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Establishing an *in-silico* ayurvedic medication towards treatment of Schizophrenia

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Abstract- RGS4 protein responsible for Schizophrenia is taken from NCBI's *Entrez* database; its 3D structure is determined by homology modelling. Ashwagandha, Sarpagandha and Mandukparni (Thankuni) were selected from Indian Ayurvedic medication. Their active component's 3D structures were established. Their combination is found to dock with RGS4 protein, hence establishing a remedy.
Keywords- schizophrenia, drug designing, Ayurvedic medication, modelling, docking

Introduction

Schizophrenia is gaining importance as an important topic of research. Neuroscientists are facing enormous difficulties in treating schizo (& neuro-patients) since they with psychotic trend have their own world of hallucinations and delusions. This study explores the application of Ayurvedic medicinal plants to treat schizophrenia related disorders. Schizophrenia is a severe mental disorder characterized by delusions, hallucinations, incoherence and physical agitation; it is classified as a "thought" disorder while Bipolar Disorder is a "mood" disorder. It is estimated that 1 percent of the world's population has schizophrenia. While there is evidence that genetic factors have a role in developing schizophrenia, other unknown causes play a significant part as well. Schizophrenia is a severe, lifelong brain disorder. Schizophrenia is a psychiatric diagnosis that describes a mental illness characterized by impairments in the perception or expression of reality [1]. Scientists are studying genetic factors in schizophrenia. It appears likely that multiple genes are involved in creating a predisposition to develop the disorder. In addition, factors such as prenatal difficulties like intrauterine starvation or viral infections, perinatal complications, and various nonspecific stressors, seem to influence the development of schizophrenia. However, it is not yet understood how the genetic predisposition is transmitted, and it cannot yet be accurately predicted whether a given person will or will not develop the disorder. Several regions of the human genome are being investigated to identify genes that may confer susceptibility for schizophrenia. Basic knowledge about brain chemistry and its link to schizophrenia is expanding rapidly.

Neurotransmitters, substances that allow communication between nerve cells, have long been thought to be involved in the development of schizophrenia. It is likely, although not yet certain, that the disorder is associated with some imbalance of the complex, interrelated chemical systems of the brain, perhaps involving the neurotransmitters dopamine and glutamate. This area of research is promising. There have been dramatic advances in neuroimaging technology that permit scientists to study brain structure and function in living individuals. Many studies of people with schizophrenia have found abnormalities in brain structure (for example, enlargement of the fluid-filled cavities, called the ventricles, in the interior of the brain, and decreased size of certain brain regions) or function (for example, decreased metabolic activity in certain brain regions). It should be emphasized that these abnormalities are quite subtle and are not characteristic of all people with schizophrenia, nor do they occur only in individuals with this illness. Microscopic studies of brain tissue after death have also shown small changes in distribution or number of brain cells in people with schizophrenia. It appears that many (but probably not all) of these changes are present before an individual becomes ill, and schizophrenia may be, in part, a disorder in development of the brain [2, 3]. A great deal of effort has been put into molecular genetic studies of schizophrenia, which attempt to identify specific genes which may increase risk. A 2003 review of linkage studies listed seven genes as likely to increase risk for a later diagnosis of the disorder. Two recent reviews suggested that the evidence was strongest for two genes known as dysbindin (DTNBP1) and neuregulin (NRG1), and that a number of other genes (such as COMT [catechol-O-methyltransferase], RGS4 [Regulator of G protein signaling 4], PPP3CC [human calcineurin A gamma subunit gene], ZDHHC8 [zinc finger, DHHC-type containing 8], DISC1 [Disrupted in schizophrenia 1], and AKT1 [V-akt murine thymoma oncogene homolog 1]) showed some early promising results [4]. Schizophrenia was ranked the 'third most disabling disorder' - mostly because it not only involves hallucinations and delusions, but also leads to depression, confusion, lack of motivation and strained personal relationships. In recent years, the importance of behavior therapy and support nets has been stressed, in addition to the continuous

dosage of a routine schizophrenic drug in minimum dose [2]. Career counseling, housing assistance and education are extremely helpful to a schizophrenic's integration into society. No one is sure what causes schizophrenia, but our genetic makeup and brain chemistry probably play a role. Medicines can relieve many of the symptoms, but it can be safely used as adjuvant with herbal therapy [5]. In human brains, kalirin is the brain protein needed to build the dense network of highways, called dendritic spines, which allow information to flow from one neuron to another. Northwestern scientists have found that without adequate kalirin, the frontal cortex of the brain of a person with schizophrenia only has a few narrow roads. The information from neurons gets jammed up like rush hour traffic on an interstate highway squeezed to a single lane [6].

Regulator of G protein signaling 4

One of the gene associated with schizophrenia is RGS4, which maps to the putative linkage region on chromosome 1q22. It was targeted for genetic analysis following a microarray-based gene expression study in which decreased RGS4 expression was found in schizophrenic postmortem brain. Independent evidence for association between schizophrenia and a haplotype at the 5′end of the gene was found in 2 samples from the US, and while it did not provide significant evidence alone, a sample from India that was included added to the overall level of support. Positive findings have subsequently been reported by several other groups, but the level of support for each has been modest and the pattern of association different among samples. RGS4 is a negative regulator of G protein–coupled receptors. However, the evidence that RGS4 modulates activity at certain serotonergic and metabotropic glutamatergic receptors while its own expression is modulated by dopaminergic neurotransmission is of relevance to its possible role in schizophrenia. Moreover, RGS4 interacts with ErbB3, which may be of relevance as ErbB3 is a NRG1 receptor whose expression is downregulated in schizophrenic brains [4]. Regulator of G protein signaling 4 or RGS4 is a protein which regulates G protein signaling. A number of studies associate the RGS4 gene with schizophrenia, while some fail to detect an association. RGS4 is also of interest as one of the three main RGS proteins (along with RGS9 and RGS17) involved in terminating signalling by the mu opioid receptor, and may be important in the development of tolerance to opioid drugs. Regulator of G protein signaling (RGS) family members are regulatory molecules that act as GTPase activating proteins (GAPs) for G alpha subunits of heterotrimeric G proteins. RGS proteins are able to deactivate G protein subunits of the Gi alpha, Go alpha and Gq alpha subtypes. They drive G proteins into their inactive GDP-bound forms. Regulator of G protein signaling 4 belongs to this family. All RGS proteins share a conserved 120-amino acid sequence termed the RGS domain. Regulator of G protein signaling 4 protein is 37% identical to RGS1 and 97% identical to rat Rgs4. These proteins negatively regulate signaling upstream or at the level of the heterotrimeric G protein and are localized in the cytoplasm. [7,8]. In Ayurveda, Schizophrenia is known as a type of "Unmaad". Ayurvedic medicine, developed in India more than 3000 years ago, is one of the oldest medical systems known to man. It is a complete and holistic science of healthy balanced living which views each person as an individual, with a unique mind-body constitution and set of life circumstances. Ayurvedic medicine has been used in the treatment of mental health problems in India since its advent in c.1000 BC. It is now being used either on its own or in conjunction with antipsychotic medication [9, 10]. Sarpagandha (*Rauvolfia serpentina*) is a famous tranquilizer and antipsychotic herb of India for the treatment of paranoia and schizophrenia, as well as a substance that controls hypertension. Sarpagandha is considered as one of the best-known plant drugs in the world. The roots contain major alkaloidal constituents, notably reserpine, rescin-namine and deserpidine, which are used for medicinal purpose in India. The root of Sarpagandha that carries immense medicinal properties is widely used even in the countries overseas in the treatment of high blood pressure and as a sedative and tranquillizing agent. For centuries, Ayurveda and Unani practitioners reckon the root of Sarpagandha as a hypnotic and sedative, for reducing high blood pressure, and for treating various central nervous system disorders, both psychic and motor, including anxiety, psychosis, schizophrenia, epilepsy and insomnia. The tribal inhabitants of southern and eastern Bihar take the powdered roots orally as an antidote to snake venom. Extracts of the roots of Sarpagandha plant are valued for treatment of intestinal disorders, particularly diarrhoea and dysentery, and also as an anthelmintic [11]. *Centella asiatica* is a small herbaceous annual plant of the family Apiaceae, native to Asia. Also known as Gotu kola it has been used as a medicinal herb for thousands of years in India, China and Indonesia. Its ability to heal wounds, improve mental clarity, and treat skin conditions such as leprosy and psoriasis were important reasons for its extensive use. As per Ayurveda Gotu kola herb is one of the chief herbs for revitalizing the nerves and brain cells; hence primarily known as a brain food in India. It has been used for the purposes like boosting memory, wound healing, a mild diuretic, increasing concentration, alertness, as well as

anti-anxiety and anti-stress. It has also been used for centuries in the treatment of liver and kidney problems. The active component of the plant asiaticoside is used here [12]. Withanolides, derived from Indian herbal medicine Ashwagandha (Withania Somnifera), are currently being explored for their brain regenerative properties. The compound is isolated from fresh roots of the plant. Works by Schliebs R (1997) et.al [13] and Tohda, C (2000) et.al [14] establishes the brain regenerative property of withanolides.

Methodology
Softwares/Web Servers used

1) modeller9v5
2) Swiss-PdbViewer v4.01
3) ACD/ChemSketch
4) RAMPAGE
5) ArgusLab 4.0.1
6) HEX 5.1
7) PATCHDOCK
8) Yasara View

The Schizophrenia causing protein, human rsg4, was retrieved from NCBI's Entrez database accession AAH00737 for this work. To find out the cellular mechanism that mediated this particular disorder, homology modelling is carried out using the software Modeller9v5 software. A 3-D structure is essential to study the interaction between the proteins. Homology modeling studies were done using modeller9v5 using the following three templates (homologous proteins to our query in the RCSB's pdb database):
1EZTA (Rattus norvegicus), 2ODED (Homo sapiens), and 2OJ4A (Homo sapiens). Five models (3D structures) of the rgs4 protein were generated by the software 9v5. The models generated by Modeller is analysed by Rampage Ramachandran Plot server and the best stable model is selected.

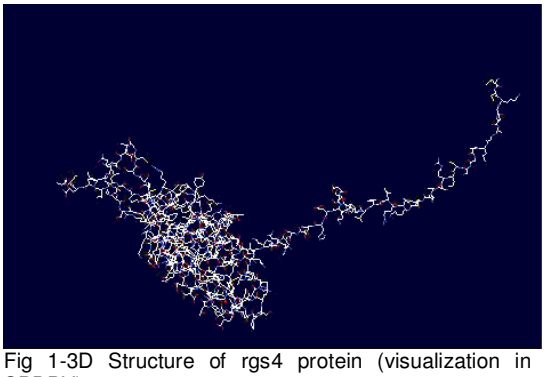

Fig 1-3D Structure of rgs4 protein (visualization in SPDBV)

For proposed treatment three known principal alkaloids from Sarpagandha, Mandukparni (Thankuni) and Ashwagandha were selected. Authors propose that combined effect of the three herbs will be effective in treating/controlling schizophrenia patients. The chemical structure of reserpine (principle alkaloid of Sarpagandha), asiaticoside (principle alkaloid of Mandukparni) and withanolide (principle alkaloid of Ashwagandha) were drawn using ACD/Chemsketch software. Again, the structures of reserpine, asiaticoside and withanolide were merged as one structure (proposed treatment). This combination is converted to 3d of *.pdb structure using the software Arguslab and submitted to HEX software along with rgs4 protein for docking studies.

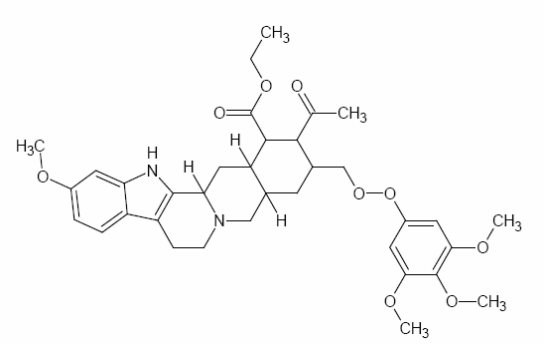

Fig 2- Chemical structure of reserpine drawn using ACD/Chemsketch software

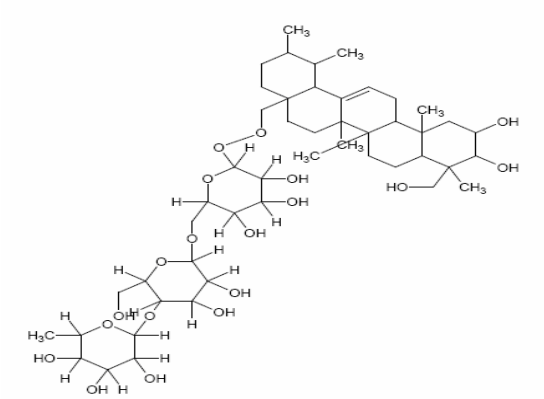

Fig 3- Chemical structure of asiaticoside drawn using ACD/Chemsketch software

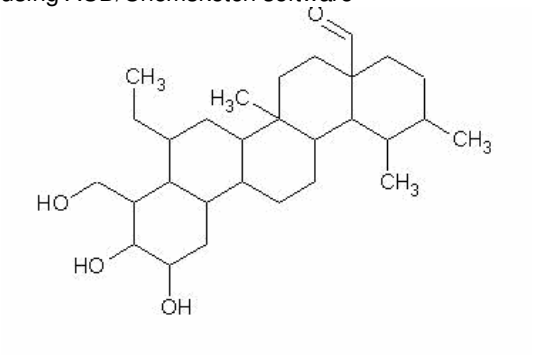

Fig 4- Chemical structure of withanolide drawn using ACD/Chemsketch software

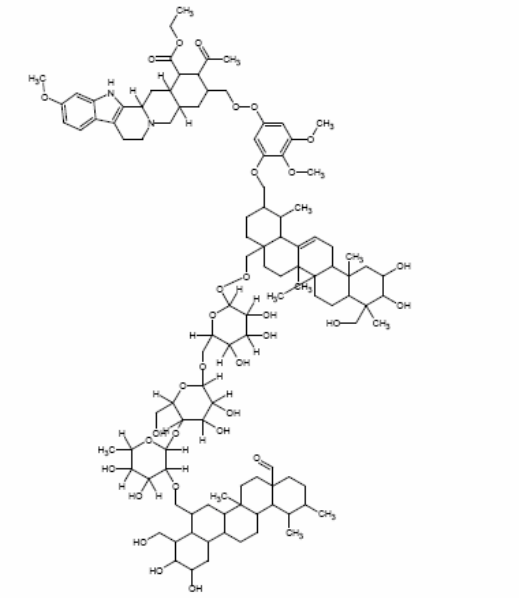

Fig 5- Combined Structure of reserpine, asiaticoside & withanolides

Result

The rsg4.pdb protein obtained after homology modeling was analyzed with Ramachandran Plot server. The results obtained are as follows:

Model 1
Number of residues in favoured region (~98.0% expected: 197 (97.0%)
Number of residues in allowed region (~2.0% expected): 5 (2.5%)
Number of residues in outlier region : 1 (0.5%)
Model 2
Number of residues in favoured region (~98.0% expected): 196 (96.6%)
Number of residues in allowed region (~2.0% expected): 7 (3.4%)
Number of residues in outlier region : 0 (0.0%)

Model 3
Number of residues in favoured region (~98.0% expected): 194 (95.6%)
Number of residues in allowed region (~2.0% expected): 9 (4.4%)
Number of residues in outlier region : 0 (0.0%)

Model 4
Number of residues in favoured region (~98.0% expected): 196 (96.6%)
Number of residues in allowed region (~2.0% expected): 6 (3.0%)
Number of residues in outlier region : 1 (0.5%)

Model 5
Number of residues in favoured region (~98.0% expected): 195 (96.1%)
Number of residues in allowed region (~2.0% expected): 8 (3.9%)
Number of residues in outlier region : 0 (0.0%)

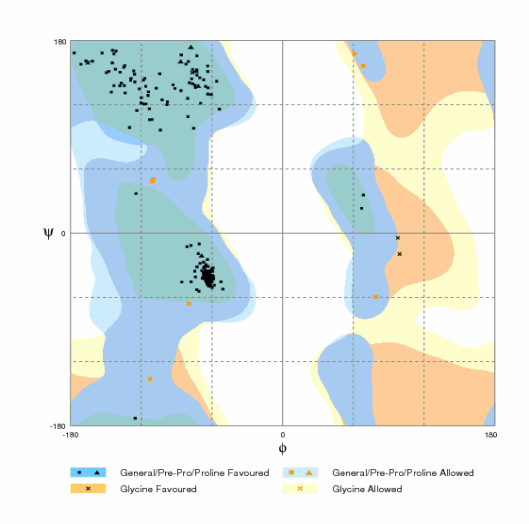

Fig 6- Ramachandran Plot analysis if Model 2

Hence as per Ramachandran Plot Analysis, Model 2 was chosen as the best model (no residue in the outlier region and maximum residue in favoured region). This model gets docked successfully with reserpine, asiaticoside and withanolide combination by HEX software.

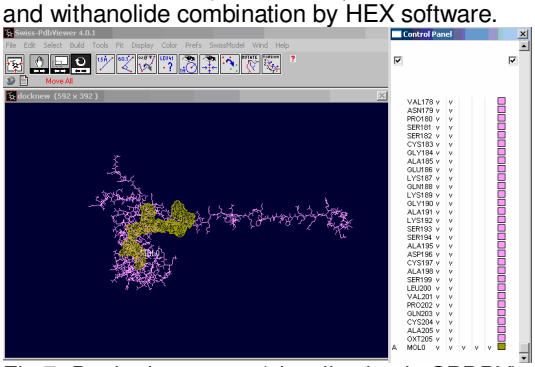

Fig 7- Docked structure (visualization in SPDBV)

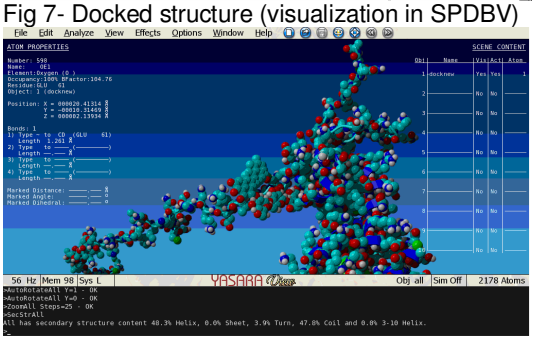

Fig 8- Secondary structure content of the Docked Structure (visualization in Yasara view software)

Conclusion

The successful docking of rg4.pdb protein with reserpine, asiaticoside and withanolide combination proves that the combination can be effective in the treatment of Schizophrenia.

(Verified by the Patchdock server which gave the docking score as 11776).

Discussion

This *in-silico* ayurvedic work highlights two important landmark achievements towards this disorder: (i) mutation as one of the cause of Schizophrenia and identification of the mutant gene & its corresponding protein, and (ii) application of Indian ayurvedic medication towards treatment/cure of this disorder. These achievements should prove to be a boon towards schizophrenia research. In the last part of 20th century and towards the beginning of 21st century scientists made an important breakthrough in understanding the genetic causes of schizophrenia, which affects one in 100 people. Several studies have identified and established multiple mutations in many genes which are found to be a critical trigger for the condition. Among several studies, a study by scientists at the Julius-Maximilians-University of Wuerzburg, Germany investigated that genes on human chromosome 22 elucidated the genetic background of dominantly inherited catatonic schizophrenia, which was characterized by acute psychotic episodes with hallucinations, delusions, and disturbed body movements. Again, the UCI researchers and their collaborators studied the CAG repeats in the new gene, hSKCa3 which is responsible for schizophrenia. These studies establish mutations as one of the causes of Schizophrenia. Schizophrenia is one of the most burning problems of the globe. Usually the schizophrenic patients are thrown mercilessly to mental asylums neglecting their pain above all they are ill-treated. There are reports that several intelligent people are prey to this disorder. Henceforth the authors wish successful cure towards this disorder and also hope that they may return to the normal life. Ayurvedic herbs like Sarpagandha, Mandukparni (Thankuni) and Ashwagandha are used over decades to treat mental disorders and they are mentioned in Charaka samhita. Henceforth authors wish to highlight the application of ayurvedic medicinal plants to treat schizophrenia.

Dedication

This work is dedicated to Divine Lord Sri Sri Thakur Anukul Chandra, Satsang, Deoghar who has brought back several lost Ayurvedic medications/formulations to mankind.

References

[1] Robert Laynton Mysticism, Madness And Mania (2009) *An Exploration Of Experiences Of God And Mental Disorde*r, 27-41.

[2] Schizophrenia: National Institute of Mental Health (2009) 1-7.

[3] Peter J. Weide, Patricia L. Scheifler, Joseph P. McEvoy, Allen Frances, and Ruth Ross (1999) *Reprinted from J Clin Psychiatry*, 60(11), 1-8.

[4] George Kirov, Michael C. O'Donovan and Michael J. Owen (2005) *The Journal of Clinical Investigation*, 115(6), 1440-1448.

[5] Russell L Blaylock (2008) *Medical Veritas*, 5(182), 1727–1741.

[6] *Traffic Jam in Brain Causes Schizophrenia Symptoms Scientists create first mouse to develop disease as teenager, just like humans: Northwestern University* (2009), 1-2

[7] Susanne Hollinger and John R. (2002) *Pharmacol Rev*, 54(3), 527–559.

[8] Joshua W. Buckholtz et.at., (2007) *The Journal of Neuroscience*, 27(7), 1584 – 1593.

[9] Vishesh Agarwal, Akhil Abhijnhan and Prakash Raviraj (2009) *The Cochrane Library,* 4(CD006867), 1-37.

[10] Subbarayappa and Subbarayappa B.V. (2001) *Journal Of Biosciences*, 26(2), 135–143.

[11] *Sarpagandha from Hand Book on Medicinal & Aromatic Plants*: *NEDFi* (2005) 51-55.

[12] Product Brochure by Indena R.T.M. *Centella Asiatica Selected Triterpenes: A Highly Standardized Natural Remedy for the Maintenance of an Healthy Venous System, (Date Unknown).*

[13] Schliebs R., Liebmann A., Bhattacharya S.K., Kumar A., Ghosal S., Bigl V. and Paul Flechsig (1997) *Neurochem Int*, 30(2), 181-90.

[14] Tohda C., Kuboyama T. and Komatsu K. (2000) *Neuroreport*, 11(9), 1981-5.

