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

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Abstract

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder. The underlying molecular mechanism lead to α-synuclein aggregation, progressive and selective loss of dopaminergic neurons, and intracellular inclusions (known as Lewy bodies (LBs)). Several genetic and environmental factors may lead to PD. Ecological factors can influence the onset of most sporadic PD cases via genetically mediated pathways. Rotenone (a mitochondrial complex one inhibitor, a naturally occurring toxin, a pesticide) reproduces PD's neurochemical, neuropathological, and behavioral features. Rotenone increased aggregation and phosphorylation of α-synuclein and attenuated the phosphorylation of AKT and GSK3beta. GSK-3β /AKT is involved in multiple signaling pathways. The results suggest that the AKT /GSK3beta signaling pathway mediated the rotenone-induced a-synuclein accumulation. The inhibition of GSK-3β by lithium results in the increase of β-catenin levels to enhance dopaminergic cell viability and then the reduction of accumulation and phosphorylation of α-synuclein by promoting autophagy Lithium inhibit neuronal apoptosis via increasing the BCl2 protein level, Preservation of mitochondrial function with LiCl treatment confirmed that the damaged mitochondria were cleared through autophagy (mitophagy). Via increased level of LC3. Finally, we can conclude that GSK-3beta inhibitors can provide new insights into biology and a possible therapeutic avenue for further investigating Parkinson's disease.

Introduction

Parkinson's disease is the second most common neurodegenerative disorder characterized by losing dopaminergic neurons (DA) in the substancia nigra and forming cytoplasmic inclusion bodies containing alpha-synuclein Lewy Bodies. These neurons typically produce dopamine which functions in the direct and indirect pathways of the nigrostriatal pathway.[1] The direct pathway stimulates voluntary movements, while the indirect pathway inhibits these movements.[2] When about 70% of neurons are lost, the communication between brain and muscle cells weakens, resulting in classic motor symptoms. When dopamine is unable to be created or released by degenerating neurons due to Lewy bodies, the receiving end of the pathways, the striatum, has more inhibitory motor action. This explains the slow movements and other motor symptoms seen in PD patients.[3] The etiology of this disease remains to be clarified but is considered the result of a combination of genetics and environmental factors, and where aging also has a crucial role. PD cases are divided into sporadic and familiar. Familiar issues carry a heritable disease mutation in genes referred to as PARK genes and account for 5–10% of all the PD cases.) Lewy Body, containing aggregated -synuclein, ubiquitin, and other misfolded proteins, is the hallmark pathological feature of PD. The ubiquitin-proteasome system and the autophagy-lysosomal pathway are the two most important cellular mechanisms for protein degradation. [4-6] Autophagy is responsible for the bulk degradation of cytosolic proteins and organelles non-specific. Mitophagy is a crucial pathway for maintaining mitochondrial health and, ultimately, maintaining neuronal health. An impaired mitophagy accumulates damaged mitochondria, leading to neuronal death and consequent neurodegeneration.[6] Mitochondria are multifaceted. They are involved in cellular physiological processes, including cell fate, differentiation, proliferation, and apoptosis [7,8]. The administration of rotenone, a well-known complex one inhibitor, induces PD-like symptoms in-vitro and in-vivo.[9] rotenone increases ROS production, which mediates cytochrome C release and caspase-dependent cell death. [10,11] Rotenone has been demonstrated to induce activation of GSK-3 (glycogen synthase kinase 3[11] GSK-3 signaling pathway play key roles in the regulation of different aspects of neural development, such as neurogenesis, proliferation, and neural differentiation. GSK-3 is also involved in the regulation of receptors trafficking and synaptic plasticity [12,13] GSK-3β can regulate cell survival and apoptosis by controlling mitochondrial complex I activity and ROS production it regulates oxidative phosphorylation by inhibiting NADH (complex I), which is the leading site of ROS formation, [14] PI3K/Akt, negatively regulates GSK-3b, the activation of PI3K/Akt pathways might be associated with neuroprotection. dysregulation of

the PI3K/Akt signaling pathway is involved in PD pathology. [15,16] α-synuclein is a substrate for GSK-3β phosphorylation. GSK-3β inhibition decreases α-synuclein protein expression Overexpression of α-synuclein inhibits TH activity and decreases dopamine biosynthesis. Its overexpression and accumulation impaired mitochondrial function by increasing oxidative stress by increasing autophagy. These aggregated proteins can be removed easily. This can be done via autophagy enhancers or GSK inhibitors. LiCl is a potent inhibitor and upstream activator of GSK and AKT. It can induce autophagy via the mTOR independent pathway.[17] This study summarized the pathways that may link GSK-3β to PD. Then we reviewed the evidence that explains GSK-3β inhibitors can rescue dopaminergic neurons and improve their pathogenesis through a prosurvival mechanism with a feasible therapeutic approach for PD.

Materials And Methods

Cell lines, culture, and reagents-

SH-SY5Y cell line was obtained from NCCS, Pune. All the cells were 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% CO2. The cells were maintained at the log phase. As the cells became confluent, they were split after treatment with Trypsin-EDTA. 2',7'-Dichlorofluorescin diacetate (DCFDA) (#D6883) and LiCl (#L4408) from Sigma-Aldrich, Mito-tracker, (Invitrogen™ M7512), were purchased for immunofluorescence studies, LC3B Antibody (Cell Signaling, #2775), GSK-3β Antibody (Cell Signaling, #9315), a-Synuclein (cell signaling, #2642), Bcl-2Antibody (Cell Signaling, # 4223) AKT Antibody (Cell Signaling, # 4685) b-catenin Antibody (Cell Signaling, #8480) GAPDH Antibody (Cell Signaling, #5174) were used. For Western blots, Rotenone was given 24 hrs. Before assessing the cell damage. For MTT assay, Reactive oxygen species, & Mitochondrial membrane potential (MMP) detection, apoptosis analysis. LiCl was initially dissolved in dH_2O before adding to DMEM. The total duration of LiCl treatment is 48hrs in which cells were pre-treated with LiCl for 24 hours and then with rotenone for another 24hr.

MTT assay and cellular morphology observation -

we first studied the cytotoxic effect of the drugs. Ten thousand cells per well (10*103 cells/well) were seeded in 96 well plates after incubation of 24 hrs. Cells were treated dose-dependently with 1mM to 100mM LiCl for 48hrs and with rotenone 25nM to 1micro M for 24 hrs. Cytotoxicity was measured as per the manufacturer's protocol. After the treatment, MTT was added 20m lit (5mg/ml) to the wells, then incubated in a humidified incubator at 37 °C for two hrs. To allow the formation of purple formazan crystal. These crystals dissolved in 100 microliters of DMSO, and absorption was taken at 570 nm.

MMP, ROS, assessments-

SH-SY5Y Cells were harvested and re-suspended in PBS, then immediately stained with Mito-tracker (stock 1mM solution in DMSO to working 500mM) for Mitochondrial membrane potential ROS DCFH-DA (10 M, Invitrogen), and incubated at 37 °C for 30 min in darkness. After washing twice with ice-cold PBS, the samples were subject to a FAC Scan Flow Cytometer (BD). Data were analyzed by FSC express version 3.0 (De Novo Software, Los Angeles, CA, USA)

Apoptosis -

The rate of cell death was analyzed by flow cytometry following annexin5-FITC/propidium iodide (Invitrogen molecular prob, USA). According to this method, cells were seeded in 6 well plates at semi confluent stage cells were treated with LiCl (10mM,30mM) for 48 hrs followed by rotenone 100nM for 24 hrs. Cells were harvested by 0.25% trypsin and washed with PBS; then, cells were treated with FITC conjugated Annexin and incubated at 37oc for 15 min in darkness, add 2ml 1x binding buffer centrifuge at 6000 rpm for 5 min at room temp. resuspend the cell in 200ul in buffer add 5 ul PI 5-15 min on ice data were analyzed by BD-FACS Caliber instrument (BD- biosciences, 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 1mM Mito-Tracker® stock solution to the final working concentration of 100nM, then remove the media from the dish and add prewarmed (37°C) staining solution containing Mito-Tracker® probe and incubate for 15 min under growth conditions. After staining, wash the cells in a fresh, pre-warmed buffer or growth medium. 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 minutes at four °C. Cells were counterstained with DAPI after rinsing with one × PBS. The coverslips were mounted with DABCO (Sigma) over pre-cleaned slides, observed using fluorescence Microscopy fluorescence measured using ImageJ software, and statistical analysis was done using GraphPad Prism 9 software.

Western Blot –

cells treated with rotenone alone and combine with LiCl (10mM, 30mM) were collected after 48 hrs by washes with PBS. Cell lysis was done with ice-cold RIPA buffer (Sigma Aldrich, USA). The cell lysate was kept for 20 min followed by gentle vortexing in between, then centrifugation was done on 12000rpm 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 equal quantity 50mg was used for western blot analysis protein was resolved electrophoretically on 10% SDS-PAGE gel then transferred on to PVDF membrane (Millipore, USA). PVDF membrane was blocked with 5% non-fat milk dissolved in TBST containing 0.5% tween 20. after blocking, the membrane was incubated with primary antibody GSK-3beta, AKT, Bcl-2, and LC-3B, beta-catenin, and GAPDH antibodies overnight at $4^{O}C$; the membrane was further incubated with their respective secondary antibody conjugated to ALP for 3 hrs after that membrane was exposed to BCIP-NBT solution (America USA). The relative intensity of bands was determined densitometrically by using the Quantity One software (Quantity One, Hercules, CA, USA). All data from independent experiments were expressed as the ratio to optical density values of the corresponding controls for statistical analyses.

Statistical analysis-

Numerical data was represented graphically as mean ± standard deviation (SD). The student's t-test (paired for cell lines) was applied to compare the control-treatment groups using GraphPad Prism 9 software (La Jolla, USA). $P \leq 0.05$ was considered statistically significant and was two-sided.

Results

Autophagy Enhancers ameliorate SH-SY5Y survival against rotenone-induced toxicity.

We first want to examine whether LiCl, which is known to be a potent autophagy inducer, has any significant role in cell survival under standard culture conditions. Analyses indicate that LiCl below 50mM shows no toxic effect with a half-maximal inhibitory concentration (IC50) ranging between (45 to 60mM). Rotenone treatment resulted in cell death with an IC50 200nM, so we have taken 100nM drug concentration for further experiments. We have found that rotenone treatment for 24 hrs has more intercellular distance, less synaptic connection, and a shortening of neurites shrinking the cell body than control. We then measured whether LiCl could prevent rotenone induce toxicity. And we found that 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. MTT assay indicated that rotenone drastically lowers the viability of the cells by 60 % while pre-treatment with LiCl [fig 1].

LiCl attenuates Rotenone-induced oxidative stress and mitochondrial dysfunction:

MMP reduction was seen when treated with rotenone for 24 hr. It decreases the fluorescent intensity of the Mito-tracker. Mito tracker red is a red-fluorescent dye that stains mitochondria in live cells. Its accumulation depends upon potential membrane Reduction of MMP means a lower ability to produce ATP. When treated with LiCl for 48 hrs, it significantly inhibits the mitochondrial injury induced by rotenone. Mitochondrial damage triggers oxidative stress by ROS formation. LiCl treatment for 48 hrs (24hrs before rotenone) significantly reversed ROS production. We measured the DCFDA florescence's intensity taking H_2O_2 as a positive control. [fig2]

Cellular damage or Damaged Mitochondria removed via autophagy Enhancement:

The association between Autophagy and removal of damaged mitochondria or any DNA damage can be seen via immunostaining with Mito-tracker and acridine orange. Acridine Orange is a cell-permeant nucleic acid binding dye that emits green fluorescence when bound to dsDNA, also been used as a lysosomal dye. The results show that when we treat cells with LiCl, the fluorescence that appears in the cytoplasm is punctuated or very much intact around the nucleus in the case of acridine orange, and the fluorescence Mito-Tracker is high. But when the cells are treated with rotenone alone, it is defused in the cytoplasm. Co-localization of Mito-Tracker Red and acridine orange indicated that the turnover of mitochondria might be within auto phagolysosomes. [fig3]

Autophagy enhancement is associated with a decrease in apoptosis rate. Neuroprotection and removal of aggregated protein:

A relationship between a-synuclein-induced neurotoxicity and a decrease in the cytoplasmatic levels of beta-catenin has been proposed. Also, the inhibition of glycogen synthase kinase (GSK-3beta through LiCl), a central modulator of the pathway, protects neurons from a-synuclein damage. Interestingly, during the progression of PD, it has been described that active GSK-3beta is found in neuronal cell bodies and neurites, co-localizing with pre-neurofibrillary tangles observed in disease brains. The ratio of LC3/2 with rotenone treatment was decreased as Compared with control; it was higher in the group with the co-treatment of LiCl and rotenone. Lithium has been shown to induce a concentration-dependent increase in the Bcl2 protein level. It plays a prominent anti-apoptotic role. In the case of LiCl, autophagy shows a prosurvival effect because it gradually removes the damaged mitochondria via mitophagy and the aggregated protein. Multiple pathways regulate autophagy, but the critical pathway is AKT/GSK, and LiCl being a potent inhibitor of GSK 3beta, is common in most of them. GSK is the target of AKT, and rotenone attenuates phosphorylation of both; GSK activation through Rotenone depresses autophagy and increases the alpha-aggregation [fig4], which LiCl reduces.

Discussion

Neurodegenerative disease may be summarized as a dynamic and triangulated process that comprise irregulated interrelated prospect that influences each other like protein aggregation, neuronal dysfunction, and neuronal death. Parkinson's disease is a neurodegenerative disease where the loss of dopaminergic neurons occurs. The neuronal structural framework is a dynamic system contributing to neuronal plasticity. The relative requirement for plasticity in outgrowing neurites versus stabilized axons is reflected in their cytoskeletal composition.[18] When the number of dopamine-producing cells in the substantia nigra falls to less than 20–40%, then the symptoms of PD such as bradykinesia (slowness and minimal movement), rigidity, resting tremor, and postural instability appear. Furthermore, it is also revealed that some environmental (such as exposure to pesticides or a toxin in the food supply) and genetic factors might be involved in the pathogenesis of certain forms of PD. [19] As a mitochondrial complex I inhibitor, rotenone has been extensively used for modeling PD in recent years. In this study, rotenone-induced mitochondrial dysfunction is evidenced by the increased production of ROS and the decreased MMP, which may lead to the activation of the mitochondrial apoptotic pathway and protein aggregation [20-22]. Autophagy has been proposed as an endogenous, antioxidant, protective pathway that can clear accumulated ROS. Protein aggregation is regulated by its conformation and phosphorylation, and dephosphorylation changes the conformation of these proteins. That leads to altered biological function.[23] Protein accumulation and oxidative stress are pathologically pronounced in neurodegenerative diseases. Enhancing autophagy could scavenge aggregated proteins and increase ROS [24]. In the previous studies, inhibited autophagy was accompanied by accumulated mitochondrial mass and protein aggregation [25,26]. Thus, we think LiCl might reduce mitochondrial load and protein aggregation. Lithium is also neurotrophic, and most of the studies show that it significantly enhances neurites extensions neurite branching that leads to the establishment of a neuronal network, i.e., increases synaptogenesis. [27]

However, multiple direct and indirect pathways have been known. Still, the most common is that LiCl is a potent inhibitor of GSK 3b. It is well explained that GSK-3b dysregulation involves various cellular processes that eventually promote neurodegenerative pathology diseases PD. In addition to GSK-3b , lithium also affects the activity of other protein kinases/phosphatases, such as AKT, Bcl-2, tau, synuclein, β-catenin, etc. [28-32] α-synuclein abundantly found in the nervous system its role is the production of dopamine through the interaction with tyrosine hydroxylase [TH], its rate-limiting enzyme responsible for overexpression α-synuclein inhibit TH activity and decrease dopamine biosynthesis. of the conversion of tyrosine to L-3,4-dihydroxyphenylalanine in the dopamine synthesis pathway. [33-35] GSK-3β is co-localized with a-Synuclein in the halo of Lewy bodies (LBs); the authors suggested that GSK-3b localization in the LBs may let it interact with substrates in LBs and play a pathological role [36]. It has been shown

that overexpressing a- Synuclein in transgenic mice is associated with elevation of active GSK-3b , suggesting that accumulation of a-Synuclein might be the causative for GSK-3b increment seen in PD brain [37]. Lithium reduces GSK-3β activity by increasing the inhibitory phosphorylation of GSK3β. Through direct activation of the Akt pathway. The activation of Akt modulates Bcl-2 associated death protein (Bad) (a pro-apoptotic protein of the Bcl-2 family) [38,39]. These results suggest that GSK3 activity is related to α-synuclein aggregation through impaired autophagy. Therapeutic concentrations of the GSK-3β inhibitor led to the increase in β-catenin levels that promotes β-catenin transcriptional activity, which enhances dopaminergic cell viability, and then the reduction of α-synuclein. β-catenin training is necessary for the early development of the nervous system, especially in the formation of neural crest and neural tube inhibition of GSK promotes axon formation and its branching. [40-42] The autophagy-lysosomal pathway influenced the degradation of intracellular proteins, including α-synuclein and p-tau. Akt/GSK pathway can regulate autophagy. [43] so the GSK/AKT pathway may involve in the pathogenesis or playing a vital role in which LiCl may have therapeutic potential, but the possibility requires elaborated investigation of Reactive Oxygen Species [Fig5]

Conclusion

The pathogenesis of PD is a complex process, and multiple events, including oxidative stress, mitochondrial dysfunction, protein aggregation, and neuroinflammation, mediate the loss of dopaminergic neurons. Understanding the signaling processes that regulate the pathogenesis of PD is critical for developing novel therapeutics for PD treatment. Because of the prominent co-relation of GSK-3b and a-synuclein, this kinase was mainly associated with many neurodegenerative diseases. However, evidence implying the involvement of GSK-3b in shared cellular damage pathways such as Autophagy, ER-stress, mitochondrial dysfunction, and apoptosis has changed this view and cleared that aberrant GSK-3b. The cellular and molecular mechanisms of the protective effects of GSK-3β inhibition on dopaminergic neurons in pathogenic conditions require further elucidation. They may provide a potential efficient target for treating PD by blocking the pathogenic pathway. Taken together, these findings indicated that lithium was able to attenuate cell apoptosis, inhibit ROS production, and MMP decrease induced by rotenone in SH-SY5Y. Therapeutic dose, induced cytoskeleton protein changes to promote neuritogenesis lithium regulate GSK3b directly and through more complex network effects affecting more than one molecular target at a time. We conclude that rotenone-induced a-synuclein aggregation is mediated by the AKT/GSK3b signaling pathway. Pharmacologically induction of autophagy by lithium may raise a new hope for novel disease-modifying strategies in PD

Declarations

Conflicts of Interest:

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Figures

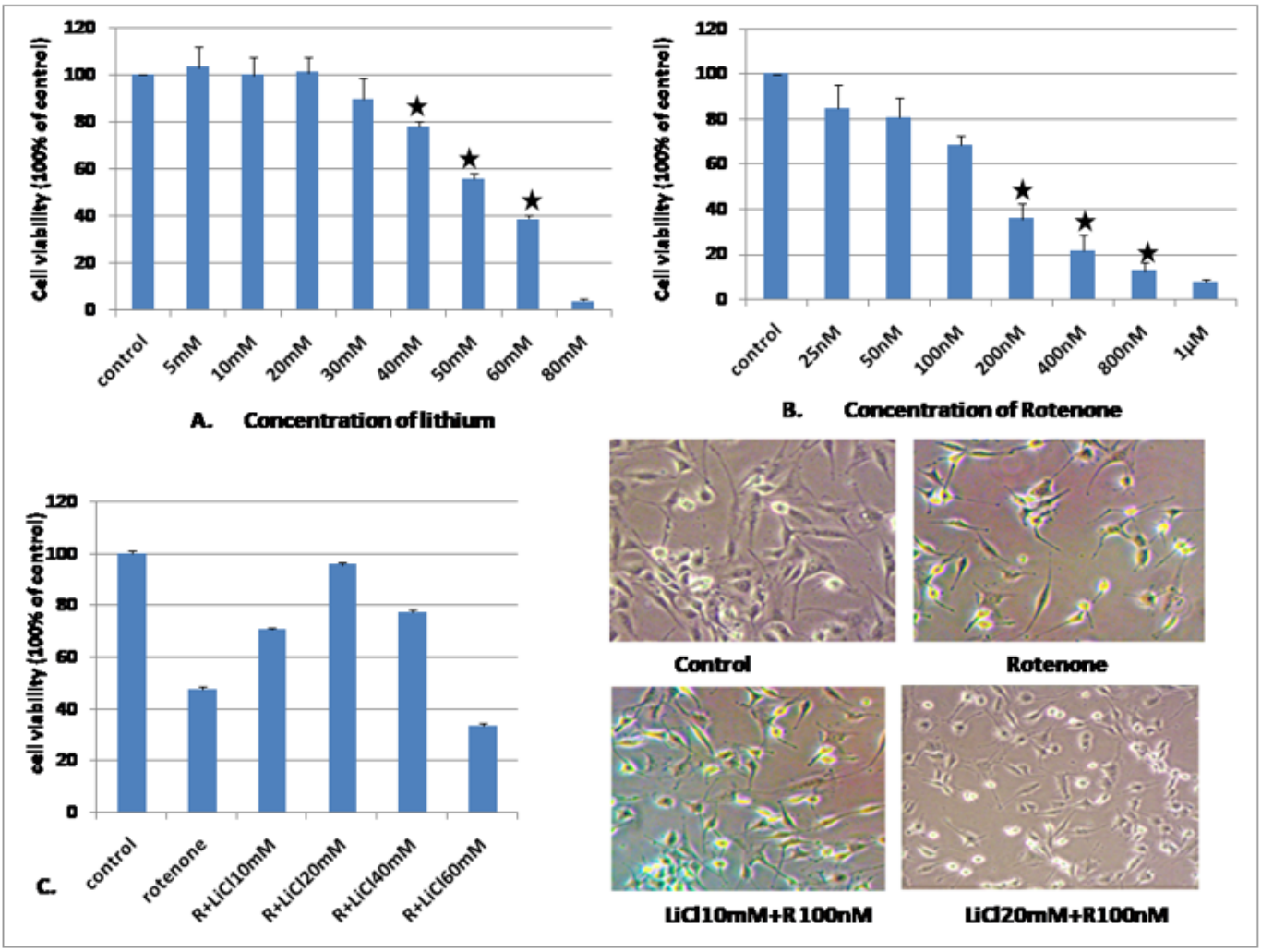

Figure 1

Cell viability was measured by MTT assay after SH- SY5Y cells treated with lithium for 48 hr. at indicated concentrations with or without rotenone exposure. (A) Lithium exposure (0–80 mM) had done to measure cell survival of SH-SY5Y cells under standard culture conditions for 48 hrs. IC50 is 50 mM(B) Rotenone exposure (25-1000nM) had done to measure cell survival of SH-SY5Y cells under normal culture conditions for 24hrs. IC50 is 200 nm. below the IC50 value(100nM) was taken for the rest of the experiments (c) Lithium dose-dependently protected SH-SY5Y cells from rotenone-induced cytotoxicity. *P < 0.05 as compared with control, two concertations in which maximum cells were surviving were finally taken (10mM & 20mM) or the rest of the experiments, and the morphological difference can be seen. between control and treated cells

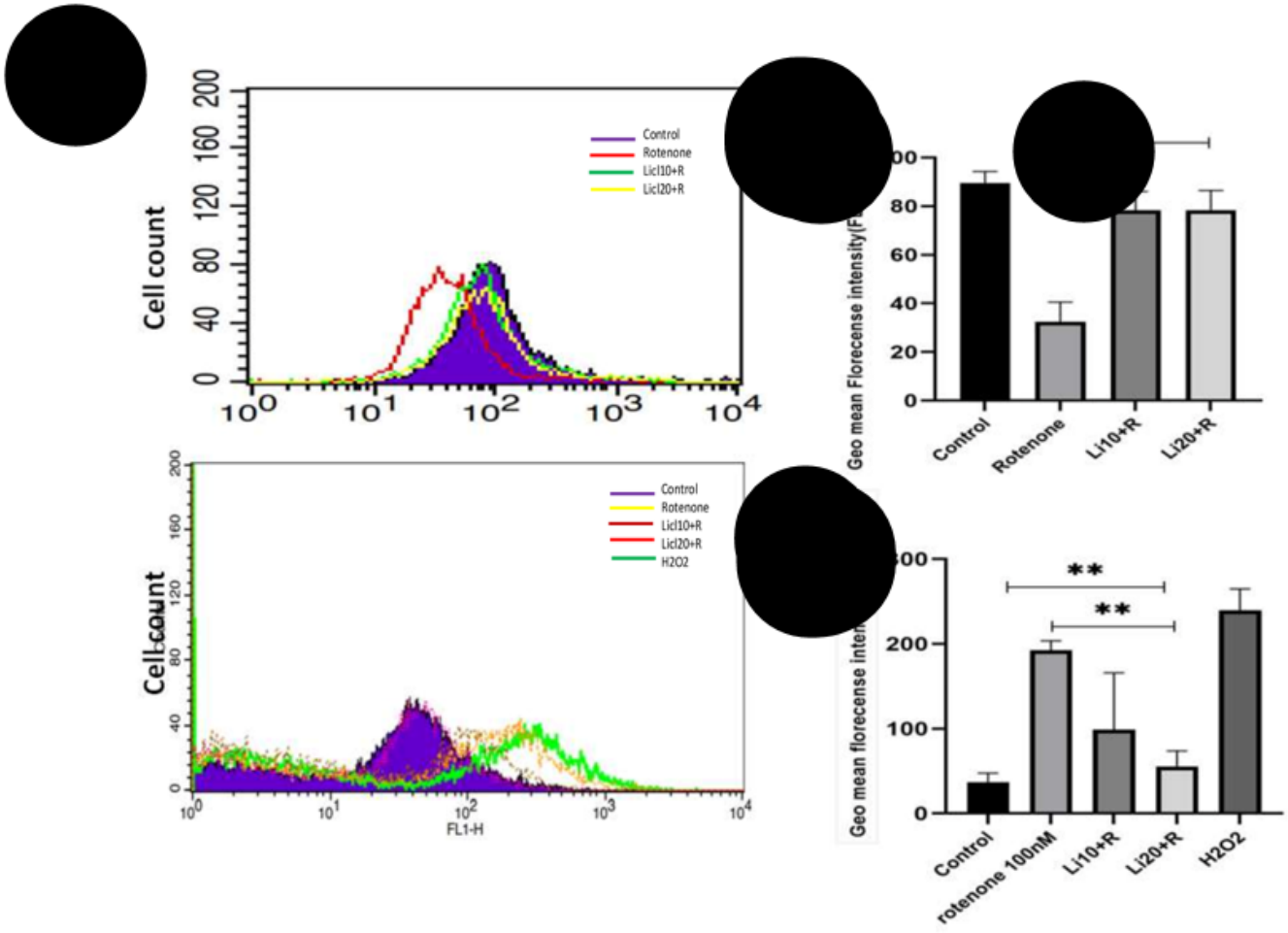

Figure 2

MitoTracker red CMXRos at a final concentration of 500 mM was used to visualize the mitochondrial transmembrane potential. The fluorescence intensity of DCFH was measured by flow cytometry in all groups. the geo means fluorescence intensity was expressed. (A) *$P < 0.05$ as compared with Rot with LiCl + Rot group; these are overly histogram of SH-SY5Y from center to the left shows the decrease in MMT and right shift shows an increase in MMT so we can see rotenone can cause a decrease in MMT but when treated with LiCl MMT recovers (b) **P < 0.05 as compared with Rot with LiCl + Rot group, These are histogram of SH-SY5Y caused a right shift of peak in SH-SY5Y. This indicates an increase in ROS generation in treated groups. LiCl can effectively decrease ROS level H2O2 treated sample groups are taken as a positive control.

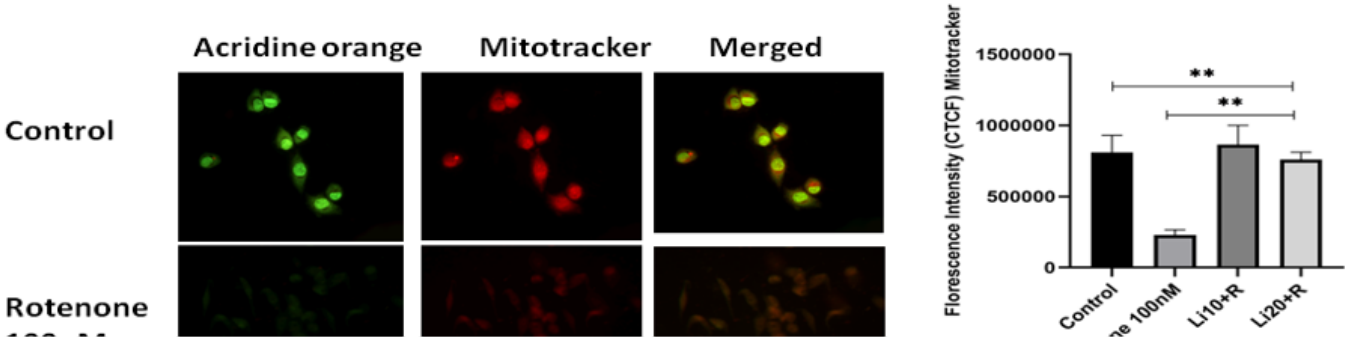

Figure 3

Autophagy enhancers can remove damaged mitochondria by increasing autophagy; cells are subjected to immunofluorescence 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 40X magnifications under a fluorescence microscope. Fluorescence intensity was measured via image J Data are presented as mean ± SD, and p-value ≤0.05 is considered significant.

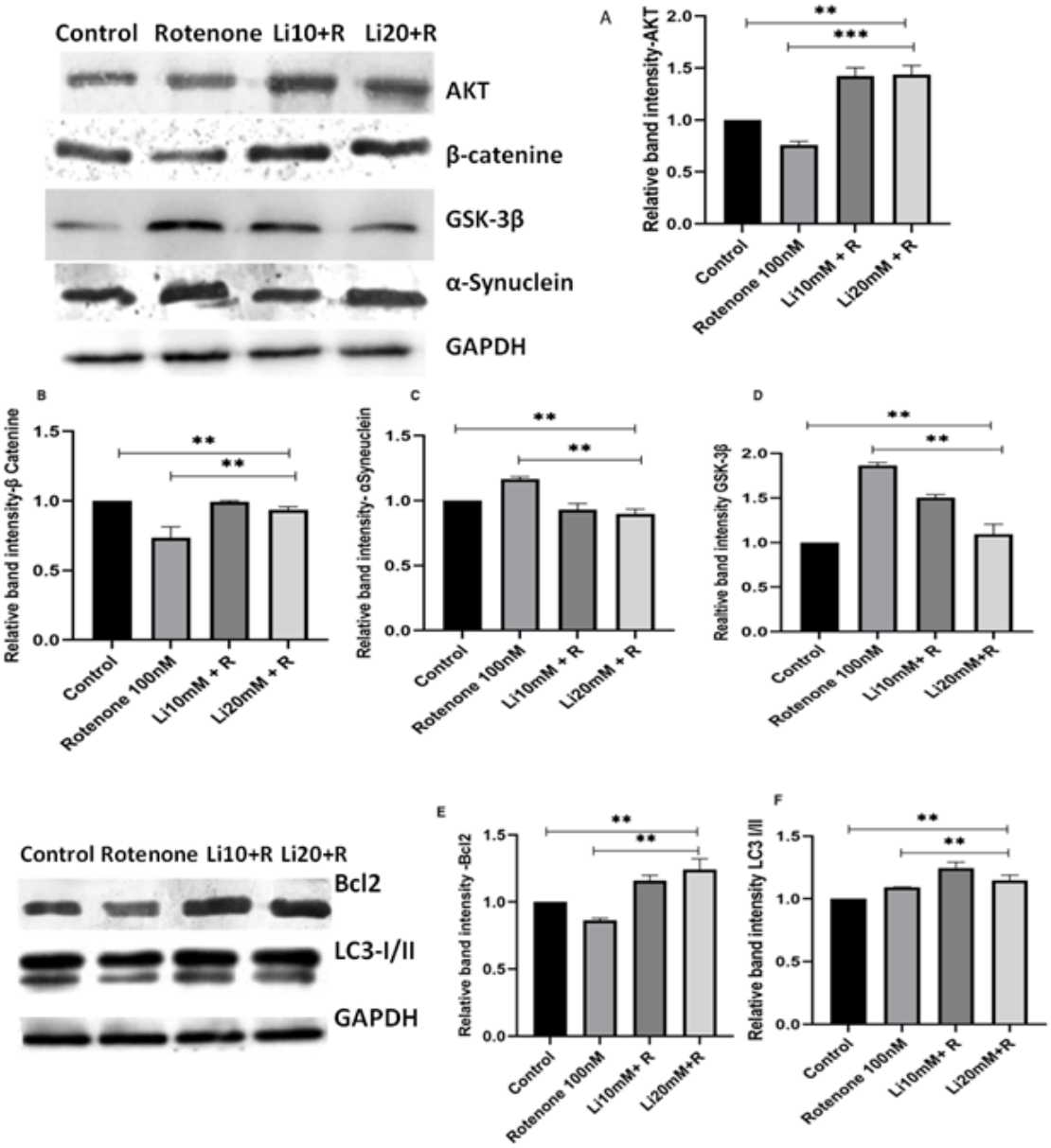

Figure 4

(A) AKT is at the crossroads of cell death and survival, playing a pivotal role in multiple interconnected cell signaling; LiCl increases the activity of AKT. protein level. **p < 0.05 compared with the rotenone treated cells. (B) Wnt/β-catenin signaling pathway can be activated by GSK3β antagonists LiCl it prevented cell death induced by rotenone by increasing. b-catenin. LiCl treated cells protein level. **p < 0.05 compared with the rotenone treated cells. Rotenone increased (C)a-synuclein protein levels and increased the (D)GSK activity, but on treatment with LiCl, it reduces and inhibits the aggregation, which prevents Lwi body formation. LiCl also reduces rotenone-induced cell death by increasing proapoptotic proteins like(E) Bcl-2, which further increases Autophagy and removes the aggregated protein and dysfunctional mitochondria that can be measured by (F)LC3 protein level. The relative intensity of the protein band was normalized to GAPDH by Image J software (**P < 0.05; ***P < 0.01). All data presented as means ± SD from three independent experiments.

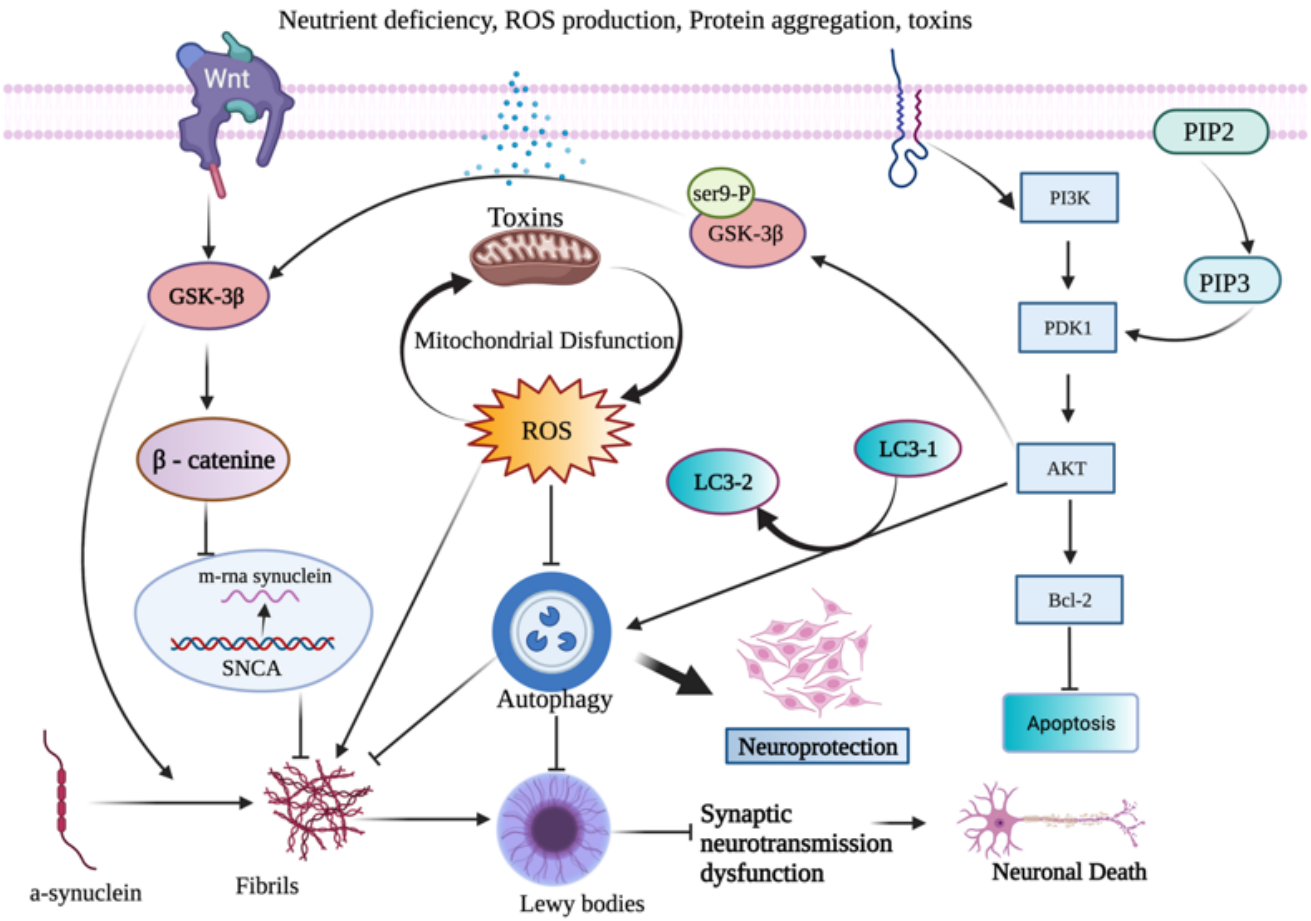

Figure 5

Schematic representation illustrating the plausible mechanism(s) for restoring Synuclein-induced oxidative stress and neurodegeneration through the activation of autophagy and GSK-3b/AKT signaling pathway. Different cell surface receptors activate PI3K, which activates PIP2 and PIP3. That causes the co-recruitment of the 3-phosphoinositide dependent protein kinase-1 (PDK1) [44] and Akt to the cell membrane and the activation of Akt by PDK1. Activated AKT phosphorylates GSK at ser9 to inactivate it inactivated GSK-3β. AKT also promote Autophagy which removes damaged mitochondria and decrease oxidative stress to the cell and aggregated protein Cytosolic GSK-3β phosphorylates the α-synuclein, leading to their aggregation, which contributes to cell injury by oxidative stress & Lewy body formation by inhibiting synaptic neurotransmission, which causes neuronal death β -catenin which inhibits the SNCA gene to produce mRNA of α-synuclein to promote cell survival.[45] Activated GSK -3β enable α -Synuclein protein to form aggregates fibril that will form Lewy body Akt also promote BCL-2 activation, which inhibits cell apoptosis.

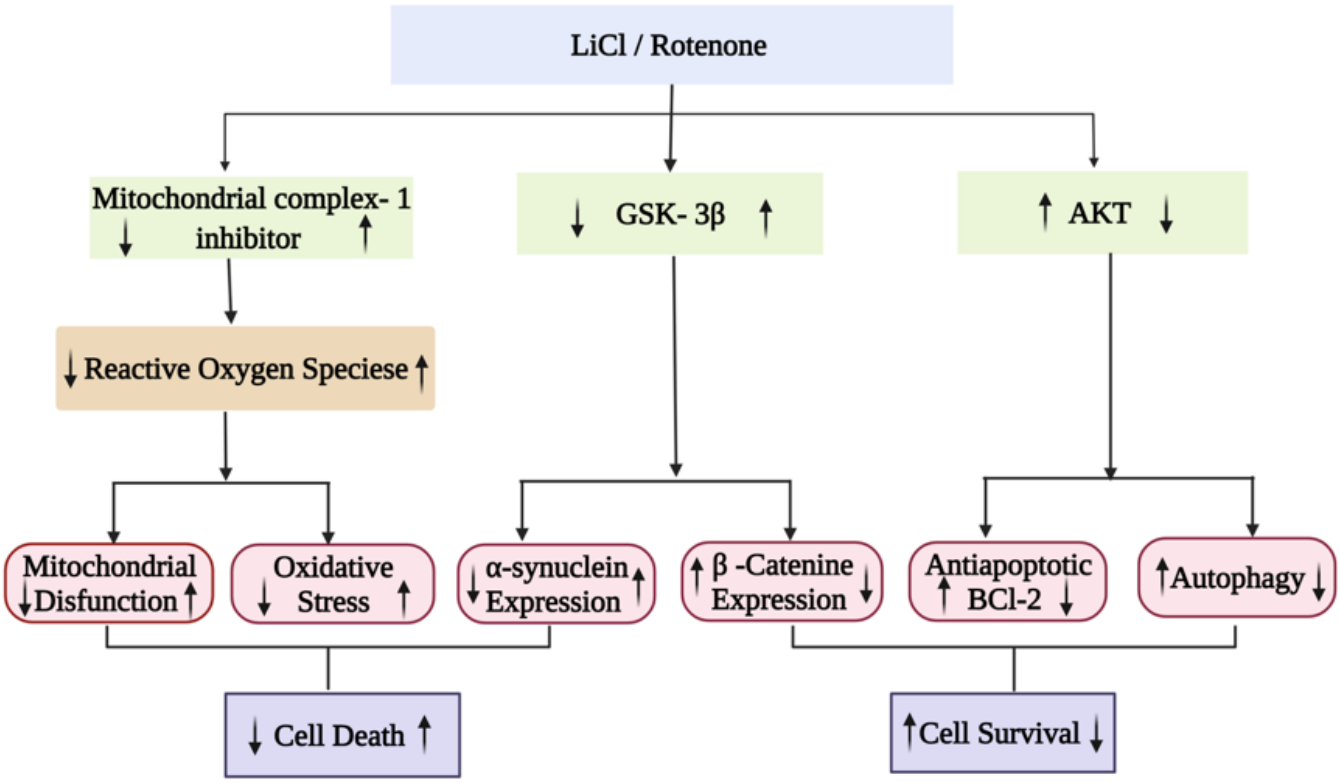

Figure 6

Flowchart of results: [Fig6]. Schematic diagram showing the role of Rotenone and LiCl. Rotenone acts as a mitochondrial complex 1 inhibitor and increases the activity of GSK-3b and dephosphorylate AKT, which leads to an increase in reactive oxygen species and causes mitochondrial dysfunction, which causes oxidative stress to the cell and increases in synuclein expression, which causes Lewy body formation and ultimately cell death. Were as LiCl inhibits GSK-3 and increases the phosphorylation of AKT, which increases the Antiapoptotic protein BCL-2 activity and inhibits apoptosis, and promotes Autophagy which can eliminate dysfunctional mitochondria and aggregated protein. On the other hand, Inhibition of GSK-3b increases b-catenin activity which enables cell survival by inhibiting excessive m-RNA formation of Synuclein; GSK also inhibits the accumulation of a-synuclein protein to form Lewy body and promote cell survival.