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Reverse Docking Approach Reveals the Negative Effect of Caffeine Toxicity on Glutamate GluR2 Receptor

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Abstract

In the past decade, the consumption of caffeine has increased exponentially throughout the world. Caffeine is now not only limits to coffee but is present in several desserts and other beverages as well. Caffeine is a psychostimulant that helps in improving your performance but its excessive consumption can also affect the cognitive functions of the Central Nervous System (CNS) negatively. The present research is an attempt to understand the mechanism of caffeine action in blocking central nervous system receptors thereby affecting brain function adversely. The reverse docking approach of computational biology has been implied to visualize the interaction of several neurotransmitter receptors with caffeine. Reverse molecular docking is an approach for determining the effect of a ligand on a range of receptors. The binding energy of the receptors with caffeine is considered for determining the best receptor-ligand complex. A list of 7 different neurotransmitters was identified through a literature study and taken into consideration in the current research. The molecular interaction of the human neuro-receptors was seen with caffeine using AutoDock4.0 to study the impact of caffeine on several biological processes of the human brain. The molecular docking approach identifies glutamate gluR2 receptor to be adversely affected by caffeine toxicity thereby affecting the neurotransmission process in the human nervous system.

Keywords: Caffeine, Glutamate GluR2, Neurological Disorders, Reverse Docking

1. Introduction

Caffeine, present in the pulp of coffee[1], is a psychostimulant of the methylxanthine class and is the most widely taken globally among people of all ages and from various cultural and regional backgrounds[2]. Caffeine (1,3,7-trimethylpurine-2,6-dione) is made up of eight carbon atoms, ten hydrogen atoms, four nitrogen atoms, and two oxygen atoms. Most caffeine is consumed in coffee but caffeine is also present in food, beverages, energy

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drinks, and drugs[3]. According to Ingraham and Ferdman[4], more than two billion cups of coffee are consumed per day worldwide. Caffeine consumption also affects the cognitive function of its consumers differently. Studies have demonstrated that caffeine can help in learning, reducing stress levels, lowering the rate of anxiety, and enhancing the memory of several activities. Additionally, it has also been shown to improve performance in several activities. When eaten in tiny dosages, caffeine has also been demonstrated to reduce users' levels of anxiety and enhance their mood. It is crucial to research how caffeine affects people's cognitive function given how many individuals use it daily throughout the world[5].

Most of the human body functions such as perception, homeostasis, and cognitive behaviour. These functions are controlled by neural cells (also known as a neuron) that generate and propagate electrochemical signals that help in the release and diffusion across synaptic junctions of neurotransmitters that mediate the exchange of those signals between the neural cells. The CNS processes the information from and delivers the information to the peripheral nervous system from one neuron to another through synapses. Thus, it has an essential role in the transmission of information through the central and peripheral nervous systems played by neurotransmitters. Neurotransmitters are essential components in the nervous system for proper brain function, and their dysfunction is involved in several mental disorders[6]. Neurotransmitters can be Acetylcholine, serotonin, dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline), glutamate, Histamine and γ-aminobutyric acid (GABA), Delta opioid[7]. When any kind of disturbance occurs in the homeostasis of neurotransmitters[8] in the brain due to some overconsumption of phytochemicals, it can cause several neurological disorders such as Anxiety disorders, GABA-transaminase deficiency, Parkinson's disease, Epilepsy, Alzheimer, Schizophrenia, Autism Spectrum Disorder, Chronic stress, mood disorder, Major Depression and Brain development disorders.

One of the excitatory neurotransmitters in the brain is Glutamate (Nonessential amino acid) is recycled and made by glial cells in the brain. Glial cells convert glutamate to glutamine, which is converted back again into glutamate when delivered back to the terminal area of nerve cells. Mostly the CNS neurons generally use Glutamate as a major neurotransmitter because they increase neuronal excitability and decrease relaxation. Glutamate Receptor (GluR2) is a part of Group II of metabotropic which are located pre, post, and extra-syntactically throughout the CNS are slow excitatory transmission which has been identified in only a few brain regions, such as the olfactory bulb and cerebral cortex. As the signal travels along the nerve cell, the electric charge of a signal cause vesicles of neurotransmitter i.e., glutamate is found in synaptic vesicles in nerve terminals from where it can be released by exocytosis in the fluid-filled spaces where glutamate binds to the next signal receiving receptor on a nerve cell. After binding glutamate triggers the action in the next nerve cell, and this communication continues from one nerve to another nerve cell. Maintaining the optimum level in the extracellular fluid is essential, as a high level of Glutamate can lead to cell death[9]. So, the Glutamate transporter is located on the outside of the neuron and Astrocytes remove excess Glutamate.

As Glutamate plays a major role in shaping learning and memory, Energy source for brain cells, a Chemical messenger, a sleep-wake cycle manager, neuronal development, and synaptic plasticity[10] (Ability of the brain to change and adopt new information effectively while communicating between two neurons). Glutamate has two major types of receptors ionotropic and metabotropic. Ionotropic glutamate receptors (iGluRs) such as N-Methyl-d-Aspatate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptor, are ligand gate ion channel allowing the passage of Na+ and K+, and in some cases small amounts of Ca2+, that stimulates fast excitatory neurotransmission[11]. Whereas metabotropic Glutamate Receptor (mGluRs) is G- proteins coupled receptors that have been categorized into three groups that function to modulate cell excitability and synaptic transmission via second messenger signaling pathways.

2. Methodology

The present study involves the use of several bioinformatics tools and techniques for the identification of caffeine toxicity. Figure 1 illustrates the complete methodology used in the present study.

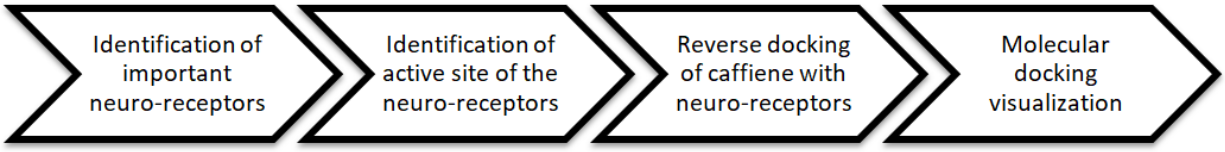


Figure 1. The summarized workflow of the current study.

2.1 Receptor Identification

An intensive literature study was done to identify the major receptors involved in the functioning of the human brain. We identified 10 major receptors which are involved in the human nervous system and play a key role in the proper functioning of the brain. These receptors are adenosine A1 and A2A[12], dopamine D2 and D3[13], GABA-B[14], histamine H3, glutamate gluR2[15], M2 muscarinic acetylcholine[16], delta opioid, and serotonin 5HT1[17]. The 3D structure of these 10 brain receptors had been identified and downloaded from the Protein Data Bank (PDB)[18].

2.2 Active Site Prediction

Active sites of the listed 10 receptors have been predicted by using the online Prank Web (https://prankweb.cz/) and Protein Plus web server (https://proteins.plus/)[19]. The active site predicted through these tools helps in identifying the exact binding pocket for carrying out rigid docking studies.

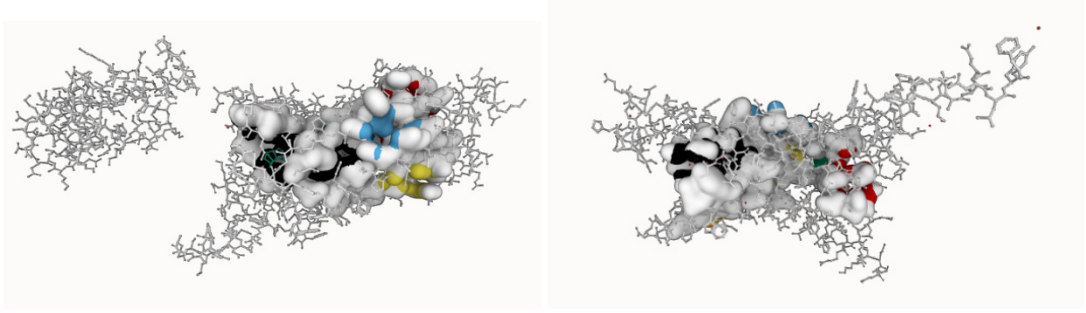
2.3 Molecular Docking and Visualization

An approach of molecular docking strategy is taken into consideration where the chemical structure of caffeine was downloaded from PubChem, and the three-dimensional structure of the protein is downloaded from PDB. Docking predicts the preferred orientation or conformation of a ligand molecule to a protein when bound to form a stable complex with overall minimum energy. While knowing the location of binding sites in protein before docking starts then it significantly increases docking efficiency[20]. The reverse docking technique has been performed using a tool known as AutoDock 4.0. The reverse docking approach is carried out between one ligand and 10 different neuro–receptors. Several poses were obtained with better binding modes and intermolecular interactions inside the receptor active site in the different runs of docking[21]. The poses with the most acceptable active site and lowest RMSD (Root Mean Square Deviation) refine values and the same binding mode of the ligand were selected.

3. Results

3.1 Active Sites Identification

The identification of active sites is a key step in the reverse docking approach to facilitate targeted docking of the ligand with the receptor. The active sites of neuro-receptors were predicted using Prank Web[22]. Figure 2 shows the predicted active site of major neuro-receptors involved in the study.



(A) (B)

Figure 2 to be continued...

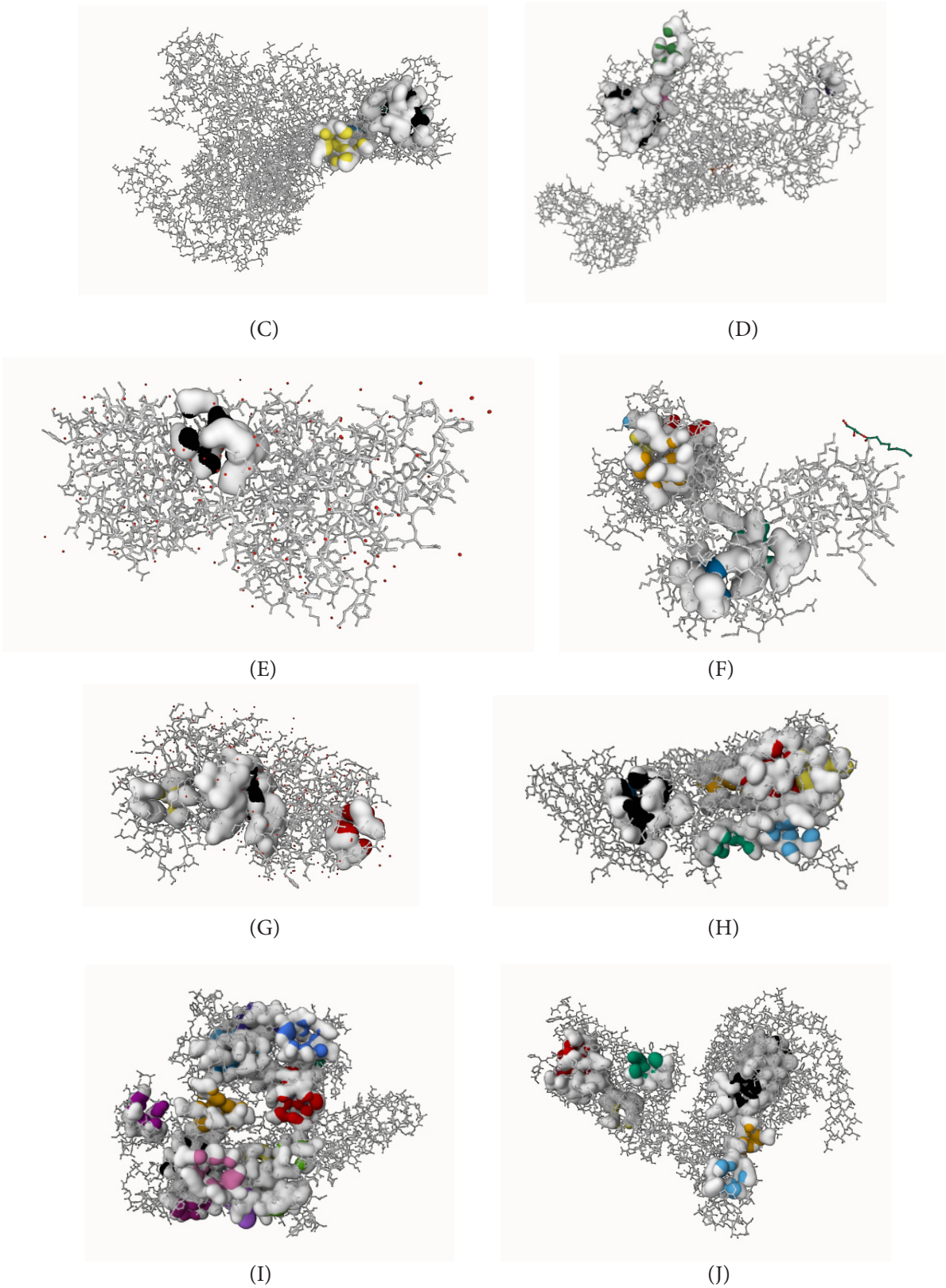


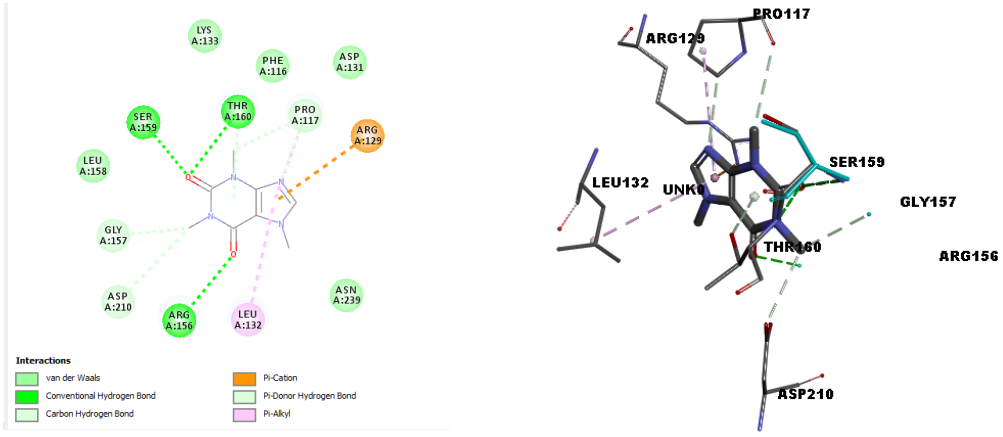
Figure 2. Predicted active site of neuroreceptors **(A)** Adenosine A1 **(B)** Adenosine A2A **(C)** Dopamine D2 **(D)** Dopamine D3 **(E)** GABA-B **(F)** Histamine H3 **(G)** Glutamate GluR2 **(H)** M2 muscarinic acetylcholine **(I)** Delta opioid **(J)** Serotonin 5HT1.

3.2 Molecular Docking Analysis and Visualization

Molecular docking is carried out between the native ligand and the active site of the target protein. Autodock4.0[23] successfully docked caffeine to receptors to form the complex. Out of 10 neuro-receptors of the brain Glutamate GluR2 was found to be the most stable due to the lowest RMSD value (-6.02 kJ/mol) as shown in the Table 1. The 2D and 3D protein-ligand interactions were visualized using discovery studio[24]. Figure 3 shows the molecular docking complexes of glutamate gluR2 receptor with caffeine. Interestingly, important interactions of Glutamate GluR2 can be found between the atoms and the residues of amino acids such as Arg129, Pro117, Leu132, Unk0, Ser159, Thr160, Gly157, Arg156, and Asp210. Which directly participates in the neurological mechanism. The ligand-receptor complex is stabilized mainly by hydrogen bonds and hydrophobic and alkyl interactions. All the docked poses generated by each docking exhibited well-established bonds with one or more amino acids in the binding pocket of the different receptors[25]. The top-ranked pose, i.e., Glutamate GluR2 with the lowest docked binding affinities and high stability is generally used as a standard selection.

Table 1. Binding energy of the neuro-receptors with caffeine along with its interacting amino acids

S.No.	Receptors	Binding energy	Residues
1	GABA - B	-5.81	Leu151, Thr39, Tyr390, His127, Met59, Ile121, Pro125, Gly124
2	Adenosine A2A	-4.26	Ile100, Phe44, Ala97, Ile124
3	Adenosine A1	-4.56	Phe1276, Asn1359, Leu1355, Met1285
4	DopamineD2	-4.26	Ile122, Cys118, Phe390, Val115, Thr119
5	Dopamine D3	-4.56	Val111, Phe346, Asp110, His349, Phe345, Ile183, Val189, Ser192, Ser196, Cys114
6	Histamine H1	-5.34	Thr1034, Leu1032, Leu1033, Tyr1024, Tyr1025, Gly1023, Ser1036, Lys1035
7	Serotonin 5HT1B	-5.08	Phe180, Pro183, Ile180, Tyr208, Thr209, Pro184, His205, Unk0, Asp204, Thr203
8	Glutamate GluR2	-6.02	Arg129, Pro117, Leu132, Unk0, Ser159, Thr160, Gly157, Arg156, Asp210
9	M2muscarinic acetylcholine receptor	-5.51	Trp155, Ser107, Tyr104, Val111, Unk0, Ala194, Trp400, Tyr403, Ala191, Asn404
10	Delta opioid receptor	-5.18	Tyr3083, Gly307, Trp274, Ile304, Unk0, Ala48, Met132, Asp128



(a) (b)

Figure 3. Molecular docking interaction of glutamate gluR2 receptor with caffeine visualized using discovery studio. **(a)** 2D structure interaction. **(b)** 3D structure interaction.

4. Summary

In this study, we looked into the possibility that excessive caffeine consumption in daily life could lead to neurological disorders. The majority of the population is currently consuming more across a range of ages. It has been demonstrated to be connected to both advantageous and detrimental effects on health[26]. In healthy adults and adolescents, excessive caffeine consumption has been linked to a number of harmful health effects, including nervousness, irritability, nausea, cardiovascular symptoms, sleep disturbances, osteoporosis, and gastric ulcers, according to scientific research[27]. Recently, excessive use of energy drinks has been linked to deaths in the United States, Canada, and different other countries[28]. The possibility that caffeine could have negative effects on human health has attracted the attention of this review. While docking has undergone significant development in recent years, it has also emerged as a useful tool in various branches of structural biology and computer-aided drug design. This method is used to examine how consuming too much caffeine affects the human body. Additionally, a focus on some significant molecular interactions and their stability of occurrence with the receptor was revealed by analysis of the docked ligand with protein receptors.

5. Conclusion

Our docking results reveal how caffeine molecules bind to the neuro-receptors in our brains, which can impair normal function. The glutamate gluR2 receptor and caffeine complex showed the strongest interaction energy and maximum stability. Numerous central nervous system circuits are significantly excitatory due to the actions of glutamate GluR2 receptors. The overconsumption of caffeine disrupts the normal functioning by binding to the glutamate GluR2 receptor instead of glutamate itself, which causes mental fatigue, insomnia, low energy, trouble concentrating, disruptions in memory and learning, the possibility of stroke due to disruptions in neuronal signaling, an unhealthy sleep cycle, mood disorders like major depressive disorder, and neurodevelopmental disorders.

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