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Anthraquinone from Edible Fungi *Pleurotus ostreatus* Protects Human SH-SY5Y Neuroblastoma Cells Against 6-Hydroxydopamine-Induced Cell Death—Preclinical Validation of Gene Knockout Possibilities of PARK7, PINK1, and SNCA1 Using CRISPR SpCas9

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Abstract
Parkinson's disease (PD) results from the degeneration of the nervous tissue brought about by ecological and hereditary components which affects nerve cells in the brain. It is the world's second most normal neurodegenerative issue, which can essentially weaken the personal satisfaction, make reliance, and trigger untimely mortality of affected people. The commonness pace of PD is 0.5–1% among individuals in the age group of 65–69 years and 1–3% among those 80 or more. Clinical appearances incorporate bradykinesia, tremors, unbending nature, and postural unsteadiness; spectrums of non-motor symptoms include psychological hindrance and passionate and behavioral brokenness. In this study, 6-OHDA-induced neurotoxicity was analyzed for various cytotoxicity analyses. The genes identified were PINK1 (PTEN-induced kinase 1), PARK7 (Parkinsonism-associated deglycase) and SNCA 1 (alpha synuclein1) validated using CRISPR spcas9 genome editing tool. In this study, Anthraquinone isolated from *Pleurotus ostreatus* was treated against a dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), which induced neurotoxicity in SH-SY5Y cells. Experimental groups in SH-SY5Y neuroblastoma cells were treated with anthraquinone (50 nM) and 6-OHDA (100 nM). MTT and ROS assays were performed to assess the cell viability and oxidative stress within the cells, followed by mixed-member proportional (Mitochondrial membrane potential), dual staining, and immunoblotting. 6-OHDA-induced cell death in SH-SY5Y cells was dose-dependently attenuated by treatment with anthraquinone. The genes responsible for mutation were studied and the mutated RNAs knockout possibilities was studied using CRISPR spcas9 genome editing tool. Treatment with anthraquinone attenuated the level of oxidative stress and reduced the mitochondrial dysfunction associated with 6-OHDA treatment. Immunoblot analysis carried out with apoptotic markers showed that cytochrome C and caspase-3 expression increased significantly in anthraquinone-treated cells, whereas 6-OHDA-treated group showed a significant decrease when compared with an experimental control group. The mutated genes PARK7, PINK1, and SNCA1 were analyzed and found to exhibit four gene knock possibilities to treat PD. Reports demonstrate that other than following up on the biosynthesis of dopamine and its metabolites,

these mixes counteract D2 receptors' extreme touchiness. It is proposed that further examinations need be directed to better understand the activity of the bioactive mixes circulated in these edible fungi *Pleurotus ostreatus*. The gene knockout possibilities identified by CRISPR SpCas9 will pave a way for better research for PD treatment.

Keywords Anthraquinone · SH-SY5Y · neuroblastoma · Dopamine · 6-OHDA · CRISPR

Background

Parkinson's diseases are a long-term gradually intensifying disorder, which is concurred with neurological disorder [1]. Compared with the auxiliary neurodegenerative disorder, Parkinson's disease was predicted to be about 9 million populations worldwide by 2030 [2]. Initial occurrence of Parkinson's disease (PD) was identified with mental impairment related to substantial neuron dopamine impairment of midbrain [3]. Pesticide exposure is also being considered as one the provoking agent for PD and this confirmed by in vitro and in vivo epidemiological studies [4]. 6-hydroxydopamine (6-OHDA), dopamine, hydrogen peroxide, and paraquat are a well-known neural lethal pesticide with high lipophilic nature, thus capable of crossing blood brain barrier, without any cellular transporter [5]. *Pleurotus ostreatus*, an oyster mushroom, is disclosed to have numerous health benefits for diabetes, cancer, hypertension, and arthritis [6]. GC-MS analysis of *Pleurotus ostreatus* revealed several phytoconstituents which were shown to have medicinal properties. Anthraquinone derivatives were shown to target inflammatory pathway, thereby preventing neurodegenerative disease [7, 8]. Anti-neuro degenerative properties of various other medicinal plants like *Citrus aurantium* treats oxidative stress, and ethyl acetate extract of *Eclipta prostrata* was shown to possess excellent antioxidant capacity and attenuate SH-SY5Y cell death by blocking apoptotic signalling cascade caused by 6-hydroxydopamine [9]. By various research conducted on plants, it is proven that majority of them naturally possess medicinal properties; few among them are *Coriander sativum* and its bioactive phytochemicals announced for a wide scope of organic activities including anticancer, neuroprotective, anxiolytic, sleep inducing, anticonvulsant, pain relieving, calming, and antidiabetic properties [10]. For the past one decade, extensive research has indicated that nutraceuticals were derived from spices prevents neurodegeneration [11]. Hence, evident from the present study, anthraquinone from *Pleurotus ostreatus* could be also used as a potent agent against neurological disorders.

In the present PD hereditary qualities classification, 18 explicit chromosomal regions, additionally called chromosomal locus, are named PARK (to mean their putative connect to PD) and numbered in sequential request of their distinguishing proof (PARK1, PARK2, and PARK3). Notwithstanding being a fragmented rundown of known PD-related qualities, this arrangement framework, lamentably, has various irregularities. It includes affirmed loci, just as those for which linkage or affiliation could not be imitated (non-confirmed). The causative quality has not yet been recognized for the majority of the loci nor do the majority of the distinguished qualities contain causative or sickness deciding changes (i.e., varieties in a portion of these qualities are viewed as hereditary hazard variables expanding the hazard to create PD as opposed to being an adequate reason). At long last, one locus, PARK4, was assigned as a novel chromosomal locale related with PD [12, 13] yet was later observed to be indistinguishable with PARK1 (SNCA-related PD). It is vital that a portion of the loci have been distinguished by hereditary linkage examination in huge families, some dependent on the

known capacity of the protein result of the quality they contain, yet others have been set up by genome-wide affiliation studies performed on a populace level.

Methods

Chemicals

6-hydroxydopamine (6-OHDA), heat-inactivated FCS (fetal calf serum), Dulbecco's modified eagle's medium (DMEM), penicillin, glutamine, trypsin, streptomycin, EDTA, MTT, and rhodamine123 (Rh123) were procured from Gibco and Sigma Aldrich (India). Other antibodies like Bcl-2, caspase-3, and β-actin antibodies were obtained from Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. Diagnostic grade chemicals were used for the further analysis.

Neuronal Cell Culture

Human neuroblastoma cell line (SH-SY5Y) was procured from NCCS, Pune, India. The SH-SY5Y cells (passage numbers 10–14) were used and the doubling time (3–4 days) was constant during this passage numbers. Later, the initial growth of cells was done in Dulbecco's modified eagle's medium (DMEM) with 1% antibiotic-antimycotic mixture and 10% FBS (fetal bovine serum) in 5% CO2-humified incubator at 37 °C.

Neuronal Cytotoxicity Assessment

The preliminary cell toxicity assay was done separately using various escalating concentrations of 6-hydroxydopamine (6-OHDA) and anthraquinone to optimize the dosage for the experimental group (data not shown). Further, to check the effect of anthraquinone against 6-OHDA-induced toxicity, $3X10^3$ SH-SY5Y cells were seeded in 96-well culture plate (Corning cell culture) and the neuroprotective effect of anthraquinone was evaluated by pretreatment of SH-SY5Y cells with different concentrations of anthraquinone (5 nM, 10 nM, 20 nM, and 50 nM) for 2.5 h and finally nurtured with 6-hydroxydopamine (6-OHDA) (50 nM) for 24 h. These doses were selected based on the preliminary cell viability assay conducted. After the incubation of about 24 h, the cells were treated with about 10 μL of MTT solution for 4 h at 37 °C. The dark blue formazan crystals formed in intact cells were dissolved by adding solubilization solution DMSO (100 μL) into each well and incubated overnight. The absorbance was measured at 570 nM in a microplate ELISA reader. All experiments were performed in triplicates. The results obtained from this toxicity assay were used to fix the effective single dose of anthraquinone against 6-hydroxydopamine (6-OHDA)-induced toxicity in further experiments.

Reactive Oxygen Species Assessment

The cells (1×10^5cells/well in 6-well plates) were undergone anthraquinone (50 nM) pretreatment for 2 h followed by incubation with 6-hydroxydopamine (6-OHDA) (100 nM) for 24 h. After that, the cells were incubated with 100 μL of DCFH-DA (2,7 diacetyl dichloro fluorescein). DCFH-DA is proficient in penetrating into the intracellular matrix of cells where this oxidation forms a fluorescent dichlorofluorescein for 30 min at 37 °C and PBS is used for

washing and finally washed in to fluroslide and viewed using a fluorescent microscope (Perkin Elmer, USA).

Mitochondrial Membrane Potential Assessment

6-OHDA treatment induces propagation and extricates mitochondrial membrane loss. Although the proliferative cells are more defiant to 6-OHDA (neurotoxin), the initial mitochondrial depolarization occurs in proliferating rather than neuron-like differentiated cells. Mitogreen (green-fluorescent mitochondrial dye) is a fluorescent cationic dye, used in the determination of membrane potential of mitochondria. After the reactive oxygen species assessment, the media containing 1×10^5 cells/well in 5 plates were changed to fresh culture media DMEM containing 1 μL of mitogreen (5 m mol/L) and followed by 30-min incubation at room temperature. Then, the fresh medium was used to replace the loading solution and cells were imaged using fluorescence microscope. Green color indicates the depolarized mitochondrion which the orange color, and red fluorescence indicates the polarized mitochondrion.

AO/EB Dual Staining

A dual staining technique using acridine orange (AO) and ethidium bromide (EB) was used to verify the apoptosis by fluorescence microscopy. After Mitochondrial membrane potential assessment, PBS was used to wash the cells and seeding of cells (1×10^5) was carried out in 5 plates; used medium was changed to fresh culture medium containing 100 μg/mL of AO and EB stains. Excess dye was removed by washing with warm 1X PBS followed by incubation for 30 min at room temperature. Fluorescence intensity was measured at 535 nM and the cellular morphology of apoptosis was photographed using fluorescence microscope.

Western/Immunoblot Analysis

Immunoblotting analysis was performed on 30 μg of protein extracts from the SH-SY5Y cell line. Protein extracts were separated by 12% SDS-PAGE and electro-transferred to PVDF membranes (Bio-Rad, USA). The membranes were blocked with 5% non-fat dry milk in TBS containing 0.1% Tween-20 for 1 h. The following primary antibodies were used: anti-caspase-3 (1:1000; Santa Cruz Biotechnology), anti-cytochrome C (1:1000; Santa Cruz Biotechnology), anti-Bcl-2-associated X protein (Bax) (1:500; Santa Cruz Biotechnology), anti-B cell lymphoma (Bcl-2) (1:500; Santa Cruz Biotechnology), and anti-β-actin antibody (1:1000; Santa Cruz Biotechnology). Then membranes were then washed thoroughly in TBS-0.1% Tween-20 and incubated for 1 h with the horseradish-conjugated secondary antibodies from Santa Cruz Biotechnology, Inc. at a dilution of 1:5000. Specific bands for each protein were detected using Super Signal chemiluminescence kit (Thermo Fisher Scientific, Rockford, IL). β-Actin was served as loading control. The intensities of the protein bands were quantified using ImageJ software (NIH, Bethesda, USA).

Statistical Analysis

All observed readings were represented as the standard deviation (SD) of mean ± SD. Mean deviation analysis was carried out using sigma plot. Other than one-way ANOVA, two-way

ANOVA was utilized for estimation of the difference. CRISPR spcas9 genome editing device was utilized to approve the possible outcomes of gene knockout in PARK7, PINK1, and SNCA 1. The product additionally utilized in looking at human interpretation framework in PD. At first, the instrument dissected the essential grouping in choosing the early coding locale, trailed by distinguishing proof of potential targets. These objectives were contrasted with the 36,845,672 destinations for ascertaining the azimuth score obtained by each gene. Hence, the applicable RNAs were chosen for quality gene knockout.

Results

Effect of Anthraquinone and 6-Hydroxydopamine (6-OHDA) on SH-SY5Y Cells

Cells treated with the escalating concentrations of anthraquinone and 6-OHDA were reviewed for toxicity. Anthraquinone against 6-OHDA-induced toxicity showed expanded cell viability to 90 ± 2.38% of control in the presence of 50-nM dose. Based on this data, the dose for treatment, 50 nM anthraquinone and 100 nM 6-OHDA were chosen for the further experiments (Fig. 1a).

Effect of Anthraquinone on ROS in 6-OHDA-Induced Toxicity

6-OHDA-induced oxidative stress was reversed by the treatment of cells with anthraquinone. This exceptional variation in SH-SY5Ywas measured using spectrofluorometer. DCFDA, a fluorescent dye, was used for the analysis of reactive oxygen species in SH-SY5Y cells. Thus, the ROS produced by 6-OHDA was decreased by the administration of 50 nM anthraquinone as shown in Fig. 1b.

Effect of Anthraquinone on Reduction of Mitochondrial membrane potential Against 6-OHDA-Induced Toxicity

This was determined by a decreased fluorescence intensity, as correlated to the control-untreated cells. Pretreatment of cells with 50 nM anthraquinone showed increased in Mitochondrial membrane potential that is similar to control, indicating that treatment with 6-OHDA decreased the Mitochondrial membrane potential production which was reversed by administration of anthraquinone (Fig. 1c).

Effect of Anthraquinone on Apoptosis in 6-OHDA-Induced Toxicity

Acridine orange and ethidium bromide were used to determine the apoptosis of SH-SY5Y cells. 6-OHDA-induced cells exposed orange-colored luminescent apoptotic bodies, when correlated to control and anthraquinone-treated cells. Dual staining was then examined under a fluorescent microscope. No significant apoptosis was noticed in the negative control group. Staining was localized asymmetrically within the cells. Anthraquinone treatment increased the number of early-stage apoptotic cells. Late-stage apoptotic cells with orange nuclear EB staining were also detected. Necrotic cells also increased in volume and showed as uneven orange-red fluorescence as shown in Fig. 1d. Anthraquinone treatment significantly protected the 6-OHDA-induced nuclear damage. Figure 1e indicates the comparison of four different groups using DAPI staining from the nuclear

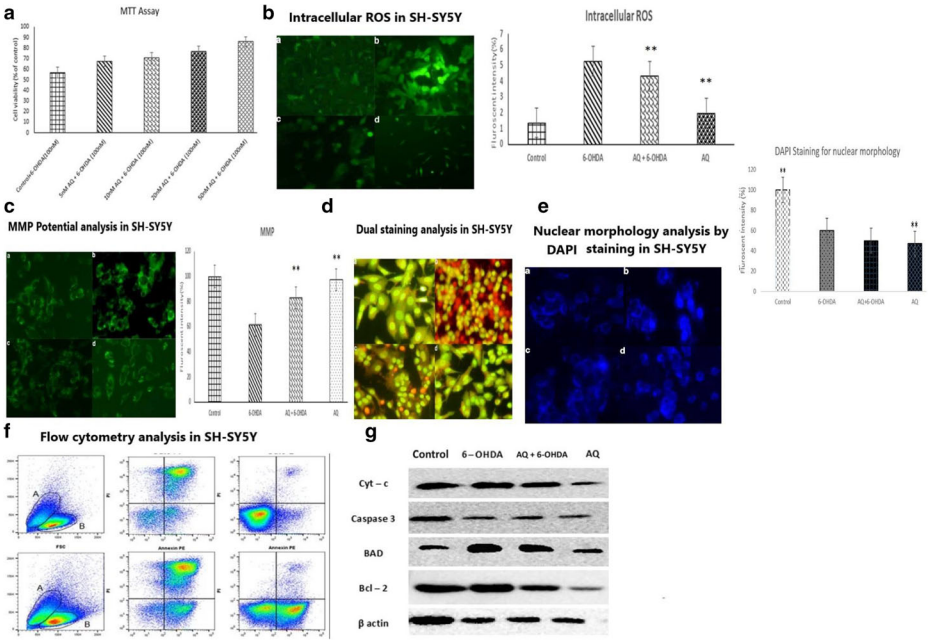

Fig. 1 **a** Neuronal cytotoxicity assessment using MTT assay with different concentrations of anthraquinone (5 nM, 10 nM, 20 nM, and 50nM) against 6-OHDA-induced toxicity was measured at 570 nm. Values are expressed as mean ± SD of triplicate experiments. **b** AQ reduces cellular ROS formation. (a) Positive control: intracellular image of SH-SY5Y cells without any treatment; (b) necrotic control: SH-SY5Y cells treated with 6-OHDA, increases ROS; (c) experimental control: SH-SY5Y cells treated with AQ, decreases ROS leading to cell viability; (d) extract alone: SH-SY5Y cells treated with AQ alone also attenuated the level of oxidative stress. **c** AQ reduces Mitochondrial membrane potential activity. (a) Positive control: intracellular image of SH-SY5Y cells without any treatment; (b) necrotic control: SH-SY5Y cells treated with 6-OHDA induces mitochondrial dysfunction; (c) experimental control: SH-SY5Y cells treated with AQ, reduces the mitochondrial dysfunction associated with 6-OHDA treatment; (d) extract alone: SH-SY5Y cells treated with AQ alone increases more viability in cells without any Mitochondrial membrane potential activity. **d** Anti-apoptotic effect of AQ against 6-OHDA-induced toxicity. (a) Positive control: intracellular image of SH-SY5Y cells without any treatment; (b) necrotic control: SH-SY5Y cells treated with 6-OHDA; (c) experimental control: SH-SY5Y cells treated with AQ showed early apoptotic cells, nucleus shows yellow-green fluorescence by acridine orange (AO) staining and late apoptotic cells; the nucleus of cell shows orange fluorescence by EB staining; (d) extract alone: SH-SY5Y cells treated with AQ also show similar results like experimental group. **e** Nuclear apoptosis is indicated by condensation and fragmentation in (b) which is reversed in (c and d) groups by AQ administration to the SH-SY5Y cells. **f** Flow cytometry analysis in SH-SY5Y neuroblastoma cells conducted for various staining. **g** Western blot analysis of the expression of apoptotic proteins. BAD, caspase 3, and cytochrome C increased and

morphology of the cells. Complete analysis of the staining was carried out using flow cytometry which is represented in Fig. 1f.

Effect of Anthraquinone on Apoptotic Gene Expression

Protective effect of anthraquinone on 6-OHDA-induced apoptosis was analyzed by expression of the protein, by using pro- and antiapoptotic markers. Anthraquinone-treated cells showed the release of cytochrome C marker into cytosol by the expression of BAD, caspase 3 (Fig. 1g). Thus, caspase-3 may also represent a commonplace integration point and, hence, may also constitute an attractive target for antiapoptotic remedy in PD. In this context, it is interesting to note that caspase-3 activation

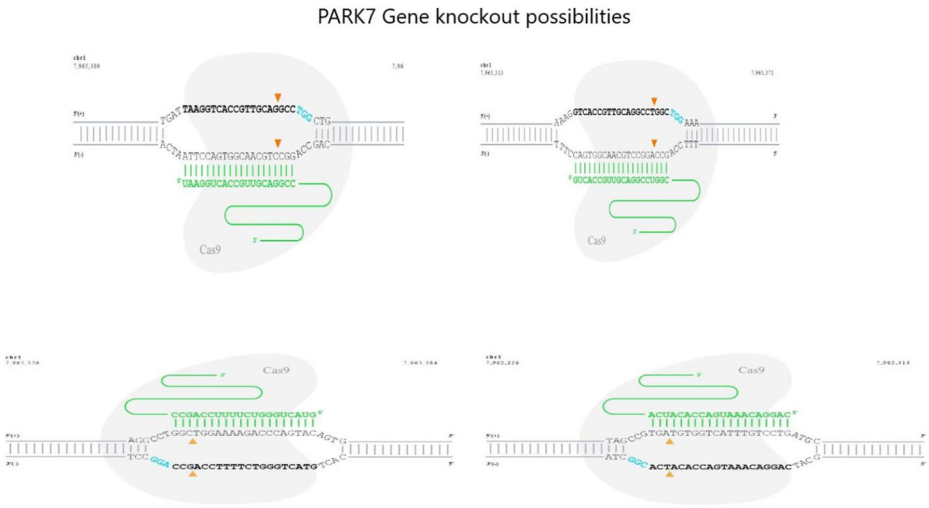

Fig. 2 Gene knockout possibilities predicted by the CRISPR Spcas9 genome editing tool for PARK7

precedes chromatin condensation and the final breakdown of the mobile, a stage at which neuroprotective strategies are probable to fail.

Discussion

Reactive oxygen assessment of cells also indicates that other 20% of nigral neurons are also affected by stress [14]. MTT assay done in SHSY-5Y cell line clearly stated 6-OHDA (100 nM) treatment induced about 50% cell death, when incubated at a short time interval of 24 h, in which some cells are viable and some cells are not viable indicating dead cells [15]. The maximum cell viability obtained by MTT assay by anthraquinone against 6-OHDA-induced neurotoxicity was about (86%) in a dose-dependent manner. Apoptosis was studied by dual staining, DAPI staining, and nuclear

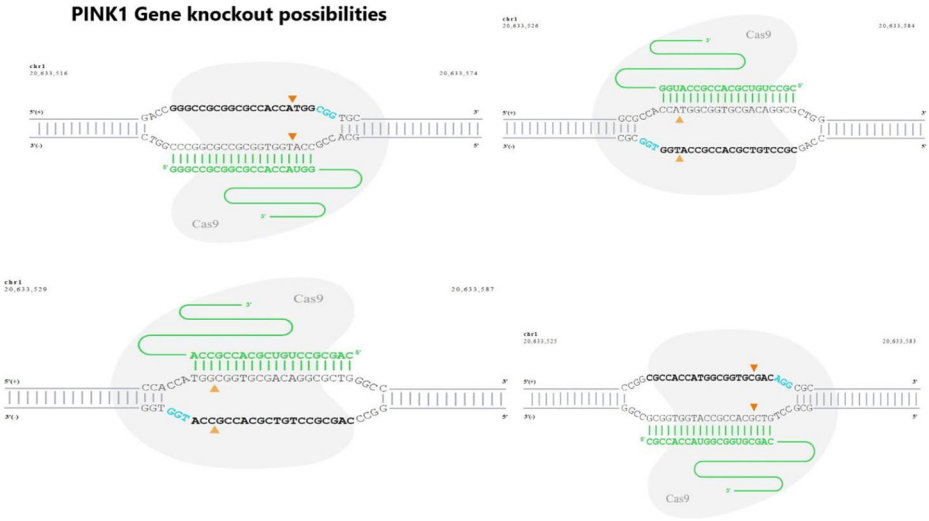

Fig. 3 Gene knockout possibilities predicted by the CRISPR Spcas9 genome editing tool for PINK1

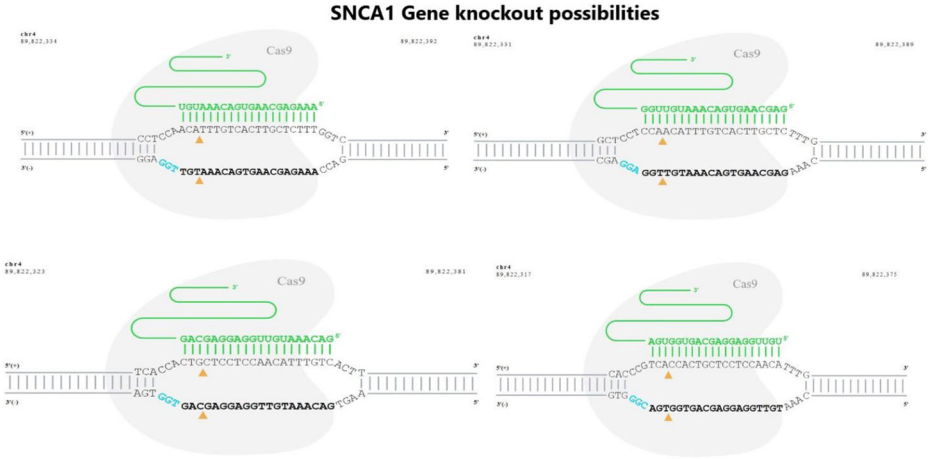

Fig. 4 Gene knockout possibilities predicted by the CRISPR Spcas9 genome editing tool for SNCA1

staining. Dual staining is capable to distinguish the normal and damaged cells where normal cells uptake acridine orange and appear green while the ethidium bromide enters only through the damaged cells thus appearing red. The differences in the apoptosis formation have been seen in the drug-treated cells while less or nil apoptosis have been seen in the control. Anthraquinone-pretreated cells emit more green fluorescence than 6-OHDA in dual staining. MTT assay and dual staining confirmed that anthraquinone inhibited the apoptosis which was induced by 6-OHDA. Similar results were seen in curcumin-treated PCl2 and human umbilical vein endothelial cells (HUVEC) from amyloid β (1-42) which correlates the results obtained by anthraquinone treatment in 6-OHDA-induced neurotoxicity [16–19].

Increased production of ROS is seen in in vitro and in vivo models of PD, where 6-OHDA is induced [20]. Besides there is also an overproduction of ROS by 6-OHDA, which imbalances the mitochondrial membrane potential [21] and mitochondrial transition pore permeability and disparity in cellular antioxidant systems [22], leading to cell death. Mitochondrial electron transport mechanism in catalyzing the NADH is ubiquinone oxidoreductase in the neuron inhibited by 6-OHDA by

Fig. 5 The 2.5 kilobase sets (kb) upstream of the interpretation begin site and the 5′ untranslated exons that upgrade articulation of the PARK7, PINK1, and SNCA1 quality in SH-SY5Y cells

the increased production of ROS [23]. Thus, the toxicity of cells is induced by 6-OHDA. It was also found that curcumin also exhibits the similar property when compared with vitamin E. Dopaminergic neurons are degenerated by oxidative stress due to 6-OHDA in SHSY5Y cells [24]. Due to increased oxidative stress, the Mitochondrial membrane potential is decreased steadily to the apoptosis of cells. Similar changes were analyzed in Mitochondrial membrane potential by any Rh123 showing 6-OHDA-treated decreased Mitochondrial membrane potential production, where anthraquinone-treated cells reversed Mitochondrial membrane potential effect. Oxidative stress resulted apoptosis to increase the production of cytochrome c from mitochondria to the cytoplasm [25]. Various antioxidants had been proven to inhibit the production of oxidative compounds associated with many sicknesses [26–28]. Oxidative stress plays pivotal position within the pathophysiology of metals bringing about toxicity [29, 30].

Antiapoptotic proteins like Bcl-2 and proapoptotic protein BAD are the main factors related to the permeability of the mitochondrial membrane. These factors release cytochrome C, whereas the antiapoptotic factors like BCl-2 inhibit the factor responsible for releasing cytochrome C into cytosol. BAD is responsible for promoting apoptosis by the release of heterodimers [31]. Anthraquinone exhibited downregulation of BAD to an antiapoptotic effect. In this study, expression of caspase 3 significantly increased in 6-OHDA-treated cells, which conveys that apoptosis is activated in both intrinsic and extrinsic pathways.

It has been recognized that 2.5-kilobase (kb) sets upstream begins the interpretation, and the 5′ untranslated exons may upgrade the articulation of the SCN1A quality in SH-SY5Y cells, a human cell line got from a neuroblastoma. So also, PINK1 is included with mitochondrial quality control by distinguishing harmed mitochondria and focusing on explicit mitochondria for debasement. Solid mitochondria keeps up a film potential that can be utilized to import PINK1 into the internal layer where it is divided by PARL and cleared from the external film. Seriously harmed mitochondria needs adequate layer potential to import PINK1, which at that point amasses on the external film. PINK1 then enrolls parkin to focus on the harmed mitochondria for corruption through autophagy [32]. Due to the nearness of PINK1 all through the cytoplasm, it has been proposed that PINK1 capacities as a "scout" to test for harmed mitochondria. Protein deglycase DJ-1, otherwise called Parkinson sickness protein 7, is a protein which in people is encoded by the PARK7 quality.

The gene knockout of PARK7, PINK1, and SNCA 1 using genome designing CRISPR programming was utilized. The outcomes have been obtained for the capability controller PARK7, PINK1, and SNCA 1. Since the translation elements complete metabolism is controlled by these genes in the PD, CRISPR spcas9 editing tool was utilized to interrupt down the practicable outcomes to knockout in vitro. It was an in silico way to address, distinguish, and approve the counter PD genes shown by way of the interpretation analysis on PARK7, PINK1, and SNCA 1 (Figs. 2 and 3). The four counselled RNAs, for high-quality knockout for the restraint of PD proven by way of PARK7, PINK1, and SNCA 1 by using people, is given in Figs. 4 and 5. Therefore, it is apparent that the SH-SY5Y metabolism in cellular level is just like that of humans in approving the clinical cellular examinations [33]. Recently, this machine has accrued extending idea as an appropriate and fundamental genome-making plans unit and has transformed the present scientific era in gene editing. This technique is validated for its transformative packages in transcriptional bothering, epigenetic guideline, base adjusting, excessive-throughput inherited screening, and time of animal or cellular models for sickness evaluation. For the remedy of human diseases, genomic level editing is enhancing to cope with validation analysis of genes and it is becoming a promising technique. As an essential and programmable nuclease-primarily based genomic adjusting instrument, the continually interspaced quick palindromic (CRISPR)/CRISPR-related protein nine (Cas9) framework gadget has liberally progressed the potential to take off precise enhancements within the human genome.

Quick improvement of CRISPR-based advances has broadened its utility scope and progressed CRISPR-primarily-based medicinal drugs in preclinical validation. The usage of the CRISPR structure over the span of the cutting-edge was 2 years, together with its development and application in base editing, information trade and epigenetic adjusting, genomic-scale screening, and cellular and early life shape remedy [34, 35]. Finally, the potentialities and movements related to the use of CRISPR/Cas9 progresses remains rising.

Conclusion

In summary, this study clearly states that anthraquinone isolated from *Pleurotus ostreatus* could be used as an effective agent against neurodegenerative disease associated with PD during aging. In conclusion, our findings successfully established that anthraquinone isolated from *Pleurotus ostreatus* could be used as an effective agent against neurodegenerative disease associated with PD. Further in silico assessments on CRISPR spCas9 also gave an evident in altering the transcriptional structure of PINK1, PARK7, and SNCA1without losing its property.

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Compliance with ethical standards

Consent for publication All authors solely agree for publishing the research

Abbreviations *AQ*, Anthraquinone; *PD*, Parkinson's disease; *CRISPR*, Consistently interspaced short palindromic rehashes; *6-OHDA*, 6-hydroxydopamine; *PARK7*, Parkinsonism-associated deglycase; *PINK1*, PTEN-induced kinase 1; *SNCA 1*, Sodium voltage-gated channel alpha subunit 1

References

1. Hirsch, E., Graybiel, A. M., & Agid, Y. (1988). Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature., 334*, 345–348.
2. Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., & Kieburtz, K. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology., 68*(5), 384–386.
3. Greenamyre, G. T., & Hastings, T. G. (2004). Parkinson's-divergent causes, convergent mechanisms. *Science, 304*, 1120–1122.
4. Chaudhuri, K. R., & Schapira, A. H. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol, 8*, 464–474.
5. Aggarwal, B. B., Sundaram, C., Malani, N., & Ichikawa, H. (2007). Curcumin: the Indian solid gold. *Adv. Exp. Med. Biol., 595*, 1–75.
6. Dexter, D. T., & Jenner, P. (2013). Parkinson disease: from pathology to molecular disease mechanisms. *Free Radical Biology & Medicine, 62*, 32–44.
7. Hancock, D. B., Martin, E. R., Mayhew, G. M., Stajich, J. M., Jewet, R., Stacy, M. A., Scott, M. L., Vance, J. M., & Scott, W. K. (2008). Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. *BMC Neurology, 8*, 6–11.
8. Priyadarshi, A., Khuder, S. A., Schaub, E. N., & Shrivastava, A. (2000). A meta-analysis of Parkinson's disease and exposure to pesticides. *Neurotoxicology., 21*(4), 435–440.

9. Dhanalakshmi, C., Manivasagam, T., Nataraj, J., Thenmozhi, A. J., & Essa, M. M. (2015). Neurosupportive role of vanillin, a natural phenolic compound, on rotenone induced neurotoxicity in SH-SY5Y neuroblastoma cells. *Evidence-based Complementary and Alternative Medicine, 5*, 626–028.
10. Nammi, B., Lodagala, K. M., & Behara, R. B. (2003). The juice of fresh leaves of *Catharanthus roseus* Linn. Reduces blood glucose in normal and alloxan diabetic rabbits. *BMC Complementary and Alternative Medicine, 3*, 8–9.
11. Bindhu, J., & Das, A. (2017). Evaluation edible oyster mushroom (Pleurotus ostreatus) on oxidative stress and neurological cognitive disorder in streptozotocin diabetic rat. *Clin Inv (Lond), 7*, 177–186.
12. Singleton, A., & Hardy, J. (2011). A generalizable hypothesis for the genetic architecture of disease: pleomorphic risk loci. *Human Molecular Genetics, 20*(R2), R158–R162.
13. Farrer, M. J., Hulihan, M. M., Kachergus, J. M., et al. (2009). DCTN1 mutations in Perry syndrome. *Nature Genetics, 41*(2), 163–165.
14. Tamilselvam, K., Braidy, N., Manivasagam, T., Essa, M. M., Prasad, N. R., Karthikeyan, S. R., Thenmozhi, A. J., Selvaraju, S., & Guillemin, G. J. (2013). Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. *Oxidative Medicine and Cellular Longevity, 3*, 24–29.
15. Cannon, J. R., Tapias, V. M., Na, H. M., Honick, A. S., Drolet, R. E., & Greenamyre, J. T. (2009). A highly reproducible rotenone model of Parkinson's disease. *Neurobiology of Disease, 34*(2), 279–290.
16. Dauer, W., & Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron., 39*(6), 889–909.
17. Ganguli, M., Chandra, V., Kamboh, M. I., Johnston, J. M., Dodge, H. H., Thelma, B. K., Juyal, R. C., Pandav, R., Belle, S. H., & DeKosky, S. T. (2000). Apolipoprotein E polymorphism and Alzheimer disease: the indo-US cross national dementia study. *Archives of Neurology, 57*, 824–830.
18. Fetoni, A. R., Paciello, F., Mezzogori, D., Rolesi, R., Eramo, S. L., Paludetti, G., & Troiani, D. (2015). Molecular targets for anticancer redox chemotherapy and cisplatin-induced ototoxicity: the role of curcumin on pSTAT3 and Nrf-2 signalling. *British Journal of Cancer, 113*, 1434–1444.
19. Sirisidthi, K., Kosai, P., Jiraungkoorskul, K., & Jiraungkoorskul, W. (2016). Antithrombotic activity of turmeric (Curcuma longa): a review. *Indian J Agric Res.*, 101–106.
20. Abe, Y., Hashimoto, S., & Horie, T. (1999). Curcumin inhibition inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol Res., 9*, 41–47.
21. Mao, J. J., Powell, J., Water, C. L., Keen, H. H., Schmitz, M. E., & Gershwin, E. J. (1999). The influence of cocoa procyanidins on the transcription of interlukin-2 in peripheral blood mononuclear cells. *International Journal of Immunotherapy, 15*, 23–29.
22. Yong-Kee, C. J., Sidorova, E., Hanif, A., Perera, G., & Nash, J. E. (2012). Mitochondrial dysfunction precedes other sub-cellular abnormalities in an in vitro model linked with cell death in Parkinson's disease. *Neurotoxicity Research, 21*(2), 185–194.
23. Marella, M., Seo, B. B., Matsuno-Yagi, A., & Yagi, T. (2007). Mechanism of cell death caused by complex I defects in a rat dopaminergic cell line. *The Journal of Biological Chemistry, 282*(33), 24146–24156.
24. Ding, H. Q., Gao, J., Zhu, H. R., Xiong, Y., & Liu, J. (2008). Mitochondrial dysfunction enhances susceptibility to oxidative stress by down-regulation of thioredoxin in human neuroblastoma cells. *Neurochemical Research, 33*(1), 43–50.
25. Moon, K. H., Lee, J. H., Park, D., Geum, K., & Kim, K. (2005). Mitochondrial membrane depolarization and the selective death of dopaminergic neurons by rotenone: protective effect of coenzyme Q (10). *Journal of Neurochemistry, 93*, 1199–1208.
26. Muthuraman, P., Ramkumar, K., & Kim, D. H. (2014). Analysis of dose-dependent effect of zinc oxide nanoparticles on the oxidative stress and antioxidant enzyme activity in adipocytes. *Applied Biochemistry and Biotechnology, 174*(8), 2851–2874.
27. Ramakrishnan, R., Elangovan, P., & Pari, L. (2017). Protective role of tetrahydrocurcumin: an active polyphenolic curcuminoid on cadmium-induced oxidative damage in rats. *Applied Biochemistry and Biotechnology, 183*(1), 51–69.
28. da Rocha, P. D. S., Campos, J. F., Nunes-Souza, V., do Carmo Vieira, M., de Araujo Boleti, A. P., Rabelo, L. A., dos Santos, E. L., & de Picoli Souza, K. (2017). Antioxidant and protective effects of Schinus terebinthifolius raddi against doxorubicin-induced toxicity. *Applied Biochemistry and Biotechnology*, 1–16.
29. Isenberg, J. S., & Klaunig, J. E. (2000). Role of the mitochondrial membrane permeability transition (MPT) in rotenone-induced apoptosis in liver cells. *Toxicological Sciences, 53*(2), 340–351.
30. Doran, E., & Halestrap, A. P. (2000). Cytochrome C release from isolated rat liver mitochondria can occur independently of outer membrane rupture: possible role of contact sites. *The Biochemical Journal, 348*, 343–350.

31. Kim, H. Y., Chung, J. M., & Chung, K. (2008). Increased production of mitochondrial superoxide in the spinal cord induces pain behaviors in mice: the effect of mitochondrial electron transport complex inhibitors. *Neuroscience Letters, 447*, 87–91.
32. Clark, J., Clore, E. L., Zheng, K., Adame, A., Masliah, E., & Simon, D. K. (2010). Oral N-acetylcysteine attenuates loss of dopaminergic terminals in alpha-synuclein overexpressing mice. *PLoS One, 51*, 23–33.
33. Dairam, A., Limson, J. L., Watkin, G. M., Antunes, E., & Daya, S. (2007). Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male Wistar rats. *Journal of Agricultural and Food Chemistry, 55*, 1039–1044.
34. Kim, H., Ham, S., Jo, M., Lee, G. H., Lee, Y.-S., Shin, J.-H., & Lee, Y. (2017). CRISPR-Cas9 mediated telomere removal leads to mitochondrial stress and protein aggregation. *International Journal of Molecular Sciences, 18*, 2093.
35. Reczek, C. R., Birsoy, K., Kong, H., Martinez-Reyes, I., Wang, T., Gao, P., Sabatini, D. M., & Chandel, N. S. (2017). A CRISPR screen identifies a pathway required for paraquat-induced cell death. *Nature Chemical Biology, 13*(12), 1274–1279.

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