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Research Article

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Anti-human Glioma Cancer Potentials of Neobavaisoflavone as Natural Antioxidant Compound and Its Inhibition Profiles for Acetylcholinesterase and Butyrylcholinesterase Enzymes with Molecular Modeling and Spin Density Distributions Studies

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Abstract

Introduction: In this study, the anti-human glioma cancer potentials of Neobavaisoflavone as natural antioxidant compound and its inhibition profiles for Acetylcholinesterase and Butyrylcholinesterase enzymes with molecular modeling and spin density distributions studies were investigated.

Material and Methods: To investigate the antioxidant properties of Neobavaisoflavone, the DPPH test was performed in the presence of butylated hydroxytoluene as control. The cell viability of Neobavaisoflavone was low against common human glioma cancer cell lines, i.e. LN-229, U-87, and A-172 cell lines without any cytotoxicity effect on the normal cell line.

Results: Neobavaisoflavone inhibited half of the DPPH in the concentration of 125 µg/mL. The best anti-human glioma cancer effects of Neobavaisoflavone against the above cell lines was in the case of LN-229 cell line. Also, important anti-human glioma cancer capacities of Neobavaisoflavone against popular human glioma cancer cell lines are linked in this study. IC_{50} values Neobavaisoflavone, 63.87 nM for AChE and 112.98 nM for BChE were calculated with % Activity-[Inhibitory] graphs. Values inhibitor dissociation constant of the enzyme, Ki, were found as 2.08±0.31 nM for AChE and 135.03±26.38 nM for BChE.

Conclusion: According to the above results, Neobavaisoflavone may be administrated for the therapy of diverse kinds of human glioma cancers in humans. Also, molecular modeling calculations were made to compare the biochemical activities of the neobavaisoflavone molecule against enzymes. In these calculations, the enzymes used are acetylcholinesterase and butyrylcholinesterase, respectively. After molecular docking calculations, ADME/T analysis was performed to examine the properties of neobavaisoflavone molecule to be used as a drug in the future. Then, various parameters for the anti-oxidant activity of the neobavaisoflavone molecule were calculated.

Keywords: Neobavaisoflavone; antioxidant; human glioma cancer; enzyme inhibition; molecular modeling

1. Introduction

The chemotherapeutic materials are a special type of drugs and supplements that are used to treat several types of cancers such as glioma cancer in human [1-3]. The present chemotherapeutic drugs/supplements have many side effects for body, so that mostly the patients die due to the side effects of the chemotherapeutic drugs/supplements instead of cancer [2-4]. It is important to design new chemotherapeutic medications due to the strong side effects of chemotherapy drugs such as nausea, weakness, hair loss, vomiting, diarrhea, weight loss, and mouth sores [5,6]. Scientific results revealed that antioxidant compounds have excellent anti-cancer properties. Their results are stated to be positive or negative, based on the characteristics of antioxidant compounds in terms of physical-chemistry, scale, concentration, and composition [7-9].

There is no definitive cure for Alzheimer's disease like Parkinson's disease. Current treatments are aimed only at minimizing the effects of the disease or improving the quality of life. For this purpose, AChE inhibitors (Donepezil, Rivastigmin) are used to ensure a better quality of life for patients. These common medications may have side effects such as gastrointestinal disorders and hepatotoxicity. For this reason, in recent years, natural AChE inhibitors, which have both a high inhibitory capacity and are reliable, have been considered. They are widely used in AChE inhibitors in agriculture and medicine. Because both disciplines are directly related to living organisms, interest in inhibitors in the natural products group is increasing day by day to eliminate side effects [10-12].

It is seen in recent studies made with theoretical calculations that there are similar results with experimental studies [13-15]. This situation can be used to synthesize more active and effective molecules in theoretical studies before experimental studies. Therefore, theoretical studies are an important guide to experimental studies [16]. Among the theoretical studies conducted, one of the most widely used methods to compare the biological activities of molecules is molecular docking. Molecules with the highest biological activity are found by using the molecular docking method to compare the activities of molecules in terms of enzymes. With these

calculations, experimental studies are directed. The molecular docking method gives a lot of information about the many parameters of molecule calculated before the experimental procedures to be carried out [17].

Anti-oxidants inhibit the initiation or development of oxidative chain reactions. They are compounds that prevent or delay the oxidation of biological molecules such as lipids, proteins or DNA. In order to examine the anti-oxidant properties of the neobavaisoflavone molecule, calculations were made using the gaussian software package. Many parameters about the neobavaisoflavone molecule were calculated. Comparison with other molecules can be made by using numerical values of these parameters about the anti-oxidant activity of this molecule [18,19].

We also determined the Neobavaisoflavone in the anti-human glioma cancer studies against common human glioma cancer cell lines i.e., LN-229, U-87, and A-172 cell lines *in-vitro*. The best result was achieved in case of LN-229 cell line. Also, we investigated the inhibition effects it on cholinesterase enzymes and also molecular modeling studies.

2. Materials and Methods

2.1. Determination of anti-human glioma cancer effects of Neobavaisoflavone

In this research, the following cell lines have been used for investing the cytotoxicity and anti-human glioma cancer effects of the Neobavaisoflavone using the common cytotoxicity test i.e., MTT assay:

I) Normal cell line: HUVEC.

II) Glioma cancer cell line: LN-229, U-87, and A-172.

15 ml of RPMI 1640 medium containing 10% FSC (10 mg/ml penicillin and 100 mg/ml streptomycin) in a culture flask, placed in a CO2 incubator for 2 hours to equilibrate the medium. Under safe conditions (using insulated gloves and goggles) the frozen cell vial was removed from the nitrogen storage tank. In order to avoid the possibility of explosion of the vial (due to the possible entry of liquid nitrogen into the vial), loosen the lid, after disinfecting the outer surface of the vial with 70% alcohol, under the hood to remove nitrogen gas. Close the vial

lid again and immediately melt it in a pan at 37 ° C. The melting process should be completed in about 1 minute and the cells should be avoided from overheating. The medium was added dropwise to the vial and then its contents were taken out and centrifuged with the medium in 15 cc sterile test tubes. After centrifugation, the supernatant was removed and the cells were suspended again in the medium and transferred to a pre-prepared flask containing the medium and FBS and incubated [20-24].

Cell line used in RPMI 1640 medium containing penicillin (100 IU / ML), streptomycin (100 IU / ML), glutamine (2 mmol) and 10% fetal bovine serum (FBS). They were incubated at 37 ° C and in an atmosphere containing 0.5 CO_2. Cells began to grow in 75 cm^2 T-flasks in 15 ml medium with an initial number of 1-2 × 10^6 cells. After three days and covering the flask bed with the cell, the adhesive layer to the bottom of the flask was separated enzymatically using trypsin-verson and transferred to a sterile test tube for 10 minutes at 1200 rpm. The cells were then suspended in a fresh culture medium with the help of a Pasteur pipette and the suspension was poured into 100-well plate flat wells (for cell culture) using an 8-channel sampler of 100 µl. One column of wells was kept cell-free and as a plank containing only culture medium. In another column, it was considered to contain culture medium and healthy cells and in other columns, it was considered to contain culture medium and cell line cells. One of these columns, which contained culture medium and cells and did not contain Neobavaisoflavone, was considered as a control [20-24].

The plates were incubated in the incubator for 24 hours to return the cells to normal from the stress of trypsinization. After this time, suitable dilutions of the prepared Neobavaisoflavone (0-1000 µl / ml) and 100 µl of each dilution were added columnarly to the plate wells (Thus, the final concentration of the studied compound in the wells was halved. Therefore, the concentrations were prepared twice as much to reach the final concentration after being added to the well). The cells were incubated for 37 hours at 37 ° C and 5% CO2 in the atmosphere. After 72 hours, 20 µl of MTT solution (5 mg/ml) was added to each well. The plates were incubated for 3 to 4 hours and then the residue was removed and 100 µl of DMSO was added to each well to dissolve the resulting formazan. After 10 minutes, using shaking the plates, the optical absorption of Formazan at 570 nm was read using a plate reader. Wells containing cells without Neobavaisoflavone were considered as control and the

optical density of wells without cells and only culture medium were considered as blank. The percentage of cell viability was calculated using the following formula [20-24]:

$$\text{Cell viability (\%)} = \frac{\text{Sample A.}}{Control\ A.} x100$$

The closer is the obtained value to the IC50 of Neobavaisoflavone, the stronger is the cell viability activity of the material. The graph of the IC50 of the Neobavaisoflavone was produced by drawing the percent inhibition curve versus the Neobavaisoflavone concentration [20-24].

2.2. Determination of the antioxidant activities of the Neobavaisoflavone

Analysis of antioxidant capacity by DPPH radical method is a well-known test for measuring the antioxidant power of various compounds. The basis of this method is based on the reduction of free radical DPPH by antioxidants in the absence of other free radicals in the environment [25-27]. A compound is generally compared to a known antioxidant compound such as Butylated Hydroxytoluene (BHT). Analysis of antioxidant capacity by DPPH method is a test that has received much attention in the fields of food, pharmaceuticals and biotechnology and is used to develop and introduce new antioxidants. The basis of this method is based on the reduction of free radical DPPH by antioxidants in the absence of other free radicals in the environment, which results in color in the environment whose intensity can be measured by spectroscopy. DPPH is a stable free radical that has an unpaired electron on one of the nitrogen bridge atoms. Radical inhibition of DPPH is the basis for assessing antioxidant capacity [25-28]:

$$DPPH^{\circ}\ (\text{purple}) + H - A \rightarrow DPPHH\ (\text{yellow}) + A^{\circ}$$

DPPH is a stable radical whose methanolic solution has a purple color that shows the highest light absorption at 519-595 nm. The basis of this method is that the DPPH radical acts as an electron acceptor of a donor molecule such as an antioxidant, thus converting DPPH to DPPH2. In this case, the purple color of the environment turns yellow, so the absorption intensity decreases to 595 nm. Antioxidant properties can be determined by measuring the decrease in adsorption intensity by spectroscopy [25-27].

In the present study to measure the antioxidant properties of the Neobavaisoflavone, 2 ml of DPPH (100 μM) dissolved in methanol with 2 ml of the Neobavaisoflavone at the concentrations of μg/ml. The resulting mixture was placed at 25 °C for 30 minutes. Then the samples absorbance was measured at 520 nm by spectrophotometer (Spectramax Gemini XS; Molecular Devices, Sunnyvale, CA) and the amount of antioxidant effect was determined by the below formula [25-28]:

$$\%\,\text{Inhibition} = \left[\left(A_{\text{blank}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100$$

The blank sample consisted of a mixture of 2 ml of Neobavaisoflavone and 2 ml of methanol and a sample containing 2 ml of DPPH and 2 ml of Neobavaisoflavone with the concentrations used was considered as negative control. BHT was also used as a positive control [25,26].

2.3. Determination of IC50 at the antioxidant test

Calculating the 50% inhibition error (IC50) is an excellent way to compare drug activity that dose in which 50% of the final activity of the drug occurs is the criterion for measurement and comparison. In this test, the amount of IC50 for different repetitions of the test was also calculated and is compared with IC50 of BHT molecule, which is an indicator of antioxidant activity. The closer IC50 is to BHT, the stronger the antioxidant activity. In the following experiments, the inhibition concentration of 50% of the Neobavaisoflavone was calculated by plotting the inhibition percentage curve against the Neobavaisoflavone concentration. In the next step, a serial dilution was prepared from each sample and 50% inhibition concentration of three separate samples was measured and its mean was calculated. All experiments were performed three times [25-27].

2.4. Qualitative Measurement

After collecting data, Minitab statistical software was used for statistical analysis. Evaluation of antioxidant results in a completely randomized design and comparison of means was Duncan post-hoc test with a maximum error of 5%. To measure the percentage of cell survival in factorial experiments with the original design of completely randomized blocks and compare the means, Duncan post-hoc test with a maximum error of 5% was

used. The 50% cytotoxicity (IC50) and 50% free radical scavenging (IC50)) was estimated with ED50 plus software (INER, V: 1.0). Measurements were reported as mean ± standard deviation.

2.5. Enzymes inhibition assays

The element method [29] was performed with a slight change in the reference. One disposable cuvette was then filled with 0.4 ml of 0.4 mg / ml DTNB, 25 µl of AChE solution (0.5 µg in 1 mM acetyl thiocoline), 425 µl of PBS, 50 µl of paraoxon in isopropanol or isopropanol. The reaction was started by adding 100 µl of acetyl thiocoline chloride at a certain concentration to evaluate Km and Vmax or 1 mM for toxicological and pharmacological investigations [30]. The absorbance was measured at 412 nm immediately after one minute. Enzyme activity was calculated by estimating the extinction coefficient of 14 = 14.150 $M^{-1}cm^{-1}$ [31].

2.6. Theoretical method

2.6.1. Molecular docking

Recent studies have shown that molecular modeling is the most commonly used assay for comparing the activities of compounds. This method is used to compare the biochemical activities of compounds against enzymes. Indeed, molecular modeling calculations were made to compare the biological activities of the neobavaisoflavone molecule. Many parameters about molecules were obtained. The numerical values of these parameters obtained as a result of the calculations provide significant information about the biochemical activities of molecules. Molecular modeling calculations to calculate the biochemical activity of the neobavaisoflavone molecule were performed using the Maestro Molecular modeling platform (version 12.2) by Schrödinger. Protein and molecules must be prepared for calculations. In the calculations made, a different process is made for the molecules at each stage. First, it was used from Gaussian software program [32-34] to obtain optimized structures of molecules, using these structures *.sdf extension files were created. In the next step, the LigPrep module [35] was used to prepare the working molecules for calculations [36-39].

2.6.2. Gaussian study

It is possible to theoretically calculate the anti-oxidant properties of the neobavaisoflavone molecule. For these calculations, more than one thermodynamic parameter of the molecules must be calculated. These thermodynamic parameters are bond dissociation enthalpy (BDE), proton affinity (PA), ionization potential (IP), proton dissociation enthalpy (PDE), and electron transfer enthalpy (ETE) [40-42].

Basically, the anti-oxidant mechanism consists of 3 important mechanisms. The first of these mechanisms is the hydrogen atom transfer mechanism (HAT).

$$R\cdot + AH \rightarrow RH + A\cdot \qquad (1)$$

In this mechanism, the free radical molecule is used to remove a hydrogen atom from the anti-oxidant. As a result of this, the anti-oxidant now becomes a free radical molecule. The bond dissociation enthalpy (BDE) of the A-H bond has to be calculated to calculate the anti-oxidative activity of the antioxidant molecule.

The second mechanism is single electron transfer-proton transfer (SET – PT). The single electron transfer-proton transfer mechanism consists of two consecutive reactions. In the first, it consists of reactions with the removal of the electron from AH, followed by the transfer of protons.

$$R\cdot + AH \rightarrow R^- + AH^{+\cdot} \qquad (2)$$

$$AH^{+\cdot} \rightarrow H^+ + A^+ \qquad (3)$$

These two mechanisms are proton dissociation enthalpy (PDE) from $AH^{+\cdot}$ cation radical (2) and ionization potential (IP) (3), respectively.

Finally, in the third and final mechanism, it again consists of two stages. These two stages are referred to as the SPLET (Sequential proton loss electron transfer) mechanism. The single electron transfer-proton transfer mechanism consists of two consecutive reactions. In the first, it consists of reactions with the removal of the electron from AH, followed by the transfer of protons.

$$AH \rightarrow A^- + H^+ \qquad (4)$$

$$A^- + H^+ + R\cdot \rightarrow A\cdot + RH \qquad (5)$$

For these two reactions, the numerical value of the enthalpy of the first reaction indicates the proton affinity (PA) of the anion. On the other hand, the numerical value of the enthalpy of the second reaction is abstraction of

electron, whose name is electron transfer enthalpy (ETE). In calculating the parameters for the anti-oxidant activities of the molecule, the enthalpies of the reactions are calculated as follows:

$$BDE = H(A^{\cdot}) + H(H^{\cdot}) - H(AH) \quad (6)$$

$$IP = H(AH^{+\cdot}) + H(e^{-}) - H(AH) \quad (7)$$

$$PDE = H(A^{\cdot}) + H(H^{+}) - H(AH^{+\cdot}) \quad (8)$$

$$PA = H(A^{-}) + H(H^{+}) - H(AH) \quad (9)$$

$$ETE = H(A^{\cdot}) + H(e^{-}) - H(A^{-}) \quad (10)$$

Gaussian software program is used to calculate the enthalpies of the reactions in the calculation of the anti-oxidant activities of the molecule. For these calculations, the necessary parameters were found by making calculations in both the gas phase and the water phase in the basis set of Hartree-Fock (HF) [43] with 6-31 ++ G (d, p).

3. Results and Discussion

3.1. Cytotoxicity and anti-human glioma cancer potentials of Neobavaisoflavone

The MTT assay is a procedure of colorimetric based on reducing and breaking of yellow tetrazolium crystals by the enzyme succinate dehydrogenase to form insoluble purple crystals. In this method, unlike other methods, the steps of washing and collecting cells, which often cause the loss of a number of cells and increase the work error, have been eliminated and all test steps from the beginning of cell culture to reading the results with a photometer are performed on a microplate, so the repeatability, accuracy and sensitivity of the test are high [20,21]. If the test is performed on cells attached to the plate, an appropriate number of cells (about 2,000 cells) must first be cultured in each of the wells. Then we select the control and test wells and add the appropriate amount of mitogen or drug to the test wells and place the plate in the incubator for the required time so that the desired substance affects the cells [20-22]. At the end of the incubation time, discard the supernatant and add 200 µl of culture medium containing half an mg/ml of MTT solution to each well and put it again in a carbon dioxide incubator for 2 to 4 hours at 37 ° C. During incubation, MTT is regenerated by one of the enzymes of the mitochondrial respiratory cycle i.e., succinate dehydrogenase. The regeneration and breakage of this ring produce purple-blue crystals of

formazan that are easily detectable under a microscope. At the end, the optical absorption of the resulting solution can be read at 570 nm and the cells number can be calculated using a standard curve [22-24].

In the current research, the cytotoxicity of Neobavaisoflavone was explored by studying its interaction with normal (HUVEC) and common human glioma cancer cell lines, i.e. LN-229, U-87, and A-172 cell lines by MTT assay for 48h. In this study, four cell lines which have been shown in Figure 1.

In all cases, the % cell viability gets reduced with increasing Neobavaisoflavone concentrations. The IC_{50} values of Neobavaisoflavone against LN-229, U-87, and A-172 cell lines were found 166, 204, and 313 µg/mL, respectively (Table 1). Thus, the best cytotoxicity results and anti-human glioma cancer potentials of our Neobavaisoflavone were observed in the case of the LN-229 cell line.

Oxidation from reactive oxygen species can cause cell membrane disintegration, damage to membrane proteins, and DNA mutation that the result is the onset or exacerbation of many diseases such as cancer, liver damage, and cardiovascular disease. Although the body has a defense system, constant exposure to chemicals and contaminants can lead to an increase in the number of free radicals outside the body's defense capacity and irreversible oxidative damage [44,45]. Therefore, antioxidants with the property of removing free radicals play an important role in the prevention or treatment of oxidation-related diseases or free radicals. Extensive molecular cell research on cancer cells has developed a targeted approach to the biochemical prevention of cancers that the goal is to stop or return cells to their pre-cancerous state without any toxic doses through nutrients and drugs. Numerous studies have been performed on the use of natural compounds as anti-cancer agents in relation to appropriate antioxidant activity [44-46]. It seems the high anti-lung adenocarcinoma properties of Neobavaisoflavone are related to its antioxidant activities. Our successful efforts to utilize Neobavaisoflavone in adenocarcinoma studies certainly shed light on future studies in this area.

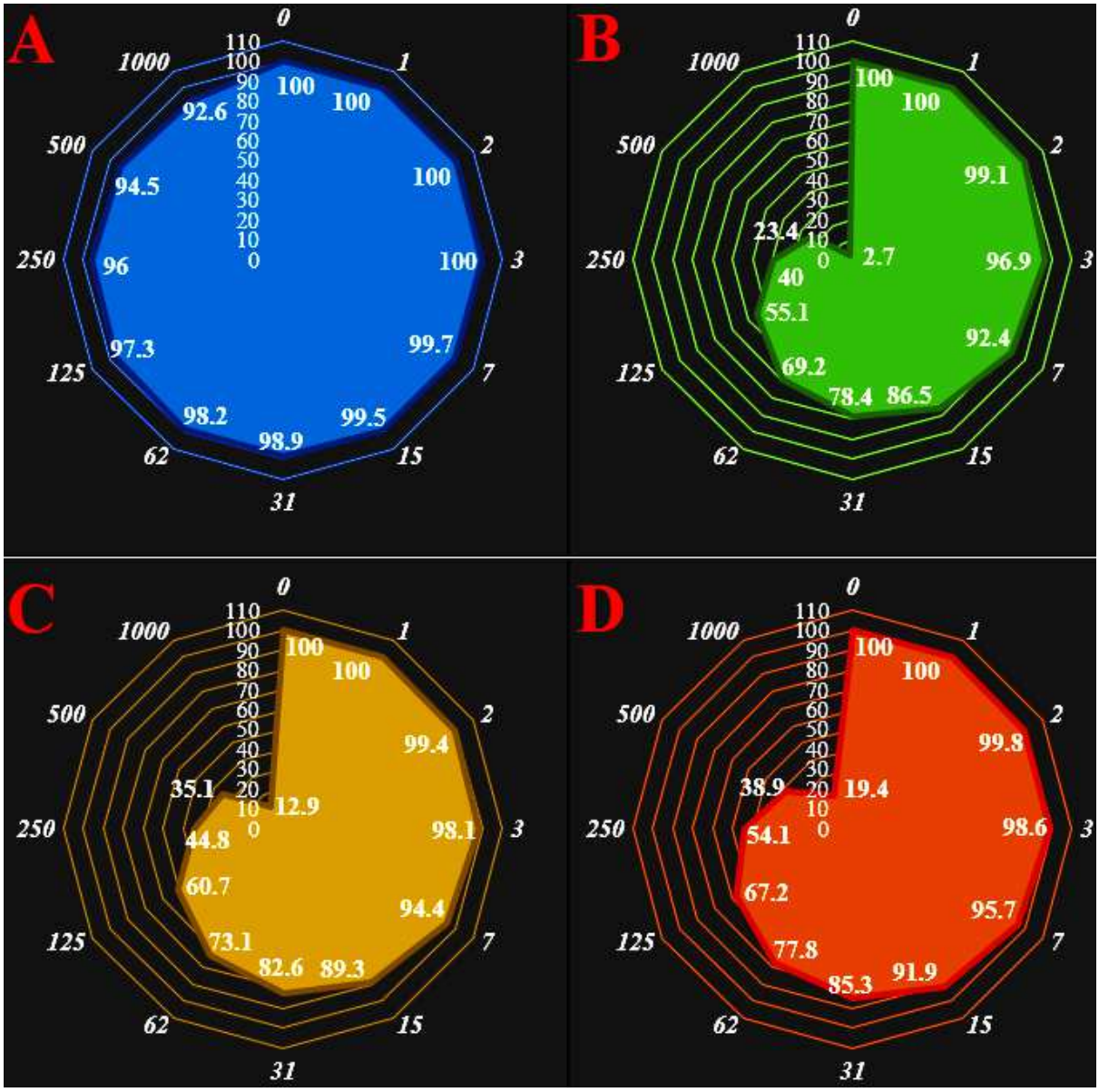

Fig. 1. The anti-human glioma cancer properties (Cell viability (%)) of Neobavaisoflavone (Concentrations of 0-1000 µg/mL) against normal (HUVEC: A) and common glioma cancer cell lines, i.e. LN-229 (A), U-87 (B), and A-172 (C) cell lines.

The numbers indicate the percent of cell viability in the concentrations of 0-1000 µg/mL of Neobavaisoflavone against several human glioma cancer cell lines.

Table 1. The IC_{50} of Neobavaisoflavone in the anti-human glioma cancer test.

	LN-229	**U-87**	**A-172**
IC_{50} (µg/mL)	166	204	313

3.2. Antioxidant capacities of Neobavaisoflavone

In this study, we assessed the antioxidant properties of Neobavaisoflavone by using the DPPH test as a common free radical. Free radicals are atoms, molecules, or ions with unpaired electrons and are therefore very active, unstable, and highly reactive. Free radicals are formed by breaking a bond of a stable molecule. Free radicals collide with other molecules to achieve stability and can separate electrons from them, as a result, they form a chain of more unstable molecules. A free radical can have a positive, negative or neutral charge [25,26]. During the body's natural metabolism or under conditions such as smoking, pollution, the entry of unnecessary chemicals into the body in any way, radiation and stress in the body produce free radicals. The most important free radical in the human body is oxygen, which can damage DNA and other molecules. Oxidative stress is the victory of free radicals over the body's antioxidant defense and is a biological attack on the body [47,48]. Antioxidants are molecules that can donate an electron to a free radical without destabilizing themselves. This stabilizes the free radical and makes it less reactive. The result of oxidative stress in the body is various degeneration, eye damage, premature aging, muscle problems, brain damage, heart failure, diabetes, cancer, and overall weakness of the immune system [25-28]. Oxygen radicals are continuously produced in all living organisms and with destructive effects, lead to cell damage and death. The production of oxidant species under physiological conditions has a controlled rate, but this production increases under oxidative conditions [26,27]. Various studies have shown that antioxidant compounds have very significant anti-cancer effects with omitting the free radicals. Herbs are rich in antioxidant compounds and reduce the risk of some chronic diseases such as cataracts, rheumatoid arthritis, memory loss, stroke, heart disease, and cancer by protecting cells and increasing the power of plasma antioxidants. Flavonoids and alkaloids commonly found in medicinal plants have high antioxidant activity [47,48].

In the recent study, the interaction between Neobavaisoflavone and DPPH might have occurred by transferring electrons and hydrogen ions [49]. The scavenging capacity of the Neobavaisoflavone and BHT at different concentrations, expressed in terms of percentage inhibition, has been shown in Fig. 2. In the antioxidant test, the IC50 of butylated hydroxytoluene and Neobavaisoflavone were 137 and 125 µg/mL, respectively (Table 2).

Antioxidant compounds show higher antioxidant effects against free radicals formation into the living system [50-57]. The antioxidant compounds have excellent redox properties and have a significant role in free radicals deactivating [51]. Previous researches have indicated that flavonoids and phenolic compounds have significant antioxidant properties [52].

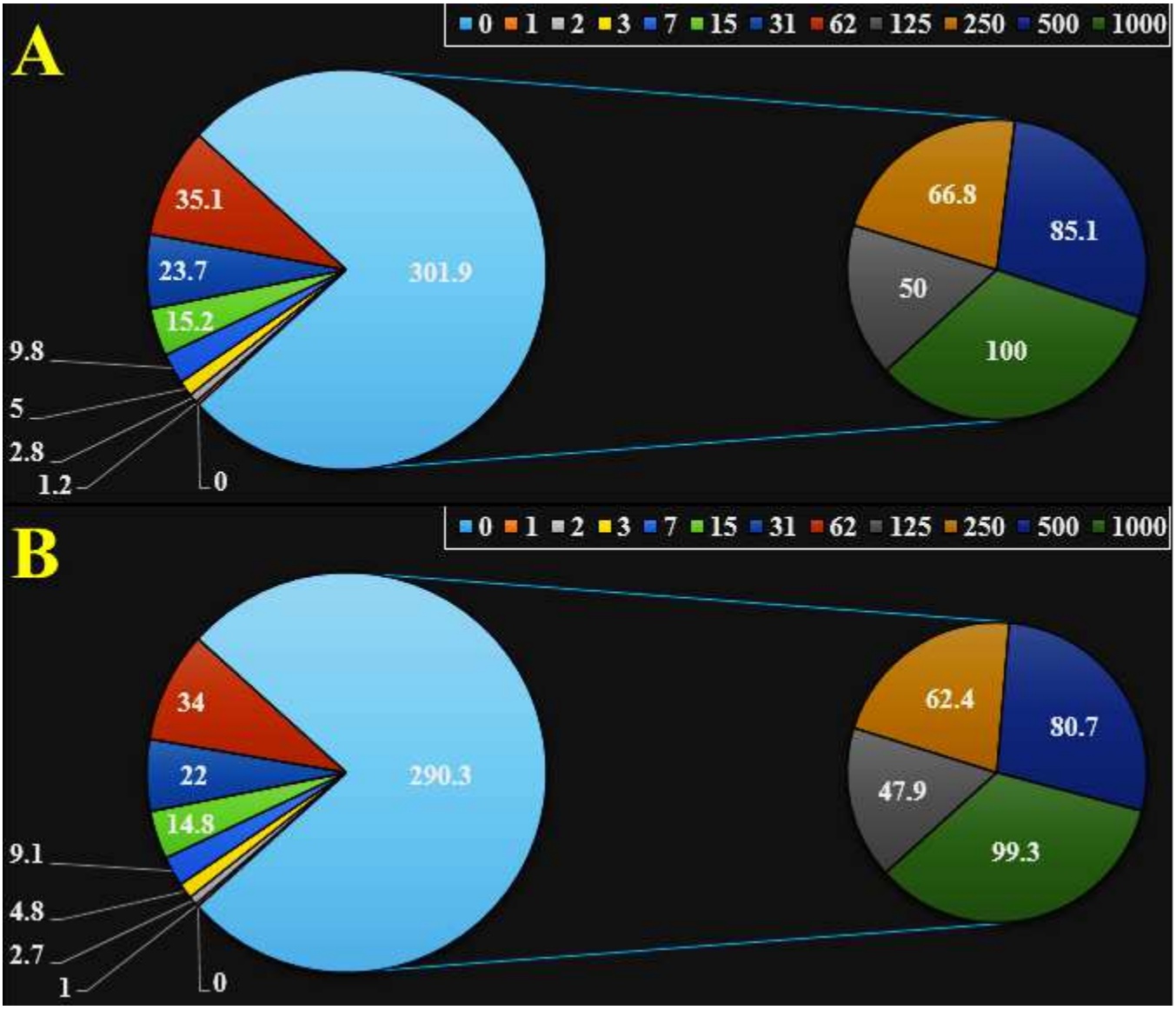

Fig. 2. The antioxidant properties of Neobavaisoflavone (A) and BHT (B) against DPPH.

The numbers indicate the percent of free radical (DPPH) inhibition in the concentrations of 0-1000 µg/mL of Neobavaisoflavone (A) and BHT (B).

Table 2. The IC_{50} of Neobavaisoflavone and BHT in the antioxidant test.

	Neobavaisoflavone	BHT
IC_{50} (µg/mL)	125	137

3.3. Enzymes Results

Neobavaisoflavone was effective inhibiting AChE as metabolic enzyme. K_i values for AChE were obtained to be 2.08±0.31 nM (Table 3). Also, the Tacrine (TAC) molecule was used as AChE enzyme control molecule; it had K_i values of 7.35±1.07 nM. Neobavaisoflavone and TAC values IC_{50} were: Neobavaisoflavone (3.13 nM) < TAC (8.67 nM) for AChE. Additionally, Neobavaisoflavone and TAC values IC_{50} were: Neobavaisoflavone (76.24 nM) < TAC (19.58 nM) for BChE. The inhibitory compound AChE is a neurotoxic molecule capable of causing central, peripheral, or peripheral and central cholinergic crises. The molecule studied in the present study could be used as a pharmaceutical product for the treatment of myasthenia gravis and AD. On the other hand, Neobavaisoflavone shown as IC_{50} and K_i values are 65.58 nM, and 78.21±6.65 (Table 3).

Table 3. The enzyme inhibition results of Neobavaisoflavone against AChE and BChE enzymes

Compounds	IC_{50} (nM)		K_i (nM)	
	AChE	BuChE	AChE	BuChE
Neobavaisoflavone	3.13	76.24	2.08±0.31	135.03±26.38
Tacrine	8.67	19.58	7.35±1.07	15.92±2.84

3.4. Molecular modeling results

It is seen in the studies conducted in recent years that theoretical studies are becoming more popular day by day. Because the results obtained as a result of experimental and theoretical studies are in great harmony. These studies give direction to experimental studies. The obtained results of the theoretical calculations are used as the most

important guide for the synthesis of more effective and more active molecules. Accordingly, theoretical studies have become widespread, providing both time and material convenience to compare the biological activities of molecules. The most widely used molecular docking program is used in calculations for this [58]. As a result of the calculations made with this program, many parameters about the biological activity of the molecule were obtained. He compared the biological activities of molecules against enzymes with these parameters. The enzymes used are Butyrylcholinesterase (BChE) (5NN0), acetylcholinesterase (4M0E) (AChE), respectively.

Table 4. Numerical values of the docking parameters of molecule against enzymes

	AChE	**BChE**
Docking Score	-7.97	-3.97
Glide ligand efficiency	-0.33	-0.17
Glide hbond	-0.47	-0.46
Glide evdw	-33.98	-14.56
Glide ecoul	-7.69	-10.49
Glide emodel	-59.00	-31.17
Glide energy	-41.67	-25.05
Glide einternal	4.76	1.95
Glide posenum	374	307

It is used to compare the numerical values of these parameters obtained as a result of theoretical calculations and biological activities of molecules. These parameters are given in Table 4. The most important parameter among many parameters obtained for the neobavaisoflavone molecule as a result of molecular docking calculations is the Docking score parameter. because this parameter of the neobavaisoflavone molecule is the numerical value of the interactions between the neobavaisoflavone molecule and the enzyme. The numerical value of this parameter of the neobavaisoflavone molecule is the most negative, and the biological activity of the molecule is

higher than other molecules [59]. As the interaction between molecule and enzyme increases, the numerical value of this parameter decreases more. These interactions have many interactions such as hydrogen bonds, polar and hydrophobic interactions, π-π and halogen [60]. These interactions of molecule with enzymes are given in Figure 3 and 4.

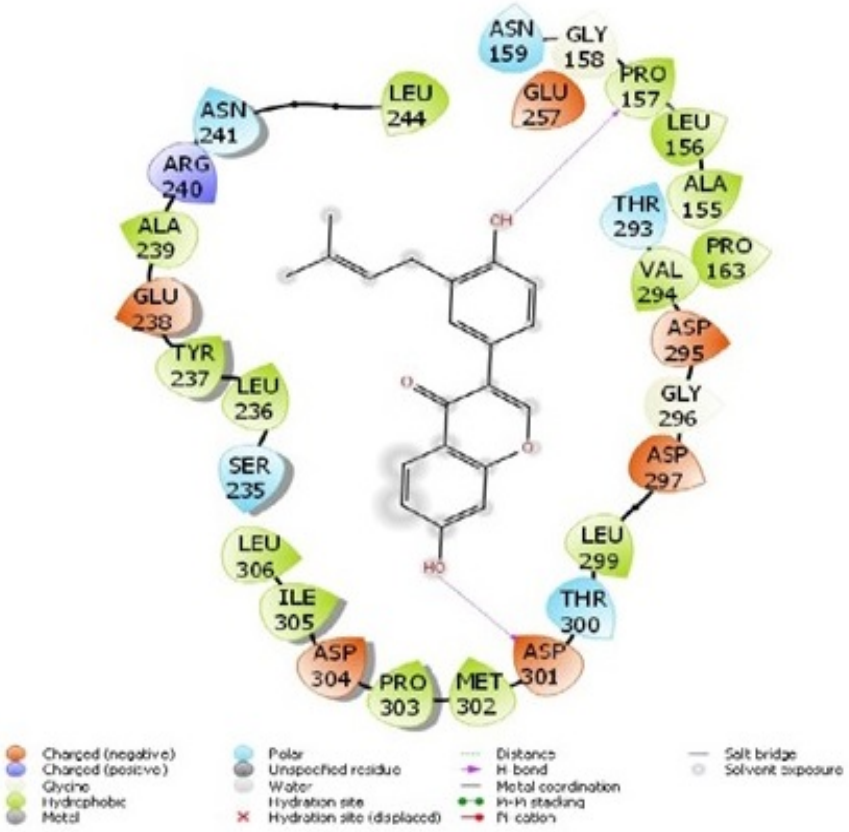

Figure 3. Presentation interactions of neobavaisoflavone with BChE enzyme.

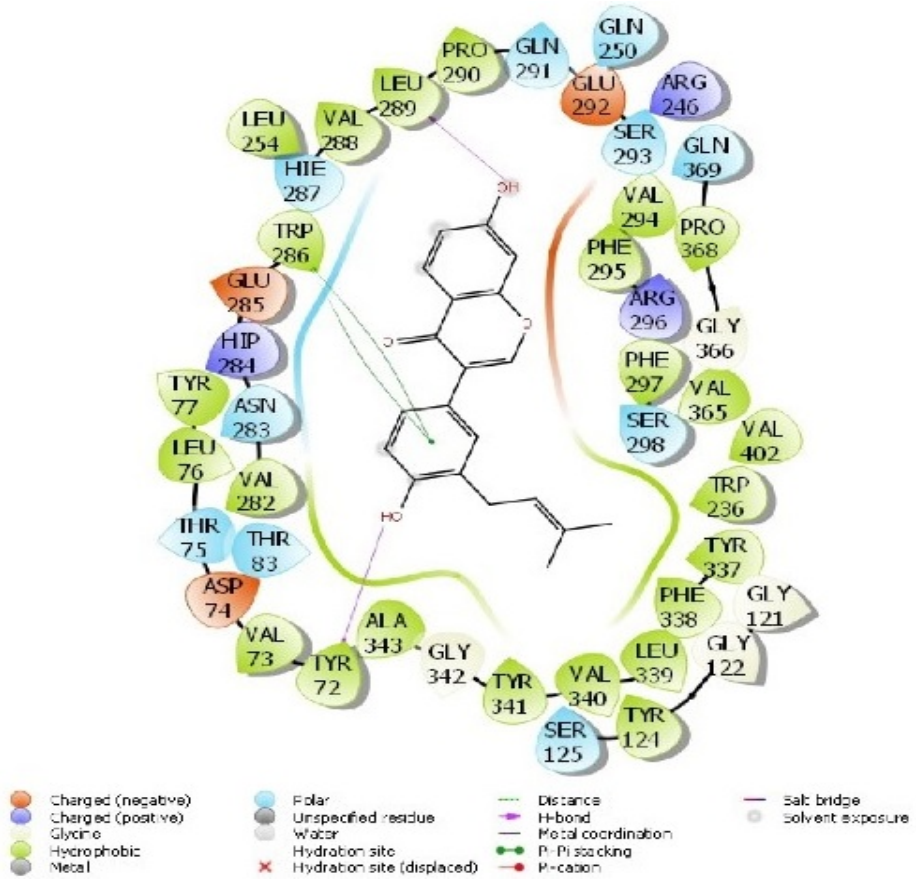

Figure 4. Presentation interactions of neobavaisoflavone with AChE enzyme

Many parameters obtained from docking calculations of neobavaisoflavone molecule were obtained. These parameters provide a lot of information about the biological activity of the molecule. The Glide hbond, Glide evdw, and Glide ecoul parameters of the neobavaisoflavone molecule provide information about the chemical interactions of the molecule with the enzyme. The Glide emodel, Glide energy, Glide einternal, and Glide posenum parameters of the neobavaisoflavone molecule provide information about the interaction pose that occurs in the interaction of the molecule with the enzyme. Detailed information about these parameters has been explained in detail in previous studies [61,62]. After comparing the biological activity of neobavaisoflavone molecule against enzymes, ADME/T analysis was performed to theoretically calculate the effects and responses of the neobavaisoflavone molecule on human metabolism [63-65].

For the anti-oxidant calculations of the neobavaisoflavone molecule, the calculations were made by removing the hydrogen in the OH bond in the molecule. The hydrogen atoms removed are shown in Figure 5. There are 2 OH bonds in the neobavaisoflavone molecule. One is oxygen atom 26 and the other is oxygen atom 16. The numerical

value of the BDE for the OH bond of the neobavaisoflavone molecule is a parameter related to the HAT mechanism of the neobavaisoflavone molecule. It was observed that the molecule with the numerical value of the smaller BDE parameter had higher radical-scavenging activity [66].

For the neobavaisoflavone molecule, in the first step of the SET – PT mechanism, the IP value of the neobavaisoflavone molecule is calculated. Calculations of molecules show that if a molecule has a lower IP value, it is known that the electron donation ability of the molecule is higher. For the neobavaisoflavone molecule, PDE values are calculated in the second step of the SET–PT mechanism. Calculations of the molecules show that if a molecule has a lower PDE value, this means an easier reaction. In the last mechanism for anti-oxidant calculations, the SPLET mechanism for neobavaisoflavone molecule consists of two steps. In the first step, the PA parameter of the neobavaisoflavone molecule is calculated. Calculations of molecules show that if a molecule has a lower PA value, that molecule has a higher proton affinity. In the second step of SPLET, ETE parameter is calculated. Calculations of the molecules show that if a molecule has a lower ETE value, that molecule indicates an easier reaction [67].

Table 5. Anti-oxidant properties of molecule

in gas phase	BDE	IP	PDE	PA	ETE
O16-H	43.09	155.20	202.39	342.20	15.39
O26-H	40.89	155.20	200.19	282.30	73.09
in water phase					
O16-H	47.39	116.04	245.84	367.19	-5.30
O26-H	41.32	116.04	239.78	303.55	52.28

Parameters of neobavaisoflavone molecule anti-oxidant properties are given in Table 5. The parameters given in this table were calculated for the neobavaisoflavone molecule both in the gas phase and in the water phase. In the

calculations made, two O-H bonds were calculated for the neobavaisoflavone molecule. As a result, it was observed that the anti-oxidant activity of the molecule from which the O-16 hydrogen was removed was higher. The most important reason for this is that the phenolate anion formed in both the gas phase and the solvent phase is more stable [68].

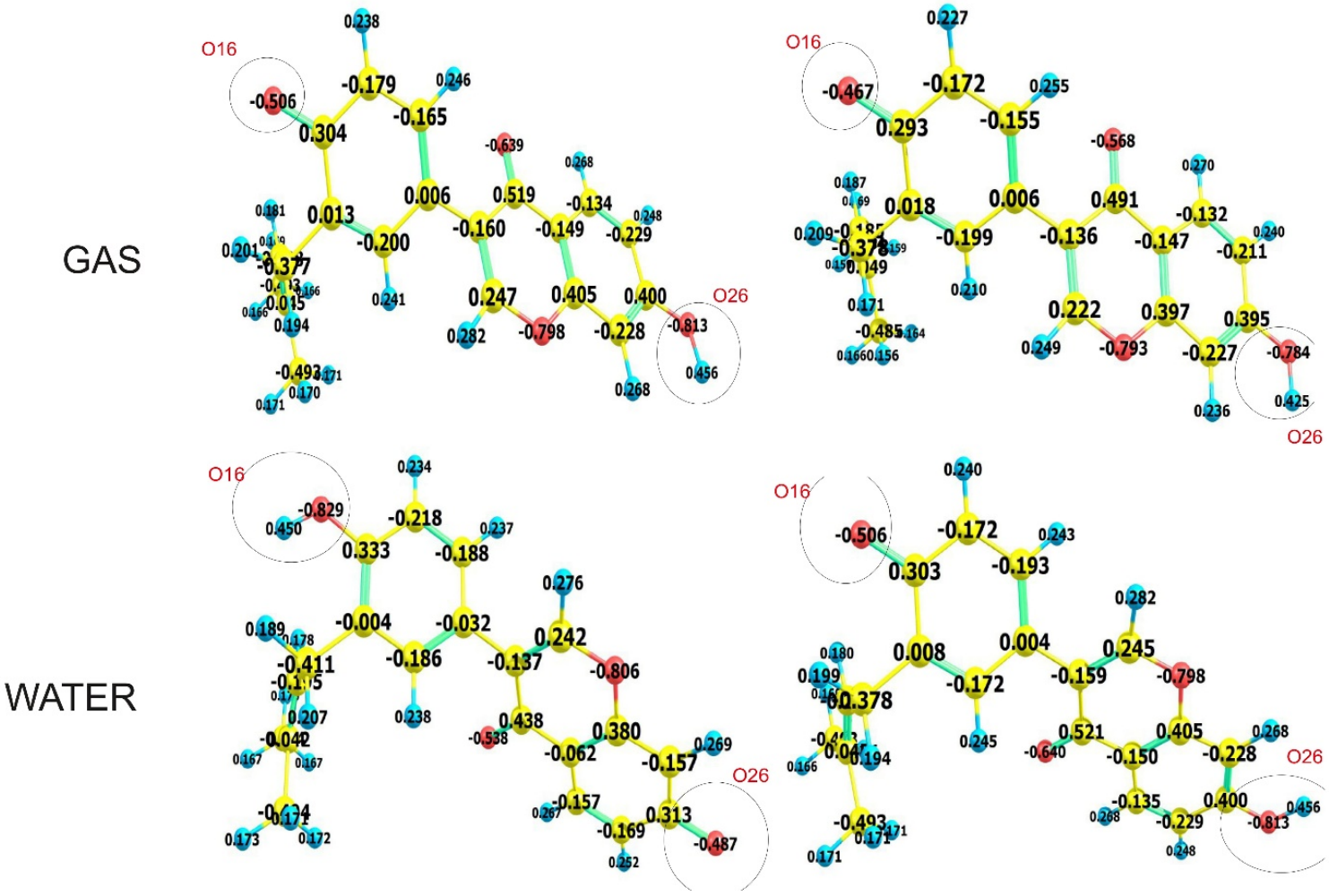

Figure 5. Spin density distributions of molecule

As a result of the anti-oxidant calculations, spin densities were calculated to examine the charge density of the neobavaisoflavone molecule. The numerical values of the spin densities of the molecule are given on the atoms in figure 5. The lower the spin density shown on the atoms in the molecule indicates more delocalization. All calculations for the neobavaisoflavone molecule show that higher delocalization means easier radical formation.

In this direction, the fact that it is more radical affects the stability of the radical and causes the formation of more stable radicals [69,70].

4. Conclusions

Neobavaisoflavone was also assessed in biological applications like radical scavenging and anticancer activities. Neobavaisoflavone showed significant cytotoxic activities against common human glioma cancer cell lines, i.e. LN-229, U-87, and A-172 cell lines. The IC_{50} values of Neobavaisoflavone against LN-229, U-87, and A-172 cell lines were found 166, 204, and 313 μg/mL, respectively. Neobavaisoflavone also exhibited good antioxidant properties, even better than the reference standard molecule. In the antioxidant test, the IC50 of butylated hydroxytoluene and Neobavaisoflavone were 137 and 125 μg/mL, respectively. Likely significant anti-human glioma cancer potentials of Neobavaisoflavone against common human glioma cancer cell lines are linked to their antioxidant activities. Biological activities of neobavaisoflavone molecule against enzymes were compared. Afterwards, a theoretical ADME/T analysis of these molecules was performed. As a result of these analyzes, it was examined with many parameters. Then, anti-oxidant activity values of neobavaisoflavone molecule were calculated. As a result of these calculations, many parameters were calculated for the neobavaisoflavone molecule. The values of these parameters in the gas phase and the water phase are compared. When the numerical values of all parameters calculated as a result of theoretical calculations are examined, they can be used in future in vivo and in vitro studies and can be used in the discovery of new drug candidates.

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***Declarations**

Availability of data and material (data transparency): N/A

Code availability (software application or custom code): N/A

Authors' contributions: All authors have had a same role in preparing, designing, doing experiments, analyzing, writing, and submitting the recent manuscript.

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Figures

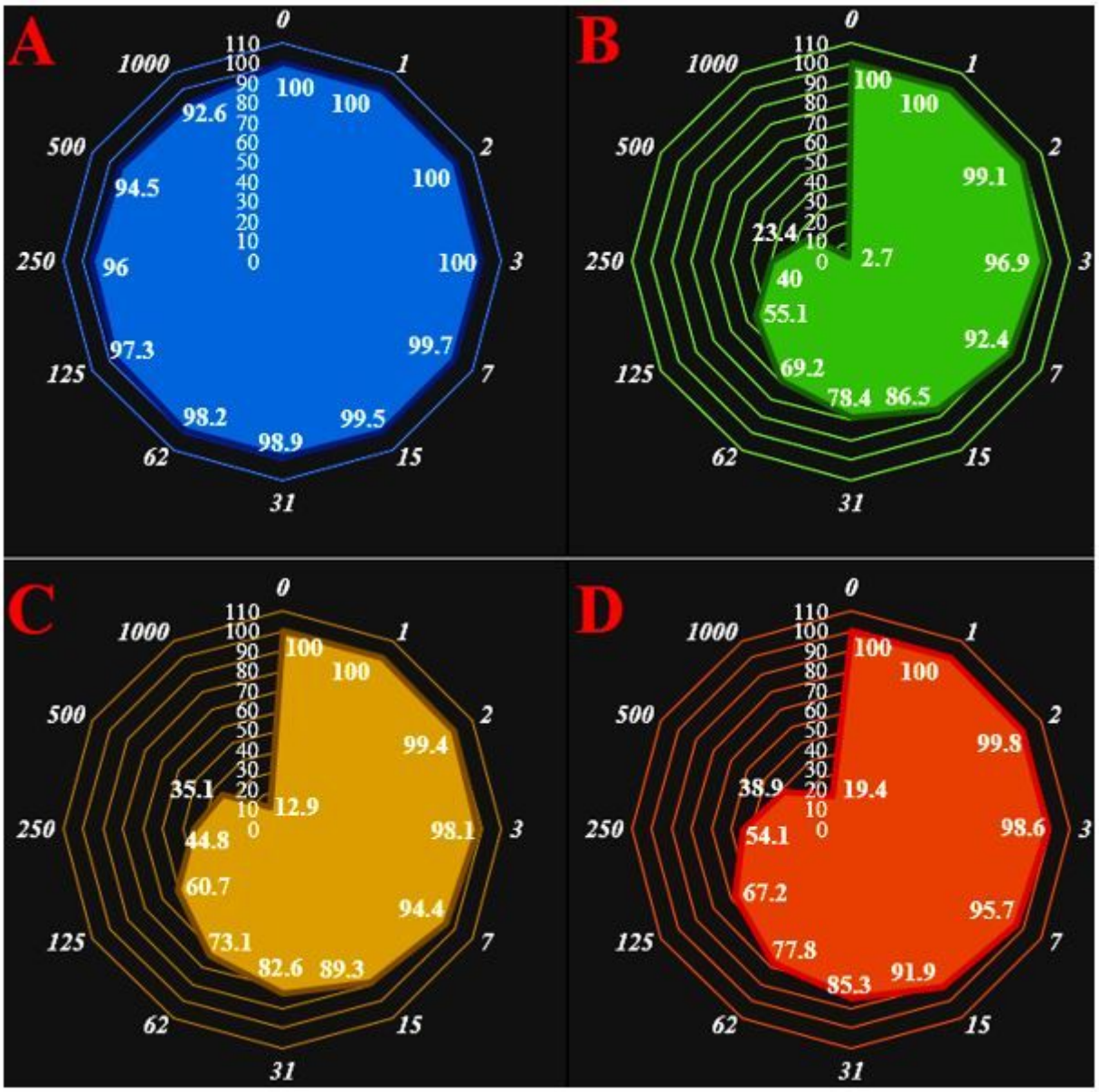

Figure 1

The anti-human glioma cancer properties (Cell viability (%)) of Neobavaisoflavone (Concentrations of 0-1000 µg/mL) against normal (HUVEC: A) and common glioma cancer cell lines, i.e. LN-229 (A), U-87 (B), and A-172 (C) cell lines. The numbers indicate the percent of cell viability in the concentrations of 0-1000 µg/mL of Neobavaisoflavone against several human glioma cancer cell lines.

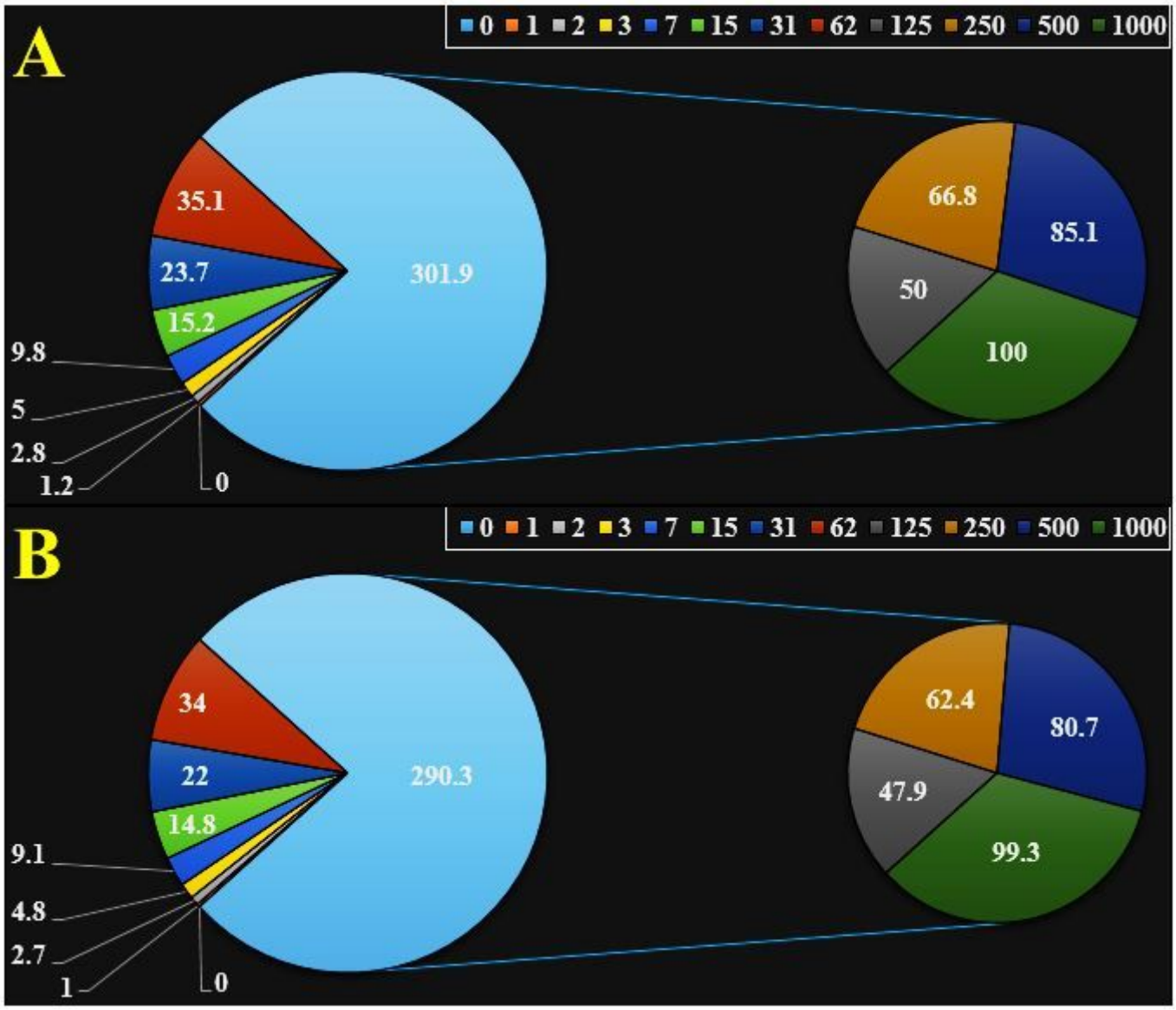

Figure 2

The antioxidant properties of Neobavaisoflavone (A) and BHT (B) against DPPH. The numbers indicate the percent of free radical (DPPH) inhibition in the concentrations of 0-1000 µg/mL of Neobavaisoflavone (A) and BHT (B).

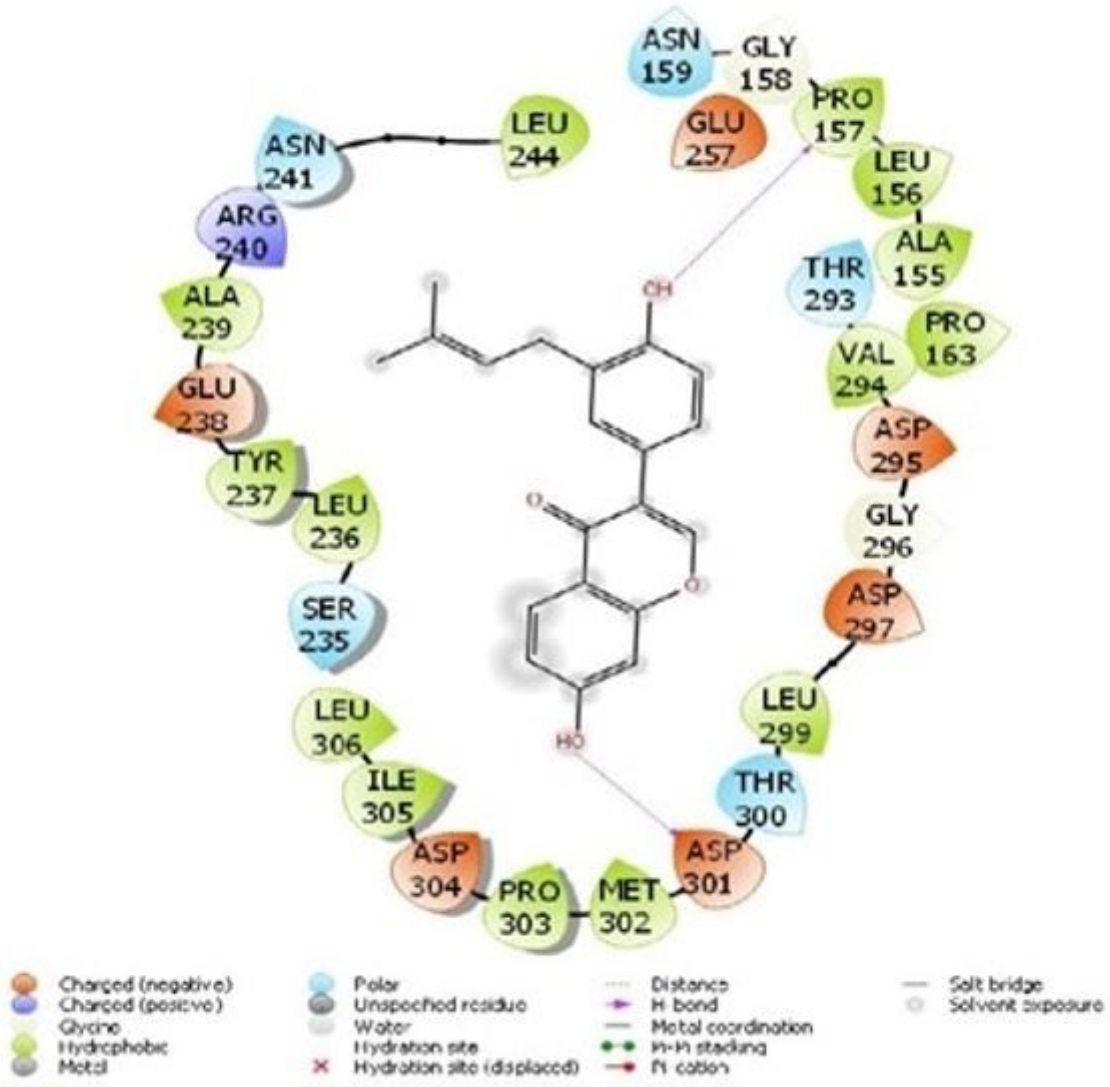

Figure 3

Presentation interactions of neobavaisoflavone with BChE enzyme.

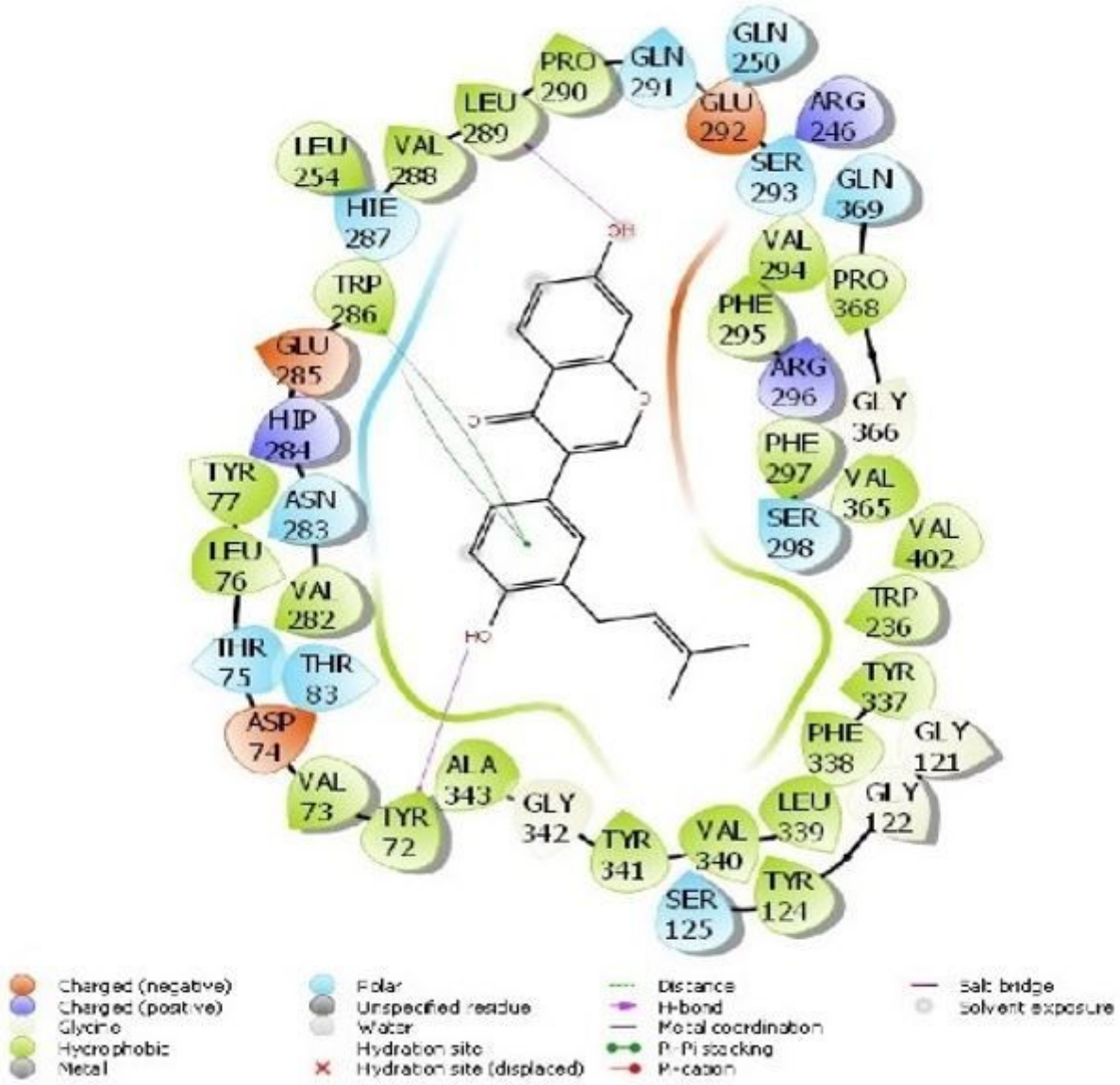

Figure 4

Presentation interactions of neobavaisoflavone with AChE enzyme

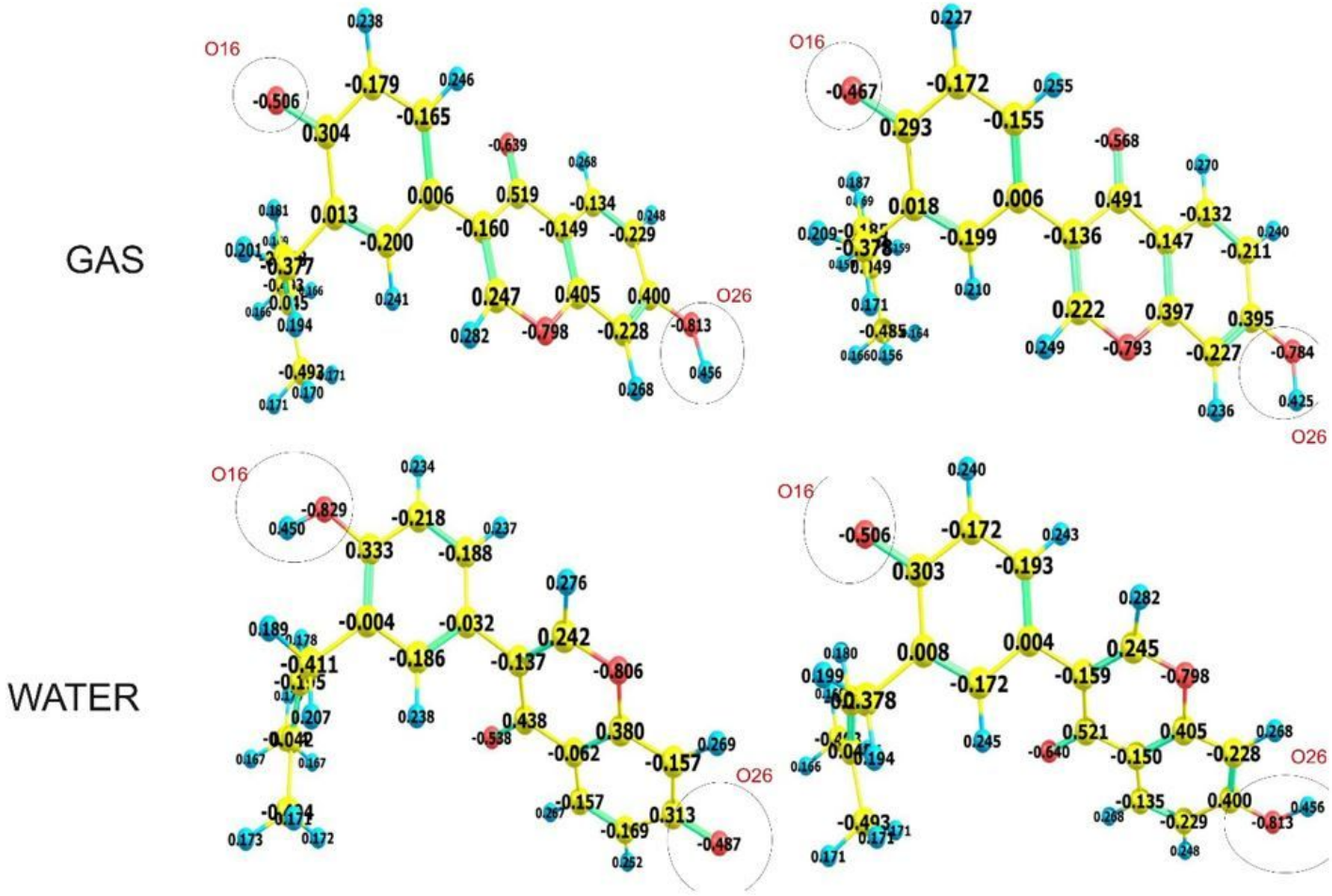

Figure 5

Spin density distributions of molecule