INSILICO EVALUATION OF KAEMPFEROL-3-O-BETA-D-XYLOSIDE FROM RICINUS COMMUNIS LINN FOR ANTI-ANXIOLYTIC EFFECT

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ABSTRACT

The aim of this study is to find the anxiolytic activity of Ricinus Communis for the glycoside compound Kaempferol-3-O-beta-D-xyloside with the 18kDa translocator protein by molecular docking. The docking study was done for Kaempferol-3-O-beta-D- Xyloside from Ricinus communis using the translocator protein (18kDa). The Translocator protein ligands are promising candidates for fast-acting Anxiolytic drugs. Ricinus communis have been widely used in the traditional medicine such as abdominal disorders, arthritis, backache, muscle aches, chronic headache, sleeplessness. Kaempferol-3-O-beta-D- Xyloside is found in the dried leaves of the Ricinus communis. Ricinus communis is classified as the most poisonous plant on earth of humans. Anxiolytic agents treat the symptoms of anxiety, fear, uneasiness, muscle tightness and fear. Anxiolytics work by targeting key chemical messengers in the brain. Docking is a method which predicts the preferred orientation of one molecule to a second when a ligand and a target are bound to each other to form a stable complex. For this analysis. Autodock tools v1.5 and auto dock v4 program and molegro molecular viewer v2.5 were used. For the present study, the crystal structure of 18kDa translocator protein were retrieved from the protein data bank (PDB) and used for the computational analysis. The ligand was docked to target protein complex 18kDa Translocator protein using autodock tools. The electrostatic map and affinity of all atoms present were computed with grid spacing of 0.35A. The result were evaluated by sorting the different

orientation of ligands with respect to Kaempferol-3-O-beta-D-Xyloside with 18kDa translocator protein interaction, which was developed by molegro molecular viewer v2.5.

INTRODUCTION

Background : Ricinus communis have been widely used in the traditional system of medicine for the treatment of diseases such as abdominal disorders, arthritis, backache, muscle aches, chronic headache, sleeplessness, etc.,. Ricinus communis have been used for a long time in the traditional medicine for the treatment of Central Nervous System ailments. The present study was carried out to study the role of Kaempferol-3-O-beta-D-xyloside which is present in the Ricinus communis leaves against the anxiolytic activity.

Molecular docking is a significant process in drug development [1]. It can act for atomic level interactions linking a compact molecule and a protein, that describe target protein binding sites in addition to expound key biochemical processes. Methodology in docking involves dual process: predicting the structure of ligand along with its location and direction within sites called pose as well as determination of binding affinity. Above steps are dealt with sampling technique and scoring schemes[2]. Before beginning the docking process, knowing where the binding site is present highly enhance the docking ability. Usually, the binding site will be recognized prior docking the ligands into it[3]. Molecular docking is a useful computational approach for predicting non-covalent interactions between drugs and proteins[4]. Molecular docking is the most widely used method. Its primary application involves structure-based virtual screening for the discovery of new active element against a specific target protein. It achieved many successes[5]. It is the component of a larger workflow of various in-silico and experimental techniques[6]. Docking, in combination with other computational tools and experimental data, could be used to study drug metabolism and extract important information[7][8][9].

Anxiety is a complicated behavioural and physiological disturbance of the body that, if left untreated, can lead to a wide range of central nervous system (CNS) problems. It's an uncomfortable emotional state that's related with uneasiness, discomfort, and worry or fear about some undetermined future threat[10]. Anxiety disorder occurs when anxiety becomes excess, and it can severely affect a person’s quality of life, as well as cause a variety of psychosomatic

diseases[2]. Among the disorders in this category are agoraphobia, specific phobias, social anxiety disorder (social phobia), panic attacks, separation anxiety disorder, and selective mutism[11]. The treatment of anxiety disorders involves the synthetic drugs such as benzodiazepines, selective serotonin inhibitors, tetra cyclic antidepressants, and others are commonly prescribed, but these have common limitations such as co-morbid psychiatric disorders and increased dose leading to intolerable side effects such as nausea, sedation, nervousness, dry mouth, pharmacological dependence, and so on[12][13]. Neurotransmitters are the chemicals present in brain that are released for allowing an impulse to travel from one nerve cell to the another nerve cell. There are about 50 known neurotransmitters[14]. The (excitatory)-Acetylcholine, nor epinephrine and (inhibitory)-Gamma Amino Butyric Acid, serotonin, dopamine are the most frequent neurotransmitters[15].

Ricinus communis belonging to the **Euphorbiaceae** family is used in this study. Ricinus communis is a common plant that is known as **Aamanakku** in Tamil language. The plant is native to India and is grown in gardens and fields throughout the country, as well as found excess in free lands. Ricinus communis native to south africa, India, Brazil, and Russia is a tiny woody tree that grows to approximately 6 metres in height. Ricinus communis stems contain anticancer, antidiabetic, and antiprotozoal properties[16]. Various parts of this plant are utilised in Indian medicine to cure inflammation and liver diseases, as well as hypoglycemia and laxative effects[17].

Castor oil is a moderate and effective purgative which is ideally suited for newborns and small children, puerperal women, and irritable disorders of the GI Tract. It is one of the highly recommended purgative which is safe to use for persistent constipation alleviation[18].

Capillary electrophoresis and amperometric detection are together used to determine the disaccharide glycosides like rutin, gentistic acid, quercetin, and gallic acid in the dried leaves of Ricinus communis linn[19]. Flavonoids like kaempferol-3-O-beta-D-rutinoside and kaempferol-3-O-beta-D-xylopyranoside were also determined[20], two alkaloids, ricinine (0.55 percent) and N-demethylricinine (0.016 percent), and six flavones were discovered in the dried leaves of Ricinus communis linn.The glycosides includes kaempferol-3-O-β-D-Xylopyranoside, kaempferol-3-O-β-D-glucopyranoside, quercetin-3-O-β-D-xylopyranoside, quercetin-3-O-β-D-glucopyranoside, kaempferol-3-O-β-rutinoside and quercetin-3-O-β-rutinoside[25]. Ricin A, B,

and C, as well as one ricinus agglutinin, are all toxic proteins found in Ricinus communis seeds[22]. The roots have been extracted of indole-3-acetic acid[23]. The pericarp of Ricinus communis fruits contain an alkaloid called ricinine[24]. The glycoside component Kaempferol-3-O-beta-D-xyloside present in the dried leaves of Ricinus communis linn has been used in this study.

MATERIALS REQUIRED

- Plant : Ricinus communis.
- Glycoside component : Kaempferol-3-O-Beta-D-Xyloside (Kaempferol-3-O-Beta-D-Xylopyranoside).
- Protein : 18kDa Translocator protein.

METHODOLOGY

In Silico Analysis :

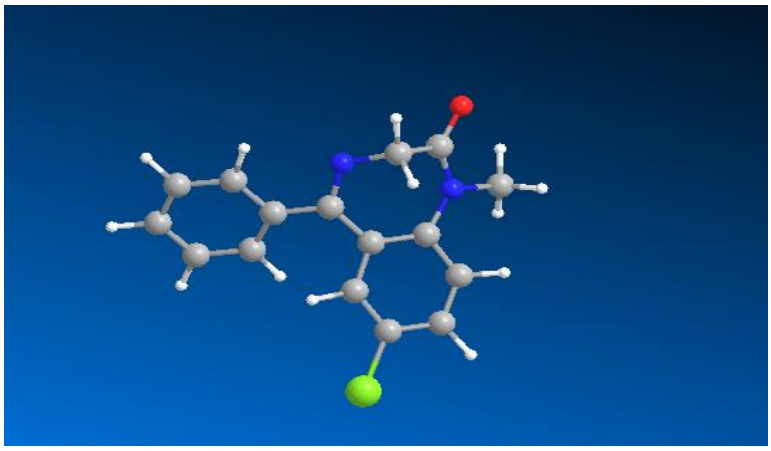
Auto dock tools v1.5 and auto dock v4 program from the scripps research institute (http://www.scripps.edu/mb/olson/doc/autodock) and Molegro molecular viewer v2.5 were installed on desktop equipped with Intel (R) Core (TM) i5 -7200u CPU @ 2.50GHz, 270 GHz processor (8.00GB RAM core CPU) and Windows 10 operating system was used. For the present study, the crystal structure 18kDa translocator protein (PDB ID: 4UC1) was retrieved from the protein data bank (PDB) and used for the computational analysis. The docking for the ligand Kaempferol-3-O-beta-D-xyloside and the standard drug Diazepam to target protein complex was done, the affinity maps and electrostatic map of atoms were computed with a grid spacing of 0.375 Ao. The result were evaluated by classifying the different orientations of ligands corresponding to Kaempferol 3 -O- beta -D- xyloside vs 18kDa translocator protein, DIAZEPAM vs 18kDa translocator protein, interactions, which was developed by Molegro molecular viewer v2.5.

RESULTS

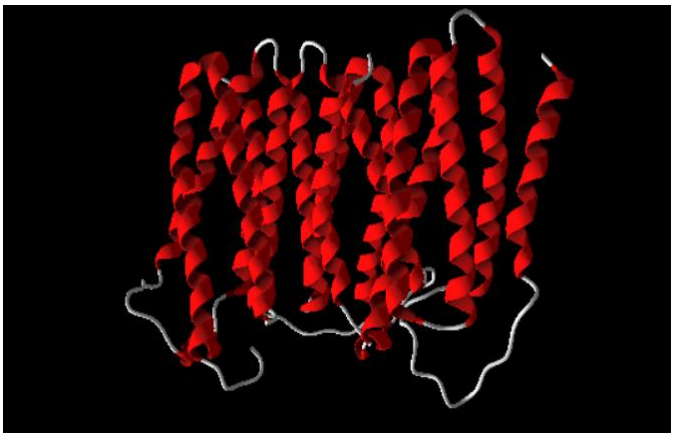
In silico analysis :

Docking analysis of interaction between Kaempferol 3 –O- beta –D- xyloside vs 18kDa translocator protein at the ligand binding domain:

3D STRUCTURE of Kaempferol 3 -O-beta -D- xyloside-(Fig1A)



DIAZEPAM-(Fig1B)



Fig(2A) 4UC1- 18 kDa Translocator protein

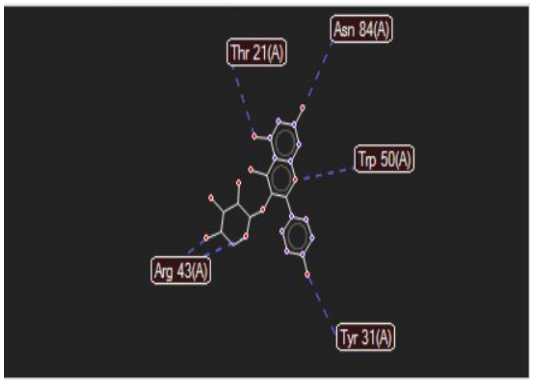
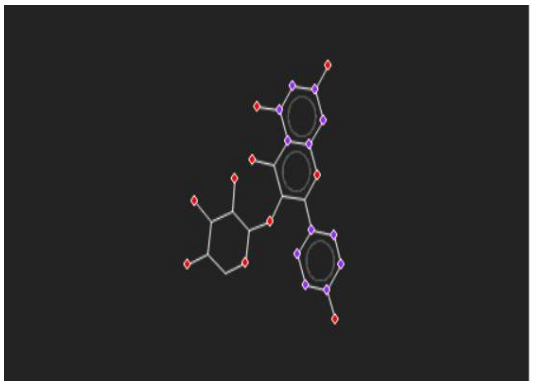
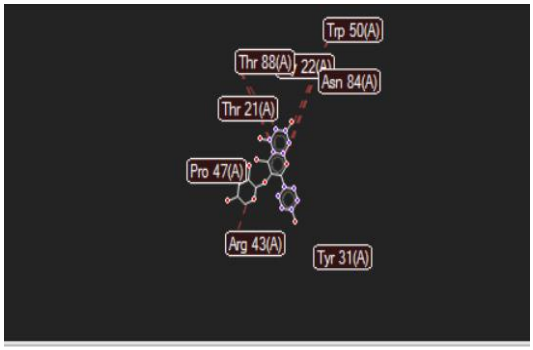


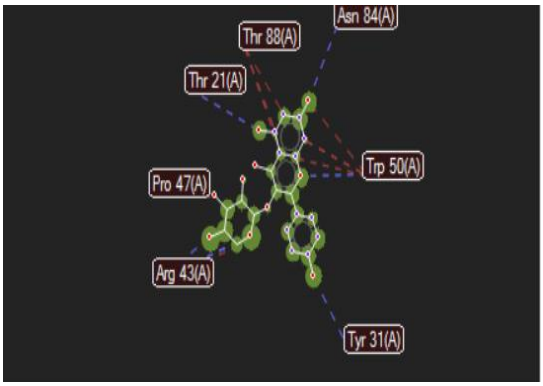
Fig-3.(A) Hydrogen bonding



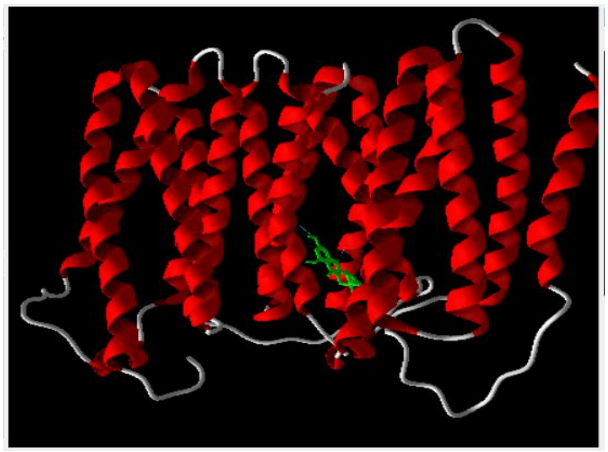
3.(B) Electrostatic interaction



3.(C) Steric interaction



3.(D) Overall interaction

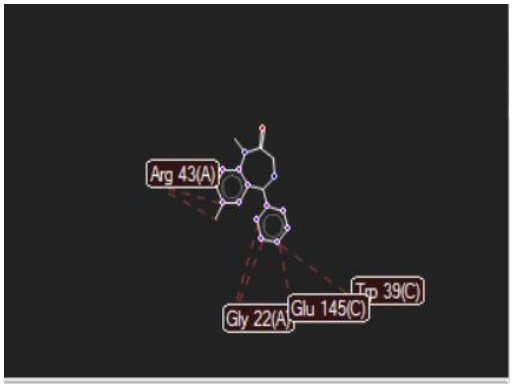


3.(E) Ligand protein binding secondary structure view

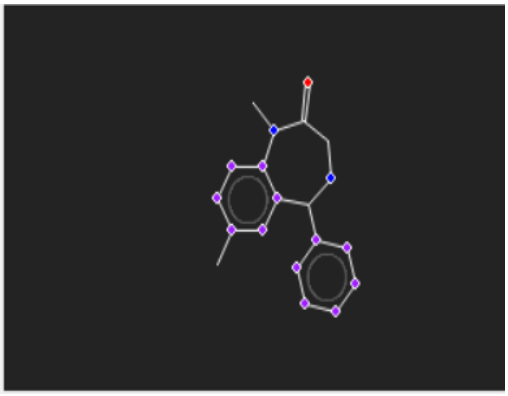
Kaempferol 3 -O- beta -D- xyloside vs 18kDa translocator protein- (Fig3)

The Fig 01 (A,B) shows the 3D structure of ligand Kaempferol 3 –O- beta –D-xyloside and Diazepam respectively. Fig 02 (A) shows the representative image of secondary structure view of the protein. Fig 3 shows the molecular docking stimulation for the ligand Kaempferol-3 –O- beta –D- xyloside and 18kDa translocator protein where Fig 3 (A) shows the hydrogen bond interactions of Kaempferol 3 –O- beta –D- xyloside at the ligand binding site of18kDa translocator protein. Fig 3 (B&C) represents the electrostatic and steric interaction between Kaempferol 3 –O- beta –D- xyloside and 18kDa translocator protein. Fig 3 (D) shows the overall interactions of Kaempferol 3 –O- beta –D- xyloside With 18kDa translocator protein, where Thr-21(A),Asn84(A), Trp50(A), Tyr31(A), Arg43(A) residue of 18kDa translocator protein was estimated to be engaged in hydrogen bonding with the ligand Kaempferol 3 –O-beta –D- xyloside which was shown in blue dotted lines. The amino acid residue of 18kDa translocator protein Asn-84, Thr-88,21, Tyr-31, Asn-81 and Trp-50,22 were involved in the stearic interaction with Kaempferol 3 –O- beta –D- xyloside. And the binding energies of Kaempferol-3 –O- beta –D- xyloside -18kDa translocator protein -10.35 Kcal/mol which was significantly lower showing the better affinity between Kaempferol 3 –O- beta –D- xyloside -18kDa translocator protein.

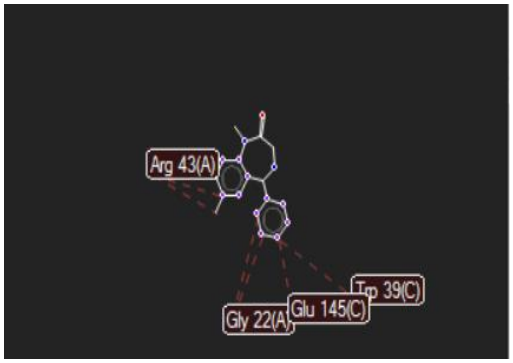
Docking study of diazepam and 18kDa translocator protein; a comparative analysis:



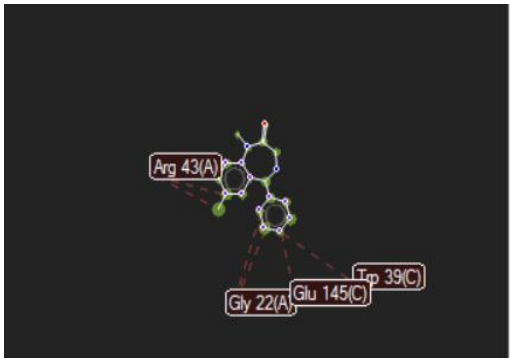
4.(A) Hydrogen bonding



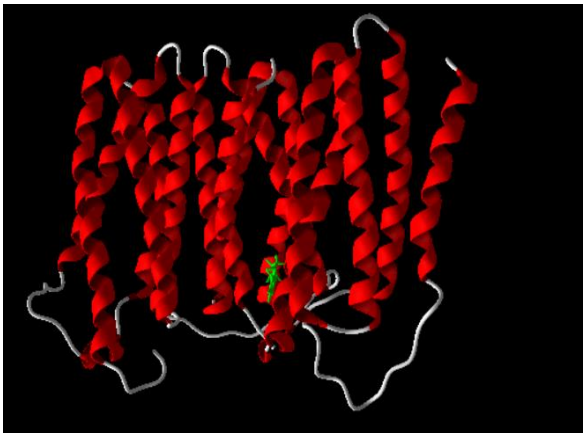
4.(B) Electrostatic interaction



4.(C) Steric interaction



4.(D) Overall interaction



4.(E) Ligand protein binding

DIAZEPAM vs 18kDa translocator protein- (Fig4)

Fig 4 shows the molecular docking stimulation for the ligand diazepam and 18kDa translocator protein, where Fig 4 (A) shows the hydrogen bond interactions of diazepam at the ligand binding site of 18kDa translocator protein. Fig 4 (B&C) represents the electrostatic and steric interaction between diazepam and 18Kda translocator protein. Fig 4 (C) shows the overall interactions of diazepam with 18kDa translocator protein where Arg-43 residue of 18kDa translocator protein was estimated to be engaged in hydrogen bonding with the ligand. The amino acid residue of 18kDa translocator protein Arg-43, Glu-145, Gly-22, and Trp-39, were involved in the stearic interaction with diazepam. The binding energies of diazepam -18kDa translocator protein -9.72 Kcal/mol. Thus the present docking stimulation study showed that the binding affinity of Kaempferol 3 -O- beta -D- xyloside was greater than that of diazepam, as observed from the lower binding energy of Kaempferol 3 -O- beta -D- xyloside with 18kDa translocator protein.

DISCUSSION

The translocator protein (TSPO) is associated in transporting cholesterol, proteins, and porphyrins into mitochondria and other organelles[30]. Because of prominent anxiolytic benzodiazepines like diazepam (Valium)[32], TSPO is recognized as a peripheral-type benzodiazepine receptor in mammals[31]. The 18-kDa translocator protein was first found in

marginal tissues as a benzodiazepine drug receptor, and it is now thought to be involved in cholesterol transport into mitochondria, the initial and rate-determining step in steroid hormone synthesis[27][28]. The Translocator proteins binds porphyrins and steroids. It have been linked to a variety of human disorders, serving as biomarkers and therapeutic targets[29]. The 18-kilodalton translocator protein, which is thought to have a role in cholesterol transport into mitochondria, is found in significant amounts in metastatic cancer, steroidogenic tissues, inflammatory and neurological illnesses like Alzheimer's and Parkinson's diseases. TSPO ligands, such as benzodiazepine medications, have been linked to apoptosis regulation and are widely employed in diagnostic imaging[26].

The 18kDa translocator protein is a benzodiazepine receptor which is involved in the synthesis of steroids. Due to the binding of drugs to the benzodiazepine receptor, it results in the relaxation from traumatic stress. When the drugs binds with benzodiazepine receptor, it helps in relieving the general stress and the stress that are caused due to the traumas. Our component Kaempferol-3-O-Beta-D-Xyloside binds to the benzodiazepine receptor whose binding energy is very low when compared to the diazepam. So our component Kaempferol-3-O-Beta-D-Xyloside can produce a promising effect in alleviating the anxiety and stress. Hence, it could act as a better Anti-Anxiolytic agent.

CONCLUSION

Molecular docking analysis of Kaempferol-3-O-beta-D-xyloside with 18kDa translocator protein was carried out. The docking study has shown that Kaempferol-3-O-beta-D-xyloside has promising effect on Anxiolytic activity. The 18kDa translocator protein is a benzodiazepine receptor which is involved in the synthesis of steroids. Due to the binding of drugs to the benzodiazepine receptor, it results in the relaxation from traumatic stress. When the drugs binds with benzodiazepine receptor, it helps in relieving the general stress and the stress that are caused due to the traumas. Our component Kaempferol-3-O-Beta-D-Xyloside binds to the benzodiazepine receptor whose binding energy is very low when compared to the diazepam. So our component Kaempferol-3-O-Beta-D-Xyloside can produce a promising effect in alleviating the anxiety and stress. Hence, it could act as a better Anti-Anxiolytic agent. Further studies can be carried out on Kaempferol-3-O-beta-D-xyloside for neuro-protective activities.

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