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IN SILICO PREDICTION OF THE MECHANISM OF LEVETIRACETAM AGAINST ALZHEIMER'S DISEASE

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ABSTRACT

Objective: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is affecting people worldwide and it is important to discover effective drug treatment for Alzheimer's disease. Levetiracetam (LEV) is an anticonvulsive drug use in epilepsy and found effective in treatment of Alzheimer's disease but the mode of action of LEV against AD is unclear. So, this study aims to explore the anti-AD mechanism of LEV. **Methods:** A database named STITCH was used to identify the targets of LEV and a protein-protein interaction network was constructed using the same database which evaluated the interaction between the targets. Furthermore, gene ontology enrichment analysis with ClueGO plugin was used. Finally docking was performed for computational analysis. **Results:** According to results shown, 48 drug targets or gene proteins were found. After analyzing these targets abundance values and by gene functional annotation, seven GO terms were found to be closely related with Alzheimer's disease. The terms found to be involved in mechanism of LEV against AD are KCNMA1, CBS, GRIN2B, CSTB, APOD, AMPH and FAS. **Conclusions:** The findings elaborate that LEV can be utilized to manage AD. However, multiple molecular mechanisms of action could be proposed for this effect. The observed core anti-AD mechanism of LEV could be the action on different genes found to be involved in AD. Furthermore, ILE97 and ALA436 are found to be interactive towards LEV. These mechanisms can be further elaborated through in-vivo studies.

KEYWORDS: Biological effects; Cytoscape; Alzheimer's disease; mechanism of action; molecular targets; Levetiracetam; STITCH.

INTRODUCTION

Levetiracetam (LEV) is an antiepileptic agent, approved by FDA in 1999 for treatment of partial epilepsy in adults.[1] LEV belongs to the pyrrolidine class of drugs and is used to treat various types of epileptic seizures.[2] LEV is widely in use and is available in different formulations such as IV infusion, extended and immediate release tablets and oral syrups. As LEV is a pyrrolidine acetamide, so it is chemically unique from other most anticonvulsive drugs. The major side effects of LEV include low energy levels, headache, and sleepiness. The mechanism of LEV is not completely understood. There is no significant and direct effect of LEV found on GABAergic system and receptors involved in Epilepsy such as adenosine (P1) receptors, ion channels, and amino acid related channels.[3] Studies suggest that LEV can show its antiepileptic effects by binding to a glycoprotein synaptic vesicle, affecting neurotransmitter release, vesicle exocytosis and fusion.[4] According to recent study and knowledge it is suggested that LEV shows its antiepileptic effects by binding to SV2A (synaptic vesicle protein 2A). LEV and its related analogues showed affinity towards SV2A protein which is found potential for anti-epileptic effects. LEV is also found to have indirect effects on GABAergic system, modulating the ions of neurons. LEV can also inhibit N-type calcium channels. But these actions of LEV are not yet clarified.[2] After oral administration LEV is absorbed almost completely and rapidly, showing peak plasma concentration within 1 hour. Food shows no effect on

LEV bioavailability but decreases the peak plasma concentration of drug by 20% and delaying it by 1.5 hours. The metabolism of LEV does not occur through CYP-P450 enzyme; rather metabolism of LEV drug occurs through acetamide group hydrolysis in blood (about 27% metabolized). Plasma half-life of LEV in adults is 7 ± 1 hour. While in elderly plasma half-life of LEV can be increased with an average of 2.5 hours probably due to reduce level of creatinine clearance. LEV is mainly excreted through kidneys in unchanged form.[5]

Alzheimer disease (AD) is one of the most common progressive neurodegenerative disorders.[6] AD is among the most common form of dementia almost involved in 60-70% of dementia cases. Dementia is a syndrome illustrated by dysfunction of numerous brain activities, which includes disturbance of language, thinking, memory, judgment, learning capacity, calculation, comprehension, and orientation. In AD mainly cognitive functions become impaired causing progressive loss of memory, learning capacity, thinking and language but consciousness is not affected. AD often starts with minor symptoms and can ends with severe damage of brain. AD cases are more common seen in people with age 65 and more. The AD pathophysiology is associated with the injury and death of those neurons which are involved in learning and memory, originating in the hippocampus of brain. After that in AD entire brain is affected by atrophy.[7]

AD is characterized pathologically by senile plaques, formed by extracellular deposits of amyloid beta proteins (Aβ) and intracellular neurofibrillary tangles (NFTs). In AD, Aβ accumulates in the cerebral blood vessels and parenchyma of brain forming amyloid plaques termed as cerebral amyloid angiopathy (CAA) and also known as congophilic angiopathy. While NFTs formation in AD are paired helical filaments with hyperphosphorylated tau proteins which causes synaptic and neuronal loss with some distinct lesions.[8] According to composition amyloid plaques are subclassified and all forms containsAβ. Aβ is an amino acid peptide which is produced by proteolytic cleavage through the action of β- and γ-secretase on APP, producing main products $A\beta_{1-40}$ and $A\beta_{1-42.}$ An excessive of $A\beta_{1-42}$ predisposes toward amyloid aggregation into oligomers and fibrils that result in formation of amyloid plaques. Another amyloid related fact which plays a role in AD pathology is that proteins which are encoded by ApoE, APP, PS1, PS2 and SorL1 are involved in amyloid processing and production. Despite of all many studies showed that amyloid plaques are not a leading cause in AD. Furthermore, in AD there is deposition of NFTs along with aggregates of hyperphosphorylated tau protein within neurons located at frontal association cortices, lateral parietotemporal region and mesial temporal lobe. Normally, this tau protein helps in microtubule assembly and is crucial for normal neuronal development and axonal growth. The importance of NFTs role in AD is explained by relationship between density and location of tau NFT and by AD dementia severity and its symptoms. Additionally, some studies showed that Aβ oligomers are harmless without the presence of tau. Typically, in AD neuronal death in the nucleus basalis of Meynert causes the decline levels of Ach, a memory neurotransmitter. Today many antiepileptic drugs are used to treat this decline of Ach. In the brainstem, loss of locus ceruleus and median raphe results in decline levels of NE (norepinephrine) and 5HT (serotonin) respectively. This disbalance of NE and 5HT in AD are thought to be involved in insomnia and dysphoria like symptoms.[9] The genotype APOE (apo-lipo-protein E) is found to be associated with AD. The core medication class for the treatment of AD is AChEIs (acetyl-cholinesterase inhibitors) whose main target is APOE. But according to the "Cholinergic Hypothesis" it is recognized that in AD cholinergic neurons are not mainly involved, so APOE do not play a major role in AD.[8]

Different studies showed effectiveness of LEV in AD but there is no such study which can explain mode of action of LEV and its signaling pathway in AD. So, in this study network pharmacology is used to identify LEV mechanism against AD, as there is need of understanding multi-target regulation of LEV. The aim of study was to explore signaling pathways of LEV and its mechanism against AD by using NP, GO enrichment and by docking of molecules. In future, this in-silico study will help in clinical trials of LEV in treatment of AD.

METHODOLOGY

To determine the mechanism of LEV against AD, STITCH database 5.0 and Cytoscape were used. In this systematic study (Figure 2) firstly, the targets of LEV were determined by constructing protein interaction network by using STITCH then the molecular mechanism of LEV that affect against AD were explored through using Cytoscape and its plugin ClueGO. Finally, docking studies were performed to examine the computational approach of selected ligand for its target proteins by using Swiss-model and Sybyl-X1.3.

Drug target identification, network construction and their analysis

Firstly, the targets of LEV were searched through an interactive web tool STITCH 5.0 database (http://stitch.embl.de/) which at present contains information about 9.6 million proteins from approximately 2300 organisms. At the same time, the interaction of drug with identified target proteins and chemicals were analyzed by using the same networking tool STITCH 5.0 database with the confidence score set at 0.15 (low confidence level) and maximum number of interactions in the 1st shell as "not more than 50 interactions".

Gene Ontology and Pathway Enrichment Analysis

To dissect targeted genes in a form of hierarchic structure, based on biological terms, GO enrichment analysis were applied. This analyzed and identified the distinctive biological properties of the potential targets of LEV. As enrichment analysis was found beneficial to investigate mechanism of LEV against AD. To evaluate and visualize the network Cytoscape software 3.4.0 was used and for determining the mechanism and pharmacodynamic effects of LEV against AD ClueGOCytoscape plugin was applied.

In this study, for ClueGO analysis, the level of significance was set at 0.05. Furthermore, medium network type option and two-sided hypergeometric test along with a Bonferroni correction was applied. As compared to the detailed and global network, medium network reveals GO terms found in the GO levels 1–8, with a medium number of genes linked and a medium percentage of uploaded genes found. Lastly, organic layout algorithm was used to visualize functional network.

Structure's preparation and docking studies

The proteins for docking have been obtained from RCSB Protein Data Bank (PDB). The following proteins were utilized (PDB Id, 2HZR (APOD), 2OCT (CSTB), 2RC3 (CBS), 3EZQ (FAS), 3NAF (KCNMA1), 4V11 (SVA2), 7DTD (SCN1A), 1ES0 (GAD2).[19-26] PDB id for two KCNJ10 and SLC1A2 proteins, were missing. To obtain the structure for those proteins, sequence was extracted from PDB and put into SWISS-Model to draw the protein structure. The proteins were prepared using the biopolymer module from Sybyl-X1.3. To initiate the docking, substructures were removed from protein along with the removal of water molecules. A well-known Anti-psychotic drug levetiracetam was docked into each of the protein. Missing hydrogens were applied into the protein model and the ligand molecule. Ligand was minimized and Gasteiger-Huckell charges were applied to optimize the ligand structure best fit for docking.

RESULTS

Drug target identification, Network Construction and Analysis

A protein-protein interaction network (PPIN) with low confidence score level i.e., 0.15, was constructed using STITCH 5.0 database (accessed in January 2021). The constructed network showed 48 nodes which represents the relevant genes or target proteins. Moreover, the obtained network showed 174 edges (lines), representing the gene pairs interactions. Network stats showed that if there is random selection of nodes for this PPIN, the expected number of edges is 81, while the PPIN enrichment *p*-value obtained is 0.00. This small PPIN enrichment *p*-value indicates that nodes are not random and observed numbers of edges are significant. Average number of linkages of a protein target in a PPIN at the threshold score is represented by average node degree, whose value in this network was obtained 7.25. While the degree of connectivity of nodes in a PPIN is represented by clustering coefficient, higher the clustering coefficient value, higher is the connectivity of obtained network. The obtained value of clustering coefficient for this network was 0.634. Furthermore, the obtained PPIN showed 44 hubs, among which the protein having highest node degree is KCNMA1=20. The subsequent proteins, having higher node degree than the average are CBS, GRIN2B, CSTB, APOD, AMPH and FAS having 18, 14 9, 8, 7 and 5 node degree, respectively. These all above mentioned proteins have been reported to be involved in AD.

Table 1: Node degree of LEV targets obtained through STITCH.

Node	Node degree	Score	Mode of interaction
KCNJ10	10	0.845	Activation
SCN1A	19	0.833	Inhibition
FAS	5	0.786	Unknown
SV2A	2	0.702	Unknown
CSTB	9	0.577	Unknown
TRAK1	5	0.423	Unknown
AMPH	7	0.414	Unknown
GAD2	18	0.391	Unknown
PPT1	9	0.372	Unknown
KCTD7	9	0.340	Unknown
CBS	18	0.334	Unknown
GLRB	5	0.314	Unknown
GPHN	8	0.313	Unknown
PRICKLE1	8	0.300	Unknown
ENSG00000268864	3	0.273	Unknown
SLC7A4	7	0.273	Unknown
SLC7A1	6	0.273	Unknown
SLC7A3	5	0.273	Unknown
SLC7A14	7	0.273	Unknown

SLC7A2	7	0.273	Unknown
SLC1A2	15	0.269	Unknown
CRP	1	0.269	Unknown
BCAT1	5	0.267	Unknown
BCAT2	5	0.267	Unknown
NHLRC1	9	0.253	Unknown
TBC1D24	3	0.252	Unknown
KCNJ1	6	0.251	Activation
KCNJ9	6	0.251	Activation
KCNJ11	10	0.251	Activation
KCNJ14	7	0.251	Activation
KCNJ5	6	0.251	Activation
KCNJ15	8	0.251	Activation
KCNJ12	3	0.251	Activation
KCNJ4	8	0.251	Activation
KCNJ3	9	0.251	Activation
KCNJ6	11	0.251	Activation
KCNJ16	12	0.251	Activation
KCNJ2	12	0.251	Activation
KCNJ8	10	0.251	Activation
KCNJ13	15	0.251	Activation
CYP2C9	5	0.247	Unknown
SUZ12	1	0.241	Unknown
KCNMA1	20	0.240	Unknown
MTHFR	8	0.239	Unknown
APOD	8	0.239	Unknown
MT2A	1	0.239	Unknown
GRIN2B	14	0.238	Unknown
PFKL	11	0.236	Unknown

Gene Ontology and Pathway Enrichment Analysis

The retrieved protein targets of LEV were examined through ClueGO-mediated enrichment analysis by employing GO terms for the annotation of the biological functions. This analysis evolved to significant enrichment of 70 GO terms, but these GO terms were classified into 13 sub-groups. These sub-groups were mainly involved in Fas signaling pathway, negative regulation of I-kappaB kinase/NF-kappaB signaling, response to antibiotic, positive regulation of neuron death, regulation of lamellipodium organization, programmed necrotic cell death, positive regulation of type I interferon production, regulation of signal transduction by p53 class mediator, negative regulation of signal transduction by p53 class mediator, double-strand break repair via nonhomologous end joining, positive regulation of apoptotic signaling pathway, extrinsic apoptotic signaling pathway via death domain receptors, regulation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway. These observations are valued in improved understanding of the mechanism of LEV against AD.

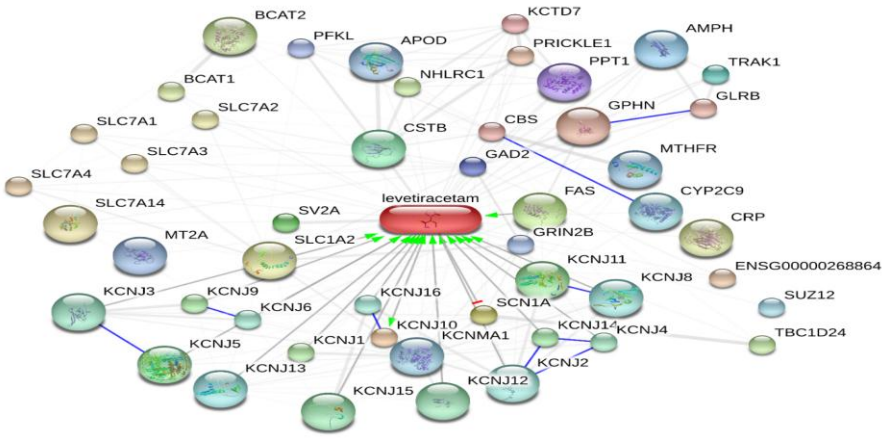

Figure 1: Action view of the protein network of LEV targets. Action type is represented by colored edges, as described here: activation (—) inhibition (—), binding (—), catalysis (-—), phenotype

(),posttranslational modification (), reaction () and transcriptional regulation (). Action effects are shown by following signs: Positive (), negative () and unspecified (). Note: KCNJ10 - potassium inwardly-rectifying channel, subfamily J, member 10;SCN1A - sodium channel, voltage-gated, type I, alpha subunit; FAS - Fas (TNF receptor superfamily, member 6); SV2A - synaptic vesicle glycoprotein 2A; CSTB - cystatin B (stefin B); TRAK1 - trafficking protein, kinesin binding 1; AMPH - amphiphysin; GAD2 - glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa); PPT1 - palmitoyl-protein thioesterase 1; KCTD7 - potassium channel tetramerisation domain containing 7; CBS - cystathionine-beta-synthase; GLRB - glycine receptor, beta; GPHN - gephyrin; PRICKLE1 - prickle homolog 1 (Drosophila); ENSG00000268864 - Uncharacterized protein; SLC7A4 - solute carrier family 7 (orphan transporter), member 4; SLC7A1 - solute carrier family 7 (cationic amino acid transporter, y+ system), member 1; SLC7A3 - solute carrier family 7 (cationic amino acid transporter, y+ system), member 3; SLC7A14 - solute carrier family 7 (orphan transporter), member 14; SLC7A2 - solute carrier family 7 (cationic amino acid transporter, y+ system), member 2; SLC1A2 - solute carrier family 1 (glial high affinity glutamate transporter), member 2; CRP - C-reactive protein, pentraxin-related; BCAT1 - branched chain amino-acid transaminase 1, cytosolic; BCAT2 - branched chain amino-acid transaminase 2, mitochondrial; NHLRC1 - NHL repeat containing 1; TBC1D24 - TBC1 domain family, member 24; KCNJ1 - potassium inwardly-rectifying channel, subfamily J, member 1; KCNJ9 - potassium inwardly-rectifying channel, subfamily J, member 9; KCNJ11 - potassium inwardly-rectifying channel, subfamily J, member 11; KCNJ14 - potassium inwardly-rectifying channel, subfamily J, member 14; KCNJ5 - potassium inwardly-rectifying channel, subfamily J, member 5; KCNJ15 - potassium inwardly-rectifying channel, subfamily J, member 15; KCNJ12 - potassium inwardly-rectifying channel, subfamily J, member 12; KCNJ4 - potassium inwardly-rectifying channel, subfamily J, member 4; KCNJ3 - potassium inwardly-rectifying channel, subfamily J, member 3; KCNJ6 - potassium inwardly-rectifying channel, subfamily J, member 6; KCNJ16 - potassium inwardly-rectifying channel, subfamily J, member 16; KCNJ2 - potassium inwardly-rectifying channel, subfamily J, member 2; KCNJ8 - potassium inwardly-rectifying channel, subfamily J, member 8; KCNJ13 - potassium inwardly-rectifying channel, subfamily J, member 13; CYP2C9 - cytochrome P450, family 2, subfamily C, polypeptide 9; SUZ12 - suppressor of zeste 12 homolog (Drosophila); KCNMA1 - potassium large conductance calcium-activated channel, subfamily M, alpha member 1; MTHFR - methylenetetrahydrofolate reductase (NAD(P)H); APOD - apolipoprotein D; MT2A - metallothionein 2A; GRIN2B - glutamate receptor, ionotropic, N-methyl D-aspartate 2B; PFKL - phosphofructokinase, liver;

Table 2: Retrieval of GO terms and their related genes via ClueGO; Corrected with Bonferroni step down.

GO ID	Go term	Term pvalue	Group pvalue	Associated Genes Found
36337	Fas Signaling Pathway	1.97E-07(1.08E-05)	1.97E-07(7.88E-07)	[CASP8AP2, FASN, SP100]
43124	Negative Regulation of I-kappaB kinase/NF-kappaB signaling	6.67E-04(4.67E-03)	6.67E-04(1.33E-03)	[CASP8, DAB2IP, IL1RL1]
46677	Response to Antibiotic	1.05E-08(6.71E-07)	1.05E-08(9.44E-08)	[CASP3, CASP8, ETS1, ADM2, TP53BP1, UQCRFS1]
1216	Positive Regulation of Neuron Death	2.21E-06(9.73E-05)	1.68E-07(8.40E-07)	[CASP3, CDC42, MAP3K5, PRKN, TP53BP1]
2743	Regulation of Lamellipodium Organization	2.42E-04(3.87E-03)	9.60E-04(9.60E-04)	[CDC42, KANK1, RAC1]
97300	Programmed Necrotic Cell Death	1.46E-07(8.32E-06)	3.57E-08(2.85E-07)	[CASP8, FADD, FASN, MAP3K5, TRAF2]
32481	Positive Regulation of Type I Interferon Production	1.12E-03(1.12E-03)	1.25E-05(3.74E-05)	[MRE11, MYD88, TLR2]
1796	Regulation of Signal Transduction by p53 Class Mediator	2.82E-09(1.83E-07)	8.23E-08(4.94E-07)	[ADM2, MRE11, NBN, PRKN, RAD50, RFFL, RNF34, SUMO1, TP53BP1]
1797	Negative Regulation of Signal Transduction by p53 Class Mediator	3.96E-08(2.50E-06)	8.23E-08(4.94E-07)	[ADM2, PRKN, RFFL, RNF34, SUMO1]
6303	Double-Strand Break Repair Via Nonhomologous End Joining	1.02E-06(4.81E-05)	8.23E-08(4.94E-07)	[MRE11, NBN, RAD50, SUMO1, TP53BP1]
1235	Positive Regulation of Apoptotic Signaling Pathway	5.17E-08(3.20E-06)	2.41E-13(3.14E-12)	[CASP8, DAB2IP, FADD, FASN, MAL, PRKN, TP53BP1, TRAF2]
8625	Extrinsic Apoptotic Signaling	1.08E-15(7.76E-14)	2.41E-13(3.14E-12)	[CASP8, CASP8AP2,

	Pathway Via Death Domain Receptors			DAB2IP, FADD, FASN, FEM1B, MAL, RFFL, RNF34, SP100, TRAF2]
1267	Regulation Of Cysteine-Type Endopeptidase Activity Involved In Apoptotic Signaling Pathway	5.23E-07(2.56E-05)	2.41E-13(3.14E-12)	[CASP8, FADD, FASN, TRAF2]

Table 3: Surflex scores of docked ligands ASR, BHMM, MISP, PHS and UPOL in the binding site of various proteins.

Protein	Docking complex	CScore[a]	Crash score[b]	Polar score[c]	D score[d]	PMF score[e]	G score[f]	Chem score[g]	Amino acid interaction
levetriacetam	APOD	4.48	-2.67	1.40	-105.479	-48.69	-203.702	-10.32	V154
	CBS	3.86	-1.14	1.32	-67.492	30.497	-161.38	-10.15	V37, L126
	CSTB	2.83	-0.22	3.44	-207.670	-11.55	-59.158	-12.44	T81, S83
	FAS	3.98	-0.87	0.93	-69.509	30.860	-143.355	-7.113	V158, E337
	GAD2	3.35	-0.45	3.32	-63.313	-24.09	-91.431	-7.915	D27, Y123, A125, P124
	KCNJ10	3.63	-1.39	3.47	-43.095	32.495	-137.884	-7.722	W24, K173, K174
	KCNMA1	4.05	-0.90	3.52	-66.737	30.063	-105.539	-6.301	R514, M513, D362
	SCN1A	3.30	-0.77	1.86	-64.296	26.706	-133.694	-8.239	V237, S1471
	SLC1A2	3.94	-0.89	1.96	-73.816	27.285	-145.166	-6.340	ILE97, ALA436
	SVA2	3.30	-0.22	3.11	-32.697	56.619	-92.223	-2.053	V358, V359, V292, L324

[a]CScore is a consensus scoring which uses multiple types of scoring functions to rank the affinity of ligands, [b]Crash-score revealing the inappropriate penetration into the binding site, [c] Polar region of the ligand, [d] D-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies, [e] PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF), [f] G-score for charge and van der Waals interactions between the protein and the ligand, [g] Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

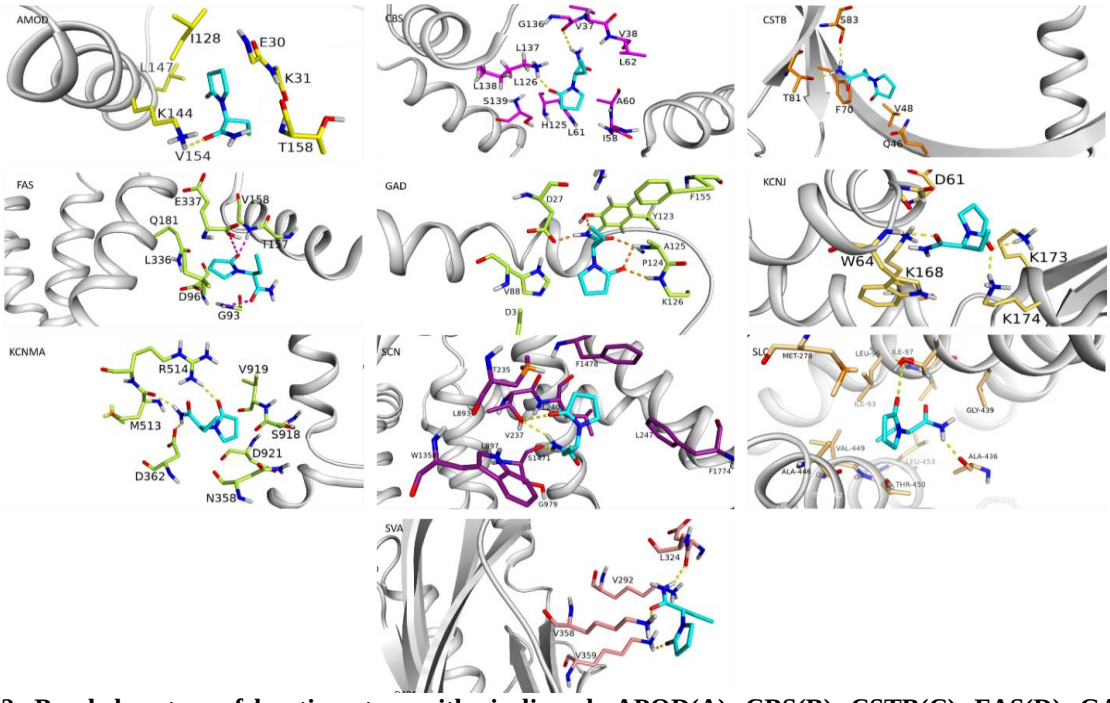

Figure 2: Bonded system of levetiracetam with six ligands APOD(A), CBS(B), CSTB(C), FAS(D), GAD2(E), KCNJ10(F), KCNMA1(G), SCN1A(H), SLC1A2(I) and SVA2(J).

DISCUSSION

This in-silico study was designed to evaluate the role and mechanism of LEV against AD. As the AD is prevailing by time, so there is urgent need of new tactics to prevent and treat it. Problems in activity of brain's function contribute to AD development and this may also cause cognitive decline due to AD.[10] LEV is a second-generation AED (antiepileptic drug) and used worldwide to treat partial seizures with or without secondary generalized seizures.[11] Different studies showed that LEV is effective in treating AD, but its mechanism is undefined, so we identified protein targets of LEV by forming protein interaction network. Furthermore, GO enrichment analysis of the screened protein targets confirmed that LEV possesses anti-AD activity.

The main hubs in PPIN, like KCNMA1, CBS, GRIN2B, CSTB, APOD, AMPH and FAS are already reported to be involved in AD. According to RGD (Rat Genome Database), KCNMA1 (potassium calcium-activated channel subfamily M alpha 1) gene is found to be involved in AD and also in idiopathic generalized epilepsy.[12] Another gene information data base Harmonizome also showed that KCNMA1 is involved in AD.[13] As PPIN shows that LEV have negative activity on KCNMA1, it is taught to be a possible mechanism of LEV against AD. Moreover, studies suggested that *KCNMA1* gene is associated with vascular dysfunction, cochlear hearing impairment, innate immunity, epilepsy, and mental retardation. By contemplating this that KCNMA1 has potentially important impact on brain's functions and mental capacity, shows that any alteration in this gene can results in individual's cognitive impairment.[14] So, LEV can help to avoid or treat cognitive symptoms of AD. Hyperhomocysteinemia is a known risk factor for AD. Hyperhomocysteinemia may occur because of mutation in gene encoding the enzymes which are involved in metabolism of homocysteine. These genes are methionine synthase (MS), methylenetetrahydrofolate reductase (MTHFR) and cystathionine beta-synthase (CBS). MS and MTHFR are positively associated with AD.[31] while the protein encoded by CBS gene acts as a homotetramer to catalyze the conversion of homocysteine to cystathionine.[15] and CBS mutation is an independent risk factor for AD.[16] Total plasma elevated levels of homocysteine (Hcy) are found to be as a risk factor for AD. The exact pathology of relation between AD and hyperhomocysteinemia (HHcy) is not clear. But the researches showed the role of hydrogen sulfide and NMDAR activation in HHcy causes dysfunction of synapse and disruption of blood brain barrier as risk developing AD.[17] LEV may show its action against AD by acting on CBS gene. NMDA (N-methyl-D-aspartate) is a channel like receptor with heteromers having three subunits which are NR1(GRIN1), NR2 (GRIN2A, GRIN2B, GRIN2C, or GRIN2D) and NR3 (GRIN3A or GRIN3B). Among which NR2 subunit is the agonist binding site for glutamate. In human brain, glutamate is the major excitatory neurotransmitter which binds to the NR2 receptor. As NMDA receptor is involved in long term potentiation of synaptic vesicle, it is thought to be involve in triggering different kinds of learning and memory.[18] Our study showed that LEV act on GRIN2B which encodes NR2 unit of NMDA receptor and may improves the memory and learning. APOD gene encodes a component of high-density lipoprotein that has no marked similarity to other apolipoprotein sequences. APOD is involved in several processes like negative regulation of protein imports into nucleus, macromolecule metabolic process negative regulation and deleterious regulation of cell matrix. Moreover, it is involved in binding of cholesterol. It is also a biomarker of AD, type 2 diabetes mellitus and gestational diabetes. In AD, APOD increases protein expression in CSF and hippocampus.[18] Our study showed that LEV may show its ant-AD effects by acting on this gene. AMPH is the gene founded to encode a protein of synaptic vesicles; this protein is associated with the synaptic vesicle cytoplasmic surface.[19] AMPH have capacity to bind with phospholipids. AMPH is thought to be involved in learning and endocytosis of synaptic vesicle that localizes to leading edge membrane.[20] FAS gene encodes that protein which is the member of the TNF-receptor subfamily. This receptor contains the death domain and exhibit a central role in physiological regulation of programmed cell death. This receptor is also involved in various pathogenic process like immune system disorders and different kinds of malignancies. The interaction of this receptor with its ligand will initiate cell death signaling pathway complexes which eventually results in apoptosis.[20] Action of LEV on AMPH gene may be a possible mechanism for its ant-AD effects. FAS gene exhibits kinase binding, identical protein binding and calmodulin binding activity. FAS is thought to be involve in various body processes like positive regulation of metabolic process of cellular proteins, signal transduction regulation, and involve in surface receptor regulation. This gene is also involved in several diseases like urinary system cancer, pre-eclampsia, hematologic cancer, cystic fibrosis, and autoimmune disease. FAS also act as a biomarker for various diseases like hematologic cancer, gastrointestinal system cancer, eye disease, autoimmune disease, and neurodegenerative disease. As per studies FAS is biomarker for Alzheimer's disease (AD).[21] Anti-AD effects of LEV may be occurred by its actions on FAS gene.

In the APOD complex, LEV is involved in the interaction of V154 residue through its oxygen present in the vicinity of cyclic structure. E30, K31, and I128 are those residues which resides parallel to the ligand position. The one of the reasons for its high binding score might because of the absolute hydrophobic and electrostatic position of this molecule corresponding to the residues resides in the vicinity. The most common residue forming intermolecular interactions is evolved to be valine. Half of the proteins are making this interaction with valine includes CBS, FAS, SCN1A and SVA2,

where out of four interactions three bonding acted through valine. In CBS, V37 and L126 forms hydrogen bonding with ligand molecule where leucine does not involve in any of the binding pattern except SVA2. In SVA2 only valine and leucine are forming hydrogen interactions through V358, V359, V292 and L324. CSTB has the lowest scores among docked complexes. T81 and S83 are the two residues responsible for docking interactions. Other than CSTB, serine is only involved in hydrogen bond interaction in SCN1A where another binding residue is V237 along with S1471.

FAS are forming intermolecular interactions through the V158 and E337 residues. GAD2 and KCNMA1 forms different binding interactions through multiple binding residues like D27, Y123, A125, P124 and R514, M513, D362, respectively. In KCNJ10, two lysine residues are involved in hydrogen binding K173, K174 other than W24. SLC1A2 is another protein which exhibits moderate docking score for the selected ligand molecule. ILE97 and ALA436 are the only two residues which are responsible for the intermolecular interaction with levetiracetam.

Our study has proposed several mechanisms for anti-AD effect of LEV and revealed that this drug significantly involves targeted genes to decrease the symptoms of AD. The anti-AD activity of LEV has evaluated as a preclinical pattern. This study suggests that there are several possible mechanisms of LEV to treat AD which can be further explore through preclinical and clinical trials in evidence-based medicine. by targeting different genes involved in AD.

CONCLUSIONS

The network pharmacological studies, genes analysis is used to understand the drugs, their targets, and effects. In current study, databases and study of interactions are used for analyzing mechanism of action through network pharmacology. In this study, for understanding anti-AD mechanism of a chemical drug LEV, system pharmacology studies are applied. The layout of this study included gene targets, networks, and pathways. GO molecular studies showed that LEV have different pathways involved in its therapeutic actions. Some genes targets of LEV like KCNMA1, CBS, GRIN2B, CSTB, APOD, AMPH and FAS are found to be associated in AD, which indicates that LEV may show its anti-AD effects by acting on these targets. The results aided to understand the molecular mode of LEV, providing some evidence on its potential clinical use in AD. The limitation of such type of computational studies is that this article describes only the retrieval and annotation of the documented drug molecules. However, these mechanisms can further be elaborated through docking and molecular dynamics simulation. Furthermore, in vivo studies are needed which will further help in confirmation and identification of anti-AD mechanism of action of LEV.

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