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Dubious Action of Atrazine as an Endocrine Disrupting Agent: An *In silico* Approach

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Abstract

Atrazine is chlorine containing chemical compound which is widely used as an herbicide. However, upon its entry into the human body, it is known to affect several organs and their functions. Additionally, atrazine also represses photosynthesis in plants and causes serious disorders in animals as well. One of the most challenging actions of atrazine is its action has an endocrine disruptor. The present article makes an effort to delineate on the binding affinity of the herbicide to two human hormone receptors, and also to understand its endocrine disrupting capability adapting the computational methods.

Keywords Atrazine; Endocrine disruptor; MDV; Computational approach; Environmental toxin

Introduction

Atrazine is a chlorotriazine that comprises of a ring, referred to as a triazine ring, alongside five nitrogen atoms and a chlorine atom [1]. Immaculate atrazine is an odourless, white powder, responsive, or combustible. It is accessible in emulsifiable concentrate, wettable powder, granular and emits irritating toxic fumes when exposed to fire that include hydrogen chloride, nitrogen oxide. It can be assimilated into the body by ingestion [2]. The herbicide atrazine represses the photosynthesis in certain plants. It is water-solvent and can be transported in dissolved form [3] and is found in water bodies [4] and can be easily leached, however, can also exist in precipitate form. Approximately 1% to 6% of the connected herbicides are discharged to the water environment. Constant use of the herbicides can get to be dangerous because of expanded adsorption and diminished bioavailability over time [5].

Atrazine is a potential information disruptor in vertebrates [6]. It is exceptionally portable in both physical and amphibian biological systems. It is impervious to debasement, with a reported half-life of 95-350 days [7] and is discovered 10 to 20 times more frequently in water. The water quality detection reveals atrazine to be regarded as the most potential pesticide. Atrazine degrades slowly (1-2 years) once in the water [8] and has been shown to intrude with olfactory-related physiological procedures in numerous species [9].

Atrazine finds several ways to enter into the body. In the event, that atrazine-containing dust is breathed in, a percentage of the particles may store in the lungs. Bigger atrazine particles maybe store before coming to the lungs and be hacked up and gulped. On the off chance that human skin interacts with atrazine polluted soil or water, a little measure of it may go through the skin and enter the circulatory system. In the event, that one swallows contaminated, water, or soil containing atrazine, the majority of it will go through the covering of stomach and digestion tracts and enter the circulation system. When atrazine enters the bloodstream, it is circulated to numerous body parts and gets converted into the metabolites [10] and finds entry into certain organs or fat depositions [11]. However, atrazine does not develop or stay in the body. The vast majority of the metabolites leave the body in 24-48 hours, principally through urine, and through faeces [9].

Individuals, who dwell close to downstream localities or those who consume ground water for drinking, come in contact with these chemicals. The factory workers and the farm workers are largely exposed to atrazine in light of the fact that it is utilized as a part of agribusiness. Individuals may be presented to atrazine by coming into contact in soil that has atrazine in it. Children may be exposed to atrazine by playing on ground that contains atrazine in the similar manner children may be exposed through methods such as breathing, beverages, eating and touching [12]. When atrazine is used, some of it may enter the air while a little amount may be washed from the dirt by downpour fall and enter encompassing ranges including streams, lakes, or different channels and the remaining may relocate from the upper soil surface to more profound soil layers and enter the groundwater. As a rule, atrazine separates in the dirt over a time of one developing season. Atrazine is expelled from air for the most part by precipitation and can be blown to a much farther area through the wind. Atrazine does not have a tendency to be accumulated in living beings, for instance, green growth, micro-organisms, shell fishes, or fish, and in this manner, does not have a tendency to develop in the sustenance chain [9,13].

The potential wellbeing impacts for a person includes blockage of the heart, lungs, and kidneys, low pulse, muscle fits, weight reduction, harm to adrenal organs, cardiovascular harm, retinal degeneration, muscle degeneration and malignancy. Studies with vertebrates have demonstrated potential atrazine-connected endocrine impacts as it builds human aromatase action in human adrenocortical carcinoma

cells *in vitro* atrazine exposures [14]. Furthermore, plasma levels of testosterone in male salmon presented to 3.6 mg/L atrazine [15]. In August 2009, atrazine was conspicuously included as a potential reason for pregnancy issues, low conception weights, and menstrual issues when expended at concentrations underneath government standards [16]. Atrazine can influence wellbeing by manipulating the method of working of the reproductive system. Investigations of couples living on homesteads affected by atrazine discovered an increment in the danger of pre-term pregnancy. Atrazine influence the regenerative framework in people by a different component. It additionally brought about liver, kidney, and heart harm in creatures and humans. An expanded danger of creating mammary tumors was seen in one strain of female rats. Insufficient data is accessible to certainly state whether atrazine causes tumor in humans [17]. The chemical further influences regenerative science by emulating or estranging the activity of hormones. The natural vicinity of atrazine has been once in a while identified with regenerative unsettling influences in wild mammals [18], birds [19], reptiles [20], and fish [21]. Atrazine, hence, is known as an endocrine disruptor and acts by inhibiting cAMP specific Phosphodiesterase-4 [22].

The objective of the present investigation is to assess the binding ability of the herbicide with the male and the female hormones and further to understand its nature as an endocrine disruptor by *in silico* method.

Materials and Methods

Protein selection

The protein targets for the current investigation are the hormone receptors. Two human hormones 2IOK, female and 2AO6 of the male were imported from Protein Data Bank. The chemistry of the missing hydrogen's was corrected after the removal of the water molecules and the hetero atoms. Furthermore, the proteins were subjected to energy minimizing steps until the conjugant gradient satisfied was obtained. The targets were then imported onto the Molegro.

Ligand selection

The ligand for the present investigation is the herbicide atrazine. The structure of the atrazine was sketched on the Marvin sketch software and was saved in MOL2 format. It was later imported onto the Molegro for performing the docking.

Molecular docking

Molegro Virtual Docker (MVD) was adapted for calculating the dock scores and ligand docking studies were run on the MVD, that has as of late been presented and preferred consideration among the scientists. MVD is a quick and adaptable docking program that gives the doubtless compliance of ligand binding to a macromolecule [23]. The scoring capacity of MolDock is in light of the Piecewise Linear Potential (PLP), a rearranged potential that depends on certain parameters like fit to protein-ligand structures and scoring capacity [24,25] that is further reached out in GEMDOCK (Generic Evolutionary Method for molecular DOCK) [26] with another hydrogen holding term and charge plans.

EPLP utilizes two distinct arrangements of parameters: one for the steric (van der Waals) term in between atoms, and the other for more grounded potential for hydrogen bonds. Also, a re-ranking strategy was connected to acquire the most elevated positioned stances to expand the docking precision further. All things considered, 10 docking runs were executed to get high docking scores. MolDock naturally distinguishes potential binding sites (cavities) utilizing an adaptable cavity identification and calculation, E score and is characterized by the accompanying vitality terms:

$$E_{score} = E_{inter} + E_{intra}$$

Where, E_{inter} is the ligand-protein interaction energy:

$$E_{inter} = \sum_{i \in ligand} \sum_{j \in protein} X\left[E_{PLP}\left(r_{ij}\right) + 332.0_{qiqi}/4\, r^2 ij\right]$$

The summation keeps running over every substantial particle in the ligand and in the protein, including any cofactor atoms and water particle molecules that may be available. The second term portrays the electrostatic connections

E_{intra} is the internal energy of the ligand:

$$E_{inter} = \sum_{i \in ligand} \sum_{j \in protein} E_{PLP}\left(r_{ij}\right) + \sum A\,[1 - cos\left(mXq - q_o\right)] + E_{clash}$$

The double summation is between atom pairs in ligand, barring atom matches that are joined by two bonds or less. The second term is a torsion energy term, parameterized by hybridization sorts of the bonded atoms, while θ is the torsion edge of the bond. The last term, E_{clash}, gives a penalty of 1,000 if the separation between two atoms (more than two bonds separated) is under 2.0 Ao. In this way, the Eclash penalizes non-feasible ligand adaptations.

MVD has two docking screening algorithms; MolDock Optimizer and MolDock SE (Simplex Evolution). The default search algorithm utilized as a part of MVD is the MolDock Optimizer [27,28] which is in view of a developmental calculation. From MVD variant 1.5, an option heuristic inquiry calculation named MolDock SE (simplex advancement) is additionally actualized.

MolDock SE performs better on some parameters where the standard MolDock calculation comes up short. In like manner, the two scoring capacities, the MolDock Score and its gird based form, MolDock Score [GRID] are utilized for assessing docking arrangements. On the other hand, thorough docking computations were done utilizing both inquiry calculations alongside both scoring capacities. The five best docking arrangements were returned after every docking run [29].

Role of atrazine as an endocrine disruptor

As described in the above section, the article also makes an effort to understand the role of atrazine as an endocrine disruptor adapting the ACD/Labs software that utilizes Algorithm Version: v5.0.0.184.

Results and Discussion

Protein targets

The protein targets for the present study, 1A52 and 2AO6 were imported on to the Molegro work bench. The binding site pocket analysis was performed to understand the number of pocket present and their volume in Ao3, Figure 1.

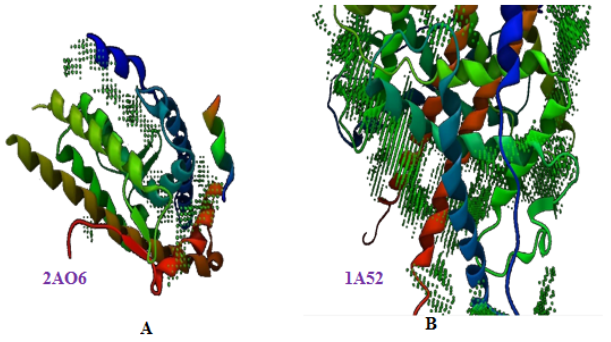

Figure 1: Proteins 2AO6 (A) and 1A52 (B) with their binding sites.

The protein 2AO6 consists of seven binding site pockets; however, the binding pocket with the highest volume, 120.32 Ao3 was preferred for performing the docking. The protein 1A52 showed 15 binding site pockets and the pocket with the volume of 2572.80 Ao3 was chosen for docking with the ligand atrazine Figure 2.

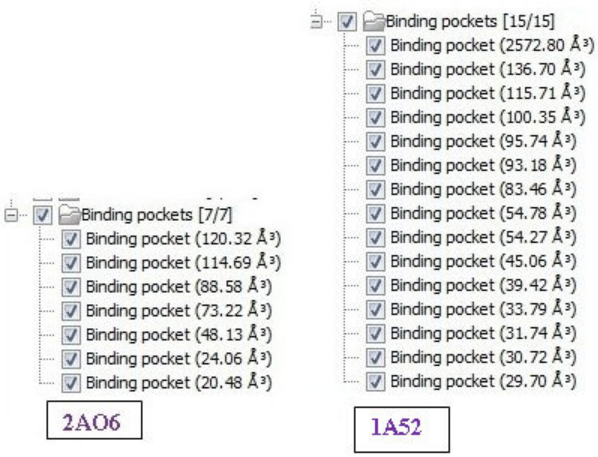

Figure 2: Binding pocket volume of the proteins.

Molecular docking

MolDock optimizer: In MVD, chose parameters were utilized for the guided differential evolutionary algorithms: number of runs=10 (by checking compel postures to cavity alternative), population size=50, maximum iterations=2000, cross over rate=0.9, and scaling element=0.5. Variation based termination scheme was chosen instead of root mean square deviation (RMSD). To guarantee the most perfect binding mode in the binding cavity, pose clustering was utilized, which prompted various binding possibilities [29].

MolDock SE: For the generation of the pose, 1500 maximum iterations were utilized by selecting a population size of 50 and were constructed incrementally from their rigid root point (Figure 3). The pose generator tests various diverse torsion points, rotations and interpretations, assesses the influenced part of the molecule and picks the value with the least energy. The poses created were added to the population if the energy value was beneath the 100.0 threshold. At every progression, no less than 10 torsions/rotations/translations were tried and the one giving the least energy was picked.

MolDock score: The ignore distance atoms option was utilized to do away with the atoms far from the binding site. Also, hydrogen bond directionality was situated to check whether hydrogen holding between potential contributors and acceptors can happen. The binding site on the protein was characterized as reaching out in X, Y and Z headings around the chosen cavity with a radius of 15 Ao.

MolDock Score [GRID]: The MolDock Score [Grid] is indistinguishable to the MolDock Score except that the hydrogen bond directionality is not considered. The lattice (grid) based scoring function gives 4-5 times rate up by pre-calculating potential energy values on an equitably dispersed cubic grid. The energy potential is assessed by utilizing tri-linear interpolation between the similar grid points. Remaining terms in the MolDock Score [Grid] adaptation (i.e., inner ligand vitality commitments and limitation punishments) are indistinguishable to the standard function scoring function. A grid resolution of 0.80 A0 was situated to start the docking procedure.

The dock scores generated revealed that the herbicide atrazine had a strong affinity towards the estrogen receptor, 1A52 with the dock score of -36.09 when compared to androgen receptor 2AO6 with a dock score of -29.20. The RMSD was additionally seen to be high for 1A52 of 139.27 while it was discovered to be 33.89 for 2AO6 which is low than the estrogen receptor. In contrast to the above the hydrogen bond score was high for 2AO6 with -9.88 and for 1A52 it was found to be -2.00. However, both the proteins had four flexible bonds each, Table 1.

The collaboration between the protein and the ligand demonstrated that the protein 2AO6 formed four hydrogen bonds with atrazine, Figure 4. The OH molecule of TRY 739 formed two hydrogen bonds while the N2 atom of LYS 905 formed remaining two hydrogen bonds. Conversely, the estrogen receptor, 1A52 could form only one hydrogen bond with the ligand atrazine, Figure 5. The amino acid residue involved was O atom of LEU 346.

Role of atrazine as an endocrine disruptor: The endocrine disrupting ability of the chemical compound atrazine was studied using the ACD/Labs software that utilizes Algorithm Version: v5.0.0.184. The results obtained were in contrast to the statement that atrazine is an endocrine disruptor as it shows no binding to the human estrogen alpha (LogRBA<-3). The *in silico* results give rise to the debate on the effect of atrazine on endocrine system however, in contrast it showed a greater effect on the other systems Figure 5, The maps generated delineates the part of individual atoms of the ligand in a shading coded way; red shading shows a positive impact towards the toxicity while green shading means the molecule or the atom has a no effect, Figure 5.

The results generated were based on the effect of the probability of Estrogen Receptor Binding and on the other systems (Table 2).

S No	Protein PDB Id	Dock score	RMSD (Ao)	Flexible Bonds	Hydrogen bond score
1	2AO6	-29.2	33.89	4	-9.88
2	1A52	-36.1	139.27	4	-2

Table 1: Dock scores.

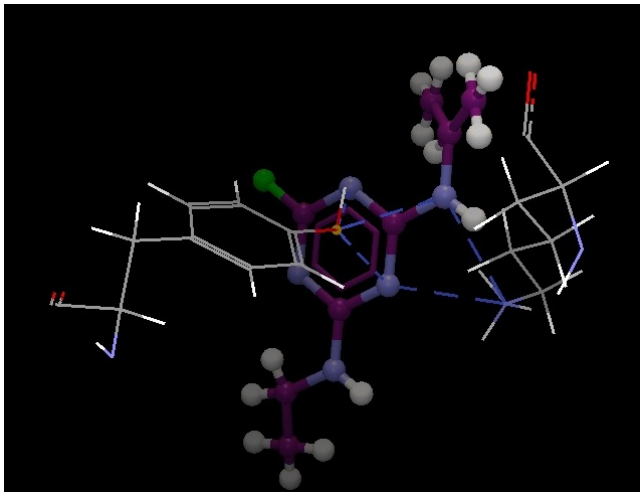

Figure 3: Dotted line indicating the hydrogen bonds between Protein and the atrazine. The amino acids residues involved are TRY 739, LYS 905.

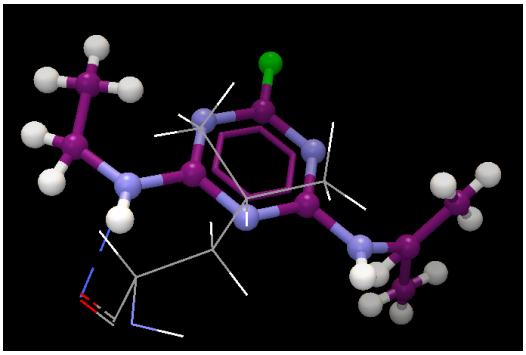

Figure 4: Dotted line indicating the hydrogen bonds between Protein and the atrazine. The amino acid residue involved is LEU 346.

Study	Parameters	Values
Probability of Estrogen Receptor Binding	Log RBA	>-3: 0.13
	Log RBA	>0: 0
Probability of Effect on other systems	Blood	0.3
	Cardiovascular system	0.26
	Gastrointestinal system	0.72
	Kidney	0.12
	Liver	0.12
	Lungs	0.12

Table 2: The results generated were based on the effect of the probability of Estrogen Receptor Binding and on the other systems.

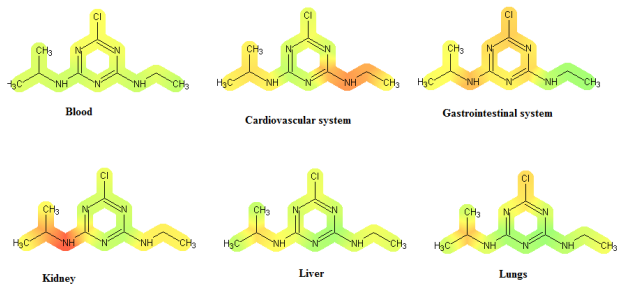

Figure 5: Probability of effect of atrazine on other systems.

Conclusion

The chemical atrazine is known to have a noteworthy effect on several biological systems in addition to the endocrine system. The present article portrays the vicinity of atrazine in distinctive circles of environment, its unfavourable impacts on human specifically to the endocrine system. Atrazine efficiently mimics the hormones and thus binds to their corresponding receptors causing serious effects. However, the *in silico* results show that the chemical compound has no binding affinity towards the estrogen receptor. In the view of the generation of much contrasting results, it is therefore, very essential to conduct extensive studies on atrazine. Also, it is recommended to use alternatives for this herbicide to curb interaction with the chemical.

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