhibition is also evaluated. The effects of the drug combinations on cell viability and acetylcholinesterase inhibition were investigated by using safe doses determined for each drug. The cytotoxic effect of drug combinations was investigated on the SH-SY5Y cell line using the RTCA method. All the individual or drug combinations were non-toxic to neuronal cells. Anti-acetylcholinesterase activities were estimated by Ellman's method yielding the inhibition percentages as 70%, 61%, 45%, and 51% for Donepezil, Etoperidone, Queuine, and Thiamine, respectively. When drug combinations were analyzed, competitive inhibition resulted for Queuine+Donepezil and Queuine+Thiamine, the enzyme inhibition percentages being diminished to 47% and 21%, respectively. A significant synergistic effect was observed for Queuine+Etoperidone with the highest inhibition of 74%. This study provides the first evidence of the nutraceutical molecule Queuine's impact on acetylcholinesterase inhibition and the synergistic effect of Queuine and Etoperidone as a potent drug combination surpassing the effectiveness of Donepezil. Queuine and Etoperidone synergism may serve as a potential AD treatment by further in vivo validations.

Keywords: Alzheimer's Disease (AD), Acetylcholinesterase (AChE), Ellman assay, Queuine, Etoperidone.

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***Corresponding author's E-mail:** nigar.carsibasi@uskudar.edu.tr

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder posing a significant challenge in identifying effective treatments due to its intricate etiology. While the exact cause of the disease is unknown, recent insights into disease progression have led to several hypotheses such as the cholinergic hypothesis which is reflected by reduced levels of acetylcholine due to overexpression of acetylcholinesterase enzyme (AChE), amyloid beta (Aβ) related cascade which involves plaque aggregation, neurofibrillary tangle formation, mitochondrial dysfunction, metal toxicity, and generation of reactive oxygen species. Current therapeutic drug options for AD patients include; Donepezil, Galantamine, and Rivastigmine which are marketed as AChE inhibitors, and Memantine which is the N-Methyl-D-aspartate receptor antagonist, as approved by the FDA. Donepezil is a synthetic drug, while Galantamine and Rivastigmine are obtained from natural sources.

Emerging studies reported a close correlation between these disease-related hypotheses. Castro and Martinez (2006) showed that other than the cholinergic function, AChE owns a secondary non-cholinergic function which is controlling Aβ deposition (1). It is suggested that the interaction of AChE with Aβ protein may enhance the aggregation of insoluble plaques in AD patients (2,3). AChE enzyme possesses two crucial binding sites: peripheral anionic site (PAS) and catalytic active site (CAS). Drugs that bind to CAS and PAS are reported to have

a dual effect of boosting acetylcholine levels and at the same time preventing Aβ plaque formation (4,5). In another study, the generation of Aβ plagues was reported to promote the glutamate receptors and increase calcium ion expression leading to neural loss (6). Deposition of Aβ aggregates may also trigger oxidative damage and inflammation contributing to AD progression (7). Hence, there is an emerging need for a dual-site multi-target drug that would impact two main concerns: AChE inhibition through interacting with the CAS and preventing Aβ plague aggregation through interacting with the PAS. Drugs that bind to the PAS of AChE may also hinder substrate binding by blocking the entrance of the gorge of the active site as well (8). Recent studies have suggested that targeting various pathways, having fewer side effects, and demonstrating long-term therapeutic effects, make natural-derived active compounds promising approaches in Alzheimer's disease (AD) treatment (9). A new road to AD pharmacological treatment is thus designing dual-binding site natural origin inhibitors. Stemming from this emergence of new generation AChE inhibitors, a recent study conducted to inhibit plaque aggregation through AChE inhibition by natural compounds curcumin and piperine demonstrated promising neuroprotective effects in SH-SY5Y cells. Furthermore, the combined use of these compounds achieved a synergistic effect with a better outcome than individual usage (10).

Nutraceuticals are derived from isolated phytochemicals, often in their pure form or as mixtures of flavonoids (e.g. Quercetin, Epigenin, and anthocyanins), phenolic acids (e.g. Catechins, Gallic Acids, curcumin, resveratrol, and epigallocatechin-3-gallate), and alkaloids (e.g. morphine, strychnine, quinine, ephedrine, and nicotine). Several studies have demonstrated the effects of natural phytochemicals on Alzheimer's disease treatment and nutraceutical-based drug discovery (11). However, more research is needed to explore the synergistic effects of these isolated metabolites. A recent study was conducted to examine the synergistic effect of herbal extracts on anti-acetylcholinesterase activity. Compounds that are extracted such as gallic acid, palmatine, berberine, etc. were tested for their inhibitory activity. Palmatine and berberine combined use was reported to have a synergistic effect by binding to the PAS region, blocking the gorge, and hindering enzymatic activity (8). In another recent study, it was demonstrated that the crude extract exhibited a promising cholinesterase inhibitory and neuroprotective effect against Aβ25-35 cytotoxicity, in comparison to the fractions containing Hinokinin and Cubebin lignan (12). Choi et al. (2013) investigated the neuroprotective effects of flavonoids against amyloid beta (Aβ) and discovered that flavonoids inhibited Aβ aggregation and Aβ-induced cytotoxicity in PC12 neuronal cells. (13). They specifically studied two isolated flavonoids, daidzein, and baicalein, and observed that while they individually exhibited similar anti-Aβ properties, their combination significantly reduced Aβ aggregation. This finding suggests the potential of baicalein and daidzein as promising approaches in developing nutraceuticals for Alzheimer's disease treatment in the future. Coqueiro et al. (2023) studied the AChE inhibitory effect of the alkaloid fraction obtained from *Piterogyne nitens*. (14). They identified three isoprenylated guanidine alkaloids (Galegine, Ptero-gynidine, and Pterogynine) from the ethanol extract of leaves of P. nitens, all of which exhibited potential anti-acetylcholinesterase activity. The highest inhibi-tion was observed with Pterogy-nidine.

Hybridization of the available drugs such as donepezil and tacrine by combining indanone and quinoline heterocyclic scaffolds was also studied. Several hybrid ligands were synthesized and tested for their cholinergic and non-cholinergic functions. Promising hybrid new leads that serve as modifying agents against AD by increasing the cholinergic function, decreasing the Aβ toxicity, and promoting neurite outgrowth were discovered (15). In another recent study individual and drug combinations of donepezil, tacrine, berberine, and galantamine were investigated and it was found that the berberine + galantamine drug combination produced the most potent synergism and reduced the total drug dose by 72% (16).

Recently, we conducted a systematic in silico strategy with in vitro evaluation to identify natural-derived molecules as potential AChE inhibitors in AD treatment. Three lead compounds namely, Queuine, Thiamine, and Etoperidone were identified as promising AChE inhibitors and interactions were compared with FDA-approved synthetic and natural source drugs namely Donepezil and Galantamine (17). Queuine and Thiamine (Vitamin B_1) are classified as nutraceuticals, while Etoperidone is a metabolite. These compounds yielded good docking scores and performed essential interactions with CAS and PAS sites of AChE that are crucial for the enzyme functioning. These interactions were prolonged as monitored by molecular dynamics simulations. Previous literature studies have already demonstrated the inhibitory effect of Thiamine on AChE (18, 19). However, Etoperidone and Queuine have not been evaluated for AChE inhibitory activity yet. Previous literature studies reported that Etoperidone was inter-linked to tau-hyperphosphorylation and Queuine was engaged in an amyloid-beta-related cascade (20-22) Hence in our previous work two new promising AChE inhibitors: Queuine and Etoperidone that may have a multi-target activity were proposed for the first time.

Considering the putative role of both CAS and PAS regions of AChE in managing disease control, it is evident that dual-binding site inhibitors may acquire importance for AD treatment. Thus, in the present study, we aim to further support our previous findings with enzyme inhibition assays and obtain a relative comparison for AChE inhibition among our lead molecules both individually and in combined use forms. The results of the present study are hoped to pave the way to propose drug or drug combinations of natural origin, namely Queuine, Etoperidone, and Thiamine, that would have dual-site binding to AChE and result in high enzyme inhibition. In short, we aim to shed light on the combined use of a PAS-effective

drug with a CAS-effective one. Would this lead to a synergistic effect which in turn would result in a boosted inhibitory action as compared to individual use of both drugs or contrary to what we expect would facilitate a competitive antagonism and hinder the individual inhibitory activities? In the present study, we tested both individuals and combinations of Queuine with other lead compounds using RTCA cell culture assay and Ellman's method for AChE assay (23) to address these issues. This research aims to propose novel drugs and drug combinations with low-risk and dual-action properties that may have therapeutic potential to address AD.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Reagents

The chemical compounds used in the experiments, namely Donepezil hydrochloride (Catalog No: D6821) and Galantamine hydrobromide (Catalog No: G1660), Thiamine hydrochloride (Catalog No: T1270), were purchased from Sigma-Aldrich. Etoperidone hydrochloride (Catalog No: sc-211494) and Queuine hydrochloride (Catalog No: sc-394021) were obtained from Santa Cruz Biotechnology. The other chemical compounds used in the study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). This study utilized SH-SY5Y (human neuroblastoma) cell lines. The SH-SY5Y cell line (CRL-2266) was provided by ATCC.

2.2. Cell Culture

After dissolving the cell line stored in a nitrogen tank for long-term use, the cells were removed from the culture dish with a 0.25 Trypsin/EDTA solution when they reached 70% density in the culture flasks. The cells were incubated in DMEM (Capricorn) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 0.1 mg/mL of penicillin at 37°C with 5% CO_2. The medium was refreshed every 48 hours, and passages were made every 5 days based on cell confluency.

2.3. Real Time Cell Analysis (RTCA) of Candidate Drugs in SH-SY5Y Cell Line

In our previous study, the optimal safe doses were determined for Donepezil, Thiamine, Queuine, and Etoperidone. In this study, we evaluated the competitive effects of Queuine in combination with Donepezil, Etoperidone, and Thiamine on cell growth and viability in cell culture using real-time cell analysis (RTCA, xCELLigence). The RTCA system is an analytical technique that enables real-time monitoring of cellular events, such as proliferation, by measuring the electrical impedance passing through microelectrodes integrated beneath specialized plates. A 16-well e-plate compatible with the RTCA device, consisting of two plates with eight wells each, was used. Initially, 100 µL of DMEM containing 15% FBS was added to cell culture to the wells to obtain the background density. Subsequently, $3x10^4$ cells were seeded in the culture medium at 100 µL. After 24 hours, the determined concentration ranges were applied individually and in combination. Previous studies on Donepezil indicated no significant impact on cell viability up to 10 µM, but a decline in cell viability was observed after reaching 15 µM (24). Referring to our previous study, we administered the safe dose of 15 µM for Donepezil on the SH-SY5Y cell line (17). Similarly, we used our previously determined safe doses on SH-SY5Y cell lines for the rest of the drugs as well. The safe doses were: Queuine (1.25 µM), Etoperidone (70 µM) and Thiamine exhibited no toxicity even at high doses. Referring to our previous study, we applied a concentration of 600 µM for Thiamine (17). To assess the synergistic effects of Queuine with Donepezil, Etoperidone, and Thiamine on cell viability, we administered a combination of Queuine at a safe concentration of 1.25 µM, Donepezil at 15 µM, Etoperidone at 70 µM, and Thiamine at 600 µM. Each selected dose was applied to two different wells. The first two wells were assigned as the control group without drug treatment. The growth curve was obtained from the data generated by the RTCA device 48 hours after drug administration. The RTCA experiments were repeated three times for each drug group.

2.4. Protein Estimation Assay

The required proteins for the Ellman method (23-25) were obtained from the SH-SY5Y cell line. A cell seeding of 1.2×10^6 cells per petri dish was performed. After 24 hours of seeding, drug administration was carried out at predetermined doses. Following a 48-hour incubation period with the drugs, the petri dishes were washed with PBS and 100 µL of RIPA cell lysis buffer were added. The proteins were collected by scraping the petri dish surface. The protein quantity was determined using the BCA (Bicinchoninic Acid) (26) method.

2.5. Acetylcholinesterase Enzyme (AChE) Inhibition Assay

Ellman's method involves the hydrolysis of acetylthiocholine iodide by AChE enzyme or butyrylthiocholine chloride by BChE enzyme, resulting in the formation of thiocholine. Thiocholine then reacts with the Ellman's reagent, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), producing a yellow-colored 2-nitro-5-thiobenzoate (TNB) which can be detected at 405 nm. The rate of yellow color production is measured at 405 nm to indicate enzyme activity. AChE activity was determined using a 96-well microplate reader (OMEGA). A 200 µL protein solution (2 mg/mL, 0.1 M phosphate buffer, pH 8.0) as enzyme source was mixed with a 100 µL solution of DTNB (3.3 mM, 0.1 M phosphate-buffered solution, pH 7.0) containing $NaHCO_3$ (6 mM), and 500 µL phosphate buffer (pH 8.0). After incubating for 3 minutes at 25 °C, AChE activity was evaluated by measuring the change in absorbance at 405 nm using a spectrophotometer. This experiment was repeated three times.

2.6. Statistical Analysis

All analysis data obtained from the RTCA device and the data read by the microplate reader (OMEGA) were documented using the MARS application. RTCA data were analyzed using GraphPad Prism 9.5.1 software to determine the significance compared to the control group. Statistical analysis was performed using one-way ANOVA with multiple comparisons using the Tukey test.

3. RESULTS AND DISCUSSION

3.1. Interactions of Drug Molecules with AChE

In our previous study, we conducted systematic simulations including virtual screening, natural database generation, molecular docking, molecular dynamics, MM-GBSA binding free energy calculations, and ADMET analysis to propose promising natural molecules with anti-acetylcholinesterase activity. The identified compounds were Thiamine, Queuine, and Etoperidone which interact with important binding sites and possess dual binding properties to CAS and PAS of AChE (Figure 1).

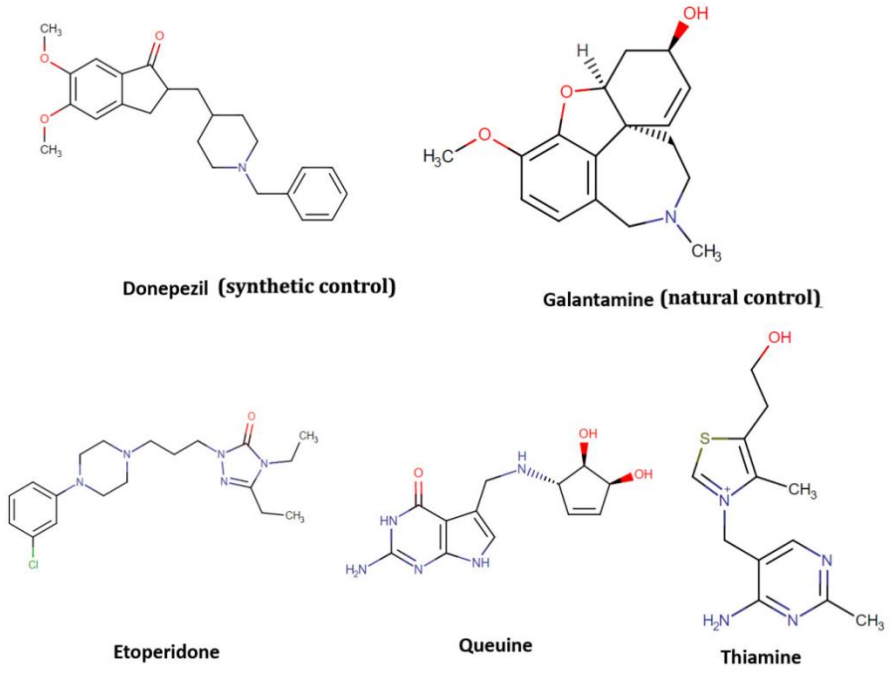

Figure 1: Structures of AChE inhibitors investigated in the present study.

The details about the methodology can be found in Girgin et al. (2023) (17). The docking scores for AChE and half inhibitory concentration (IC50) of each compound were determined on SH-SY5Y cell lines. The docking scores obtained were -10.1, -10.1, and -13.4 kcal/mol for Queuine, Thiamine, and Etoperidone, respectively which may be compared to two control benchmark drugs namely Donepezil and Galantamine yielding -14.8 and -8.1 kcal/mol, respectively. The interactions with CAS and PAS regions of AChE were also investigated by molecular docking and molecular dynamics simulations. Results revealed that Queuine interacts mainly with PAS similar to Galantamine, while Etoperidone and Thiamine interact with both CAS and PAS of AChE similar to Donepezil (Figure 2). As depicted in Figure 2, the CAS residues of AChE are His 447 and Ser 203 and PAS residues consist of Asp 72, Asp74, Tyr124, Ser 125, Trp 286, Tyr 337, and Tyr 341.

IC50 values that reflect the relative potencies of each drug were determined by in vitro cell culture assay on SH-SY5Y cells using RTCA and MTT assays. IC50 values were 70.9, 712.8, 18780.3, 222.2, and 556.0 μM for Queuine, Etoperidone, Thiamine, Donepezil and Galantamine, respectively. Among these Queuine has the highest potency with a much lower IC50 value as compared to both Donepezil and Galantamine. In light of this work, we further supported these findings with individual and combined drug use experiments on SH-SY5Y cell lines and enzyme inhibition tests to explore synergistic and/or competitive effects on anti-acetylcholinesterase activity.

3.2. RTCA Assay: Effects of the Reagents on Cell Viability

The effects of Donepezil, Etoperidone, and Thiamine as well as the combinations of Queuine with Donepezil, Etoperidone, and Thiamine on cell growth and proliferation were determined using the xCELLigence Real-Time Cell Analysis (RTCA) system on the human neuroblastoma SH-SY5Y cell line. For this purpose, SH-SY5Y cells were exposed to safe concentrations determined in our previous study: Donepezil (15 μM), Etoperidone (70 μM), and Thiamine (600 μM), as well as Queuine (1.25 μM). Additionally, the combinations of Queuine with FDA-approved Donepezil and our candidate compounds Thiamine and Etoperidone were tested at the following concentrations: Queuine with Donepezil (1.25 μM - 15 μM), Queuine with Etoperidone (1.25 μM - 70 μM), and Queuine with Thiamine (1.25 μM - 600 μM). After 48 hours of exposure, the relative cell viability was calculated. The synergistic effects of Queuine with Donepezil, Etoperidone, and Thiamine were evaluated using the xCELLigence RTCA system, and the cell index percentage graphs obtained are presented in Figure 3. In Figure 3, the cell indices are as follows: Donepezil (15 μM) 86%, Etoperidone (70 μM) 79%, Thiamine (600 μM) 88%, Queuine (1.25 μM) 92%, Queuine with Donepezil 90%, Queuine with Etoperidone 91%, and Queuine with Thiamine 101%. When comparing the RTCA results of the two

studies for the safe doses we found in our previous study and used in this study, which are Donepezil (15 µM), Etoperidone (70 µM), Thiamine (600 µM), and Queuine (1.25 µM), the results were consistent with each other (17). According to the percentage cell density graph presented in Figure 3, no significant cytotoxicity of the candidates on the cell line was observed with *P<0.05, **P<0.01, and ***P<0.001 levels of significance.

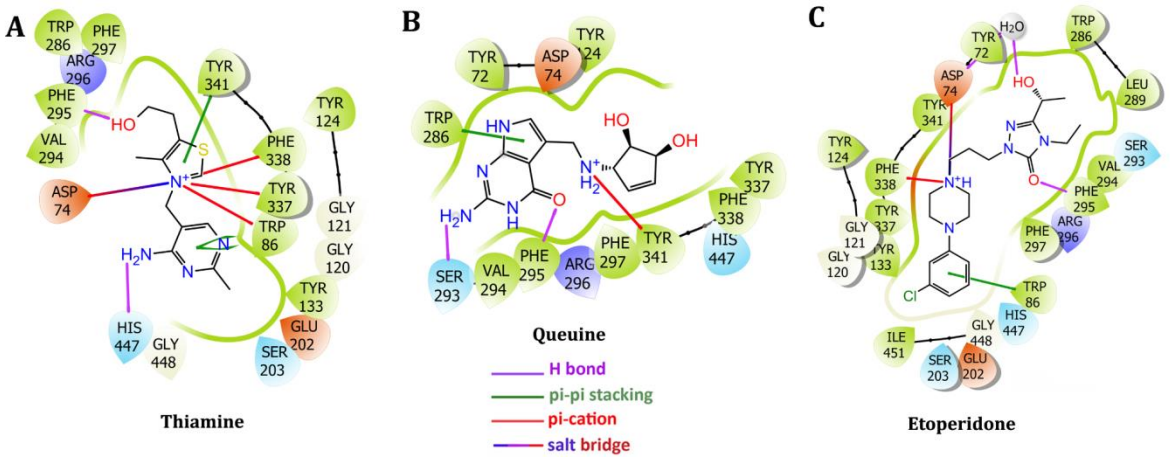

Figure 2: Binding site interactions of lead compounds Thiamine (A), Queuine (B), and Etoperidone (C) with AChE active site.

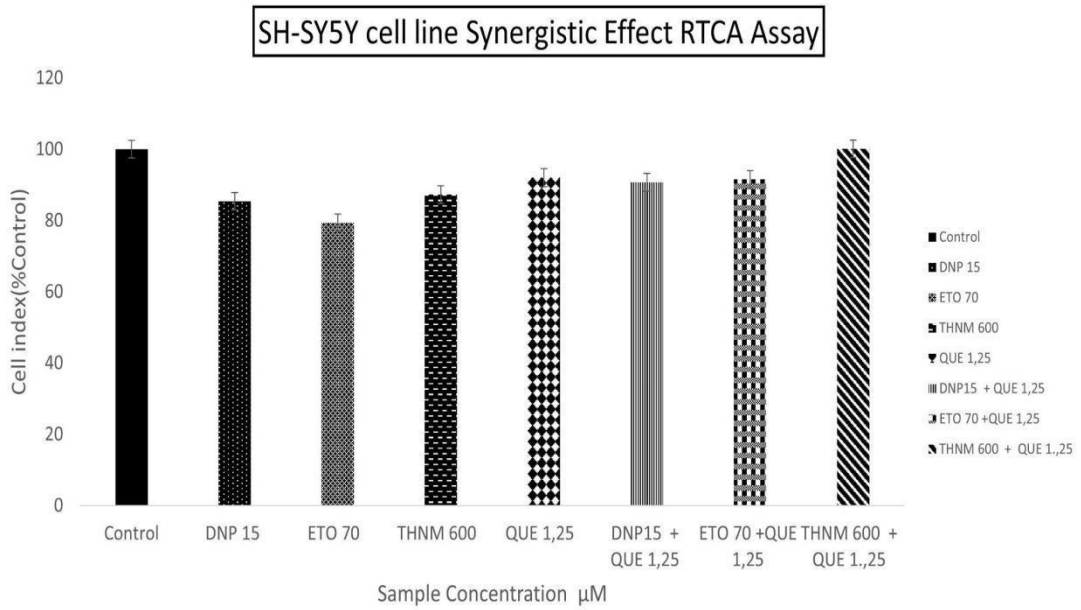

Figure 3: Viability of SH-SY5Y cells as a percentage graph compared to the control group after exposure to selected concentrations: Donepezil (15 µM), Etoperidone (70 µM), Thiamine (600 µM), and Queuine (1.25 µM). The last three columns represent the percentage graph of cell viability for the synergistic effects of Queuine with Donepezil, Etoperidone, and Thiamine. Sequentially, cell index (Control%): Donepezil (15 µM) 86%, Etoperidone (70 µM) 79%, Thiamine (600 µM) 88%, Queuine (1.25 µM) 92%, Queuine with Donepezil 90%, Queuine with Etoperidone 91%, and Queuine with Thiamine 101%. The results represent the average ± SEM of experiments repeated three times at different time points with the same concentration ranges. When compared to the control, *P<0.05, **P<0.01, and ***P<0.001 indicate significant differences.

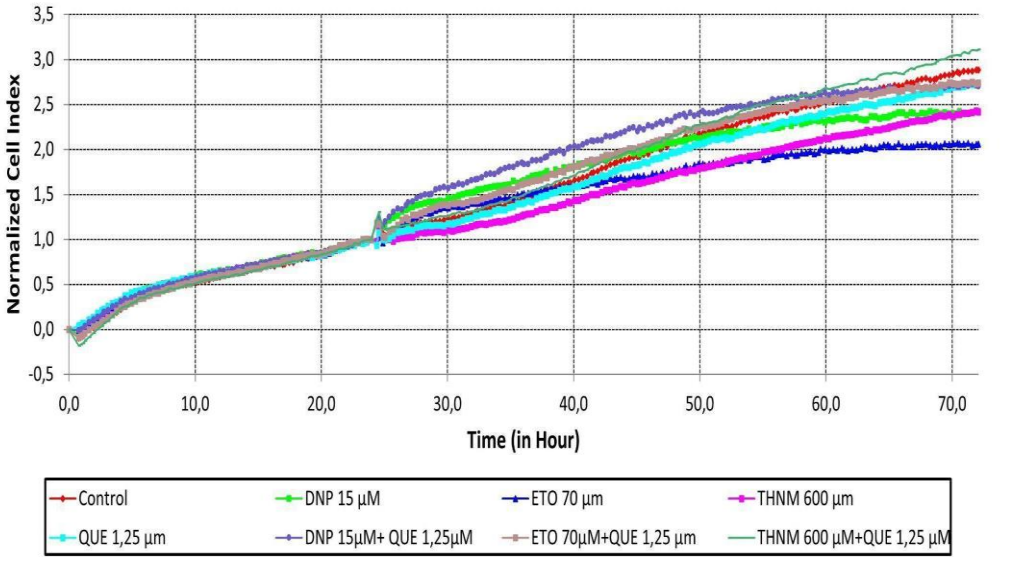

Figure 4: The results of the RTCA analysis: The cells were not treated with any drug for the first 24 hours. After 24 hours, different concentrations of drugs, both individually and in combination with Queuine, Donepezil, Etoperidone, and Thiamine at their respective safe concentrations, were applied. Following drug application, the cell index was measured continuously for 48 hours.

The cell index graphs obtained from the xCELLigence RTCA system were shown separately for Donepezil, Etoperidone, Thiamine, Queuine, and the combinations of Queuine with Donepezil, Etoperidone, and Thiamine in Figure 4. The safe dose ranges were consistent with the previous study, and no cytotoxicity was observed. Additionally, there was no significant toxic effect on cell viability and proliferation with the combination of Queuine with Donepezil, Etoperidone, and Thiamine.

3.3. Measurement of Acetylcholinesterase (AChE) Levels Using the Ellman's Method

The enzyme inhibition results are shown in Figure 5. Our synthetic control group, Donepezil achieved 70% inhibition on AChE, while our natural-sourced control group Galantamine showed 44% inhibition. Among our three lead candidates, Etoperidone, Queuine, and Thiamine provided 61%, 45%, and 51% inhibition, respectively. When Queuine combined with Donepezil, Etoperidone, and Thiamine their synergistic effect on AChE inhibition was examined, resulting in 47%, 74%, and 21% inhibitions, respectively.

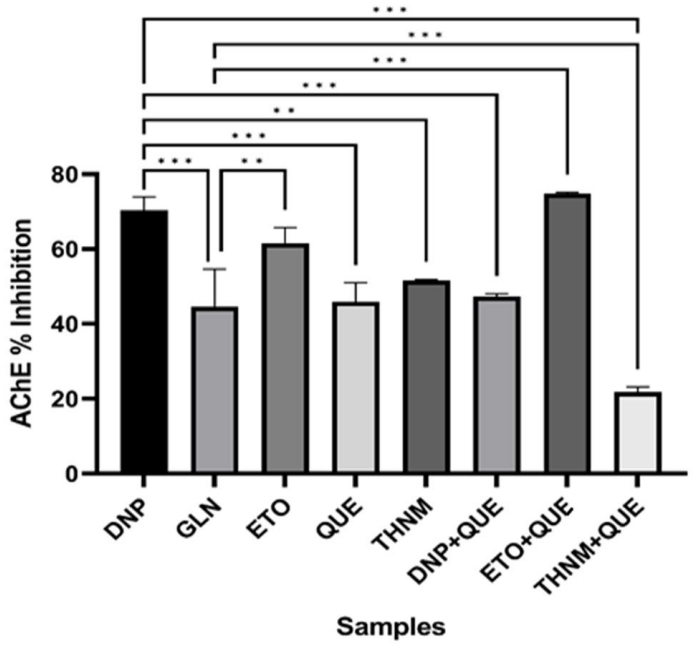

Figure 5: Percentage inhibition of AChE by study samples using Ellman's Method. Values are mean ± standard deviation, n=3. Samples were compared with controls Donepezil (DNP) and Galantamine (GLN), and the differences between the bar pairs marked with ** ($p<0.005$) and *** ($p<0.001$) were statistically significant. DNP: Donepezil; GLN: Galantamine; ETO: Etoperidone; QUE: Queuine; THNM: Thiamine.

When evaluating the percentage of AChE enzyme inhibition graphs, a significant difference was observed between Donepezil and Thiamine at ($p<0.005$). Significant differences were found between Donepezil and all candidate groups except for Thiamine at ($p<0.001$). Additionally, a significant difference was observed between Galantamine and Etoperidone at ($p<0.005$). The difference was also significant between Galantamine and the groups of Queuine+Etoperidone and Queuine+Thiamine at ($p<0.001$).

Enzyme inhibition percentages are presented in Table 1. The values reveal that Queuine and Galantamine exhibit similar AChE inhibitory activity with inhibition percentages of 45% and 44% respectively. Regarding Queuine, its impact on amyloid has been reported in a previous study conducted on the microglia cell line (22). Additionally, it is suggested that Queuine may possess cholinergic activity through AChE inhibition. These findings support us in silico studies. Hence, we propose Queuine as a candidate multi-target nutraceutical compound probably being involved both in cholinergic and non-cholinergic amyloid-related mechanisms.

Table 1: % AChE enzyme inhibition values

Samples	% AChE inhibition	SEM +/-
Donepezil	70	3.52
Galantamine	44	10.04
Etoperidone	61	4.09
Queuine	45	5.16
Thiamine	51	0.24
Donepezil+Queuine	47	0.71
Thiamine+Queuine	21	1.36
Etoperidone+Queuine	74	0.23

In clinical trials, it has been reported that Etoperidone can inhibit the reuptake of serotonin, norepinephrine, and dopamine in the central transmission of serotonin (27,28). It has been indicated that several clinical trials involving etoperidone and its derivatives (trazodone, haloperidol) are primarily focused on the treatment of sleep disorders and Alzheimer's disease (AD) (20,21,29,30). However, the precise molecular mechanism underlying this treatment has not been fully understood. In this study, the inhibition of AChE by Etoperidone is demonstrated. Etoperidone produces a comparable inhibitory action with Donepezil, the AChE inhibition values being 61% and 70% for Etoperidone and Donepezil, respectively. These results substantiate the accuracy of our previous in silico study. To elucidate the combined effect of PAS-effective and CAS-effective drugs, we conducted combined drug use experiments with Queuine specifically. It is worth noting that the combined use of Queuine with Donepezil, and Queuine with Thiamine produced a competitive inhibition by hindering the individual inhibition values. This may stem from the fact that when Queuine binds to PAS, it may prevent the entry of Donepezil and Thiamine into the active site gorge, leading to a competitive antagonism. Contrarily, we observed a significant synergistic effect for the combined use of Queuine and Etoperidone, which resulted in a boosted inhibitory value as compared to individual use of both drugs. The highest % AChE inhibition value was obtained for this combined use of Queuine+Etoperidone, which may be suggested as a promising drug therapy for AD treatment.

In previous literature studies investigating combined therapies, it has been noted that drugs binding to the peripheral anionic site (PAS) of acetylcholinesterase (AChE) could impede substrate binding by blocking the entrance to the active site gorge (8,16). Consequently, Queuine may obstruct the entry of both Donepezil and Thiamine into the gorge towards the catalytic anionic site (CAS), preventing their interaction with both CAS and PAS. This interference may hinder the individual inhibitory actions of Donepezil and Thiamine in the presence of Queuine. On the other hand, the scenario may differ with Etoperidone, as it could potentially enter the gorge before Queuine. As a result, Etoperidone might interact with the CAS, while Queuine simultaneously interacts with PAS. This dual interaction could lead to an enhanced inhibitory action. Upon analyzing the raw data from our previous molecular dynamics (MD) simulation results (17), comparing binding types and residues involved in binding interactions, we can assert the following: Queuine and Etoperidone, where a synergistic effect was observed, do not exhibit overlapping binding positions that would interfere with each other over the 100 ns MD simulation period. While Queuine shows a more active binding in the PAS region, Etoperidone demonstrates a stronger binding in the CAS. Despite the overlapping nature of these results, it is crucial to note that the synergistic effect presented in this study is highlighted for the first time, and thus its

implications need further comprehensive clarification through additional experimentation consistent with the results obtained from the Ellman method.

4. CONCLUSION

Queuine and Etoperidone were previously suggested to have neuroprotective effects in amyloid-beta plaque accumulation and tau-hyperphosphorylation, respectively (20-22). Neither of them has been mentioned as AChE inhibitors until now. In this study, we propose Queuine and Etoperidone as potential AChE inhibitors in addition to previously discovered amyloid-beta-related functions. Queuine is a nutraceutical molecule performing similar interactions and comparable enzyme inhibition values with Galantamine in the AChE PAS region. However, when their effectiveness in terms of dosages was compared, the IC50 value for Queuine was determined as 70.9 μM, while for Galantamine it was 556.0 μM. This indicates that Queuine provides its therapeutic effect at a lower dose, i.e. more potent than Galantamine. Queuine may serve as a multi-target drug that is effective both in cholinergic and amyloidogenic mechanisms and Queuine alone may serve as a strong drug candidate to replace Galantamine.

We suggest that the AChE interaction of Queuine in the PAS may be the basis of its effect on amyloid-related action. Etoperidone also has similar interactions and comparable enzyme inhibition values with Donepezil. Both can bind to CAS and PAS at the same time. However, Donepezil was found to be more potent than Etoperidone, with IC50 values being 712.8 and 222.2 μM, respectively. For this reason, Etoperidone alone is not as effective as Donepezil. Considering the synergistic effect studies, the combined use of Queuine and Etoperidone creates a significant synergistic effect, resulting in a higher enzyme inhibition than Donepezil, which also has not been reported in the literature before. Queuine and Etoperidone are of nutraceutical and metabolite origin, while Donepezil is a synthetic drug. This fact may be another advantage of substituting Donepezil with this drug combination as economic and toxicity issues are concerned. The results of the present study suggest alternative low-risk novel natural drugs and drug combinations having therapeutic potential and dual-action to combat AD.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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