ACTA SCIENTIFIC MEDICAL SCIENCES

Volume 3 Issue 2 February 2019

Research Article

Investigating Alzheimer's Disease by Studying Interaction between Acetylcholinesterase Enzyme (AChE) and Different Inhibitors including Salvation Parameter with Molecular Docking

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Received: November 12, 2018; **Published:** January 07, 2019

Abstract

Molecular modelling is a powerful and versatile toolbox that complements experimental data and provides insights where direct observation is not currently possible. This theoretical approach enables to predict the mode of interaction of a ligand with its receptor. The inhibition of enzyme Acetylcholinesterase (AChE) is an important approach in the treatment of Alzheimer's disease (AD). In this work we studied molecular interaction between Acetylcholinesterase enzyme and natural compounds from *Nigella* (p-Cymene, Thymoquinone, Carvacrol, α-Pinene, β-Pinene, Limonene…). The presence of water molecules plays an important role in the accuracy of complexes docking predictions by introduction of bulky groups causing a conformational rearrangement in the active site pocket. According to obtained results study inhibition of Acetylcholinesterase (AChE) by molecular modeling methods has been elucidated, We have observed a statistically significant overall increase in accuracy when water molecules are included during docking simulations which allows us to conclude that natural compounds from *Nigella* present better interaction of Acetylcholinesterase(AChE) Enzyme in presence of water molecules while docking and consequently can be the best inhibitor candidate to be *in vitro* and *in vivo* investigated.

Keywords: Alzheimer's Disease (AD); Acetylcholinesterase (AChE); Solvatation; Molecular Operating Environment (MOE)

Introduction

Nigella sativa (*N. sativa*) belongs to the botanical family of Ranunculaceae and commonly grows in the Eastern Europe, Middle East, and Western Asia. It is a small shrub with tapering green leaves and rosaceous white and purplish flowers. Its ripe fruit contains tiny seeds, dark black in color, known as "Habba Al-Sauda" or "Habba Al-Barakah" in Arabic and black seed in English. The seed and oil of *N. sativa* were frequently used in ancient remedies (Unani, Ayurveda, Chinese and Arabic) in Asian countries and in the middle east. The seeds of *N. sativa* have been subjected to a range of pharmacological investigations in recent years. These studies have showed a wide spectrum of activities such as antibacterial [1] antitumor [2], anti-inflammatory [3], CNS depressant and analgesic [4], hypoglycemic [5], smooth muscles relaxant, cytotoxic and immunostimulant. *N. sativa* seeds would present a potent and therapeutically interesting activity on the cardiovascular, respiratory, immune, and endocrine systems. Some of these activities have been predominantly attributed to the volatile and fixed oils. In best of our knowledge no studies on interaction between natural molecules of *Nigella* (p-Cymene, Thymoquinone, Carvacrol, α-Pinene, β-Pinene, Limonene…) and Acetylcholinesterase (ACHE) Enzyme have been done. The purpose of this study is to minimize energy of formed complex and consequently to delay its progression. In order to rationalize the properties of the inhibitors and to determine the reaction processes involving this compound. Investigating obtained results will allow development of an effective therapeutic agent for Alzheimer disease (AD) treatment.

Nigella is a genus of 18 species of annual plants in the family of Ranunculaceae, native to southern Europe, north Africa, south and southwest Asia. Common names applied to members of this genus are *Nigella*, devil-in-a-bush or love-in-a-mist. The species grow to

20–90 cm tall, with finely divided leaves; the leaf segments are narrowly linear to threadlike. The flowers are white, yellow, pink, pale blue or pale purple, with five to 10 petals. The fruit is a capsule composed of several united follicles, each containing numerous seeds; in some species (e.g. Nigella damascena), the capsule is large and inflated. The *Nigella Sativ*a, and its various other names: Herb with Spices, Hair of Venus, Beard of the Capuchins, Spider's Feet, Goat, Angel's Hair. Black cumin seeds are also called "pepper". The presence of thymohydroquinone, thymol and oxidation products of thymoquinone is also reported. The proportion of compounds can vary considerably depending on the place of harvest. Thus, an Austrian study gives the following analysis: p-cymene (38%), thymoquinone (30%), carvacrol (5-11%), α-pinene (5-14%), β-pinene (5%), limonene (4%). The main alkaloids extracted from the seeds are: nigellicin (with an indazole), nigellimine (an isoquinoline) and its derivative nigellimine N-oxide, *Nigellidine* (an indazole).

Materials and Methods

Acetylcholinesterase enzyme (ACHE)

Acetylcholinesterase (HGNC symbol ACHE), also known as AChE or acetylhydrolase, is the primary cholinesterase in the body. It is an enzyme which catalyzes the breakdown of acetylcholine and some of other choline esters functioning as neurotransmitters. AChE is found in the mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides. The structure and mechanism of action of AChE have been elucidated from the crystal structure of the enzyme. Natural studied compounds from *Nigella* in this work are given table 1.

Molecule	Name	IUPAC name	Pub ChemCID	Molar mass g/mol	Formula
1	Nigellicine	3-methyl-1-oxo-6,7,8,9-tetrahydropyridazino[1,2-a]indazole-1...	11402337	246.2619	$C_{13}H_{14}N_2O_3$
2	Nigellidine	11-(4-hydroxyphenyl)-3-methyl-6H,7H,8H,9H-10λ-pyridazino[1,2-a]indazol-10-ylium-1-olate	101253695	294.354	$C_{18}H_{18}N_2O_2$
3	Nigellimine	6,7-dimethoxyisoquinoline	20725	203.241	$C_{12}H_{13}NO_2$
4	Thymoquinone	2-Isopropyl-5-methylbenzo-1,4-quinone	10281	164.20	$C_{10}H_{12}O_2$
5	Dithymoquinone	Dithymoquinone; Nigellone	398941	328.408	$C_{20}H_{24}O_4$
6	Thymohydroquinone	2-methyl-5-propan-2-ylbenzene-1,4-diol	95779	166.220	$C_{10}H_{14}O_2$
7	Carvacrol	2-methyl-5-propan-2-ylphenol	10364	150.221	$C_{10}H_{14}O$
8	α- Thujen	4-methyl-1-propan-2-ylbicyclo[3.1.0]hex-3-ene	17868	136.2380	$C_{10}H_{16}$
9	p- Cymen	2-(4-methylphenyl)propan-2-ol	14529	150.221	$C_{10}H_{14}O$

Table 1: Physico - Chemical properties of inhibitors Naturel essential oil *Nigella Sativa for* Acetylcholinesterase (AChE).

Preparation and optimization of both enzyme and inhibitors

Download of Acetylcholinesterase (AChE) was done from PROTEIN DATA BANK under 4TVK code with three-dimensional structure obtained by X-ray diffraction under 2.3 Å resolutions. We note that Acetylcholinesterase crystallizes as a monomer (Figure 1) with 534 residues and 8377 atoms. Compounds structures were downloaded from PubChem data base. Using MOE software (Molecular operating environment) [6] we selected the active site in the enzyme and we minimized the energy of both enzyme and molecules. Energy minimizing was done under following conditions: Temperature = 300°K, pH = 7, the geometry was performed using the field strengths in the MMFF94x implanted in MOE and Hamiltonian AM1. All simulation was run using all explicit solvation model using TIP3P water molecules. Figure 2 shows the active site of the enzyme with molecule of co-crystallization. Minimized energy of ligands and their toxicity are obtained by MOE software (Table 2 and 3).

Docking and building complexes

The next step, after the construction of the ligand, is the positioning of this molecule in the active site of Acetylcholinesterase (AChE). For this, we used the Molecular Docking Module using MOE software [6]. Once the ligand -receptor complex is formed, it will adapt the most stable conformation, i.e. the lowest energy

level. The purpose of the Dock application is looking at favorable conformational binding between medium size ligands and a not so soft macromolecular target, which is usually a protein [7]. For each compound, a number of conformations called poses were generated to identify favorable binding modes. The search for binding modes is generally constrained to a small specific region of the receptor called the active site

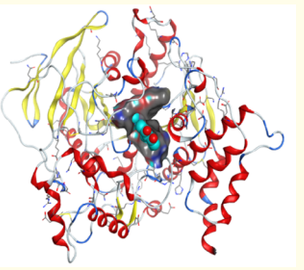
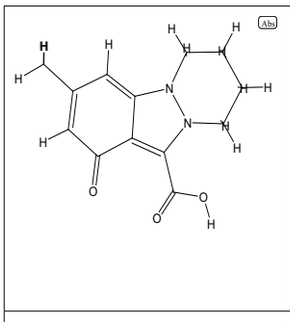
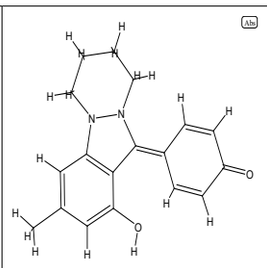
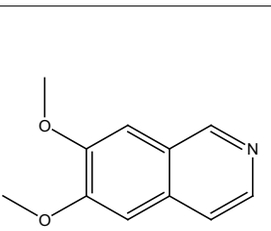
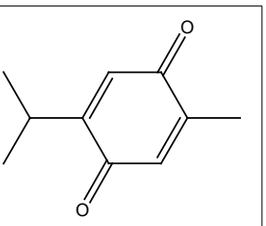


Figure 1: Simplified model of (AChE).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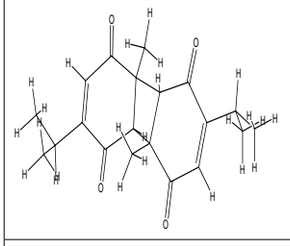
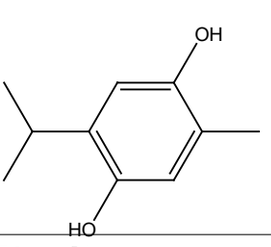
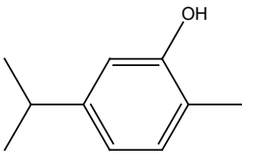
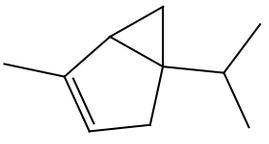
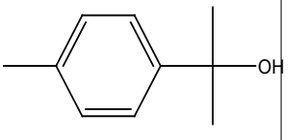
			
Ligand5		**Ligand6**	**Ligand7**
	-Hederin 		
	Ligand8	**Ligand9**	

Table 2: Natural inhibitors of Nigella used for Acetylcholinesterase (AChE) Enzyme.

Molecule	Name	Energies Kcal/mol eIUPAC name	LogP	LogS	Toxcicity
1	Nigellicine	8.03966e + 001	-0.27	-2.45	No
2	Nigellidine	1.01257e + 002	2.94	-3.71	No
3	Nigellimine	5.98134e + 001	2.56	-2.42	No
4	Thymoquinone	1.59109e + 001	1.67	-2.48	No
5	Dithymoquinone	3.74537e + 001	2.71	-3.90	No
6	Thymohydroquinone	2.32472e + 001	2.53	-2.01	No
7	Carvacrol	2.31484e + 001	2.85	-2.69	No
8	α- Thujen	2.52974e + 001	3.00	-3.44	No
9	p-Cymen	3.18855e + 001	2.53	-2.28	No

Table 3: Minimization energy of molecules Naturel For Alzheimer's disease (AD (Kcal/mol)

Log S: aqueous solubility and intestinal permeability

Log P: distribution coefficient.

These ligands are able to present a very important biological activity in accordance with the rule of Lipinski [7].

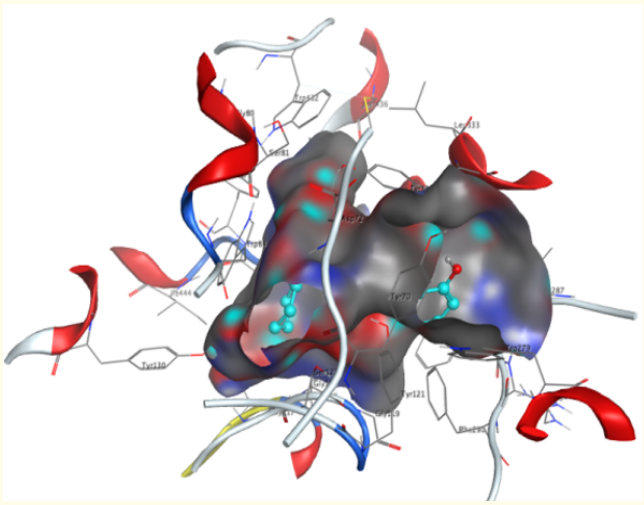


Figure 2: Site active enzyme isolated.

Results and Discussion

The results given in table 4 show that the orientation of the ligands plays a significant role for the positioning of the ligands in the active site of the enzyme, one can conclude that the introduction of bulky groups causes a rearrangement of conformation inside the cavity of the active site, which will be probably the complementarity and consequently the activity [8]. 2D molecular method of the screen has been attributed to the MOE (Molecular Operating Environment) software, which is designed to visualize the active sites of the complex (protein-ligand). The ligand is prepared and made with an improved 2D depiction layout algorithm, and protein residues version are arranged around it to indicate links spatial proximity [9]. Residues are marked with their amino acid code of 3 letters and job classification [10,11]. If there are multiple channels in the system, the positions are prefixed by the letters of the alphabet. Interactions between 2.5 Å and 3.1 Å are considered high and those between 3.1Å and 3.55Å are average. Greater than 3.55Å interactions are weak [12].

Mol	Score	Rmsd-refine	E-Conf	E-PLACE	E-SCORE1	E-REFINE	E-SCORE2
Complexe-1	-6.78561735	1.30849493	12.9737148	-77.220657	-9.9600639	-16.87837	-6.7856173
Complexe-2	-7.52602959	1.37920856	97.1041718	-71.721534	-13.945726	-16.53611	-7.5260295
Complexe-3	-6.08389187	1.42924833	49.4912567	-56.498245	-10.600860	-15.95948	-6.0838918
Complexe-4	-5.60303068	2.1676228	-5.19400358	-51.853408	-9.2292404	-17.54528	-5.6030306
Complexe-5	-5.06346941	5.43564606	-14.6155539	-52.9237709	-10.046505	-17.12595	-5.0634694
Complexe-6	-5.32762527	0.960144699	4.01593685	-54.491630	-11.006382	-9.434912	-5.3276252
Complexe-7	-5.46242237	0.8411659	10.9649925	-46.293476	-9.8267812	-10.36590	-5.4624223
Complexe-8	-4.91856861	0.627365947	36.0715103	-37.008800	-7.5579762	-8.324706	-4.9185686
Complexe-9	-5.17525864	2.06625247	27.5648022	-48.836212	-8.3772211	-12.88771	-5.1752586

Table 4: Energy balance of complexes formed with – ACHE Without Water molecules (Kcal/mol).

S: The Final Score; is the score of the last step; rmsd refine: The mean square deviation between the laying before refinement and after refinement pose; E conf: Energy Conformer; E place: Score of the placement phase; E_scor1: Score the first step of notation; E refine: Score refinement step and number of conformations generated by ligand; E_scor2: Score the first step notation; number of poses: Number of conformations [9].

Docking without water molecules

Results given in table 4 (Figure 3a and 3b) show that the complex-2 has the lowest energy (-7.52602959 Kcal/mol) and is more active than complex -1 -6.78561735 Kcal/mol).

For complex 1

Nigellicine interacts with the amino acids [TYR 121 (A) H-acceptor O2 (OH); TRP 84 (A) pi-pi (5-ring,6-ring) [5-ring) and PHE 330 (A) pi-pi 6-ring at a distance of 3.24, 3.89, 3.84, 3.74 Å, respectively (for the 1st strong interaction, 2nd, 3th and

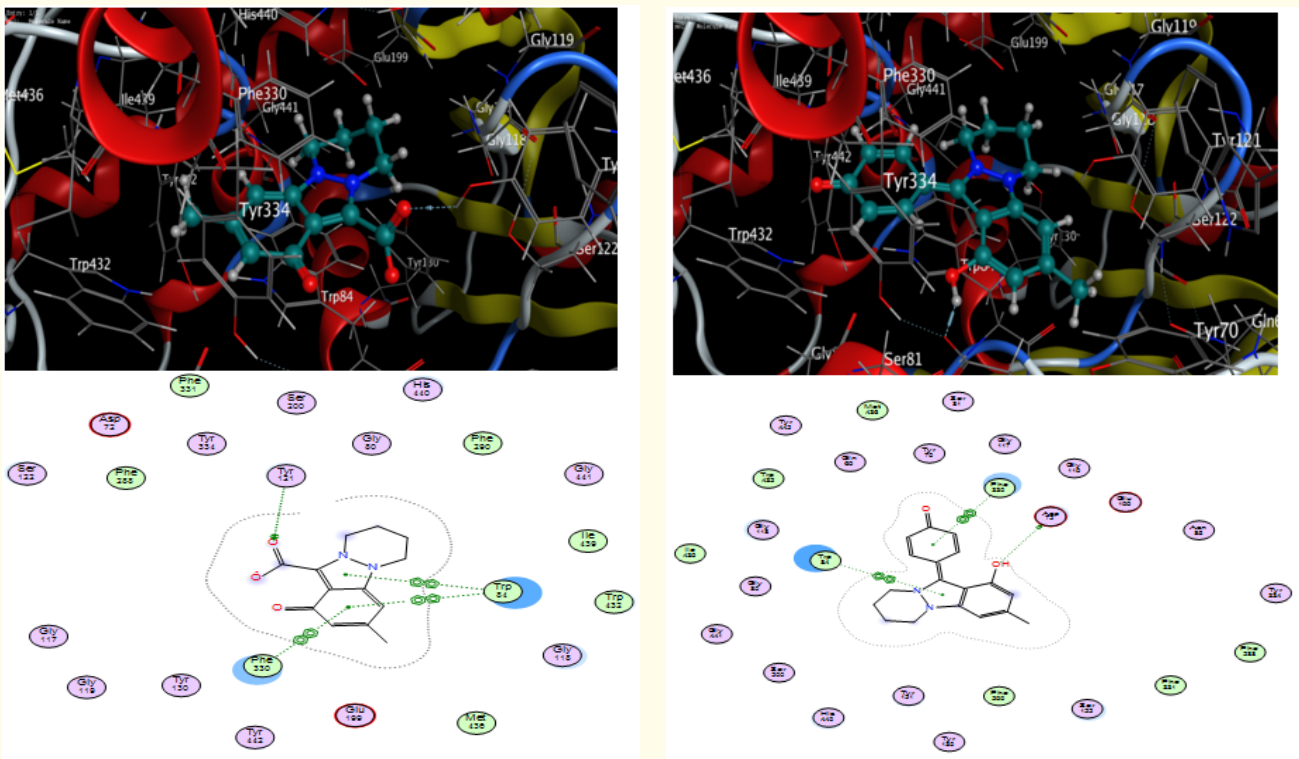


Figure a: Diagram interaction of complex-1 (AChE + Nigellicine)

Figure b: Diagram interaction of complex-2 (AChE + Nigellidine)

Figure 3: Diagrams of interactions (enzyme-ligands) 2 complexes (a,b).

4th weak interaction) this suggest that Nigellicine can inhibit Acetylcholinesterase (AChE). and interfere with [TYR 121 (A) H-acceptor O2 (OH); TRP 84 (A) pi-pi (5-ring,6-ring) [5-ring) and PHE 330 (A) pi-pi] [13].

For complex 2

Nigellidine interacts with the amino acids [ASP 72 (A) H-donor OD2 (O1); TRP 84 (A) pi-pi(5-ring); PHE 330(A) pi-pi(6-ring)] at a distance of 2.88, 3.85, 3.61 Å, respectively (for the 1st strong, 2nd, 3rd weak interaction, with the existence of three electric force PRO 84 wich suggesting that *Nigellidine* can inhibit Acetylcholinesterase (AChE) and interfere with [ASP 72 (A) H-donor OD2 (O1); TRP 84 (A) pi-pi(5-ring); PHE 330(A) pi-pi(6-ring)] [13].

Docking with water molecules

Results given in table 5 (Figure 4a and 4b) show that the complex-2 has the lowest energy -9.11354828 Kcal/mol) and is more active than complex -1 -7.56846952 Kcal/mol).

For complex 1

Nigellicine interacts with the amino acids [PHE330 TRP84 pi-pi, 6-ring) at a distance of 3.66, 3.87 Å, respectively (for the 1st and 2nd weak interaction) this suggest that Nigellicine can inhibit Acetylcholinesterase (AChE) and interfere with [PHE330 TRP84 pi-pi, 6-ring] [13].

For complex 2

Nigellidine interacts with the amino acids [ASP72, TRP84, TYP121and PHE330 (H-Donnor, H-Pi, Pi-H and, Pi-Pi) resepectevely O1, C6and 6-ring at a distance of 3.18, 4.00, 4.48 and 3.76 Å, respectively (for the 1st, 2nd, 3rd znd 4 weak interaction, wich suggesting that Nigellidine can inhibit Acetylcholinesterase (AChE) and interfere with [ASP72, TRP84, TYP121and PHE330 (H-Donnor, H-Pi, Pi-H and, Pi-Pi))] [13].

Mol	Score	Rmsd-refine	E-Conf	E-PLACE	E-SCORE1	E-REFINE	E-SCORE2
Complexe-1	-7.56846952	0.89153111	15.1734333	-68.3769302	-11.9809542	-9.78503895	-7.56846952
Complexe-2	-9.11354828	1.8248744	98.7343369	-51.698989	-14.0351686	-6.53940344	-9.11354828
Complexe-3	-7.24837112	0.71298635	51.3847694	-56.2807274	-13.7168217	-12.5217743	-7.24837112
Complexe-4	-6.81699133	2.41718721	-5.15983963	-53.9098206	-9.49168587	-17.7685337	-6.81699133
Complexe-5	-1.75505626	0.992192566	13.9917202	-50.7311096	-11.3696117	65.6222076	-1.75505626
Complexe-6	-6.92966413	1.32553411	-3.8850162	-46.5581169	-12.3904715	-13.7318163	-6.92966413
Complexe-7	-6.50787592	1.45082247	11.1317806	-59.191925	-11.6698942	-11.0453873	-6.50787592
Complexe-8	-6.05367661	0.848154187	35.8929749	-29.6925602	-7.80692625	-8.00824547	-6.05367661
Complexe-9	-6.62489462	0.735047042	27.7540646	-49.5578918	-9.8982687	-13.4204245	-6.62489462

Table 5: Energy balance of complexes formed with – ACHE with Water molecules (Kcal/mol).

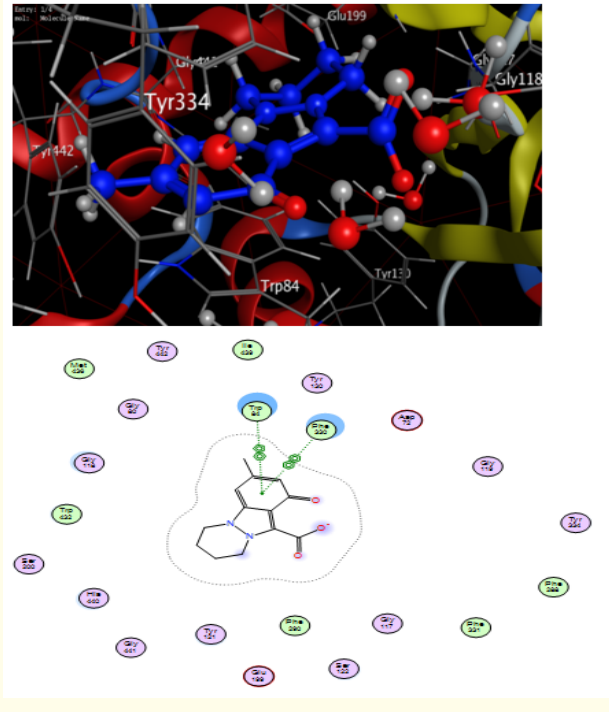


Figure a: Diagram interaction of complex-1 (AChE + Nigellicine)

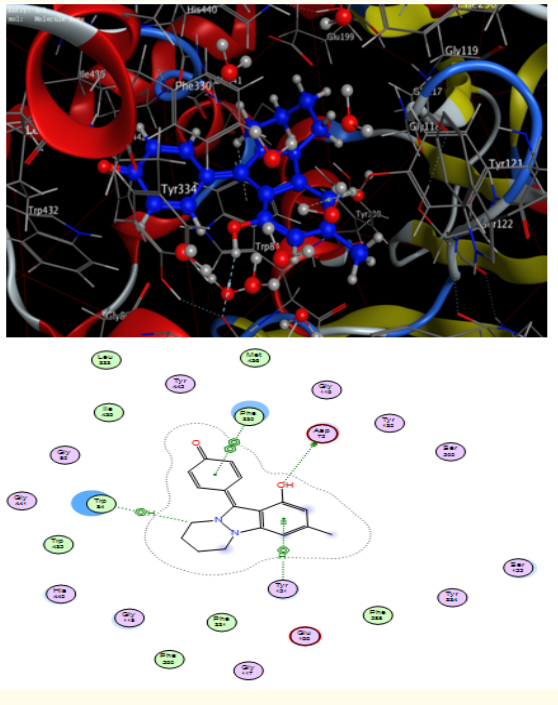


Figure a: Diagram interaction of complex-2 (AChE + Nigellidine)

Figure 4: Diagrams of interactions (enzyme-ligands) 2 complexes (a, b).

Compounds	S-score (kcal/mol)	Bonds between atoms of compounds and residues of active site					
		Atom of compound	Involved receptor atoms	Involved receptor residues	Type of interaction n bond	Distance (Å)	Energies (kcal/mol)
L1	-7.56846952	6-ring 6-ring	6-ring 5-ring	PHE330 TRP84	Pi-Pi Pi-Pi	3.66 3.87	-0.0 -0.0
L2	-9.11354828	O1 C6 6-ring	OD2 6-ring OH 6-ring 6-ring	ASP72 TRP84 TYP121 PHE330	H-Donnor H-Pi Pi-H Pi-Pi	3.18 4.00 4.48 3.76	-1.4 -0.8 -0.7 -0.0
L3	-7.24837112	6-ring 6-ring	6-ring 5-ring	PHE330 TRP84	Pi-Pi Pi-Pi	3.95 3.54	-0.0 -0.0
L4	-6.81699133	6-ring 6-ring	6-ring 5-ring	PHE330 TRP84	Pi-Pi Pi-Pi	3.72 3.80	-0.0 -0.0
L5	-1.75505626	/	/	/	/	/	/
L6	-6.92966413	O2 6-ring 6-ring	O 6-ring 5-ring	HIS440 PHE330 TRP84	H-Donnor Pi-Pi Pi-Pi	3.08 3.83 3.82	-2.6 -0.0 -0.0
L7	-6.50787592	6-ring 6-ring	6-ring 5-ring	PHE330 TRP84	Pi-Pi Pi-Pi	3.62 3.84	-0.0 -0.0
L8	-6.05367661	/	/	/	/	/	/
L9	-6.62489462	O1 6-ring 6-ring	O 6-ring 5-ring	HOH 0 PHE330 TRP84	Acceptor Pi-Pi Pi-Pi	2.86 3.63 3.79	-1.5 -0.0 -0.0

Table 6: Resultants Bonds between atoms of compounds and residues of active site in water.

When water is included present best inhibition to the evolution of the pathology studied (Alzheimer's disease (AD).). The behavior of water molecules in direct contact with the solute is very important and it is therefore crucial to ensure that not only the solute but also the first solvation layers are surrounded by a sufficient number of water molecules for ensure a realistic behavior of all the molecules of solvent (Figure 5). Refinements of certain terms of the force field describing the water molecule are imperative to ensure good results. For example, the explicit treatment of the electronic polarizability of water molecules can be included as an additional term in an empirical force field. The presence of water is sometimes para- mount to ensure a relay between the ligand and the active site [14].

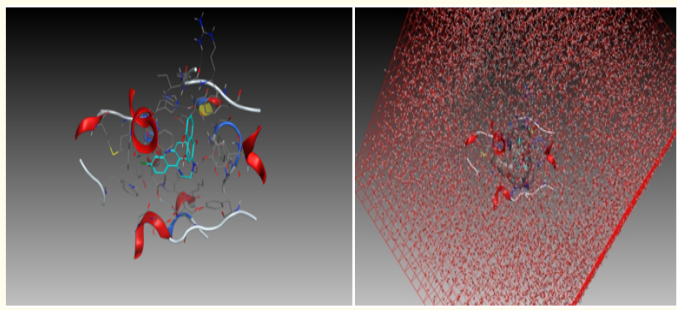


Figure 5: Solvation Ligand –Substrate in cube.

Conclusion

In this work we elucidate the inhibition of Acetylcholinesterase enzyme by molecular modeling, our results allow us to conclude that the Natural inhibitors of *Nigella* inhibitor– 2 (*Nigellidine*) can be thought as a new option in treatment on AD but needs to more multidisciplinary investigations by the pharmacologists together with neurologists.

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