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INSILICO ANALYSIS OF SCHIZOPHRNIA GENES WITH HERBAL MEDICINES.

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ABSTRACT

Schizophrenia is known as a "Fragmented mind", it is one of the most complex, chronic and challenging psychiatric disorders that affects how a person thinks, feels, behaves. It represents a heterogeneous syndrome of disorganized thoughts, delusions, hallucination, and impaired psychosocial functioning. While many factor have been associated with developing schizophrenia- including genetics, early environment, neurobiology, and psychological and social process- the exact cause of the disease is unknown. There are old treatments which use herbal medication i.e., Unani medication. We have found that the lavender-oils have promising treatments for schizophrenia but it's a slow process. So we have come up with a new idea of creating a drug which can help the person overcome this psychosis episodes. In this paper, protein-docking between those proteins which are responsible for schizophrenia and alkaloids from Sarpagandha (Rauwolfia *Serpentina*) lavender (Lavandula *stoechas*) was done. It was found that ajmalicine, one of the alkaloid interacts with all the proteins related to schizophrenia. In-vitro analysis between ajmalicine and schizophrenia proteins can help in curing this disorder. Yet to be proved, for sure short treatment to mankind to get rid of these episodes.

INTRODUCTION

Schizophrenia is a brain disorder classified as a psychosis, which affects a person's thinking ability, sense of self, and perceptions. Schizophrenia disorder gradually becomes evident during late adolescence or early adulthood stages.

The signs and symptoms of schizophrenia include false perceptions called Hallucinations. Auditory hallucinations of voices are the most common type of hallucinations in schizophrenia, but affected individuals can also experience hallucinations of visions, smells, or touch sensations (tactile). Delusions on other hand are strongly held false beliefs which also relate to schizophrenia i.e., for example, affected individuals may behave as they are historical figure or some important personality or being plotted by someone etc.

People with schizophrenia often have decreased ability to function at school, at work, and in social gatherings. Those with schizophrenia disorder have difficulty in thinking and concentration, inappropriate emotional responses, erratic speech, behavior, and difficulty with personal hygiene and everyday tasks can be a huge challenge for them to overcome. People with schizophrenia may have diminished facial expression and animation (flat affect), and in some rare cases they become unresponsive (catatonic). There are substance abuse and suicidal thoughts and actions which are common in schizophrenia patients.

There are certain movement such as tremors, facial tics, rigidity, and unusually slow movement (bradykinesia) or an inability to move (akinesia) are common in schizophrenia patients. In most cases, medicines prescribed to help control the disorder also show side-effects. However, there are some affected individuals exhibit movement abnormalities before beginning treatment with proper medication.

Psychotic disorders such as schizophrenia are different, including depression and bipolar disorder, which majorly affect emotions. However, these disorders often occur together. Individuals who exhibit strong features of both schizophrenia are often given the diagnosis of schizoaffective disorder.

Note: Some people with schizophrenia have mild impairment of intellectual function, but schizophrenia is not associated with the same types of physical & chemical changes in the brain that occur in people with dementias such as Alzheimer disease.

REVIEW OF LITERATURE

Variations in many genes are likely to contribute the risk of developing schizophrenia. In most cases, multiple genetic changes, each with a small effect can combine the increase the risk of developing the disorder. The genetic changes which are related to schizophrenia are not well understood and explained, this disease is an active area of research. The genetic changes also interact with environmental factors that are associated with increased schizophrenia risk majorly i.e., exposure to infections before birth or severe stress during childhood and many more causes etc.

The deletions or duplications of genetic material in any particular chromosome or several chromosomes can occur, which can affect multiple genes, are factors to increase schizophrenia risk. In particular, a small deletion (micro-deletion) in a region of chromosome 22 called 22q11 may be involved in a small percentage of cases of schizophrenia. Some individuals with this deletion have other features in addition to schizophrenia, such as heart abnormalities, immune system related problems, and an opening in the roof of the mouth (cleft palate), and are diagnosed with a condition called 22q11.2 deletion syndrome.

We can see that the gene & chromosome associated with schizophrenia are AKT-1(AKT1 kinase), COMT (catechol methyl-transferase), YWHAE (Tyrosin-3 monooxygenase), these are the proteins which are associated with the oncogene, breakdown of neuro-transmitters i.e., maintain the dopamine & norepinephrine levels & it also involves the direction of the movement of nerve cells.
There are few more genes which have to be considered as well in 22q11.2 gene i.e., PI4KA (phosphatidylinositol-4 kinase alpha) - this causes novel neuro-developmental syndrome with association with PAX5 (Haplo-insufficiency) & hypo-myelinating leukodystrophy. PAX5 (member of paired box) it's a transcription factor associated with abnormal posterior midbrain and cerebellum development in mice, location of pax5 is chromosome 9p13 region and also plays an important role in B-cell differentiation and also neural development. There is PAX6 also involved which acts as one of the pax neuro and pax-alpha/beta in basal bilaterian species.
NPAS3 (Neuronal PAS domain protein3) this PAS contains a transcription factor & there is a chromosomal abnormality that affect the coding potential of this gene, associated with the schizophrenia & cognitive disability expression-in brain.

Schizophrenia can lead to cancer and it has been recorded in the 2011 senses, patients with SCZD have approximate 50% increase in death rate of cancer.

In total we have 6 genes which have been the major cause for a neuropsychiatric illness i.e., schizophrenia. They are:

THE GENE (protein name)	ACCESSION NUMBER	BASE-PAIR	SWISS-MODEL NO.
NPAS3	Q8IXFO(NCBI-Protein)	933 aa	AB055962
PAX5	Q02548(UNIPROT)	391 aa	Q02548
PAX6	AAK95849(NCBI-Protein)	422 aa	2CUE(PDB)
PI4KA	AAA56839(NCBI-Protein)	854 aa	AAA56839
COMT	AAI00019(NCBI-Protein)	271 aa	4PYI(PDB)
YWHAE	P62258(NCBI- Protein)	255 aa	P62258

Table1. Schizophrenia gene.

PLANT EXTRACT

Since the golden era or the old age, we have seen that herbal medication is the 1st and abundantly used medication of all. We know that most of the medicines in the world are prepare by plants and chemicals etc. It has been found from the literature that Rauwolfia serpentine and Lavender extracts are used in the herbal medication for schizophrenia.

The Rauwolfia serpentine commonly called as Sarpagandha which is found is a large climbing or twining shrub, found in the tropical forest of Indian Peninsular region and other regions of Asia. The dried roots, leaves and rhizomes are medically important chemical constituents. The various alkaloid identified in this plant are Ajmalicine, Indobine, Rescinnamine, serpentine etc.

The genus "*Lavandula"* (Lavandula angustifolia, Lavandula stoechas and Lavandula latifolia) is the native of the lands of Mediterranean Sea and southern Europe through northern and eastern Africa and Middle Eastern countries to southwest Asia and southeast India.The 4 different lavender species have similar ethnobotanical properties and majorly the chemical constituents. These Lavender are divided into four main categories:

1. *L. angustifolia*, commonly known as English Lavender which has many pretty cultivars, habit, and blossom color.
2. *L. stoechas*, is a large plant in the lavender family with greenish-grey foliage and late blooming with a very strong odor (called French lavender also).
3. *L. latifolia*, is a Mediterranean grass-like lavender easily identified.
4. *L. intermedia*, which is the sterile cross between the two lavender species i.e., *L. latifolia* and *L. angustifolia*.

In this work, French lavender (*L. stoechas*) flower oil has been considered. The selective alkaloids which acts as a ligand are mentioned. In Unani medication, it gives us a proof that we can cure schizophrenia by using French Lavender's oil extract which is an alkaloid. The main constituents of lavender are linalool, linalyl acetate, 1, 8-cineole, and camphor. However, these constituents varies in different species accordingly. As all Lavender oil are ethnobotanical similar, (from the Family: Lamiaceae) by steam distillation, which is chiefly composed of linalyl acetate (3, 7-dimethyl-1, 6-octadien-3yl acetate), linalool (3, 7-dimethylocta-1, 6-dien-3-ol), lavandulol, 1, 8-cineole, lavandulyl acetate, and camphor.

The entire lavender oil and its major components i.e., linalool and linalyl acetate are used in aromatherapy is found to contain 51% linalyl acetate and 35% linalool measured by gas chromatography and gas chromatography-linked Fourier Transform Infrared analysis.

LIGANDS	CHEMICAL NAME	STRUCTURE (PubChem)
Linalool (lavender plant)	C10H18O	3D
1,8-cineole (lavender plant)	C10H18O	3D
Lavendulol (lavender plant)	C10H18O	3D
Lavandulyl acetate (lavender plant)	C12H20O2	3D
Ajmalicine (Rauwolfia serpentine-roots)	C21H24N2O3	3D

Table 2. Selected ligands.

PROCEDURE

We use different types of tools and website like Swiss-model, CB DOCKING, NCBI, and PUBCHEM etc., which are less time-consuming and effective way to get the results within no time.

- NCBI: The National Center for Biotechnology Information in science and health which provides access to biomedical and genomic information throughout the world.
- PUB-CHEM: It is the world's largest collection of freely accessible chemical information. Search any chemical by its name, molecular formula, structure, and other identifiers. We get the chemical and physical properties, biological activities, safety and toxicity information, patents, literature citations and much more.
- CB DOCKING: It is a protein-ligand docking method which automatically identifies the binding sites, calculates the center and size, customizes the docking box size according to the query ligands and then perform the molecular docking with Auto Dock Vina software. This tool, CB-Dock can facilitate the docking procedure and improve the accuracy by predicting the binding sites of targeted proteins using our curvature-based cavity detection approach and the binding poses of query ligands using Auto-Dock Vina accordingly.
- SWISS-MODEL: It is a fully automated protein structure homology-modelling server, accessible via the Expasy web server (Swiss PDB-Viewer).The purpose of this server is to make protein modelling easy and accessible to all life science researchers worldwide.

There are few steps to follow in order to achieve the desired results.

STEP 1:

- So as of the procedure, we use bioinformatics related tool which helps us to bind the protein and ligand.
- We need to gather all the information about the proteins, what proteins cause the schizophrenia. We gather the protein information from NCBI website and note down the accession number.
- The proteins which were not found in NCBI were available in SWISS-MODEL website
- The protein FASTA or the target sequence/UniProt KB AC sequence is taken and loaded in the SWISS-MODELLER and search for templets.
- Then it runs the templets, we get a couple of models of different identity scores. We chose the 100% identity score of 3 different models and take the best model with the best structure assessment score which has a few parameters like; Ramachandran Plot = >98%, Ramachandran Outliers = <0.2%, Rotamer outliers = <1% etc.
- Take the best identity/ structure assessment score and save it in the PDB format as MODEL1 OR MODEL2 etc.
- Now we need to take the Ligand structures in consideration, so we get back to the PUBCHEM website and place the required ligand name and we get the information and in the 2D/3D images, we consider the 3D structure which is saved in the SDF/ JSON format then we have to convert it into PDB format.
- We have to convert the SDF/JSON to MOL format by using a software called BIOVIA DISOVERY STUDIO VISUALIZER 2021.
- Chose the desired file from desktop and directly save it in PDB format which is an easy tool also make changes in our ligand or protein.

STEP 2:

- Save all the protein in PDB format and the ligand in MOL/ MOL2 format.
- Now we use the CB DOCKING to bind the ligand and protein.
- DOCKING option is provided above, click, submit the desired protein (in PDB format) and ligand (in MOL2, MOL, sdf, PDB format).
- There is a “Number of cavities for docking” available, in which u can select the number of cavities you want the ligand to dock in with.

STEP 3:

- Within no time, the results are given.
- There is a table on the right hand side, which shows the score of the ligand binding sites on the protein. As shown below:

Vina score	**Cavity size**	**Center**			**Size**		
		x	**y**	**z**	**x**	**y**	**z**
-8.3	1340	-20	0	21	22	22	35

Table 3. High energy scores from CB Docking.

1. On the left hand side we can see a 3D structure of the ligand and protein with different docking sites or different cavities present in the structure.
2. We can also cross check/ verify this 3D structure on SWISS MODEL by submitting the required queries and your results takes time of a day or so but we’ll get the entire score chart from the lowest to the highest score. We even get the 3D structure same as the CB DOCKING shows us.

RESULT

The protein-docking it was found that ajmalicine, one of the alkaloid interacts very well with all the proteins with a high energy score in relation to schizophrenia. In-vitro analysis between ajmalicine and schizophrenia proteins can help in curing for the disorder as shown below.

PROTEIN/ LIGAND	2CUE(PAX6)	4PYI(COMT)	NPAS3(AB055962	PAX5(Q02548)	PI4KA(AAA56839)	YWHAE(P62258)
LINALOOL	-3.9	-5.1	-4.5	-4.7	-5.6	-4.8
LAVANDULOL	-4.1	-5	-4.7	-4.6	-5.7	-4.7
1,8-CINEOLE	-4.5	-4.8	-5.1	-4.8	-5.9	-5.4
LAVANDULYL ACETATE	-4.4	-6	-4.9	-4.9	-5.7	-5.5
AJMALICINE	-6.5	-8.3	-7.9	-7.6	-7.5	-8.3

Table 4. Results of selected Protein and Ligand docking

a) Ajmalicine is the most affective ligand of all to be found with the high energy score.

PROTEIN/ LIGAND	2CUE(PAX6)	4PYI(COMT)	NPAS3(AB055962	PAX5(Q02548)	PI4KA(AAA56839)	YWHAE(P62258)
LINALOOL	222	536	5255	783	1154	1340
LAVANDULOL	222	536	5255	783	1154	1340
1,8-CINEOLE	222	536	5255	783	1154	1340
LAVANDULYL ACETATE	222	536	5255	783	1154	1340
AJMALICINE	222	536	5255	783	1154	1340

Table 5: Represents the cavity score of ligand and protein docking sites.

With further research through wet labs, we can find out much more efficient treatments which cures the schizophrenia patients.

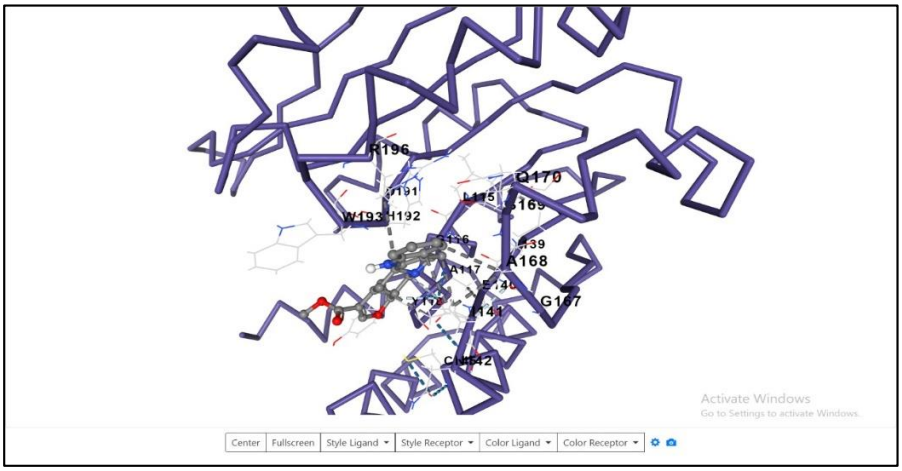

Fig:1 Interaction of COMT with Ajmalicine using CB Docking

a) This is the highest energy score found in COMT gene and Ajmalicine and also we have below, the highest energy score of YWHAE and Ajmalicine with the same (vina or) highest energy score.

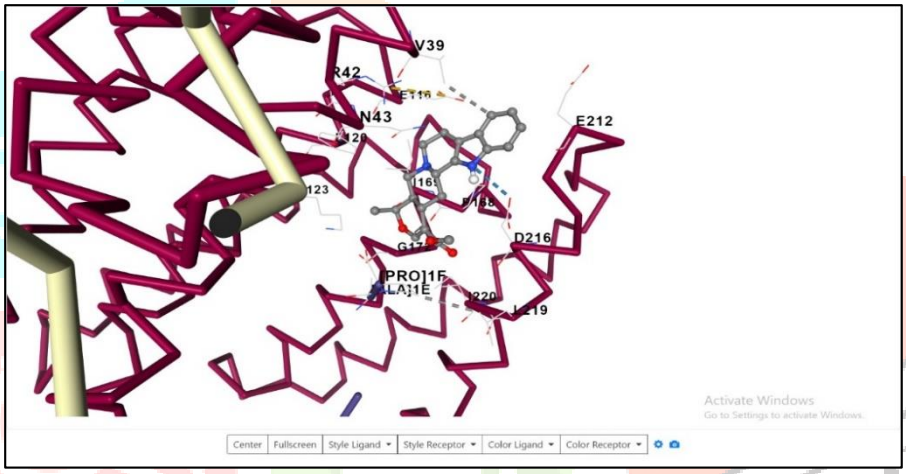

Fig: 2 Interaction of YWHAE and Ajmalicine using CB Docking.

CONCLUSION & DISCUSSION

As we know schizophrenia is caused due to the deletion or duplication in the 22 chromosome, in particular there are micro-deletions in the 22q11.2 site of the chromosome (which leads to DiGeorge Syndrome). The genes which get effected in this are

1. AKT-1 gene with AKT1 kinase which is responsible for oncogenes activation.
2. COMT gene with catechol-o-methyl-transferase enzyme which have 2 forms i.e., MB-COMT (Membrane bond) & S-COMT (soluble) which helps in breakdown of neurotransmitters (which maintains the level of Dopamine and Norepinephrine).
3. YWHAE gene (or Tyrosin-3-monooxygenase or 5-mono oxygenase activation protein ε) with the 14-3-3 epsilon which involves in neuronal migration (directing the movement of nerve cells).
4. PI4KA gene which is known as Phosphatidylinositol 4 kinase alpha which cause a novel neurodevelopmental syndrome with hypo myelinating leukodystrophy.
5. PAX 5 gene is found in the 9p13 region of the chromosome which is associated with the abnormal posterior mid-brain & cerebellum development. This alternates the expression of the gene and contributes to neoplastic transformation which is in turn is related to cancer which includes PAX 6 (all these come under the members of paired box family).

6. NPAS 3 is Neuronal PAS domain protein -3 which is a chromosomal abnormality that affects the coding potential of this gene which is associated with schizophrenia.

These alkaloids of lavender are useful for treating neurological disorders and is found to be used at herbal medication centers too. The alkaloids Linalool 1, 8-cineole, Lavandulyl acetate, Lavandulol, Ajmalicine used in treating high blood pressure.

which are used in this medication are from lavender plant and they all possess anticonvulsant, antidepressive, anxiolytic, sedative, and calming properties and almost same functionalities by effecting the Mesolimbic and mesocortical pathways in the brain.

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