Pravara Med Rev; June 2024, 16 (02), 25 - 35
DOI: 10.36848/PMR/2024/99999956000

Original article

GSK-3β inhibitor can rescue neurons through the prosurvival (autophagy) mechanism in Parkinson 's disease

Garima Gupta[1*], Deepika Joshi[1], Gopeshwar Narayan[2], Sameer Sharma[3] and Raghavendra L.S. Hallur[4]

[1]Department of Neurology, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005, India
[2]Department of Molecular and Human Genetics, Institute of Science, Banaras Hindu University, Varanasi – 221005, India
[3]Department of Bioinformatics, BioNome, Bangalore, India – 560043
[4]College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Loni-413736, Rahata Taluk, Ahmednagar District, Maharashtra, India
**Address for Correspondence Guptagarima2008@gmail.com*

Abstract:
Parkinson's disease (PD) is a pervasive chronic neurodegenerative disorder. Several genetic and environmental factors play a significant role in the progression of PD. Mainly molecular-genetic pathways are influenced by ecological factors for the onset of most sporadic cases. Rotenone (a mitochondrial complex-1 inhibitor, a naturally occurring toxin) reproduces PD's neurochemical, neuropathological, and behavioral features. GSK-3β /AKT axis is involved in many signaling pathways. Our results suggest that rotenone attenuates the phosphorylation of AKT and GSK3-β and increases the phosphorylation and aggregation of α-synuclein. In contrast, by encouraging autophagy, LiCl decreases the accumulation and phosphorylation of α-synuclein. GSK-3β/AKT signaling pathway is involved in rotenone-induced α-synuclein accumulation. GSK-3β inhibition by LiCl results in the increase of β-catenin levels which increases dopaminergic cell viability; lithium prohibits neuronal apoptosis by restoring mitochondrial function and increasing the Bcl-2 protein, which is an anti-apoptotic protein. LiCl treatment showed that the damaged mitochondria might be cleared by autophagy due to an increase in autophagy protein LC3B I/II. Thus, autophagy-enhancing drugs can provide new insights into neurobiology and a possible therapeutic avenue for further investigating Parkinson's disease and its pathology.

Keywords: GSK3-β; LiCl; Autophagy; Reactive Oxygen Species; α-synuclein

Introduction:

Symptoms of Parkinson's disease include the formation of cytoplasmic inclusion bodies called Lewy bodies, which contain α-synuclein protein. Lewy bodies lead to the loss of dopaminergic neurons (DA) in the substancia nigra. In the nigrostriatal pathway, these neurons typically produce dopamine which functions in the direct and indirect pathways [1]. The direct pathway encourages voluntary movement, whereas the indirect pathway discourages it [2]. The communication weakens between muscle cells and the brain when about 70% of the neurons are lost, resulting in motor symptoms. The molecular mechanism underlying α-synuclein protein aggregation, progressive and selective loss of dopaminergic neurons, and formation of intracellular inclusions. When dopamine cannot be created or released by dying neurons due to Lewy bodies, the receiving end of the pathways has more inhibitory motor action. It explains the other motor symptoms and slow movements seen in PD patients [3]. The etiology of PD disease is poorly understood, but it is a combination of environmental and genetic factors, where aging plays a crucial role. Two types of PD cases are classified as genetic and familial. Out of total familial cases, 5 to 10 % account for carrying a heritable mutation in the PARK genes,

The autophagy-lysosomal pathway and ubiquitin-proteasome are the two most crucial protein degradation mechanisms [4–6]. Autophagy is responsible for non-specifically degrading most of the cytosolic proteins and organelles. Mitophagy is a critical pathway for maintaining mitochondrial health and neuronal health. An impaired mitophagy accumulates injured mitochondria, leading to neuronal death, which causes neurodegeneration [6].

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

Mitochondria are multifaceted and are involved in cellular physiological processes such as proliferation, cell fate, differentiation, and apoptosis [7, 8]. The administration of rotenone, a well-known mitochondrial complex-1 inhibitor, induces Parkinson-like symptoms *in vitro* and *in vivo* [9]. Rotenone increases reactive oxygen species (ROS) production, which leads to cell death through the caspase-dependent cytochrome C release [10, 11]. Rotenone has been demonstrated to induce activation of GSK-3β (glycogen synthase kinase -3 beta). GSK-3β is involved in regulating receptor trafficking and synaptic plasticity. Also, the GSK-3β signaling pathway plays a crucial role in regulating different parameters of neural development, such as neurogenesis, proliferation, and neural differentiation [12, 13]. GSK-3β regulates cell survival and apoptosis by regulating mitochondrial complex I activity and ROS production. It also regulates oxidative phosphorylation by inhibiting NADH (complex I) which is the leading site of the ROS formation [14]. PI3K/AKT negatively regulates GSK-3β. The PI3K/AKT pathway activation might be associated with neuroprotection. It has been demonstrated that dysregulation of the PI3K/AKT signaling pathway is involved in Parkinson's pathology [15, 16] for oligomerization of α-synuclein by using GSK-3β phosphorylation as its substrate. Inhibition of GSK-3β reduces α-synuclein protein expression. Overexpression of α-synuclein reduces dopamine production and decreases TH function. Its overexpression and accumulation wreak havoc on mitochondrial function by raising oxidative stress and lowering autophagy. Autophagy-enhancing drugs can quickly remove these accumulated proteins. LiCl is a well-known autophagy enhancer and GSK-3β inhibitor. It can trigger autophagy without using the mTOR pathway [17]. The routes that may relate GSK-3β to Parkinson's disease have been investigated in this study. We have investigated whether Autophagy enhancers/GSK-3β inhibitors can rescue dopaminergic neurons and inhibit their pathogenesis via a prosurvival mechanism that can be used as a therapeutic approach.

Materials and Methods:

The SH-SY5Y cell line was obtained from NCCS, Pune, and cultured in DMEM-F12 (Cell clone) with 10% FBS (Gibco) and 1% Antibiotic cocktail (Cell clone). They were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and were maintained at the log phase. When the cells became confluent, they were split after treatment with Trypsin-EDTA. 2′,7′-Dichlorofluorescin diacetate (DCFDA Invitrogen) (#D6883) and LiCl (#L4408) from Sigma-Aldrich, Mito-tracker, (Invitrogen™ M7512), were purchased for immunofluorescence studies, anti-LC3B-I/II antibody (Cell Signaling, #2775), anti-GSK-3β antibody (Cell Signaling, #9315), anti-α-Synuclein (cell signaling, #2642), anti-Bcl-2 antibody (Cell Signaling, # 4223), anti-AKT antibody (Cell Signaling, # 4685) anti-β-catenin antibody (Cell Signaling, #8480) anti-GAPDH antibody (Cell Signaling, #5174) were used. Rotenone treatment was given for 24 h to assess the cell damage using Western blots. For MTT assay, reactive oxygen species & mitochondrial membrane potential (MMP) detection, and apoptosis analysis, LiCl was initially dissolved in dH_2O before adding to DMEM. The total duration of LiCl treatment was 48 h, during which cells were pre-treated with LiCl for 24 h and then with Rotenone for another 24 h.

MTT assay and cellular morphology observation:

To investigate the cytotoxic effect of the drugs, ten thousand cells per well (10×10^3 cells/well) were seeded in 96 well plates after incubation for 24 h cells were treated dose-dependently with 1mM to 100 mM LiCl for 48 h and with rotenone 25 nM to 1 µM for 24 h. Cytotoxicity was measured as per the manufacturer's protocol. After the treatment, MTT was added to 20 µl (5 mg/ml) to the wells and incubated in a humidified incubator at 37 °C for 2 h to allow the formation of purple formazan crystal. These crystals dissolved in 100 µl of DMSO, and absorption was taken at 570 nm.

MMP, ROS, assessments

After the treatment, SH-SY5Y cells were harvested and re-suspended in PBS, for MMP cells were immediately stained with a working solution of Mito-tracker (stock 1 M solution in DMSO to working 500 mM) and incubated at 37 °C for 15 to 20 min in darkness. For ROS, cells were stained with a 10 µM working solution of DCFH-DA (from stock 10 M), After washing cells twice with ice-cold PBS, cells were subject to a FAC Scan Flow Cytometer (BD), and emission was acquired at FL-1 channel, and data

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were analyzed by FSC express version 3.0 (De Novo Software, Los Angeles, CA, USA)

Apoptosis

Flow cytometry analyzed cell death following annexin V-FITC/propidium iodide (Invitrogen molecular prob, USA). Briefly, the cells were seeded in 6 well plates at the semi-confluent stage, and cells were then treated with LiCl (10 mM, 20 mM) for 48 h, followed by rotenone 100 nM for 24 h. Cells were harvested with 0.25% trypsin and washed with PBS then, cells were treated with FITC conjugated Annexin V and incubated at 37^{o}C for 15 min in the dark, added 2 ml 1× binding buffer, and centrifuged at 6000 rpm for 5 min at room temp. The cells were re-suspended in 200 µl of the buffer and added 5 µl of PI, incubated for 5-15 min on ice. Annexin V and PI emissions were detected in the FL1 and FL2 channels of BD-FACS Caliber (BD Biosciences, USA). The data were analyzed using FCS Express software version 3.0 (De Novo Software, Los Angeles, CA, USA)

Immunofluorescence

1000 cells were seeded on sterilized coverslips in 6 well plates (NEST, Thermo Fisher Scientific). After the drug treatment at standard conditions, the coverslip wells were washed with PBS for Mito-tracker. Dilute 1 mM Mito-Tracker® stock solution to the final working concentration of 100 nM, then remove the media from the dish and add pre-warmed (37°C) staining solution containing Mito-Tracker® probe and incubate for 15 min under growth conditions. After staining, the cells were washed in a fresh, pre-warmed buffer or growth medium for acridine orange. Dilute 1 mM stock solution to 100 µM working solution and add pre-warmed (37°C) staining solution containing acridine orange after removing media from the dish, incubate another 15 min under growth conditions, then carefully remove the medium/buffer covering the cells and replace freshly prepared, pre-warmed buffer or growth medium containing 2 – 4 % formaldehyde in complete growth medium at 37°C for 15 min at 4°C. The coverslips were mounted with DABCO (Sigma) over pre-cleaned slides and observed under a fluorescence microscope. Fluorescence intensity was measured using Image J software and statistical analysis using GraphPad Prism 9 software.

Western Blot

Cells treated with rotenone alone and combined with LiCl (10 mM, 20 mM) were collected 48 h after washing with PBS. Cell lysis was done using ice-cold RIPA buffer (Sigma Aldrich, USA). The cell lysate was kept for 20 min on ice with gentle vortexing in between, and then lysate was centrifuged at 12000 rpm for 10 min at 4^{o}C; the supernatant was collected in a fresh Eppendorf tube and sorted at -80^{o}C for further use. The protein concentration in the supernatant was measured using the standard Bradford method. 50 µg of the total protein was loaded on 10% SDS-PAGE. The electrophoresis was done at 50 volts for 4 h separated gel proteins were transferred to the PVDF membrane (Millipore, USA) using standard Western blot protocol. PVDF membrane was blocked with 5% skimmed milk dissolved in TBST containing 0.5% tween 20. After blocking, the membrane was incubated with primary antibodies anti-GSK3β, anti-AKT, anti-Bcl-2, anti-LC3B-I/II, anti-β-catenin, and anti-GAPDH overnight at 4^{o}C. The membranes were further incubated with their respective secondary antibodies conjugated to ALP for 3h, followed by exposure to BCIP-NBT solution (America USA). The relative intensity of bands was determined densitometrically using Quantity One software (Quantity One, Hercules, CA, USA). All data from independent experiments were expressed as the corresponding controls' ratio to optical density values for statistical analyses.

Statistical analysis

Numerical data were represented graphically as mean ± standard deviation (SD). The statistical difference was determined by one-way analysis of variance (ANOVA) was applied to compare the control-treatment groups using GraphPad Prism 9 software (La Jolla, USA). $P \leq 0.05$ was considered statistically significant.

Results:

Autophagy enhancers ameliorate SH-SY5Y survival against rotenone-induced toxicity:

We first investigated the effect of LiCl, a potent autophagy inducer, on cell survival under standard culture conditions. For further experiments, half-maximal inhibitory concentration (IC50) for LiCl was determined at 50mM and rotenone at 100 nM of drug concentrations. MTT assay demonstrates that

rotenone treatment for 24 hrs. drastically lowers the viability of the cells by 60%, creating more intercellular distance, less synaptic connection, a shortening of neurites, and shrinking the cell body more than the control. We then measured whether LiCl could prevent rotenone-induced toxicity. Our results demonstrate that pre-treatment with LiCl prohibits rotenone-induced cell death and causes a prosurvival effect in a dose-dependent manner, increases synaptic connection, and reduces intercellular space, extended neuritis growth, and normal cellular morphology. [**Fig. 1**]

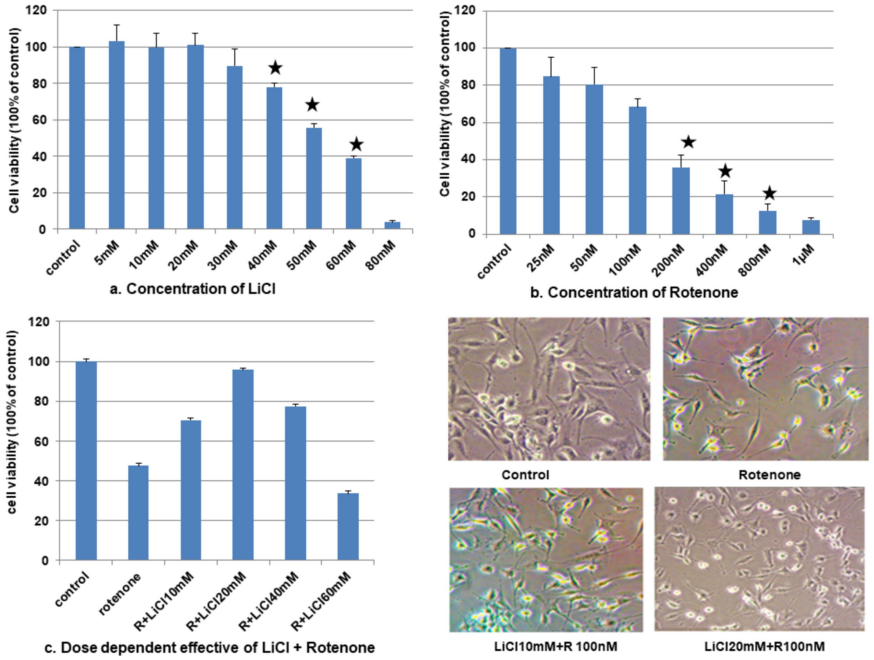

Fig.1: Cell viability was measured by MTT assay after SH- SY5Y cells were treated with LiCl for 48hrs at indicated concentrations with or without rotenone exposure. (a) Cell survival of SH-SY5Y cells following LiCl exposure (0 to 80mM) for 48 hrs. IC50 is 50mM; (b) Cell survival of SH-SY5Y cells following rotenone exposure (25-1000nM) for 24hrs. IC50 is 200nm, below the IC50 value (100nM), was used for the rest of the experiments (c) LiCl dose-dependently protected SH-SY5Y cells from rotenone-induced cytotoxicity. *$P < 0.05$ was considered significant.

LiCl attenuates Rotenone-induced oxidative stress and mitochondrial dysfunction:

MMP reduction was seen when treated with rotenone for 24hr. It decreases the fluorescence intensity of the Mito-tracker. Mito tracker red is a red-fluorescent dye that stains mitochondria in live cells. Its accumulation depends on the mitochondrial membrane potential, and the reduction of MMP suggests a lower ability of mitochondria to produce as a positive control [**Fig.2**].

ATP. When treated with LiCl for 48 h, it significantly inhibits the mitochondrial injury induced by rotenone and improves MMP.

Mitochondrial damage triggers oxidative stress by ROS formation. LiCl treatment for 48 h (before 24 h rotenone treatment) significantly reversed ROS production. We measured the DCFDA fluorescence intensity by taking H_2O_2

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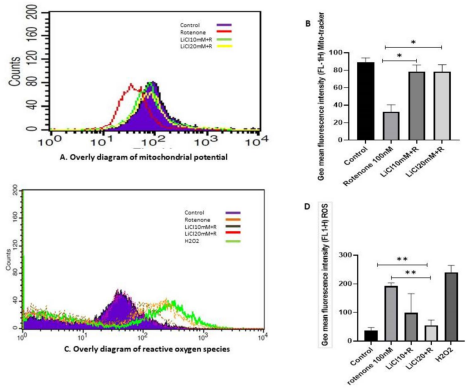

Fig.2 Mito-Tracker red CMX Ros at a final concentration of 500mM was used to visualize the mitochondrial transmembrane potential. The fluorescence intensity of DCFH was measured by flow cytometry in all groups.
The geo mean fluorescence intensity was expressed. (a) Comparison of the mitochondrial transmembrane potential of rotenone-treated cells with LiCl + Rot treatment group: Overly histogram of SH-SY5Y from center to the left showing a decrease in MMT and right shift showing an increase in MMT, suggesting that rotenone can cause a decrease in MMT but when treated with LiCl MMT recovers. (b) Graphical representation of the geo mean fluorescence intensity data of MMT * $P < 0.05$ is statistically significant. (C) Comparison of ROS generation in Rotenone treated cells with LiCl + Rot treatment group: Histogram of SH-SY5Y shows a right shift of peak in SH-SY5Y, indicating an increase in ROS generation in treated groups, and LiCl can effectively decrease ROS level. H_2O_2-treated sample groups are taken as a positive control. (D) Graphical representation of geo mean fluorescence intensity data of ROS ** $P < 0.05$ is considered statically significant.

Cellular and mitochondria damage can be minimized via autophagy enhancement:

The association between Autophagy and the minimization of mitochondrial damage or any DNA damage can be seen in the cells treated with two concentrations of LiCl (10, 20 mM) + Rotenone (100 nM). Staining was done using Mito-tracker and acridine orange. Acridine Orange is a cell-permeable nucleic acid-binding dye that emits green fluorescence when bound to dsDNA and is also used as a lysosomal dye. In contrast, Mito-Tracker is used to see the potential difference in the mitochondria. Cells stained with acridine orange showed punctuated fluorescence in the cytoplasm or very much intact around the nucleus, On the other hand, cells treated with rotenone alone show defused fluorescence. Mito-Tracker fluorescence is high on LiCl 10 mM concentration because cells with reduced mitochondrial membrane potential will fluoresce less. On the other hand, the Co-localization of Mito-Tracker Red and Acridine orange suggests that the turnover of mitochondria might be within auto phagolysosomes, indicating an increase in mitophagy. [**Fig. 3]**

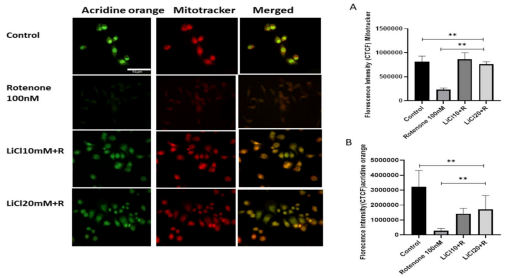

Fig.3 Autophagy enhancers may remove damaged mitochondria by increasing autophagy; cells are subjected to fluorescence detection. Significant increase in mean fluorescence intensity of (a) Mito-tracker and (b) acridine orange following the treatment of LiCl and rotenone. An increase in mean fluorescence intensity can be seen at 40× magnifications under a fluorescence microscope. Fluorescence intensity was measured using Image J and scale bar 91µM mentioned. Data are presented as mean ± SD; **p-value ≤0.001 is considered significant.

Inhibiting apoptosis and removing aggregated protein via autophagy enhancers for neuroprotection:

In the present study, it has been proposed that α-synuclein-induced neurotoxicity lead to cell death (apoptosis), and LiCl is a central modulator of the pathway that protects neurons from α-synuclein damage. Annexin V-FITC/PI apoptosis assay suggested that the percentage of the total apoptotic cell decreases after LiCl treatment compared to Rotenone. Annexin V-negative/PI-negative population was regarded as normal, healthy cells, while annexin V-positive/PI-negative and annexin V-positive/PI-positive were taken to measure early apoptosis and late apoptosis, respectively [Fig.4].

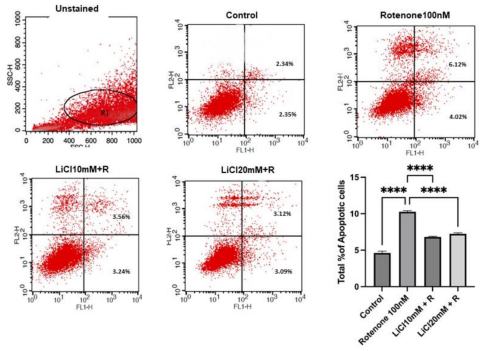

Fig.4 Cell Apoptosis as measured with Annexin V-FITC/PI assay, LiCl treatment reduces overall apoptosis in (upper right + lower right) SH- SY5Y cells after analyzing with BD-FACS Caliber (BD Biosciences, USA**)** total % of apoptotic cells were calculated. All data were presented as means ± SD (****P ≤0.01) from three independent experiments.

Interestingly, during the progression of PD, it has been described that active GSK-3β is found in neuronal cell bodies and neurites co-localizing with pre-neurofibrillary tangles observed in disease brains. Lithium has been shown to induce a concentration-dependent increase in the Bcl-2 protein level that shows a prominent anti-apoptotic role. The ratio of LC3B I/II with rotenone treatment was decreased than in control it was higher in the group with the co-treatment of LiCl and rotenone, which explains how autophagy shows a prosurvival effect because it gradually Improves the mitochondrial potential and may remove depolarized mitochondria via mitophagy and the aggregated protein as discussed above. Multiple pathways regulate autophagy, LiCl, by inhibiting GSK-3β activity and stabilizing β-catenin in the cell by preventing phosphorylation. GSK-3β activation through rotenone depresses autophagy and increases α-synuclein aggregation, which LiCl reduces [**Fig.5**].

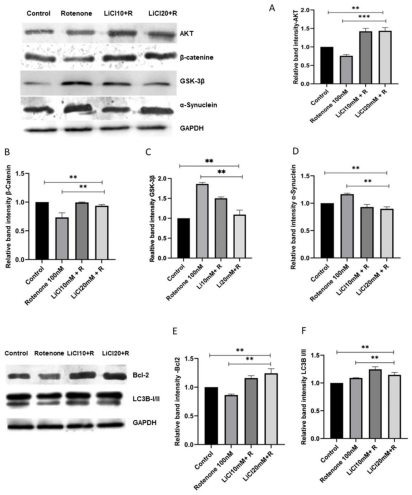

Fig.5 (a) LiCl treatment increases the AKT protein level; (b) Wnt/β-catenin signaling pathway can be activated by GSK-3β antagonists LiCl, it prevented cell death induced by rotenone by increasing β-catenin; (c, d)Rotenone increased α-synuclein protein levels and GSK-3β activity significantly, but on treatment with LiCl, it reduces and inhibits the level of α-synuclein, (e) LiCl reduces rotenone-induced cell death by increasing anti-apoptotic proteins Bcl-2 (f) Increased ratio of LC3B-I/II protein level in treated cells suggesting increased autophagy. The relative intensity of the protein band was normalized to GAPDH by Image J software (**P <0.05; ***P <0.01). All data were presented as means ± SD from three independent experiments.

Discussion:

Protein aggregation, neuronal dysfunction, and neuronal death can all be characterized as a dynamic and triangulated process that includes interrelated prospects that influence each other. Parkinson's disease is a neurodegenerative condition in which dopaminergic neurons are lost. The structural framework of the neuron is a dynamic system that contributes to the neural plasticity [18]. When the number of dopamine-producing cells in the substantia nigra falls below 20%–40%, signs of Parkinson's disease emerge, including bradykinesia (slowness and little movement), rigidity, resting tremor, and postural instability.

Environmental (such as pesticide exposure or a toxin in the food supply) and genetic factors may have a role in the pathophysiology of specific kinds of Parkinson's disease [19]. Rotenone, a common environmental toxin, has recently been widely utilized to imitate Parkinson's disease as a mitochondrial complex I inhibitor. Rotenone-induced mitochondrial dysfunction is demonstrated in this study by an increase in ROS generation and a decrease in MMP, which could lead to the activation of the mitochondrial apoptotic pathway and protein aggregation [20–22]. Autophagy has been proposed as an endogenous antioxidant and protective process that can eliminate reactive oxygen species (ROS). Protein conformation and phosphorylation control aggregation while dephosphorylation alters the conformation of these proteins, resulting in altered biological function [23]. Protein build-up, reduced mitophagy, and oxidative stress are pathologically significant in neurodegenerative illnesses. Increasing autophagy could help scavenge aggregated proteins and reduce the reactive oxygen species (ROS) [24]. In prior investigations Field, inhibited autophagy was associated with increased mitochondrial bulk and protein aggregation [25, 26]. As a result, we believe that LiCl may help to lower mitochondrial burden and protein aggregation. Lithium is also neurotrophic, and most studies suggest that it increases synaptogenesis by extending neurites and branching neurites, which leads to the formation of a neural network, i.e., increases the synaptogenesis [27].

Multiple direct and indirect paths have been identified in PD pathogenesis, one of these is GSK-3β. GSK-3β dysregulation is well understood to include a variety of cellular processes that eventually lead to neurodegenerative disorders such as Parkinson's disease. Lithium influences the activity of various protein kinases/phosphatases, including AKT, Bcl-2, Tau, α-synuclein, and β-catenin, in

addition to GSK-3β [28–32]. α-synuclein is a protein prevalent in the brain system that plays a function in the manufacture of dopamine by interacting with tyrosine hydroxylase [TH], the rate-limiting enzyme responsible for the overexpression of dopamine. When TH activity is inhibited, dopamine biosynthesis is reduced [33–35]. It is speculated that GSK-3β co-localizes with α-synuclein in the halo of Lewy bodies (LBs) which may allow it to interact with substrates in LBs and play a pathogenic role [36]. Overexpression of α-synuclein in transgenic mice has been linked to an increase in active GSK-3β, suggesting that α-synuclein accumulation could be the cause of the GSK-3β increase reported in PD brains [37]. GSK-3β activity is reduced by lithium through its inhibitory phosphorylation by activating the AKT directly. Activation of AKT promotes Bcl-2-protein to stop apoptosis which causes the death of the neurons [38, 39]. Our study suggests that the activity of GSK-3β is linked to α-synuclein aggregation via autophagy dysfunction. In earlier studies, The GSK-3β inhibitor was used at therapeutic dosages, resulting in a rise in β-catenin levels, stimulated β-catenin transcriptional activity, improved dopaminergic cell viability, and a drop in α-synuclein. β -catenin training is essential for the nervous system's early development, particularly in establishing the neural crest and tube. GSK-3β inhibition increases axon production and branching [40–42]. LC3B-I/II is involved in several stages of autophagy, including the formation of the autophagosome, a double-membrane vesicle that engulfs cellular material to be degraded. The presence of LC3B-I/II on the autophagosomal membrane is important for the proper function of autophagy. LC3B is present in two forms, LC3B-I and LC3B-II, which are both conjugated with phosphatidylethanolamine (PE) during autophagy. LC3B-II is the more heavily conjugated form and is often used as a marker of autophagic activity. LC3B-I/II is also involved in other cellular processes, such as mitophagy (the selective degradation of mitochondria) The autophagy-lysosomal pathway regulated the breakdown of intracellular proteins such as α-synuclein and p-tau because the AKT/GSK-3β route can regulate the autophagy. It's possible that the AKT/GSK-3β pathway is involved in pathogenesis or plays a key role in the therapeutic potential of LiCl. Different cell surface receptors activate PI3K, which activates PIP2 and PIP3, resulting in the co-recruitment of 3-phosphoinositide-dependent protein kinase-1 (PDK1) [43] and AKT to the cell membrane and the activation of AKT by PDK1. β-Catenin, which inhibits the SNCA to produce mRNA of α-synuclein to promote cell survival [44] A plausible mechanism is schematically shown in [**Fig.6**]

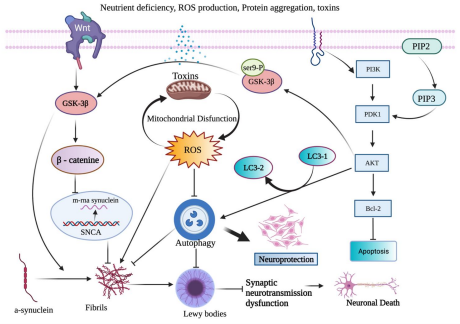

Fig.6: Schematic representation illustrating the plausible mechanism(s) for restoring α-synuclein-induced oxidative stress and neurodegeneration through autophagy and AKT/GSK-3β signaling pathway activation.

Conclusion:

Our findings suggest that LiCl can attenuate cell apoptosis, inhibit ROS production, and decrease MMP induced by rotenone in SH-SY5Y cells. A therapeutic dose of LiCl induces cytoskeleton protein changes to promote neuritogenesis, and it regulates GSK-3β directly and through a more complex network, affecting more than one molecular target simultaneously. Rotenone-induced α-synuclein aggregation is mediated by AKT/GSK-3β signaling pathway. Pharmacological induction of autophagy by LiCl may help design novel disease-modifying strategies in PD. However, further research into this is required. The role of LiCl and Rotenone is summarized in. [**Fig.**7].

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

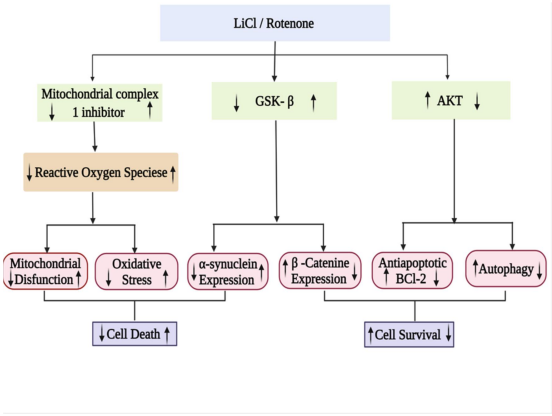

Fig.7 Schematic diagram showing the role of rotenone and LiCl. Rotenone acts as a mitochondrial complex 1 inhibitor and increases the activity of GSK-3β. It dephosphorylates AKT sequentially, increasing reactive oxygen species and causing mitochondrial dysfunction, oxidative stress to the cells, increased α-synuclein expression, Lewy body formation, and ultimately cell death. LiCl inhibits GSK-3β and increases the phosphorylation of AKT, which increases the anti-apoptotic protein Bcl-2 activity and inhibits apoptosis and promotes autophagy by increasing the ratio of LC3B-I/II protein, which can eliminate depolarized mitochondria and aggregated protein. On the other hand, inhibition of GSK-3β increases β-catenin activity, enabling cell survival by inhibiting increased transcription of α-synuclein. GSK-3β also inhibits the accumulation of α-synuclein protein to form a Lewy body and promotes cell survival.

Funding:
This research is being funded by the Ministry of Health and family welfare- department of Health and Research Under the Young Scientist (Placeholder1)to Garima Gupta; IoE faculty incentive grant of Banaras Hindu University to Gopeshwar Narayan.

References:

1. Et al.1. Young CB, Sonne J (2019) Neuroanatomy, Basal Ganglia. Treasure Island (FL)
2. Obeso JA, Rodríguez-Oroz MC, Benitez-Temino B, et al (2008) Functional organization of the basal ganglia: Therapeutic implications for Parkinson's disease. Mov Disord 23:S548–S559. https://doi.org/10.1002/mds.22062
3. Xu Q, Park Y, Huang X, et al (2011) Diabetes and risk of Parkinson's disease. Diabetes Care 34:910–915. https://doi.org/10.2337/dc10-1922
4. Shen YF, Tang Y, Zhang XJ, et al (2013) Adaptive changes in autophagy after UPS impairment in Parkinson's disease. Acta Pharmacol Sin 34:667–673. https://doi.org/10.1038/aps.2012.203
5. Xiong N, Xiong J, Jia M, et al (2013) The role of autophagy in Parkinson's disease: Rotenone-based modeling
6. Palikaras K, Daskalaki I, Markaki M, Tavernarakis N (2017) Mitophagy and age-related pathologies: Development of new therapeutics by targeting mitochondrial turnover. Pharmacol Ther 178:157–174. https://doi.org/10.1016/j.pharmthera.2017.04.005
7. Vakifahmetoglu-Norberg H, Ouchida AT, Norberg E (2017) The role of mitochondria in metabolism and cell death. Biochem Biophys Res Commun 482:426–431. https://doi.org/10.1016/j.bbrc.2016.11.088
8. Khacho M, Harris R, Slack RS (2019) Mitochondria as central regulators of neural stem cell fate and cognitive function. Nat Rev Neurosci 20:34–48. https://doi.org/10.1038/s41583-018-0091-3
9. Cannon JR, Tapias VM, Na HM, et al A highly reproducible rotenone model of Parkinson's disease. https://doi.org/10.1016/j.nbd.2009.01.016

10. Lee J, Huang MS, Yang IC, et al (2008) Essential roles of caspases and their upstream regulators in rotenone-induced apoptosis. Biochem Biophys Res Commun 371:33–38. https://doi.org/10.1016/j.bbrc.2008.03.149
11. Franco R, Li S, Rodriguez-Rocha H, et al (2010) Molecular mechanisms of pesticide-induced neurotoxicity: Relevance to Parkinson's disease. Chem Biol Interact 188:289–300. https://doi.org/10.1016/j.cbi.2010.06.003
12. Kim WY, Wang X, Wu Y, et al (2009) GSK-3 is a master regulator of neural progenitor homeostasis. Nat Neurosci 12:1390–1397. https://doi.org/10.1038/nn.2408
13. Ahn J, Jang J, Choi J, et al (2014) GSK3β, but not GSK3α, inhibits the neuronal differentiation of neural progenitor cells as a downstream target of mammalian target of rapamycin complex1. Stem Cells Dev 23:1121–1133. https://doi.org/10.1089/scd.2013.0397
14. King TD, Clodfelder-Miller B, Barksdale KA, Bijur GN (2008) Unregulated mitochondrial GSK3β activity results in NADH: Ubiquinone oxidoreductase deficiency. Neurotox Res 14:367–382. https://doi.org/10.1007/BF03033861
15. Zhang H, Mak S, Cui W, et al (2011) Tacrine(2)-ferulic acid, a novel multifunctional dimer, attenuates 6-hydroxydopamine-induced apoptosis in PC12 cells by activating AKT pathway. Neurochem Int 59:981–988. https://doi.org/10.1016/j.neuint.2011.09.001
16. Timmons S, Coakley MF, Moloney AM, O' Neill C (2009) AKT signal transduction dysfunction in Parkinson's disease. Neurosci Lett 467:30–35. https://doi.org/10.1016/j.neulet.2009.09.055
17. Sarkar S, Floto RA, Berger Z, et al (2005) Lithium induces autophagy by inhibiting inositol monophosphatase. J Cell Biol 170:1101–1111. https://doi.org/10.1083/jcb.200504035
18. R S Jope Anti-bipolar therapy: mechanism of action of lithium. Mol Psychiatry 4:117–128. https://doi.org/https://doi.org/10.1038/sj.mp.4000494
19. L B, VS G (2019) Parkinson's Disease, Lithium and Stem Cells. J Clin Lab Med 4:dx. https://doi.org/10.16966/2572-9578.126
20. Andreyev AY, Kushnareva YE, Starkov AA (2005) Mitochondrial metabolism of reactive oxygen species. Biochem 70:200–214. https://doi.org/10.1007/s10541-005-0102-7
21. Lemasters JJ (2005) Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuvenation Res 8:3–5. https://doi.org/10.1089/rej.2005.8.3
22. Céline Perier, Jordi Bové, Miquel Vila SP The rotenone model of Parkinson's disease. Trends Neurosci 26:345,346. https://doi.org/https://doi.org/10.1016/S0166-2236(03)00144-9
23. Nemani VM, Lu W, Berge V, et al (2010) Increased Expression of α-Synuclein Reduces Neurotransmitter Release by Inhibiting Synaptic Vesicle Reclustering after Endocytosis. Neuron 65:66–79. https://doi.org/10.1016/j.neuron.2009.12.023
24. Fang C, Gu L, Smerin D, et al (2017) The Interrelation between Reactive Oxygen Species and Autophagy in Neurological Disorders. Oxid Med Cell Longev 2017:. https://doi.org/10.1155/2017/8495160
25. Ganguli A, Choudhury D, Datta S, et al (2014) Inhibition of autophagy by chloroquine potentiates synergistically anti-cancer property of artemisinin by promoting ROS dependent apoptosis. Biochimie 107:338–349. https://doi.org/10.1016/j.biochi.2014.10.001
26. Giordano S, Darley-Usmar V, Zhang J (2014) Autophagy as an essential cellular antioxidant pathway in neurodegenerative disease. Redox Biol 2:82–90. https://doi.org/10.1016/j.redox.2013.12.013
27. Nciri R, Boujbiha MA, Jbahi S, et al (2015) Cytoskeleton involvement in lithium-induced SH-SY5Y neuritogenesis and the role of glycogen synthase kinase 3β. Aging Clin Exp Res 27:255–263. https://doi.org/10.1007/s40520-014-0290-3
28. Liang MH, Wendland JR, Chuang DM (2008) Lithium inhibits Smad3/4 transactivation via increased CREB activity induced by enhanced PKA and AKT signaling. Mol Cell Neurosci 37:440–453. https://doi.org/10.1016/j.mcn.2007.10.017

29. Bielecka AM, Obuchowicz E (2008) Antiapoptotic action of lithium and valproate. Pharmacol Reports 60:771–782
30. Marmol F (2008) Lithium: Bipolar disorder and neurodegenerative diseases Possible cellular mechanisms of the therapeutic effects of lithium. Prog Neuro-Psychopharmacology Biol Psychiatry 32:1761–1771. https://doi.org/10.1016/j.pnpbp.2008.08.012
31. Machado-Vieira R, Manji HK, Zarate CA (2009) The role of lithium in the treatment of bipolar disorder: Convergent evidence for neurotrophic effects as a unifying hypothesis. Bipolar Disord 11:92–109. https://doi.org/10.1111/j.1399-5618.2009.00714.x
32. Wada A (2009) Lithium and neuropsychiatric therapeutics: Neuroplasticity via glycogen synthase kinase-3β, β-catenin, and neurotrophin cascades. J Pharmacol Sci 110:14–28. https://doi.org/10.1254/jphs.09R02CR
33. Perez RG, Waymire JC, Lin E, et al (2002) A role for α-synuclein in the regulation of dopamine biosynthesis. J Neurosci 22:3090–3099. https://doi.org/10.1523/jneurosci.22-08-03090.2002
34. Yu S, Zuo X, Li Y, et al (2004) Inhibition of tyrosine hydroxylase expression in α-synuclein- transfected dopaminergic neuronal cells. Neurosci Lett 367:34–39. https://doi.org/10.1016/j.neulet.2004.05.118
35. Periquet M, Fulga T, Myllykangas L, et al (2007) Aggregated α-synuclein mediates dopaminergic neurotoxicity in vivo. J Neurosci 27:3338–3346. https://doi.org/10.1523/JNEUROSCI.0285-07.2007
36. Nagao M, Hayashi H (2009) Glycogen synthase kinase-3beta is associated with Parkinson's disease. Neurosci Lett 449:103–107. https://doi.org/10.1016/j.neulet.2008.10.104
37. Golpich M, Amini E, Hemmati F, et al (2015) Glycogen synthase kinase-3 beta (GSK-3β) signaling: Implications for Parkinson's disease. Pharmacol Res 97:16–26. https://doi.org/10.1016/j.phrs.2015.03.010
38. Alural B, Ozerdem A, Allmer J, et al (2015) Lithium protects against paraquat neurotoxicity by NRF2 activation and miR-34a inhibition in SH-SY5Y cells. Front Cell Neurosci 9:209. https://doi.org/10.3389/fncel.2015.00209
39. Jean-Martin Beaulieu 1, Raul R Gainetdinov MGC The AKT-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol Sci 8:166–172. https://doi.org/https://doi.org/10.1016/j.tips.2007.02.006
40. Zhang L, Cen L, Qu S, et al (2016) Enhancing beta-catenin activity via GSK3beta inhibition protects PC12 cells against rotenone toxicity through Nurr1 induction. PLoS One 11:. https://doi.org/10.1371/journal.pone.0152931
41. Ciani L, Salinas PC (2005) WNTs in the vertebrate nervous system: From patterning to neuronal connectivity. Nat Rev Neurosci 6:351–362. https://doi.org/10.1038/nrn1665
42. Dill J, Wang H, Zhou F, Li S (2008) Inactivation of glycogen synthase kinase 3 promotes axonal growth and recovery in the CNS. J Neurosci 28:8914–8928. https://doi.org/10.1523/JNEUROSCI.1178-08.2008
43. Singh AK, Kashyap MP, Tripathi VK, et al (2017) Neuroprotection Through Rapamycin-Induced Activation of Autophagy and PI3K/AKT1/mTOR/CREB Signaling Against Amyloid-β-Induced Oxidative Stress, Synaptic/Neurotransmission Dysfunction, and Neurodegeneration in Adult Rats. Mol Neurobiol 54:5815–5828. https://doi.org/10.1007/s12035-016-0129-3
44. Vallée A, Vallée JN, Lecarpentier Y (2021) Parkinson's disease: Potential actions of lithium by targeting the WNT/B-catenin pathway, oxidative stress, inflammation and glutamatergic pathway. Cells 10:1–16. https://doi.org/10.3390/cells10020230