RESEARCH PAPER

Transcriptomic Insights into Levetiracetam's Neuroprotective Mechanisms Against Lead-Induced Toxicity in SH-SY5Y Cells

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ABSTRACT
Levetiracetam (LEV), an antiepileptic drug of the second generation, has shown neuroprotective activity in addition to antiepileptic activity, in particular against oxidative stress-induced neuronal damage. The researchers tested the effects of finding out whether LEV can reduce the neurotoxicity effects of lead acetate in human SH-SY5Y neuroblastoma cell line via a whole-transcriptome NGS. There was an interference of gene networks concerning oxidative stress management, apoptosis, lipid metabolism, and synaptic signalling with lead acetate exposure. LEV pretreatment significantly counteracted these changes and stimulated genes involved in antioxidant protection, neuronal survival, extracellular component organization, and nicotinic plasticity and inhibited the pro-apoptotic pathway and the genes related to cholesterol biosynthesis. KEGG pathway and functional enrichment analysis showed that TGF-beta, PI3K-Akt and metal ion detoxification pathways were notably covered, and excitation-inhibition balance restoration of gene expression was restored. PPI mapping identified major hub genes linked to survival signalling and extracellular matrix stability, including as TIMP1, IGFBP5, ID1/2/3, and DCN. These data imply that LEV has transcriptional neuroprotection that enables neuroprotection due to the promotion of the detoxication processes, maintenance of synaptic integrity, and inhibition of the metal and metabolic stress responses, which underlines its repositioning as a possible treatment of neurodegenerative disorders linked to environmental neurotoxins.

Keywords: Levetiracetam, neuroprotection, lead acetate, oxidative stress, SH-SY5Y cells, transcriptomics, gene expression, Alzheimer’s disease.
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INTRODUCTION

Levetiracetam (LEV) has also been reported in the past to protect against oxidative stress-induced neurotoxicity in a wide array of seizure models [1]. With this evidence, the hypothesis of the current study was to unravel the neuroprotective effects of LEV in the neurotoxicity caused by lead acetate relying on human neuroblastoma cell line SH-SY5Y. To accomplish this, a detailed whole-transcriptomics next-generation sequencing (NGS) was conducted to determine the molecular pathways and different changes in gene expressions on LEV treatment. Levetiracetam (LEV) is a novel second-generation anti-epileptic agent and differs in its pharmacology profile as compared to the conventional agents. In addition to documented anti-seizure effect, LEV has been documented in various seizure models to inhibit oxidative stress-generated neurotoxicity [2] . To determine the neuroprotective effects of LEV against the neurotoxic effects of lead acetate in the human neuroblastoma SH-SY5Y cell line. Next-generation sequencing (NGS) whole-transcriptomics was used to study in detail the molecular processes and gene changes in reaction to neuroprotection by the LEV [3]. Lead (Pb), especially as lead acetate (PbAc), is a ubiquitous environmental neurotoxin. It passes into the brains crossing the blood-brain barrier, imitates vital ions such as calcium and zinc, interferes with neuronal communication and enzymatic activity, and ends up raising the oxidative load [4]. The multiple tissues (ranging in human mesangial cells to brain) shows that exposure to lead increases the expression of oxidative stress indicators, such as malondialdehyde (MDA) and lipid peroxidation and suppresses antioxidant defenses in favor of cell demise [5]. SH-SY5Y cells are the most appropriate human neuroblastoma cell line of study that has been well established as an in vitro tool of investigation in modes of neurotoxicity and neuroprotection. It can be particularly used to model neurodegenerative processes and dopaminergic neurons [6]. PbAc causes oxidative stress in SH-SY5Y cells through the generation of ROS and activates survival cellular pathway-mediated cell defensive mechanisms. Reflectively, on such considerations, it is

imperative to research on therapeutic chemicals capable of alleviating lead-induced oxidative neurotoxicity [7]. Levetiracetam (LEV) is a second-generation anticonvulsant suggested by piracetam and units it with conventional anti-epileptics partly due to its attractive pharmacokinetic, lack of production of hepatic enzyme, and protection. Notably, LEV has antiapoptotic effects in models of seizures, including: where it decreases indicators of oxidative damage (e.g., lipid peroxidation), as well as regulating antioxidant responses in hippocampal and other neural tissues (e.g., in sin epilepsy models) [8].

The transcriptomic studies conducted recently have indicated that the expression of the profiles related to the synaptic functioning and neuroprotection in AD may be altered by LEV, which was showed [9]. Another study, [10] furthermore revealed that LEV normalizes the expression of presynaptic proteins therefore, playing a role in synaptic health. Different studies [11,12], have concluded that LEV can protect against both hyperactivity in networks and improved cognitive results in animal and human models of AD. However, a study [13] observed that LEV did not work in tau-based models of AD indicating that responsive profile to therapeutic intervention may be altered in response to pathological substrate. Remarkably, research [14] showed that LEV reversed imbalance in the excitatory/inhibitory (E/I) system, especially in at-risk APOE-e47 genotypes in females, indicating a genotype- (and sex-) specific neuroprotective effect. Taken together these studies highlight the multidimensional mode of actions of LEV, the potential of which may not be limited to seizure control only but include also additional neuroprotective effects that can potentially be related to AD pathology.

METHODOLOGY

SH-SY5Y (human neuroblastoma) cell culturing

SH-SY5Y is the cell line of human neuroblastoma that was obtained by National Center of Cell Science (NCCS). The cells were grown in 1:1 ratio Dulbecco modified Eagles medium and Ham F-12 medium (Gibco) containing 15% fetal bovine serum (FBS) (Sigma-Aldrich), MEM non-essential amino acid and 1% penicillin (5000 µg/ml)-streptomycin (5000 IU/ml) solution (Sigma-Aldrich) as growth medium in 75 cm^2 flask. The flasks were kept at 5 % CO2 /95 % humidified air and 37 °C [15] (Singh and Kaur, 2005). It was replaced in two-day intervals (the medium was changed). Toxic effects like neurotoxicity in the SH-SY5Y cells were induced by 10 µM of Lead acetate. Neuroprotective action of SH-SY5Y cells has been experimented upon using the drug Levetiracetam. The SH-SY5Y cells grown in a proliferated form were used in the study of differential gene expression. Duplicates were prepared of each group. Cells were harvested and snap frozen in RNA later solution and shipped on dry ice to Biodeavour Research Lab, Porur, Chennai where the RNA was isolated and differential gene expressions determined using Affymetrix Clariom S Human Array to profile the expression.

RNA extraction and gene expression microarrays

Two independent experiments (biological duplicates) total RNA was extracted by using NucleoZol as per manufacturer instructions. RNA purity was examined by A260/A 280 on a NanoDropTM 2000 spectrophotometer (Thermo Scientific) and RNA concentration was measured by the OD at 260 (OD260). Quality of RNA was determined by agarose gel electrophoresis (1%) denaturing. A reverse transcription reaction was carried out with 100 ng of total RNA and taken according to the instructions of the GeneChip WT Plus reagent Kit (Affymetrix, Inc., Santa Clara, CA, USA). Amplification and synthesis of complementary RNA (cRNA) was done in-vitro through the process of transcription (IVT) by utilizing the T7 RNA polymerase (with the Affymetrix WT cDNA Synthesis and Amplification Kit). It was transcribed with template cDNA i.e. the second stranded cDNA. The purified sense strand cDNA was reverse transcribed to synthesize the cRNA to a sample using 2nd cycle primers. The affinity purified cRNA template was subsequently hydrolysed in RNase H., and purified, fragmented and biotin-labelled ss-cDNA (5.5 µg), was produced as per the Affymetrix WT End Labelling Kit. ss-cDNA was hybridized with the human transcriptome array Clariom S (Affymetrix, Inc., Santa Clara, CA, USA) in GeneChip 645 hybridization oven at 45 °C, 17 h. These arrays were washed, stained on the FS450 Fluid Station and scanned at the GeneChip Scanner 3000 7G (Affymetrix, Inc., Santa Clara, CA, USA), following the GeneChip User Guide. The raw CEL files which provide the intensity data were extracted and were analyzed. Raw CEL files were normalized with the transcriptome analysis console (TAC) software ver.4.0.1 according to the SST-robust multichip average (SST-RMA) algorithm (Thermo Fisher Scientific, Japan). The significant DEGs were taken as the transcripts that passed through the transcriptome with fold change (FC)> 1 and $P < 0.05$.

Differential gene expression analysis

Differential gene expression analysis between commonly expressed CDS of the 3 groups was done

using the DESeq2 package in the following combinations:

1. **Combination 1:** Groups 1 (Control cells) and 2 (Cells with induced toxicity by Lead acetate)
2. **Combination 2:** Groups 2 (Control cells) and 3 (Cells treated with Levetiracetam no pretreatment + lead acetate)
3. **Combination 3:** Groups 1 (Control cells) and 3 (Cells treated with Levetiracetam pretreatment + lead acetate)

The graphical representation design of the various genes as they are varied along the 'axes of biological and statistical significance (Volcano plots) were drawn using the R script.

Gene ontology and KEGG pathway enrichment analysis

The predicted list of differentially expressed genes (DEGs) was interpreted with ShinyGo 0.81 tool [16] (Ge et al., 2020). Pathway analysis and gene ontology (GO) analyses were performed. The biological pathways that had p-value below 0.05 were taken as significant. Functional enrichment of DEGs was visualized as Lollipop plot, so, it is possible to combine both expression data and results of functional assessment. The p-values less than 0.05 were found to consider. The CDS of the groups were mapped to reference canonical pathways in KEGG in order to execute the pathway enrichment analysis to determine the potential implication of the predicted CDS in biological routes.

Construction of Protein-Protein Interaction (PPI) Network and Identification of Hub Genes

The protein products of the genes were studied in database Search Tool for the Retrieval of interacting Genes/Proteins (STRING) [17] (Mering et al., 2003). The networks offered by this database are regulatory relationships between genes. Cytohubba is an easy-to-use cytoscape plug in that ranks the significance of nodes in the PPI network using varied algorithms to determine key biological components [18] (Chin et al., 2014). Genes that have a rank greater than 7 were considered as hub genes in this study and the degree algorithm was used. In view of the potential biological domains and pathways of the predicted hub genes, Gene ontology was re-analysed with KEGG pathway enrichment analysis ($P < 0.05$) through DAVID.

RESULTS

SH-SY5Y human neuroblastoma cell lines were pretreated with lead acetate and subjected to LEV-treatment in order to assess the efficacy of levetiracetam (LEV) neuroprotective against acetylene-lead-toxicity. Analysis of differential gene expression based on the next-generation sequencing (NGS) demonstrated considerable alterations of transcriptome between treatment groups. Exposure to lead acetate led to a disruption of many genes related to oxidative stresses and apoptosis as well as abnormal neuronal signalling (Table 1). Conversely, levetiracetam pretreatment mitigated most of these changes, by rescuing up-regulation expression of oxidative stress response, neuronal survival, and synaptic plasticity genes (Table 2). The up-regulation of key neuro protective as well as anti-oxidant related genes in the LEV-pretreated group as compared to lead acetate-exposed cells, and was attributed to reversal or inhibition of lead-dependent neurotoxicity (Table 3). These results align with the possible repositioning potential of levetiracetam as a neuroprotective drug and offer a mechanistic explanation behind the drug protection against neuronal damage induced by the collection of toxicity.

Table. 1: Top 50 up-regulated genes in Cells treated with Lead acetate

S. No.	Gene Symbol	Fold Change (FC) Value	Gene Name
Up-regulated genes			
1.	ID1	6.909	inhibitor of DNA binding 1, HLH protein
2.	ID2	6.863	inhibitor of DNA binding 2, HLH protein
3.	SMAD6	6.705	SMAD family member 6
4.	TIMP1	6.599	TIMP metallopeptidase inhibitor 1
5.	HTRA1	6.433	HtrA serine peptidase 1
6.	ID3	6.273	inhibitor of DNA binding 3, HLH protein
7.	MGP	6.222	matrix Gla protein
8.	ENPP2	6.102	ectonucleotide pyrophosphatase/phosphodiesterase 2
9.	IFITM3	6.089	Interferon-Induced Transmembrane Protein 3.

10.	IFITM2	5.964	Interferon Induced Transmembrane Protein 2
11.	IGFBP5	5.91	Insulin-like Growth Factor Binding Protein 5
12.	IFI6	5.678	Alpha Inducible Protein 6
13.	DCN	5.614	Decorin
14.	CRABP1	5.56	Cellular Retinoic Acid Binding Protein 1
15.	EDNRA	5.366	Endothelin Receptor Type A
16.	IGFBP3	5.329	Insulin-like Growth Factor Binding Protein 3
17.	DLX5	5.22	Distal-less Homeobox 5
18.	ATP5D	5.147	ATP Synthase
19.	SGK1	4.992	Serum/Glucocorticoid Regulated Kinase 1
20.	DKK1	4.967	Dickkopf WNT Signalling Pathway Inhibitor 1
21.	IGFBP7	4.955	Insulin-like Growth Factor Binding Protein 7
22.	DKK2	4.93	Dickkopf WNT Signalling Pathway Inhibitor 2
23.	C7	4.926	Complement Component 7
24.	FN1	4.904	Fibronectin 1
25.	ANXA2	4.885	Annexin A2
26.	ARSJ	4.829	arylısulfatase family, member J
27.	S100A11	4.696	S100 Calcium Binding Protein A11
28.	SELM	4.469	Selenoprotein M
29.	ESD	4.382	Esterase D
30.	DCPS	4.376	decapping enzyme, scavenger
31.	SNAI2	4.334	Snail Family Transcriptional Repressor 2
32.	TNC	4.331	Tenascin C
33.	MT1B	4.303	Metallothionein 1B
34.	TIMM13	4.296	Translocase of Inner Mitochondrial Membrane 13
35.	FILIP1L	4.28	Filamin A Interacting Protein 1 Like
36.	CCL2	4.264	C-C Motif Chemokine Ligand 2
37.	MT2A	4.251	Metallothionein 2A
38.	HTR2B	4.242	5-hydroxytryptamine receptor 2B
39.	TPM1	4.229	Tropomyosin 1 (alpha)
40.	GPC3	4.202	Glypican 3
41.	SRSF2	4.196	Serine and arginine rich splicing factor 2
42.	TMEM243	4.176	Transmembrane Protein 243
43.	SPCS1	4.173	Signal Peptidase Complex Subunit 1
44.	AATF	4.156	Apoptosis Antagonizing Transcription Factor
45.	MSX2	4.142	msh homeobox 2
46.	SERPINF1	4.111	Serpin family F member 1
47.	SNAI1	4.082	Snail family transcriptional repressor 1
48.	BANF1	4.081	Barrier to Autointegration Factor 1
49.	GPX4	4.052	Glutathione Peroxidase 4
50.	NDUFC2	4.034	NADH:ubiquinone oxidoreductase subunit C2
Down-regulated genes			
51.	CNTNAP5	-4.777	Contactin-associated-like protein 5
52.	RTL1	-4.395	Retrotransposon-like 1
53.	CHRM3	-4.331	Cholinergic receptor, muscarinic 3
54.	CHGB	-4.285	Chromogranin B
55.	SORBS2	-4.158	Sorbin and SH3 domain-containing protein 2
56.	IGF2	-3.975	insulin like growth factor 2
57.	RFTN1	-3.939	Raftlin, lipid raft linker 1
58.	PGD	-3.796	Phosphogluconate dehydrogenase

59	HIST1H1C	-3.749	Histone Cluster 1 H1 Family Member C (Histone H1.2)
60	CHGA	-3.731	Chromogranin A
61	HMGCS1	-3.677	3-hydroxy-3-methylglutaryl-CoA synthase 1
62	CHD4	-3.656	Chromodomain Helicase DNA Binding Protein 4
63	VSNL1	-3.605	Visinin-like 1 (also known as VILIP-1)
64	NOL6	-3.46	Nucleolar protein 6
65	ZNF608	-3.399	Zinc Finger Protein 608 (a KRAB-C2H2 family transcription factor)
66	RCC2	-3.356	Regulator of Chromosome Condensation 2
67	ACLY	-3.325	ATP citrate lyase
68	CPLX2	-3.265	Complexin 2
69	HNRNPA3	-3.24	Heterogeneous Nuclear Ribonucleoprotein A3
70	CLSTN2	-3.234	Calsyntenin-2
71	GRM8	-3.173	Glutamate Metabotropic Receptor 8
72	DHCR24	-3.089	24-Dehydrocholesterol Reductase (also known as seladin-1)
73	DNAJC14	-3.076	DnaJ Heat Shock Protein Family (Hsp40) Member C14
74	DALRD3	-3.067	DALR Anticodon Binding Domain Containing 3
75	SAMD4A	-3.059	Sterile Alpha Motif Domain Containing 4A
76	GFRA3	-3.048	GDNF family receptor alpha 3
77	EHD3	-3.002	EH Domain-Containing Protein 3
78	DLK1	-2.992	Delta Like Non-Canonical Notch Ligand 1
79	RAB27B	-2.971	Member RAS Oncogene Family
80	NECTIN1	-2.961	Nectin Cell Adhesion Molecule 1
81	MLEC	-2.953	Malectin
82	TPO	-2.914	Thyroid Peroxidase
83	AMPD2	-2.898	Adenosine monophosphate deaminase 2
84	CEP128	-2.861	Centrosomal Protein 128kDa
85	CASC3	-2.844	Cancer Susceptibility Candidate 3
86	RBM12B	-2.841	RNA Binding Motif Protein 12B
87	BMPR1B	-2.836	Bone Morphogenetic Protein Receptor Type 1B
88	DPYSL2	-2.825	Dihydropyrimidinase-Like 2
89	TRPV1	-2.799	Transient Receptor Potential Cation Channel Subfamily V Member 1
90	KCNH1	-2.798	Potassium Voltage-Gated Channel Subfamily H Member 1
91	CBLN2	-2.788	Cerebellin 2 Precursor
92	GLYR1	-2.782	Glyoxylate Reductase 1 Homolog
93	KDM2B	-2.779	Lysine demethylase 2B
94	ZBTB9	-2.765	Zinc Finger and BTB Domain Containing 9
95	HNRNPH3	-2.76	Heterogeneous Nuclear Ribonucleoprotein H3
96	AGO1	-2.682	Argonaute RISC Component 1
97	GTPBP1	-2.659	GTP Binding Protein 1
98	GCH1	-2.649	GTP Cyclohydrolase 1
99	WDR55	-2.637	WD Repeat Domain 55
100	SOD2	-2.634	Superoxide Dismutase 2, Mitochondrial

Table 2: Top 50 up-regulated genes in Cells induced with toxicity by Lead acetate and no pretreatment of LEV

S. N	Gene Symbol	Fold Change	Gene Name

o.		(FC) Value	
Up-regulated genes			
1.	SMAD6	6.719	SMAD family member 6
2.	TIMP1	6.564	TIMP Metallopeptidase Inhibitor 1
3.	ID1	6.426	Inhibitor of DNA Binding 1, HLH Protein
4.	ENPP2	6.13	Ectonucleotide Pyrophosphatase/Phosphodiesterase 2
5.	IGFBP5	6.125	Insulin-like Growth Factor Binding Protein 5
6.	HTRA1	6.12	HtrA serine peptidase 1
7.	GH1	5.841	Growth Hormone 1
8.	MGP	5.782	Matrix Gla Protein
9.	ID2	5.717	Inhibitor of DNA binding 2, HLH protein
10.	ID3	5.693	inhibitor of DNA binding 3, HLH protein
11.	IFITM3	5.646	interferon induced transmembrane protein 3
12.	DLX5	5.562	distal-less homeobox 5
13.	IFITM2	5.516	interferon induced transmembrane protein 2
14.	C7	5.347	Complement Component 7
15.	ATP5D	5.255	ATP synthase
16.	CRABP1	5.24	Cellular Retinoic Acid Binding Protein 1
17.	TAGLN	5.141	Transgelin
18.	DCN	5.108	Decorin
19.	EDNRA	4.656	Endothelin Receptor Type A
20.	GH1	4.64	Growth Hormone 1
21.	S100A11	4.618	S100 Calcium Binding Protein A11
22.	AK5	4.596	Adenylate Kinase 5
23.	HTR2B	4.569	5-Hydroxytryptamine Receptor 2B
24.	SELM	4.567	Selenoprotein M
25.	IGFBP3	4.533	Insulin-Like Growth Factor Binding Protein 3
26.	GH1	4.505	Growth Hormone 1
27.	MT1B	4.453	Metallothionein 1B
28.	NAA38	4.451	N(alpha)-acetyltransferase 38, NatC auxiliary subunit
29.	DKK2	4.431	Dickkopf WNT Signalling Pathway Inhibitor 2
30.	NTRK3	4.335	Neurotrophic Receptor Tyrosine Kinase 3
31.	IDS	4.323	Iduronate 2-sulfatase
32.	AATF	4.304	Apoptosis Antagonizing Transcription Factor
33.	SERPINF1	4.274	Serpin Family F Member 1
34.	NDUFB11	4.221	NADH:ubiquinone oxidoreductase subunit B11
35.	SNAI1	4.154	Snail Family Transcriptional Repressor 1
36.	TIMM13	4.115	Translocase of Inner Mitochondrial Membrane 13
37.	FN1	4.088	Fibronectin 1
38.	RAMP2	4.01	Receptor Activity Modifying Protein 2
39.	VIM	4.004	Vimentin
40.	TEKT4P2	3.997	Tektin 4 Pseudogene 2
41.	PSME1	3.989	Proteasome Activator Subunit 1
42.	SLC39A4	3.982	Solute Carrier Family 39 Member 4
43.	SLC27A6	3.97	Solute Carrier Family 27 Member 6
44.	SGK1	3.961	Serum/Glucocorticoid-Regulated Kinase 1
45.	ESD	3.955	Esterase D
46.	MSX2	3.936	Msh Homeobox 2
47.	GH1	3.935	Growth Hormone 1
48.	NOA1	3.919	Nitric Oxide Associated 1
49.	FAM127B	3.905	Family with Sequence Similarity 127 Member B
50.	TCOF1	3.901	Treacle Ribosome Biogenesis Factor 1
Down-regulated genes			

51.	CNTNAP5	-5.733	contactin associated protein-like 5
52.	CHRM3	-5.713	cholinergic receptor, muscarinic 3
53.	HMGCS1	-5.121	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
54.	CLSTN2	-4.645	calsyntenin 2
55.	CHGB	-4.521	chromogranin B
56.	CHRM3	-4.489	Muscarinic Acetylcholine Receptor M3
57.	RTL1	-4.383	retrotransposon-like 1
58.	SORBS2	-4.325	sorbin and SH3 domain containing 2
59.	PGD	-4.256	phosphogluconate dehydrogenase
60.	DHCR24	-4.237	24-dehydrocholesterol reductase
61.	IGF2	-4.231	insulin-like growth factor 2
62.	VCAM1	-4.097	vascular cell adhesion molecule 1
63.	RFTN1	-3.77	raftlin, lipid raft linker 1
64.	CHGA	-3.694	chromogranin A
65.	DHCR7	-3.682	7-dehydrocholesterol reductase
66.	RAB27B	-3.63	member RAS oncogene family
67.	ACLY	-3.609	ATP citrate lyase
68.	DLK1	-3.559	delta-like 1 homolog
69.	SOD2	-3.54	superoxide dismutase 2, mitochondrial
70.	PVRL1	-3.497	poliovirus receptor-related 1 (herpesvirus entry mediator C)
71.	GFRA3	-3.482	GDNF family receptor alpha 3
72.	RCC2	-3.449	regulator of chromosome condensation 2
73.	ZNF608	-3.415	zinc finger protein 608
74.	CBLN2	-3.369	cerebellin 2 precursor
75.	CHD4	-3.339	chromodomain helicase DNA binding protein 4
76.	SCD	-3.239	stearoyl-CoA desaturase (delta-9-desaturase)
77.	CPLX2	-3.212	complexin 2
78.	TNFRSF9	-3.21	tumor necrosis factor receptor superfamily, member 9
79.	HNRNPA3	-3.209	heterogeneous nuclear ribonucleoprotein A3
80.	NOL6	-3.098	nucleolar protein 6 (RNA-associated)
81.	HSPA1A	-3.076	heat shock 70kDa protein 1A
82.	ZBTB9	-3.071	5.253
83.	DAG1	-3.045	dystroglycan 1 (dystrophin-associated glycoprotein 1)
84.	PRSS12	-3.03	protease, serine, 12 (neurotrypsin, motopsin)
85.	BMPR1B	-3.023	bone morphogenetic protein receptor type IB
86.	DPYSL2	-2.983	dihydropyrimidinase-like 2
87.	CCL5	-2.975	chemokine (C-C motif) ligand 5
88.	AMPD2	-2.962	adenosine monophosphate deaminase 2
89.	EHD3	-2.962	EH domain containing 3
90.	CLCN6	-2.959	chloride channel, voltage-sensitive 6
91.	WDR6	-2.954	WD repeat domain 6
92.	GRM8	-2.941	glutamate receptor, metabotropic 8
93.	HMGCR	-2.932	3-hydroxy-3-methylglutaryl-CoA reductase
94.	LSS	-2.921	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
95.	DNAJC14	-2.917	DnaJ (Hsp40) homolog, subfamily C, member 14

96.	SHPK	-2.909	Sedoheptulokinase
97.	MCL1	-2.903	myeloid cell leukemia 1
98.	KDM2B	-2.9	lysine (K)-specific demethylase 2B
99.	MLEC	-2.896	Malectin
10(	WDR55	-2.856	WD repeat domain 55

Table 5: Top 50 up-regulated genes in cells treated with pretreatment of LEV + Lead acetate

S. No.	Gene Symbol	Fold Change (FC) Value	Gene Name
Up-regulated genes			
1.	TIMP1	7.109	TIMP metallopeptidase inhibitor 1
2.	ID2	6.951	Inhibitor of DNA binding 2
3.	ID1	6.903	Inhibitor of DNA binding 1
4.	HTRA1	6.653	HtrA serine peptidase 1
5.	MGP	6.423	Matrix Gla protein
6.	ID3	6.367	Inhibitor of DNA binding 3, HLH protein (bHLHb25)
7.	IGFBP5	6.26	Insulin-like growth factor binding protein 5
8.	SMAD6	6.237	SMAD family member 6
9.	ENPP2	6.136	Ectonucleotide pyrophosphatase/phosphodiesterase 2
10	IFITM3	6.028	Interferon-inducible transmembrane protein 3
11	DLX5	5.876	Distal-less homeobox 5
12	CRABP1	5.79	Cellular retinoic acid-binding protein 1
13	DCN	5.745	Decorin
14	EDNRA	5.534	Endothelin receptor type A
15	ATP5D	5.49	ATP synthase
16	DKK1	5.404	Dickkopf WNT signalling pathway inhibitor 1
17	IFITM2	5.369	Interferon-inducible transmembrane protein 2
18	IGFBP3	5.218	Insulin-like growth factor binding protein 3
19	OR2T2	5.123	OR2T2 olfactory receptor family 2 subfamily T member 2
20	SGK1	4.922	Serum/glucocorticoid-regulated kinase 1
21	DKK2	4.903	Dickkopf WNT signalling pathway inhibitor 2
22	HIST1H2AG	4.887	Histone cluster 1 H2A family member G
23	S100A11	4.875	S100 calcium-binding protein A11
24	MT1B	4.849	Metallothionein 1B
25	ANXA2	4.837	Annexin A2
26	TAGLN	4.835	Transgelin
27	C7	4.806	Complement component 7
28	A2M	4.759	Alpha-2-macroglobulin
29	SELM	4.758	Selenoprotein M
30	ESD	4.749	Esterase D
31	IGFBP7	4.686	Insulin-like growth factor binding protein 7
32	MT2A	4.583	Metallothionein 2A
33	MT1A	4.568	Metallothionein 1A
34	SNAI2	4.553	Snail family transcriptional repressor 2
35	RGS2	4.535	Regulator of G-protein signalling 2
36	NDUFB11	4.531	NADH:ubiquinone oxidoreductase subunit B11
37	TMEM243	4.474	Transmembrane protein 243
38	SERPINF1	4.469	Serpin peptidase inhibitor

39	NDUFC2	4.456	NADH:ubiquinone oxidoreductase subunit C2
40	TIMM13	4.434	Translocase of inner mitochondrial membrane 13 homolog
41	MT1L	4.409	Metallothionein 1L
42	RAMP2	4.371	Receptor Activity-Modifying Protein 2
43	COX4I1	4.334	Cytochrome c oxidase subunit 4I1
44	DYNLL1	4.3	Dynein light chain LC8-type 1
45	AK5	4.29	Adenylate kinase 5
46	HTR2B	4.255	5-hydroxytryptamine (serotonin) receptor 2B
47	HIGD2A HIG1	4.245	hypoxia inducible domain family member 2A
48	FAM127B	4.228	Family with sequence similarity 127, member B
49	GPC3	4.21	Glypican 3
50	PRDX3	4.182	Peroxiredoxin 3
Down-regulated genes			
51	CNTNAP5	-5.488	contactin associated protein like 5
52	CHRM3	-4.526	cholinergic receptor, muscarinic 3
53	RTL1	-4.476	retrotransposon-like 1
54	CHGB	-4.368	chromogranin B
55	SORBS2	-4.29	sorbin and SH3 domain containing 2
56	PGD	-4.021	phosphogluconate dehydrogenase
57	VSNL1	-3.887	visinin like 1
58	IGF2	-3.851	insulin-like growth factor 2
59	RFTN1	-3.846	raftlin, lipid raft linker 1
60	CHGA	-3.776	chromogranin A
61	CHRM3	-3.549	cholinergic receptor, muscarinic 3
62	RCC2	-3.539	regulator of chromosome condensation 2
63	NOL6	-3.529	nucleolar protein 6 (RNA-associated)
64	ZNF608	-3.511	zinc finger protein 608
65	HMGCS1	-3.458	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
66	CHD4	-3.457	chromodomain helicase DNA binding protein 4
67	CPLX2	-3.384	complexin 2
68	GFRA3	-3.345	GDNF family receptor alpha 3
69	WDR6	-3.33	WD repeat domain 6
70	EHD3	-3.287	EH domain containing 3
71	DHCR24	-3.245	24-dehydrocholesterol reductase
72	DLK1	-3.167	delta-like 1 homolog
73	AMPD2	-3.163	adenosine monophosphate deaminase 2
74	ACLY	-3.151	ATP citrate lyase
75	DNAJC14	-3.143	DnaJ heat shock protein family (Hsp40) member C14
76	GRM8	-3.139	glutamate receptor, metabotropic 8
77	HNRNPA3	-3.116	heterogeneous nuclear ribonucleoprotein A3
78	CEP128	-3.076	Centrosomal protein 128 kDa
79	CLSTN2	-3.055	Calsyntenin-2
80	ZBTB9	-3.035	Zinc Finger and BTB Domain Containing 9
81	ZNF326	-3.031	Zinc Finger Protein 326
82	MLEC	-3.028	Malectin

83	KDM2B	-3.027	Lysine Demethylase 2B
84	GLYR1	-3.019	Glyoxylate Reductase 1 Homolog
85	DPYSL2	-3.001	Dihydropyrimidinase-Like 2
86	RAB27B	-2.979	Member RAS Oncogene Family
87	BMPR1B	-2.974	Bone Morphogenetic Protein Receptor Type 1B
88	TRPV1	-2.951	Transient Receptor Potential Cation Channel Subfamily V Member 1 (Capsaicin receptor)
89	AGO1	-2.928	Argonaute RISC Component 1
90	SAMD4A	-2.892	Sterile Alpha Motif Domain Containing 4A
91	HSPA1A	-2.851	Heat Shock Protein Family A member 1A
92	GTPBP1	-2.851	GTP-binding protein 1
93	NECTIN1	-2.844	Nectin Cell Adhesion Molecule 1
94	MCL1	-2.819	Myeloid Cell Leukemia Sequence 1
95	KLF7	-2.785	Kruppel-like Factor 7
96	CBLN2	-2.784	Cerebellin 2 Precursor
97	FAM163A	-2.759	Family With Sequence Similarity 163 Member A
98	KCNH1	-2.749	Potassium Voltage-Gated Channel Subfamily H Member 1
99	CASC3	-2.722	Cancer Susceptibility Candidate 3
10	WDR55	-2.712	WD Repeat Domain 55

Volcano plot

A volcano plot is a scatterplot used extensively in the analysis of the differentially expressed genes (DEGs) in a high-throughput experiment like an RNA-Seq analysis or a microarray run. It is both statistically significant and large, and hence makes it a large scaled visual tool to discover the genes of interest. X axis; Log_2 (Fold Change) — represents the magnitude of gene expression change between two conditions. Y axis; $-log_{10}$(p-value) or $-log_{10}$ (adjusted p-value) — indicates the statistical significance of that change. Genes that are having high fold change with low p-values can be appear on the high corners of the plot. Top right: up-regulated genes. Upper left: the highly down-regulated gene. The evolution of genes towards the center and base of the plot will tend to have small or not significant changes (Figure 1 & 2).

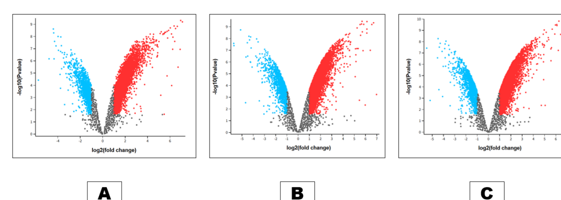

Figure 1: Volcano plots that represented the placement of the differentially expressed genes in (A) treatment of the cells with lead acetate, (B) treatment of the cells with LEV following treatment of the cells with lead acetate, and (C) pre-treatment of cells with LEV before subjecting them to lead acetate. log_2 fold change is on the x-axis and $-log_{10}$ (P-value) is on the Y-axis. The red dots identify highly up-regulated genes and the blue dots denote highly down-regulated genes and the grey ones denote insignificance of gene expression changes.

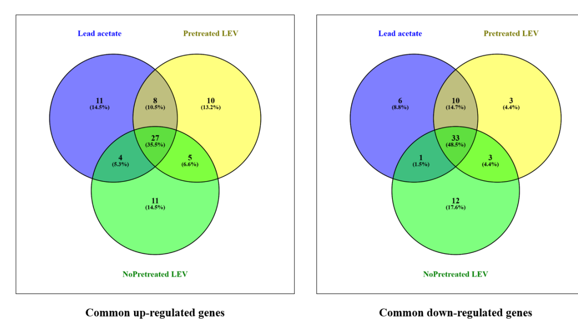

Figure 2: Venn diagrams depicting the overlap of differentially expressed genes among Lead acetate, Pretreated LEV, and No-Pretreated LEV groups. (A) Common up-regulated genes, showing 27 genes (35.5%) shared across all three conditions, along with unique and pairwise shared genes in each group. (B) Common down-regulated genes, with 33 genes (48.5%) shared among all conditions. Percentages indicate the proportion of total differentially expressed genes within each set.

Functional enrichment analysis

The functional enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database to draw insight into biological context of the differentially expressed genes (DEGs). The result of the analysis assists in finding signalling and metabolic pathways which are enriched and statistically significant level above the occurrence in the gene list. The list of DEGs was uploaded to an enrichment analysis web tool (Enrichr) and enrichment performed against the KEGG pathways database. The pathways enriched with adjusted p-values (e.g. Benjamini-Hochberg_FDR) < 0.05 were identified as significantly enriched. The key pathways up-regulatted in the dataset implying and significantly involved in the biological response or condition investigated. The presentation of the results was through bar plots of the most significantly enriched pathways together with the count of the associated genes and level of significance (Figure 3). This analysis helps deduce information on the molecular processes and regulatory pathways that these DEGs are related to and understanding on the biological implications.

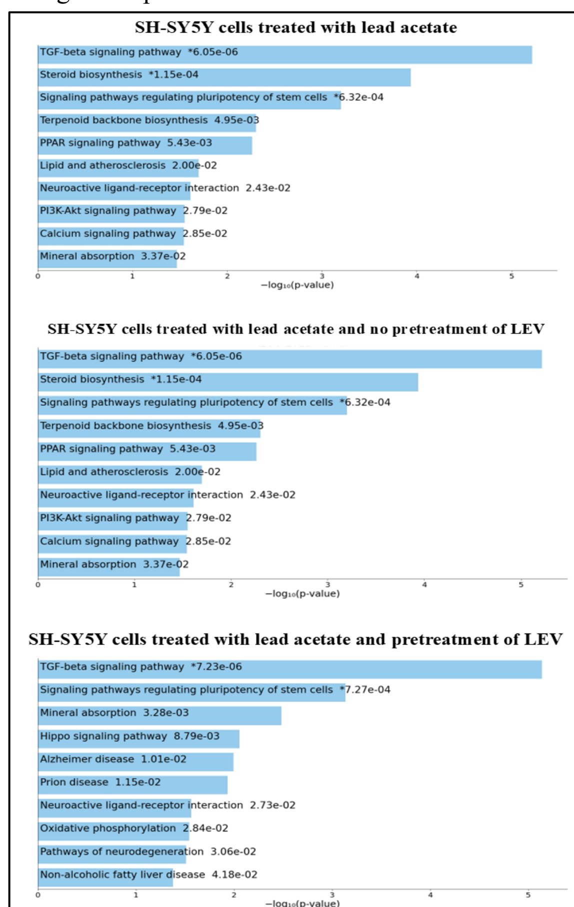

Figure 3: KEGG pathway enrichment analysis of differentially expressed genes in SH-SY5Y cells under different treatment conditions. The x-axis represents the $-\log_{10}(p\text{-value})$, indicating the statistical significance of pathway enrichment. Key pathways such as TGF-beta signalling, steroid biosynthesis, PI3K-Akt signalling, and pathways regulating pluripotency of stem cells were notably enriched, with variations observed across treatment conditions.

Gene Ontology (GO) Analysis

Gene Ontology (GO) was performed to detect any overrepresented biological processes involved in the differentially expressed genes (DEGs). Compared to GO, analysis breaks down the genes into three main categories, which are: Biological process (BP), Molecular Function (MF) and Cellular Component (CC). Consequently, it helps to bring understanding between the valuable functions of sets of genes. This DEG list was uploaded to the Enrichr web-based enrichment analysis tool (https://maayanlab.cloud/Enrichr/), which takes as input a list of gene identifiers and statistically examines them to identify enriched functional categories using a series of curated gene set libraries.

Regulation of Lead acetate in SH-SY5Y Cell Line

Lead acetate exposure in SH-SY5Y cells triggers distinct transcriptional changes affecting multiple biological pathways (Figure 4). Up-regulated genes are mainly associated with developmental regulation, vitamin metabolism, and extracellular matrix organization, indicating possible compensatory or protective cellular responses to toxic insult. Conversely, down-regulated genes cluster around lipid and cholesterol biosynthesis, metabolic pathways, and neurotransmitter signalling, suggesting disruption of membrane lipid homeostasis and synaptic function. On a cellular level, at the cellular component level, lead acetate treatment increases expression of genes located in endoplasmic reticulum lumen and collagen containing extracellular matrix and lowers the expression of nuclear and vesicular transport system components. Molecular role analysis reveals what have gained insulin like growth factor binding and cadherin binding and lost tRNA binding, G protein-coupled receptor activity and activity of hormones in the up regulated genes as compared to down regulated genes. All in all, these alterations indicate the neurotoxic effects of lead acetate on its metabolic, structural and signalling pathways in neural cells.

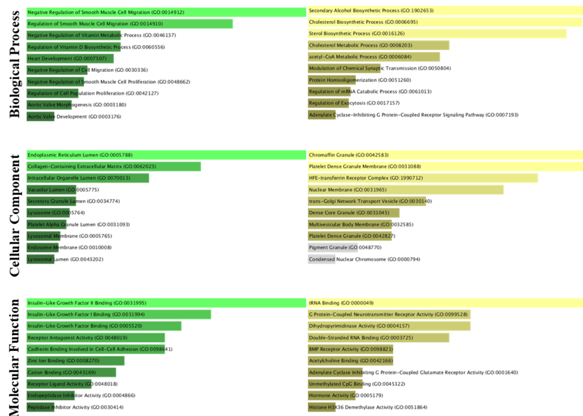

Figure 4: Differential expression of genes in SH-SY5Y cells with lead acetate using Gene ontology (GO) enrichment analysis. The categories to which the enriched GO terms are classified in the analysis are Biological Process, Cellular Component, and Molecular Function. The green bars denote a very highly enriched GO terms in up-regulated genes and yellow bars mark down-regulated genes. The x-axis is log scale with a scale level of -log10, or the statistical significance of enrichment. The top up-regulated processes are negative regulation of smooth muscle cell migration, regulation of vitamin D biosynthetic process, and aortic valve morphogenesis whereas the top down-regulated processes comprise of cholesterol biosynthetic process, acetyl-CoA metabolic process, and regulation of exocytosis.

Regulation of No Pretreated LEV in SH-SY5Y Cell Line

In the absence of LEV pretreatment, lead acetate exposure in SH-SY5Y cells induces significant transcriptional changes impacting growth regulation, cardiovascular-related development, and intracellular signalling pathways. The up-regulated genes are enriched in processes promoting cell migration, proliferation, and insulin-like growth factor signalling, suggesting a cellular attempt to repair or remodel tissue under toxic stress. The observed up-regulation of PI3K/AKT signalling-related terms points to activation of survival pathways. Conversely, down-regulated genes are heavily concentrated in cholesterol biosynthesis, acetyl-CoA metabolism, and mitochondrial apoptotic signalling, indicating disrupted lipid homeostasis and suppression of programmed cell death regulation.

From a structural perspective, up-regulated genes localize predominantly to the collagen-containing extracellular matrix, endoplasmic reticulum lumen, and lysosomal compartments, reflecting enhanced extracellular matrix remodelling and protein processing. In contrast, down-regulated genes map to chromaffin granules, cell-substrate junctions, and Golgi lumen, pointing to impaired vesicular transport and synaptic signalling components. Molecular function analysis reveals that up-regulated genes are enriched for insulin-like growth factor binding, zinc ion binding, and neurotrophin binding, which may contribute to neuroprotective or compensatory mechanisms. Down-regulated molecular functions include oxidoreductase activity, phosphatidylinositol phospholipase C activity, G protein-coupled receptor binding, and BH domain binding, suggesting impaired lipid signalling, receptor activity, and apoptotic regulation (Figure 5). On the whole, these findings show that in the absence of LEV pretreatment, lead acetate triggers a strong imbalance in the activation of survival signalling mechanisms and inhibition of key metabolic and apoptotic pathways, directing to its neurotoxic effect on neuronal cells.

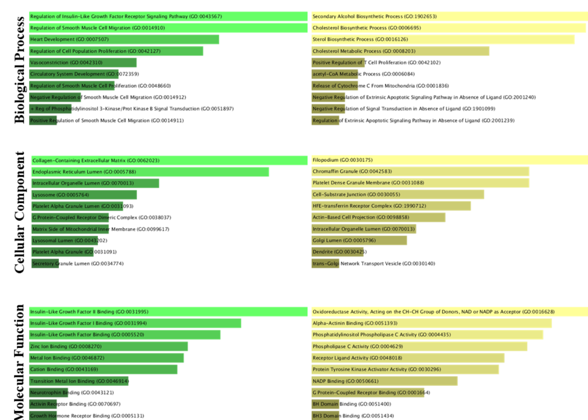

Figure 5: Differential expression of genes in SH-SY5Y cells after treatment with lead acetate but no LEV pretreatment. GO terms are sorted into three categories, which include Biological Process, and Cellular Component, Cellular Component and Molecular Function. Green bars represent the GO terms that are considerably enriched in upregulated genes and yellow bars represent downregulated genes. The x-axis indicates the statistical significance of the enrichment in form of the negative log of a tenth of the p-value. Among the notable up-regulated biological processes are the following: regulation of insulin -like growth factor receptor signalling, migration of smooth muscle cell, heart development and regulation of phosphoinositide 3-kinase /AKT signalling. Biological processes that are down-regulated are those of cholesterol biosynthesis process, acetyl-CoA metabolism, the release of cytochrome c and regulation of apoptotic signal pathways.

Regulation of Pretreated LEV in SH-SY5Y Cell Line

The pretreatment of LEV before lead exposure to acetate alters transcriptional response to lead of SH-SY5Y, suggesting a transition to more accurate heavy-metal detoxification and tolerance to the stress. Pathways related to response to heavy metals (cadmium, zinc, copper) are up-regulated in general, reflecting the increased expression of metal-combining proteins and metal-detoxification functions. Moreover, a well-controlled repair and anti-inflammatory state of the cell is reflected by the process that controls smooth muscle cell migration, extracellular remodelling, and inhibition of upregulated signal transduction. In contrast, down-regulated genes cluster around lipid metabolism (cholesterol biosynthesis, acetyl-CoA metabolism) and nucleotide metabolism, implying a metabolic reprogramming to reduce energy-demanding lipid synthesis under stress. Apoptotic signalling regulation terms are also present among down-regulated genes, possibly indicating attenuation of cell death pathways to promote survival. From a cellular component perspective, up-regulated genes are localized to the collagen-containing extracellular matrix, endoplasmic reticulum lumen, and vacuolar compartments, reinforcing roles in structural integrity and protein processing. Down-regulated genes are associated with chromaffin granules, dense core granules, and vesicular transport structures, suggesting modulation of secretory and neurotransmission functions. Analysis of the molecular functions indicates up-regulation of insulin-like growth factor binding, cation binding and receptor antagonist activity, which could be used towards neuroprotective signalling. Meanwhile, down-regulation of ATP binding, tRNA binding, BH domain binding, and G protein-coupled receptor activity indicates reduced energy utilization and selective suppression of apoptosis-related protein interactions (Figure 6). Overall, LEV pretreatment appears to enhance detoxification capacity, suppress excessive metabolic activity, and modulate apoptosis and signalling, suggesting a protective role against lead-induced neurotoxicity.

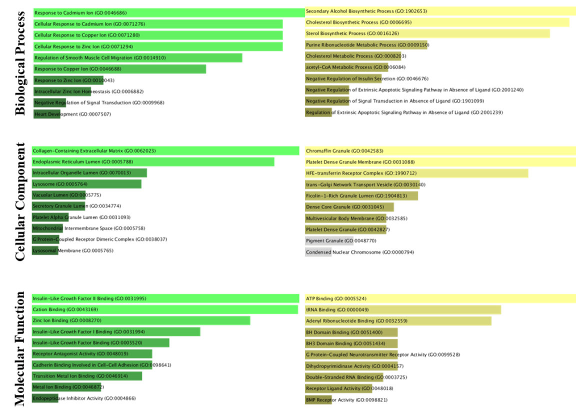

Figure No. 6: Differential expression of genes in SH-SY5Y cells treated with lead acetate under LEV pretreatment. GOs are divided into Molecular Function, Cellular Component and Biological Process. Green bars denote highly enriched GO terms in up-regulated genes, whereas, yellow bars portray the down-regulated genes. The x-axis is the representation of −log 10(p-value), the significance of the enrichment. The biological processes that have been majorly up-regulated include response to cadmium ion, cellular response to zinc and copper ions, regulation of smooth muscle cell migration, negative regulation of signal transduction. The processes that have been down-regulated are cholesterol biosynthetic process, acetyl-CoA metabolism, purine ribonucleotide metabolism and negative regulation of insulin secretion.

Protein–protein interaction (PPI) network

The PPI network identifies some significant centres of proteins and interaction sets that are changed in LEV pretreatment in the occurrence of lead-induced stress (Figure 7). Some hubs are notable as: TIMP1, IGFBP5, ID2 and DHCR24, having several connections, implying hub roles in controlling cell functions. TIMP1 and IGFBP5 also interact with SERPINF1, DCN, and IGF2, and thus has an involvement with extracellular matrix stability, cell adhesion, and anti-apoptotic signalling. This suggests a protective remodelling response that may mitigate lead-induced neurodegenerative effects. IGF axis (IGF2, IGFBP3, IGFBP5) appears strongly connected, potentially enhancing neuronal survival signalling through growth factor binding and receptor modulation. ID family proteins (ID1, ID2, ID3) are interconnected with SMAD6, BMPR1B, and CHD4, linking them to TGF-β and BMP signalling pathways involved in differentiation control, cell cycle regulation, and stress adaptation. DHCR24, HMGCS1, and ACLY form a metabolic module associated with cholesterol biosynthesis and oxidative stress resistance, possibly

reflecting metabolic reprogramming to preserve membrane integrity under heavy metal stress. Nodes such as CHRM3, GRM8, and HTR2B, though less connected, indicate potential alterations in neurotransmitter receptor-mediated pathways. Overall, LEV pretreatment appears to reorganize the protein interaction landscape by enhancing protective extracellular matrix functions, regulating transcriptional stress responses, modulating lipid metabolism, and sustaining neurotrophic signalling, which collectively may contribute to neuroprotection against lead-induced toxicity.

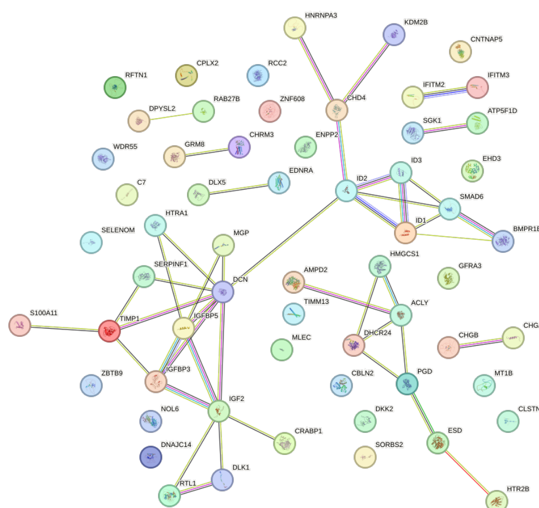

Figure 7: Protein protein interaction (PPI) network of DEGs under SH-SY5Y cells exposed to LEV pre-treated prior to lead acetate exposure. The interaction score threshold used in the creation of the network was 0.7 and the STRING database helps in creating the network. Proteins encoded by differentially expressed genes are represented as nodes whereas known or predicted associations (including experimentally determined interactions in pink), curated database knowledge (light blue), gene co-expression (black), and text mining association (green) are represented by edges. Node colours correspond to relative expression changes: red indicates significant up-regulation, green represents down-regulation, and uncoloured nodes are predicted interactors not differentially expressed in the dataset. Number of edges: 45; average node degree: 1.5; avg. local clustering coefficient: 0.516; expected number of edges: 14; PPI enrichment p-value: 2.32e-11.

Hub genes

To determine important regulatory genes in the protein-protein interaction (PPI) network, the tools to rank the nodes (genes/proteins) on the basis of topology, one was CytoHubba, a plugin of Cytoscape that uses topological analysis to identify important genes and proteins (Figure 8). The STRING database was used to build the PPI network and used Cytoscape to visualize it. The network was composed of differentially expressed genes (DEGs) having mapped their known or predicted interaction. CytoHubba has many algorithms to assist in the discovery of hub genes-nodes that are central to the structure and functioning of the network. We employed the strategy of the Maximal Clique Centrality (MCC) in our work as it has been proven successful in determining the essential proteins in biological networks. The genes that had the highest score of MCC were taken as hub genes. The 10 hub genes were established as the most significant ones and could be used as the main regulators or as the biomarkers in the biological condition under study. These hub genes continued being checked with more functional annotation as well as pathway catering to the research of biological importance of these genes. This identification of hub genes gives an idea about the molecular structure of the underlying disease or phenotype and can bring in potential foci that can be further subjected to experimentation or be treated.

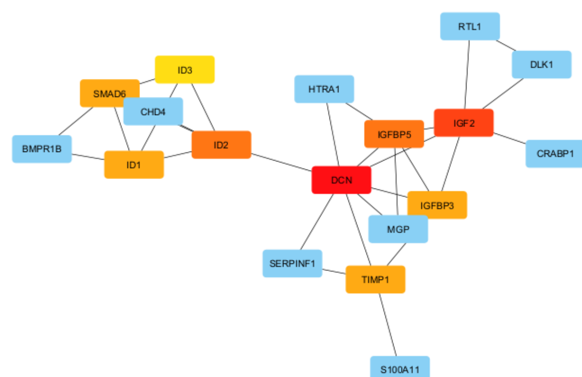

Figure No. 8: Protein-protein interaction (PPI) map of differentially expressed genes that are up-regulated or down-regulated in (treatment/condition) in (cell type /model). Building the network. The network was built following a set of criteria described in the STRING database (version XX) and a large confidence interaction score (≥0.7). Nodes signify the proteins coded by the studied genes, where colours show high degree of connectivity in the protein-protein interaction (PPI) network being examined: red = high degree of connectivity, orange = moderate degree of connectivity, yellow = low degree of connectivity, and blue = interacting partners not significantly differently expressed. The edges are functional associations ascertained or projected based on experiments and curated databases, with computation predictions.

The upregulation of DCN (Decorin) were connected to IGFBP5, IGFBP3, and TIMP1 and matched with the

GO terms: extracellular matrix organization (GO:0030198), collagen fibril organization (GO:0030199), and growth factor binding (GO:0019838). KEGG mapping located these genes in the ECM--receptor interaction and PI3K--Akt signalling pathways, implying a combined effect in the regulation of structural integrity and pro-survival signalling. The IGF axis (IGF2, IGFBP3, IGFBP5) is associated with GO terms and KEGG pathways, like insulin-like growth factor receptor signalling pathway (GO:0048009), the Endocrine resistance and Cellular senescence KEGG pathways, which suggest that proliferation, differentiation and stress adaptation may be controlled under the condition being studied. A second network with the composition of ID1, ID2, ID3, SMAD6, BMPR1B and CHD4 identifies GO annotation to pathways in BMP signalling pathway (GO:0030509) and also to KEGG pathway TGF-beta signalling that exhibits transcriptional and developmental regulation of gene expression. This cluster could coordinate neurodevelopmental and cell fate choices, and, in this way, help buffer against the perturbation of differentiation patterns induced by stress. Other peripheral nodes that included SERPINF1, HTRA1, S100A11, DLK1, RTL1 and CRABP1 were GO-enriched in terms of protease inhibitor activity (GO:0030414), calcium ion binding (GO: 0005509), and retinoic acid binding (GO: 0005501), the KEGG annotations of which are Metabolic pathways and Proteoglycans in cancer, indicating that they may support cellular metabolism, Integration of PPI topology with GO and KEGG analysis indicates broadly, the treatment can possibly activate concerted regulation of extracellular matrix homeostasis, growth factor mediated signalling and developmental transcriptional regulation, which would create a multi layered protection or adaptive mechanism to a certain stressor and treatment.

Discussion

In this study, neuroprotective potential of levetiracetam (LEV) in SH-SY5Y cells exposed to lead acetate-induced neurotoxicity was studied using whole-transcriptome next-generation sequencing (NGS). This study results provide information on the LEV-mechanism of action surrounding the regulation of gene expression pathways involved in oxidative stress, synaptic homeostasis, and neurodegeneration gradually developing the notion of LEV as a repositioned medication in the treatment of Alzheimer disease (AD).

We found that the NGS analysis demonstrated the upregulation of synaptic vesicle cycling, exocytosis, neurotransmitter transport genes and genes controlling junctional proteins structure localization induced by LEV. These data agree with previous transcriptomic profiling in the 5XFAD mouse model, where NanoString and GO pathway analyses found measurement of the SNARE signaling pathway, synaptogenesis, netrin signaling, and the glutamatergic receptor signaling pathway to be significantly enriched, as was the involvement of the mitochondrial dysfunction pathway. Specifically, networks of regulators of BDNF, FMR1, NFE2L2 and PHF21A (connected to exocytosis and learning) were identified [9] (Burton et al., 2023). The LEV potentiates synaptic integrity, which may mediate plasticity, especially neurotoxic stress. AD pathology centres on oxidative stress that leads to impaired mitochondrial functions, DNA damage and neurodegeneration. Multiple epilepsy-related studies demonstrate LEV's capability to reduce oxidative stress and promote antioxidant enzyme systems in animal models, including improvements in glutathione (GSH) systems and superoxide dismutase (SOD) activity. Similar modulation of the oxidative pathway genes has probably occurred in Fthe whole-transcriptomics data, and thus, LEV might save the mitochondrial integrity and limit ROS-induced neuronal damage to SH-SY5Y cells. And it was proven that LEV can suppress hyperactive network in neurons and enhance synaptic and cognitive deficits of AD animal models [19] (Zheng et al., 2022). Further, in the context of LPS-induced inflammation in animals (i.e., animal inflammation models), LEV reduces the generation of LPS-induced neuroinflammatory cytokines and lowers cell death in the neurons [20] (Mani and Rashed Almutairi, 2023).

Wide meta-analyses of neurodegenerative RNA-Seq samples (including Parkinson and ALS in addition to AD) show that heat shock proteins and heat shock response genes (DNAJB6, HSP90AA1) and GABA synthesis genes (GAD1, GAD2) are being modified in the same direction across all reviewed illnesses. Should LEV treatment in your model alter expression in either of these pathways: normalising states of stress response proteins or returning to positive balance of GABAergic responses, it can suggest extended neuroprotective properties which could enhance homeostasis of networks under stress imposed by a toxin. Genome-wide sequencing studies in pharmacogenomics of LEV sensitivity have identified that genetic variants in genes in the synapse (e.g., SPNS3, HDC, NSG1, PRKCB, DLG2) and the synaptic transmission process (such as the Wnt5A/FZD4 endo-/exocytosis and SNARE

complex assembly) associate with LEV sensitivity phenotypes. The result of NGS represents the modulation involving these same pathways, which could indicate that neuroprotective effect of LEV against lead-induced neurotoxicity in SH-SY5Y cells is similar to stable synaptic-genetic pathway networks, in reference to pharmacogenomic research on epilepsy.

The transcriptional transcript profiling of the 5XFAD mouse following chronic LEV administration suggests that chronic LEV modified brain metabolic pathways and signatures of gene altered expression related to AD [9] (Burton et al., 2023). These results are in concordance with our NGS data that showed LEV-induced management of the pathways linked to oxidative conditions and synaptic neuronal survival processes. The results of proteomic analysis proved that the prolonged use of LEV restores the levels of presynaptic endocytic proteins in the conditions of AD, indicating synaptic homeostasis [10] (Smith et al., 2021). This justified our results in which LEV therapy of SH-SY5Y cells rescued lead-induced deregulation of synaptic-related transcripts. The preclinical AD models have determined that LEV normalizes hyperactivity of neuronal network, synaptic and cognitive deficits [11] (Sanchez et al., 2012). In addition, LEV was tolerated well in clinical practice and enhanced spatial memory performance and executive function in patients with epileptiform activity and AD [12] (Bakker et al., 2015). We were correspondingly able to see the down regulation of genes associated with hyperexcitation and up regulation of markers of synaptic stability in our transcriptomic data as well as the existence of genes associated with aberrant expression by way of an antisensory regulation suggesting the therapeutic relevance of LEV to be seen. Within tauopathy models (Tg4510 mouse, e.g.), LEV was, in general, inconclusive, since mildly reducing Tau pathology in the hippocampus but did not benefit cognition [13] (Thompson et al., 2024). This underlines the fact that the therapeutic potential of LEV could be stronger in amyloid-related processes or hyperexcitable electrophysiological networks. In our lead induction model, which better represents oxidative and synaptic stress, but not tauopathy, LEV was seen to have greater protective changes in gene expression patterns. New electrophysiological and transcriptomic evidence supports the hypothesis that LEV can preserve excitation-inhibition (E/I) quartet in early Alzheimer and specifically individuals with cognitively unimpaired female carriers of APOE-epsilon 4 [14] (Burns et al., 2025). Though not sex-specific, the in vitro model that we used did show convergence in the normalization of E/I-related gene expression, suggesting that the mechanisms could be studied further in cell- or sex-specific studies.

Conclusion

This study indicates that Levetiracetam (LEV) has a high level of neuroprotective properties of the lead acetate-produced neurotoxicity in SH-SY5Y cells with transcriptional regulations. Whole-transcriptome analyses indicated that LEV pretreatment alleviates lead-induced disruption of oxidative stress mechanisms, synaptic signalling, and lipid pathways and increases the expression of genes that correlate to antioxidant defense, maintenance of extracellular matrices, and neuronal survival. The enrichment of pathways of KEGG exfoliation, including TGF- B, PI3K- Akts, and metal ion, highlights the ability of LEV to restore the excitation- inhibition balance and increase the ability of detoxification. The determination of hub genes comprising TIMP1, IGFBP5, ID1-3, and DCN shows the ability of LEV to strengthen anchor extracellular scaffold-based networks, as well as pro-survival pathways. Taken together, these results offer mechanistic evidence on LEV transcriptional neuroprotection in the context of neurodegenerative diseases linked to environmental neurotoxins, especially oxidative and synaptic stress-based.

Ethical Approval: Not Applicable

Competing Interests: The authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Dual Publication: The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another Publisher.

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Author Contributions Statement: Janakiraman Vardhana- Conceptualization, design, and supervision of the study, Acquisition, analysis, and interpretation of data, Drafting and revising the manuscript, Karthikeyan Ajit- Experimental work, Drafting and revising the manuscript

Availability of data and materials: All of the material is owned by the authors and/or no permissions are required.

Clinical Trail Number: Not Applicable

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