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A Possible Role of Rosmarinic Acid against CD2-Associated Protein for the Treatment of Multiple Sclerosis through *in Silico* Approach

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ABSTRACT

Multiple sclerosis is a chronic inflammatory neurodegenerative disorder which directly affects Central Nervous System (CNS). People with MS suffer with an episodic reversible memory loss during the initial stages and later it leads to the neurological deterioration. Number of research and studies has been done on the natural compounds and phytochemical compounds in order to develop the particular drug for the treatment of MS *in-vivo* &*in-vitro*. The present study focuses on the inhibitory effect of Rosmarinic acid against the effect of CD2 Associated protein with the help of Molecular Docking. Molecular Docking basically screens the ligand and the target protein and shows the interaction between them on the basis of the minimum binding affinities and drug likeliness properties. In our research, docking was performed between CD2-Associated protein and selected ligands with the help of docking software. Ligands were selected on the basis of their minimum Binding affinities and finally by their drug likeliness properties. Rosmarinic acid (BA- -5.6) was the resultant ligand of our recent study. It showed the perfect interaction with CD2-Associated protein. Therefore, we may conclude that Rosmarinic acid may act as a compound which may be used as a drug for the treatment of multiple sclerosis fromfurther *in vitro* and *in vivo*studies in future.

KEYWORDS: *Multiple Sclerosis, Central Nervous System, Rosmarinic acid, Docking, Binding affinities*

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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, chronic auto immunological neurologic disease of our Central Nervous System (CNS) [1, 2].The myelinated axons in the CNS are affected in MS which leads to the destruction of axons and myelin at varying degree [3, 4]. There are unpredictable course of MS. MS patients basically suffers from episodic reversible neurological deficits at the initial stages of disease, which further leads to progression in neurological deterioration. Apart from several studies, still the cause of MS is unknown, but it has been observed that it involves the combination of nongenetic trigger factors such as metabolism, environmental factors and a virus and genetic susceptibilities which together results in a self – sustaining autoimmune disorder which ultimately leads to recurrent immune attacks on the CNSand a genetic susceptibilities [5].

Based on course of disease, neurologists grouped patients into four categories [2]:

- **Relapsing–remitting MS:** It is one of the most general forms of MS with which around 85% of population is affected by MS. It covers the symptoms such as relapses or exacerbations which are followed by remission periods.
- **Secondary progressive MS:**It develops in some patientswith relapsing–remitting disease. The treatment done by disease- modifying agents in many patients can help to modify such progression. It does not depend on periods of remission and the disease course still continues.
- **Primary progressive MS:** approximately of about 10% of MS population is affected. From the beginning itself the symptoms of the disease get

worsen. Lack of relapses or remissions followed by occasional plateaus rarely. The most resistant form of MS towards drugs.

- **Progressive-relapsing MS:** This form is the rare form of MSwhich effect of about less than 5% of MS population. This also lacks the period of remission.

MS do not have any single diagnostic test. There are few evidence which are based on the diagnostic of MS and they are: the white matter of CNS must have at least two different lesions (plaques or scars); at least two different episodes in the disease course (the time dissemination criterion); and there must be chronic inflammation of the CNS. The presence of one or more symptoms may determine that the person is suffering from MS [2]. Typically people of age between 20 to 40 years suffer from MS; occasionally, it present in late middle age or in childhood [6]. CD2-associated protein (CD2AP), an adaptor protein which is associated with various membrane proteins which include polycystin-2, being mutated in type 2 autosomal dominant polycystic kidney disorder and nephrin, which is mutated in congenital nephrotic syndrome of the Finnish type. It is proved that both protein functions in maintenance maintenance of the integrity of the nephrons [14, 15].

It has been proved that according to Crespo-Bujosa, [7] report MS has three types of categorized symptoms that are Physical, Neurocognitive and Psychological. Since from time it has been proved that the phytochemical and natural compounds are coming in picture for making drug for MS as proper cure and diagnose is still unknown. It has been proved that Curcumin is considered as the phytochemical compound which has traditionally been used to treat inflammatory disorders and healing wounds [8]. According to the number of experiments conducted it has been proved till now that Curcumin act as an antioxidant by protecting the brain against various oxidative stressors, act as an anti-aging agent [9]. A plant named *Hypericum perforatum* belongs to Hypericaceae family has been proved to be used in making traditional medicines in treating mild and moderate depression, and also has been used as wound healing agent, antioxidant and anti-inflammatory agent [10]. Some other natural products such as hemp, evening primrose, *Ginko biloba* has been proved to have the antioxidant and anti-inflammatory properties which directly act on the CNS [11-13].

Molecular docking is one of the emerging tools which are used in drug designing and drug discovery. The drugs are designed on the basis of technique of computer-assisted drug design (CADD). Molecular docking is the most frequent virtual screening method, most commonly at the place when there is availability of 3D structures of the selected target protein and ligand. Both the structures of protein-ligand complexes along with binding affinity between ligand and protein is considered as important information for lead optimization for designing drug. There are two steps involved in docking process: to predict the position and confirmation of ligand molecule along with its orientation with ligand sites and their assessment with the binding affinity. We studied these steps in detail in our theory section. Protein- ligand docking is one of the important and frequently used molecular docking due to its therapeutic applications in modern structure-based drug design.

In our research we studied about three natural compounds named pulegone, rosmarinic acid and allo ocimene. The main aim of our current was to analyze the interaction of the ligands we selected against CD-2 Associated protein for the treating Multiple Sclerosis with *in silico* docking approach.

MATERIALS AND METHOD

Identification of protein target

CD2-associated protein (CD2AP), an adaptor protein which is associated with various membrane proteins which include polycystin-2, being mutated in type 2 autosomal dominant polycystic kidney disorder and nephrin, which is mutated in congenital nephrotic syndrome of the Finnish type. It is proved that both protein functions in maintenance maintenance of the integrity of the nephrons [14, 15]. In our research it was reported that CD2-associated protein shows its involvement in pathogenesis of Multiple Sclerosis. The structure of CD2 associated protein from UniProt (https://www.uniprot.org/) having good resolution was selected. The selected protein 3D structure having resolution 1.58 Å with PDB ID 4X1V was retrieved from RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) in .pdb format. Then water molecules were deleted followed by addition of hydrogen molecules. Brookhaven National Laboratories established PDB in year 1971. PDB is the universal tool containing all the structural data of biological macromolecule [16].

Retrieval of Ligand

Our recent Research focuses on three natural compounds named Rosmarinic Acid, Allo ocimene and Pulegone.

Rosmarinic acid is one of the common ester which is formed by caffeic acid and lactic acid which is accumulated in various plant species [17, 18]. Allo ocimene have shown the healing property against MS.

Pulegone is a monoterpene which is found in essential oils made from plants of the Labiatae family [19]. These three ligands were chosen on the behalf of the literature. The 3D structures of the ligands were retrieved from PubChem, an online database (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format. Then compounds in .sdf format were converted to .pdb format through Online SMILES Translator (https://cactus.nci.nih.gov/translate/). The converted files in .pdb format were further downloaded and later were used to run in different softwares and tools.

Virtual Screening

Virtual screening of ligands were performed through PyRx software. PyRx basically screens the ligands which have minimum binding energy with the selected protein target. This software runs on .pdbqt format. Initially, protein molecule was loaded in the PyRx screen and .pdf format files of the ligand were converted into .pdbqt format. In addition to this step the ligand molecules which were in .sdf format were downloaded. The energies from the selected ligands were minimized at first and then they were converted to .pdbqt format. Further docking was carried out between ligand molecules and protein targets. The results were analyzed on the basis of the ligands minimum binding affinities.

Drug Likeliness Property Analysis

After analyzing the binding affinity drug likeliness property was checked for the selected ligands which was based on Lipinski's Rule of Five which states the following:

- Ligands molecular weight must be less than 500 Dalton.
- High Lipophilicity (expressed as LogP (partition coefficient) less than 5)
- Number of Hydrogen Donors must be less than 5.
- Number of Hydrogen bond acceptors must be less than 10.
- Only one rule should violate among the above 4.

Final docking step was conducted on the screened ligand which show drug like properties. We analyzed Lipinski's rule of five through SwissADME (http://www.swissadme.ch/.) an online server. The SMILE notations of the selected screened ligands were copied from PubChem and were pasted in SwissADME for the analyses of Lipinski's rule of five. The resultant ligands which were following Lipinski's rule of five were further taken for final docking. Final docking step was performed through AutoDock Vina.

Docking through AutoDock Vina using MGL Tools

The protein target was loaded which was in .pdb format on the graphical window of AutoDock Vina. Further the water molecules were deleted followed by addition of polar hydrogen atoms along with Kollman charges into the protein target molecule. The final resultant protein molecule was converted to .pdbqt format. The selected ligands which were having less binding energy and were also following Lipinski's rule of five were then imported on the graphical window. Through this step all the ligands were converted into .pdbqt format from .pdb format. Afterwards, both the protein molecule and the final selected ligand in .pdbqt format were loaded on AutoDock Vina graphical window. Further the boundaries along with dimensions of the Grid box were adjusted as shown in Figure 1 for final docking

After preparing protein and ligand molecule docking was performed by using Command Prompt and then analyzed the final result.

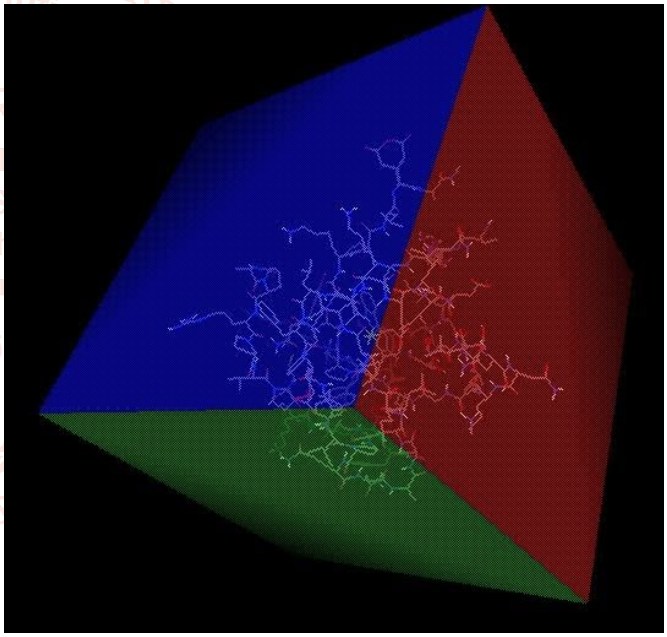

Figure 1: Grid Box

Structural Visualization through PyMOL

PyMOL is the freely available software tool which was used for visualizing the structure of protein and ligand molecule along with their interaction. When docking step through AutoDock Vina was completed, the output file with a name output.pdbqt was automatically saved. The output.pdbqt and protein.pdbqt file were loaded on the graphical screen of PyMOL 2.4. The final interaction between the ligand and protein were visualized.

RESULT AND DISCUSSION

CD2-Associated protein (PDB ID 4X1V) having resolution 1.58Å was downloaded from Protein Data Bank as shown in Figure 2. The 3D structures of Pulegone (CID: 442495), Allo ocimene (CID: 5368821) and Rosmarinic acid (CID: 5281792) were retrieved from PubChem in .sdf format as shown in Figure 3. It was further converted to .sdf format and the structure was downloaded in .pdb format.

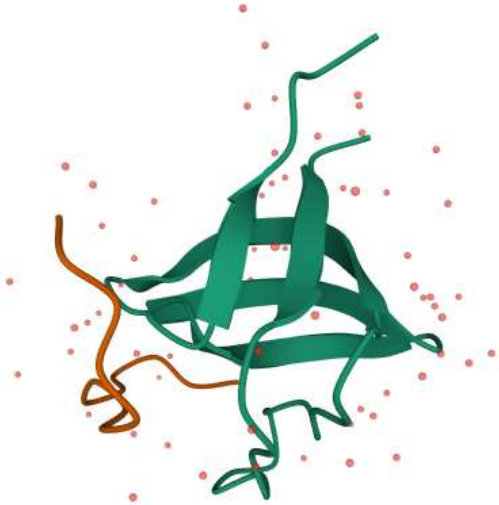

Figure 2: 3D Structure of CD2-Associated protein

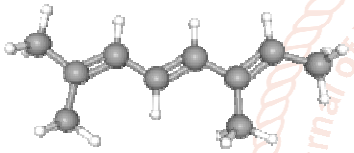	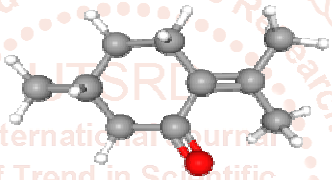	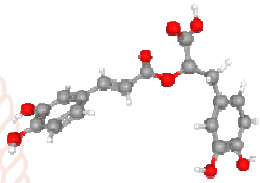
ALLO OCIMENE **CID : 5368821** **MF : C10H16** **MW: 136.23 g/mol**	**PULEGONE** **CID: 442495** **MF: C10H16O** **MW: 152.23g/mol**	**ROSMARINIC ACID** **CID: 5281792** **MF: C18H16O8** **MW: 360.31g/mol**

Figure 3: 3D Structure of Ligand

Virtual screening of the following ligands named Pulegone, Allo ocimene and Rosmarinic acid was done via PyRx Software. The binding affinities of the selected ligands Pulegone, Allo ocimene and rosmarinic acid are shown in Table 1. Binding energies of the compounds Pulegone, Allo ocimene and rosmarinic acid are shown in Table 2.

TABLE 1: Ligand Binding Affinity, RMSD upper bond, RMSD lower bond and mode.

LIGAND	BINDING AFFINITY	MODE	RMSD Upper Bond	RMSD Lower Bond
PULEGONE	-5.5	0	0	0
ALLO OCIMENE	-4.8	0	0	0
ROSMARINIC ACID	-5.6	0	0	0

TABLE 2: Binding Energy of Natural compounds

NATURAL COMPOUNDS	BINDING ENERGY
PULEGONE	-5.5
ALLO OCIMENE	-4.8
ROSMARINIC ACID	-5.6

The ligands based on their minimum Binding energy were selected. Pulegone and Rosmarinic acid were the final selected ligands from PyRx results. The ligands were further screened on the basis of Drug likeliness property. This property analysis was performed with the help of SwissADME. The selected ligands were later analyzed on the basis of Lipinski's Rule of Five as shown in Table 3. After complete analysis Allo ocimene was the only resulted ligand which was having minimum binding energy with our protein molecule and also it also followed the Lipinski's Rule of Five.

LIGAND	MOLECULAR WEIGHT	LOG Po/w	H-DONAR	H-ACCEPTOR	LIPINSKI
PULEGONE	152.2.g/mol	2.20	1	0	YES, 0 VOILATION
ROSMARINIC ACID	360.31g/mol	0.90	8	5	YES, 0 VOILATION

TABLE 3: Drug Likeliness Property of Ligand

Finally the docking between CD2-Associated protein (PDB ID: 4XIV) and Rosmarinic acid (CID: 5281792) was performed via AutoDock Vina software. As per the results, total 9 poses having different binding affinities, Root Mean Square lower bound, Root Mean Square upper bound were observed as shown in Table 4. Final interaction between the selected Ligand molecule and the Protein target was observed in PyMOL.

Mode	Binding Affinity (K cal/mol)	Distance from best mode	
		RMSD lower bound	RMSD upper bound
1	-9.6	0	0
2	-9.5	21.225	22.145
3	-9.5	20.158	21.456
4	-9.4	19.257	22.146
5	-9.4	22.879	25.143
6	-9.4	22.187	25.357
7	-9.4	21.457	23.571
8	-9.3	23.192	24.127
9	-9.3	24.137	25.163

TABLE 4: Auto Dock Vina Result

CONCLUSION

Several studies have been carried out to study the development of drug for the treatment of Multiple Sclerosis. Various neuroprotective compounds have been studied with other phytochemicals for treatment of this disease as there is no particular drug and the diagnostic method for treating MS. In our study, we worked on different natural compounds such as rosmarinic acid, Pulegone and Allo Ocimene and a protein CD2 -Associated protein. Molecular Docking was performed on the basis of binding affinity and drug likeliness property of the natural compound in association with selected protein. Rosmarinic acid was the only protein which showed the best interaction with the target protein. Therefore, we may consider rosmarinic acid as the natural compound which may be used as a drug for treatment of MS.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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