Neurotoxicity Research (2019) 36:764–776
https://doi.org/10.1007/s12640-019-00048-4

ORIGINAL ARTICLE

Attenuation of Endoplasmic Reticulum Stress, Impaired Calcium Homeostasis, and Altered Bioenergetic Functions in MPP^+-Exposed SH-SY5Y Cells Pretreated with Rutin

Adaze Bijou Enogieru[1] · William Lloyd Haylett[2,3] · Hayley Christy Miller[4] · Francois Hendrikus van der Westhuizen[4] · Donavon Charles Hiss[1] · Okobi Eko Ekpo[1]

Received: 29 January 2019 / Revised: 3 April 2019 / Accepted: 11 April 2019 / Published online: 4 May 2019

Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder that affects approximately 1% of the population over the age of 65 years. While treatment options for PD are limited, reports show that plant-derived bioactive compounds such as rutin possess numerous pharmacological benefits, including antioxidant and antiapoptotic activities. This study aimed to investigate the potential role of rutin in MPP^+-treated SH-SY5Y neuroblastoma cells, an established cell model of PD. Our findings reveal increased concentrations of Ca^{2+} and endoplasmic reticulum (ER) stress as well as impaired mitochondrial membrane potential and bioenergetic status in SH-SY5Y cells treated with MPP^+ only. This is demonstrated by a significant reduction in the expression levels of BiP, significantly reduced basal respiration, maximal respiration, and spare respiratory capacity as well as a significant increase in the expression levels of CHOP; however, these effects were significantly attenuated following pretreatment with rutin. Also, rutin significantly improved basal and compensatory glycolysis as a response to an impaired oxidative phosphorylation system triggered by MPP^+, characterized by deficient ATP production. In conclusion, our findings provide the first evidence on the ability of rutin to maintain Ca^{2+} homeostasis, inhibit ER stress, and protect the mitochondria in MPP^+-treated SH-SY5Y cells.

Keywords Parkinson's disease · Rutin · ER stress · Oxidative phosphorylation · Glycolysis

Abbreviations

6-OHDA	6-Hydroxydopamine
ATP	Adenosine triphosphate
BiP	Binding immunoglobulin protein

Highlights

- Rutin protects against dysregulated Ca^{2+} homeostasis in SH-SY5Y cells treated with MPP^+.
- Rutin maintains mitochondrial membrane potential in SH-SY5Y cells treated with MPP^+.
- The protective effect of rutin against MPP^+-induced toxicity involves the inhibition of ER stress.
- Rutin mitigates impaired oxidative phosphorylation in SH-SY5Y cells treated with MPP^+.
- Rutin improves glycolysis in SH-SY5Y cells treated with MPP^+.

✉ Okobi Eko Ekpo
oekpo@uwc.ac.za

[1] Department of Medical Biosciences, University of the Western Cape, Robert Sobukwe Road, Private Bag X17, Bellville 7535, South Africa

[2] Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

[3] Division of Endocrinology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

[4] Focus Area for Human Metabolomics, Faculty of Natural Sciences, North-West University, Potchefstroom, South Africa

Ca^{2+}	Calcium
CHOP	CCAAT-enhancer-binding protein homologous protein
DMEM	Dulbecco's modified Eagle medium
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
JC-1	5 , 5 ′ , 6 , 6 ′ - T e t r a c h l o r o - 1 , 1 ′ , 3 , 3 ′ - tetraethylbenzimidazolocarbo-cyanine iodide
MMP	Mitochondrial membrane potential
MPP^+	1-Methyl-4-phenylpyridinium
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OXPHOS	Oxidative phosphorylation
PBS	Phosphate-buffered saline
PD	Parkinson's disease
SEM	Standard error of the mean
SNpc	Substantia nigra pars compacta
UPR	Unfolded protein response
UPS	Ubiquitin proteasome system

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain, resulting in depletion of dopamine in the striatum. Clinically, its cardinal motor symptoms include bradykinesia, tremor at rest, postural instability, and rigidity while non-motor symptoms include autonomic and sensory disturbances, cognitive, sleep, and neuropsychiatric disorders (Park and Stacy 2009). There are numerous reports on the epidemiology of PD, but most reports agree that PD prevalence increases with age. For example, it has been reported that approximately (all per 100,000) 41 cases occur in every 40–49-year-olds; 107 in every 50–59-year-olds; 173 in every 55–64-year-olds; 428 in every 60–69-year-olds; 425 in every 65–74-year-olds; 1087 in every 70–79-year-olds; and 1903 in ages older than 80 (Pringsheim et al. 2014).

It is thought that PD is caused by complex interactions between pathophysiological mechanisms that result in dopaminergic neuronal death (Michel et al. 2013). These proposed mechanisms include impairment of intracellular Ca^{2+} homeostasis, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction. For example, it has been demonstrated that cytosolic Ca^{2+} levels have to be sustained within limited concentrations for optimal survival of dopaminergic neurons in the SNpc, whereas disruption of Ca^{2+} homeostasis resulting from ER and mitochondrial dysfunction reduce neuronal survival in PD (Bezprozvanny 2009; Michel et al. 2013). Moreover, a growing body of evidence indicates that the ER plays a vital role in cell signaling, thus mediating many neurodegenerative processes (Cai et al. 2016; Egawa et al. 2011; Ghribi et al. 2003). The ER is a large Ca^{2+}-storing organelle essential for regulating protein translation, membrane folding, and protein secretion. An impairment of the ER or Ca^{2+} homeostasis leads to the accumulation of unfolded/misfolded proteins in the ER lumen, thereby causing ER stress (Paschen and Mengesdorf 2005). In response, cells activate the unfolded protein response (UPR), increasing ER chaperones such as binding immunoglobulin protein (BiP) which activates an ER-associated degradation pathway that is essential to alleviate ER stress and ultimately improve cell survival (Boyce and Yuan 2006; Hiller et al. 1996). However, sustained activation of the UPR due to severe ER dysfunction results in programmed cell death (Harding et al. 2002).

Similarly, it is well established that mitochondrial dysfunction plays a key role in the development of PD (Hang et al. 2015). Impairment of the mitochondrial electron transport chain and defects in the regulation of mitochondrial dynamics are widely reported in PD disease models (Koyano et al. 2013; McCoy and Cookson 2012; Requejo-Aguilar and Bolaños 2016; Schapira et al. 1990). These changes are associated with ATP deficiency; however, to compensate for mitochondrial dysfunction, neurons may upregulate glycolysis as a low-efficient mode of energy production in PD models (Requejo-Aguilar and Bolaños 2016). Thus, any process damaging mitochondria may lead to metabolic switching intended to compensate for their diminished ability to produce ATP.

Suitable models for investigating the pathobiological mechanisms of PD are necessary in the search for novel pharmacological targets and disease-modifying therapies. Although cellular models cannot fully reproduce the complexity of PD, the development of a stable and reliable cellular model that mimics the dopaminergic cell death in the SNpc is necessary to provide valuable insights for validation in animal models and/or PD patients (Blandini and Armentero 2012; Ravid and Ferrer 2012). In this regard, the human neuroblastoma SH-SY5Y cell line has been widely used in the field of neurodegeneration, mostly to generate various toxin-induced PD models (Korecka et al. 2013; Lopes et al. 2010; Xie et al. 2010). These toxins include 1-methyl-4-phenylpyridinium (MPP^+) (Ito et al. 2017), paraquat (Van der Merwe et al. 2017), 6-OHDA (Magalingam et al. 2013), and rotenone (Li et al. 2012).

Several potentially therapeutic compounds targeting the proper regulation of Ca^{2+} homeostasis, ER stress, and mitochondrial functions have been investigated in numerous PD models (Song et al. 2010; Van der Merwe et al. 2017; Wang et al. 2018). One such compound is rutin, an antioxidant and bioflavonoid, widely found in many fruits and vegetables (Yang et al. 2008). Early studies reported that rutin strengthens the capillaries of blood vessels due to its high radical scavenging activity and antioxidant capacity (Couch 1943). Other pharmacological activities include cytoprotective (La Casa et al. 2000; Magalingam et al. 2016), anticarcinogenic (Perk

et al. 2014; Webster et al. 1996), anti-inflammatory (Guardia et al. 2001; Yoo et al. 2014), and antidiabetic effects (Ghorbani 2017).

Based on previous reports of the pharmacological effects of rutin and its potent antioxidant properties, for the first time, we provide evidence on the role of rutin on MPP^+-induced changes in mitochondrial membrane potential and ER stress as well as impaired intracellular Ca^{2+} homeostasis and bioenergetic status in SH-SY5Y cells.

Materials and Methods

Cell Culture and Reagents

SH-SY5Y neuroblastoma cells, initially purchased from the American Type Culture Collection (ATCC, Rockville, MA), was kindly provided by Dr. AM Serafin (Division of Radiobiology, Stellenbosch University, South Africa). SH-SY5Y cells were maintained in Dulbecco's modified Eagle medium (DMEM) (Life Technologies, USA), supplemented with 10% fetal bovine serum (FBS) (Life Technologies, USA), and 1% penicillin-streptomycin (Lonza, USA), at 37 °C and 5% CO_2 in a humidified incubator. In the cell culture experiments, fresh stock solutions of MPP^+ (Sigma Aldrich, St. Louis, MO, USA) were prepared in unsupplemented DMEM and used at a final concentration of 1 mM. Rutin ($C_{27}H_{30}O_{16}$; purity ≥ 94% HPLC) was purchased from Sigma-Aldrich (St. Louis, MO, USA) without further purification, and fresh stock solutions were prepared in dimethyl sulfoxide (DMSO, Life Technologies, USA) and diluted in culture media for final concentration ranges of 25 μM, 50 μM, and 100 μM.

Cell Viability and Toxicity Assays

The Cell Counting Kit-8 (CCK-8; Sigma Aldrich, St. Louis, MO, USA) was utilized in this study based on its ability to permit precise assays by employing extremely water-soluble tetrazolium salt WST-8 to produce a water-soluble formazan dye, thus allowing sensitive colorimetric assays for the determination of viable cells. SH-SY5Y cells were seeded at a density of 5×10^3 cells/well in 96-well plates and were allowed to attach for 24 h. Cells were pretreated with complete culture media supplemented with 25 μM, 50 μM, or 100 μM concentrations of rutin for 4 h and then treated with 1 mM of MPP^+ for a total of 48 h. These concentrations and time period were selected based on our findings from cytotoxicity screening and dose–response curves (results not shown). After treatment, 20 μl of CCK-8 solution was added to each well of the plate and incubated for 4 h. The optical density (OD) was obtained at 450 nm using a Glomax Multi-Detection Microplate Reader (Promega, USA).

The trypan blue dye exclusion assay was utilized in this study based on its ability to quantify the population of dead cells present in a cell suspension. In this assay, dead cells with damaged membranes permit entry of the dye and cell staining (Altman et al. 1993). SH-SY5Y cells were seeded at a density of $1.6 \times 10^4/cm^2$ in 60-mm dishes and were allowed to attach for 24 h. After that, supernatants were discarded, and cells were treated as described in the "Cell Viability and Toxicity Assays" section. Adherent cells were detached by trypsinization, pelleted, and resuspended in fresh media. A total of 10 μl of cell suspension was added to an equivalent volume of 0.4% trypan blue dye (Sigma Aldrich, St. Louis, MO, USA) and loaded into a BioRad TC20™ automated cell counter. Toxicity was expressed as the percentage of total cell count that were dead cells.

Measurement of Intracellular Calcium

Fluo-4 Direct Calcium Assay Kit (Life Technologies, USA) was utilized to detect intracellular Ca^{2+} activity. SH-SY5Y cells were seeded at a density of 5.0×10^3/well in 96-well plates and were allowed to attach for 24 h under standard incubation conditions. After that, supernatants were discarded, and cells were treated as described in "Cell Viability and Toxicity Assays" section. Measurements of intracellular Ca^{2+} were performed according to manufacturer's instructions with fluorescence intensity measured using the Glomax Multi-Detection Microplate Reader at the wavelength parameters of 490 nm excitation and 510–570 nm emission.

Mitochondrial Membrane Potential Analysis

JC-1

SH-SY5Y cells seeded at a density of $1.5 \times 10^4/cm^2$ in 100-mm dishes were allowed to attach for 24 h. Following treatment with rutin and MPP^+, adherent cells were dislodged with a cell scraper and centrifuged at 3000 rpm for 5 min, and the cell pellets were resuspended in DMEM. Cells were then incubated with 10 μg/ml of the diluted JC-1 solution (Life Technologies, USA) for 15 min at room temperature, centrifuged, and resuspended in 500 μl PBS. Stained cell suspensions were analyzed using a BD FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and BD CellQuest PRO software (Becton Dickinson, Franklin Lakes, NJ, USA) to measure red and green fluorescence, with a total of 10,000 events collected for each sample. Fluorescence acquisition, compensation, and data analysis were performed according to the manufacturer's instructions.

Rhodamine123

Rhodamine123 was used as a complementary measurement of MMP. After rutin and MPP^+ treatment, adherent cells were dislodged with a cell scraper and centrifuged, and the cell pellets resuspended in DMEM. Rhodamine 123 (Sigma Aldrich, St. Louis, MO, USA) was added at a final concentration of 10 μM. After incubation for 15 min, cells were vortexed and centrifuged, and pellets were resuspended in 500 μl PBS. Stained cell suspensions were analyzed using an Accuri flow cytometer (BD Biosciences Pharmingen, San Diego, CA, USA). A total of 10,000 events was collected for each sample.

Western Blot

Western blots were used to evaluate the effects of rutin treatment on protein markers of ER stress. Following the appropriate treatment regime, whole-cell lysates were extracted in passive lysis buffer and protein concentrations quantified with a Bradford protein assay. Equal protein amounts of cell lysates were electrophoresed on 4–20% Mini-Protean® SDS-PAGE gels (Bio-Rad, USA) followed by transfer to PVDF membranes. The membranes were blocked in 5% milk-TBST before incubation with the relevant primary antibody at 4 °C overnight. These included BiP rabbit monoclonal antibody (1:1000; Cell Signalling Technology, USA), CHOP mouse monoclonal antibody (1:1000, Cell Signalling Technology, USA), and loading control GAPDH rabbit polyclonal antibody (1:1000; Santa Cruz Biotechnology, USA). Thereafter, membranes were incubated with appropriate HRP-conjugated secondary antibodies (1:5000, Santa Cruz Biotechnology, USA) and visualized using a SuperSignal® West Pico Chemiluminescent Kit (Thermo Scientific, USA). ImageJ software (http://imagej.nih.gov/ij/) was utilized to quantify western blots by means of densitometric measurements.

The Seahorse XF Cell Mito Stress and Glycolysis Rate Assay

To evaluate the effect of rutin pretreatment on the bioenergetic status of SH-SY5Y cells treated with MPP^+, the Seahorse extracellular flux (XF^e96) analyzer (Agilent, Santa Clara, USA) was used to perform two different assays, namely the XF cell Mito stress test and the glycolytic rate assay. The Seahorse XF analyzer is capable of simultaneously measuring pH and oxygen in real-time to assess the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), indicators of glycolysis and oxidative phosphorylation (OXPHOS) respectively (Pelletier et al. 2014).

SH-SY5Y cells seeded at 3×10^3 cells/well were allowed to adhere to the XF cell culture plate overnight. The following day, cells were pretreated with rutin and then exposed to 1 mM of MPP^+ for a total of 48 h. Thereafter, all cell culture media were replaced with XF base medium (without phenol red) supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, and 10 mM glucose. The XF base media used for the Glycolytic Rate Assay were further supplemented with 5 mM HEPES. Following the exchange of media, plates were incubated for 1 h at 37 °C in a non-CO_2 incubator. Thereafter, the plate was loaded into the Seahorse XF^e96 analyzer where standard procedures were followed for the individual assays, as recommended by the manufacturer.

For the Mito Stress Test, basal respiration was initially measured followed by successive injections of 1 μM oligomycin (ATP synthase/complex V inhibitor), 0.25 μM FCCP (uncoupling agent/MMP disruptor), and 1 μM rotenone/antimycin A (complex I/III inhibitor). For the glycolytic rate assay, basal glycolysis was measured followed by two injections: firstly 1 μM rotenone/antimycin A followed by 100 mM 2-deoxyglucose. Plates were normalized to cell number using the CyQUANT Cell Proliferation Assay kit (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. All data were processed using Wave version 2.40 and Seahorse XF report generators were used to calculate various mitochondrial parameters, including basal respiration, maximum respiration, ATP-coupled respiration, and spare respiratory capacity according to established protocols (Brand and Nicholls 2011).

Statistical Analysis

GraphPad Prism Software V7 was used for all statistical analyses (www.graphpad.com/scientific-software/prism/). Data are expressed as mean with standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test was performed to determine statistical significance ($p < 0.05$).

Results

Rutin Attenuates Cell Toxicity in MPP^+-Treated SH-SY5Y Cells

To evaluate the protective activity of rutin on MPP^+-induced SH-SY5Y cell toxicity, cells were pretreated with three concentrations of rutin (25, 50, and 100 μM) before being exposed to 1 mM MPP^+ as described earlier. Our findings show that whereas treatment with MPP^+ significantly decreased cell viability ($p < 0.0001$), all concentrations of rutin significantly enhanced ($p < 0.0001$, Fig. 1A) cell viability. Also, our findings showed that treatment of SH-SY5Y cells with MPP^+ resulted in a significant increase ($p < 0.0001$, Fig. 1B) in cell toxicity; however, pretreatment of SH-SY5Y cells with rutin significantly reduced toxicity at concentrations of 25 μM ($p = 0.0016$), 50 μM ($p = 0.0025$), and 100 μM ($p = 0.0001$), respectively.

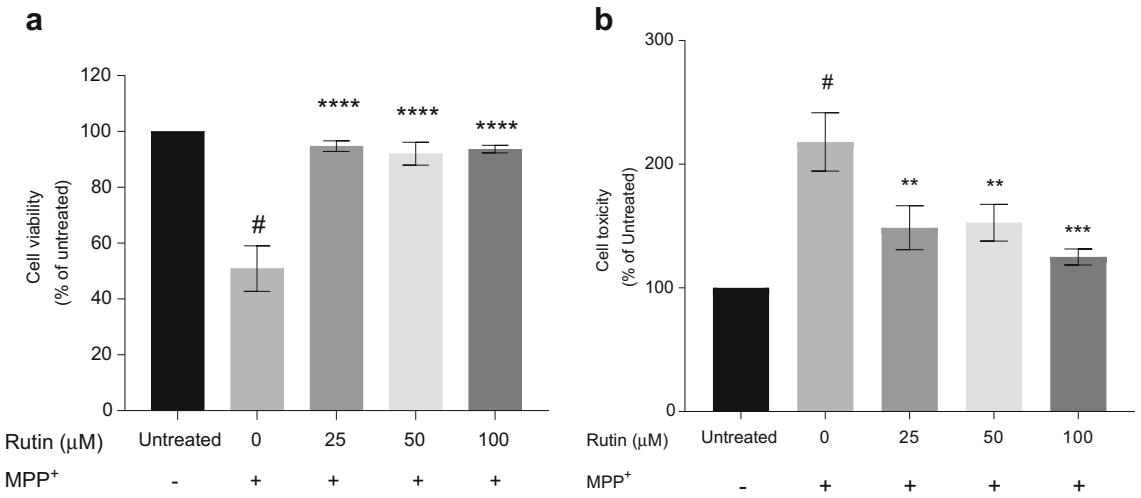

Fig. 1 Cell viability (**A**) and toxicity (**B**) in treated and untreated SH-SY5Y cells. Bars represent the mean ± SEM from three independent experiments. $^{\#}p < 0.0001$ vs untreated SH-SY5Y cells; $^{**}p < 0.005$, $^{***}p < 0.0005$, and $^{****}p < 0.0001$ vs SH-SY5Y cells treated with MPP^+ only

Rutin Attenuates MPP^+-Induced Dysregulation of Calcium Homeostasis in SH-SY5Y Cells

Previous reports indicated that Ca^{2+} dysregulation is associated with PD pathogenesis (Surmeier et al. 2012; Zaichick et al. 2017). To gain a better understanding on the role of rutin in the regulation of Ca^{2+} homeostasis, the level of intracellular Ca^{2+} in treated and untreated SH-SY5Y cells was investigated. Results showed that treatment of SH-SY5Y cells with MPP^+ resulted in a significant increase in intracellular Ca^{2+} levels ($p < 0.0001$, Fig. 2). However, this increase in Ca^{2+} levels was significantly attenuated following pretreatment with rutin at 25 μM ($p = 0.0004$), 50 μM ($p = 0.0004$), and 100 μM ($p < 0.0001$), respectively.

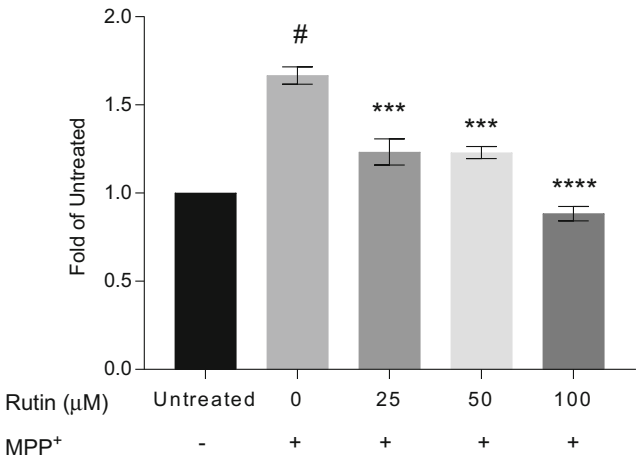

Fig. 2 Effect of rutin on MPP^+-induced dysregulation of Ca^{2+} homeostasis in SH-SY5Y cells. Bars represent the mean ± SEM from three independent experiments. $^{\#}p < 0.0001$ vs untreated SH-SY5Y cells; $^{***}p < 0.0005$ and $^{****}p < 0.0001$ vs SH-SY5Y cells treated with MPP^+ only

Rutin Attenuates ER Stress in SH-SY5Y Cells Treated with MPP^+

To detect levels of ER stress in SH-SY5Y cells, we measured two protein markers of ER stress, namely BiP and C/EBP homologous protein (CHOP). Activation of BiP is essential for regulating protein folding and the initiation of UPR signaling in the ER (Gorbatyuk and Gorbatyuk 2013). Findings revealed that treatment of SH-SY5Y cells with MPP^+ resulted in a significant decrease in the expression of BiP ($p = 0.0019$, Fig. 3A). Conversely, the expression levels were significantly increased following pretreatment with rutin at 25 μM ($p = 0.0082$), 50 μM ($p = 0.0370$), and 100 μM ($p = 0.0078$), respectively.

Similarly, CHOP is a transcription factor generally activated during ER stress (Choi et al. 2011). Findings showed that treatment of SH-SY5Y cells with MPP^+ resulted in a significant increase ($p < 0.0001$, Fig. 3B) in the expression of CHOP, indicating a marked elevation of MPP^+-induced ER stress. In SH-SY5Y cells pretreated with rutin, the expression level of CHOP was significantly decreased at concentrations of 25 μM ($p < 0.0001$), 50 μM ($p < 0.0001$), and 100 μM ($p < 0.0001$), respectively, signifying that rutin pretreatment prevented against ER stress in the cells.

Rutin Rescues Mitochondrial Membrane Potential in SH-SY5Y Cells Treated with MPP^+

Having established that rutin attenuates Ca^{2+} dysregulation and ER stress, the effect of rutin and MPP^+ treatment on mitochondrial membrane potential (MMP) was investigated, given that rutin might protect cells from MPP^+ toxicity at the mitochondrial level owing to its established antioxidant effects (Magalingam et al. 2013). MMP was assayed in SH-

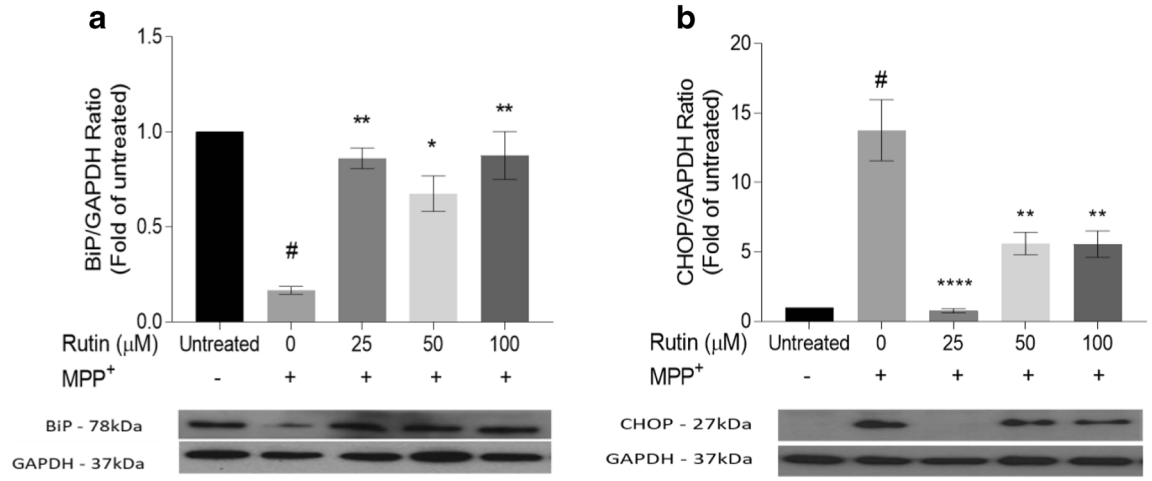

Fig. 3 Rutin prevents MPP^+-induced ER stress in SH-SY5Y cells. (**A**) Protein expression of BiP following treatment. (**B**) Protein expression of CHOP following treatment. Data is presented as the ratio of the target protein and GAPDH loading control. Bars represent the mean relative band intensity ± SEM for three independent experiments. $^{\#}p < 0.0001$ vs untreated cells; $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{****}p < 0.0001$ vs cells treated with MPP^+ only

SY5Y cells by means of two complementary potentiometric mitochondrial probes, JC-1 and Rhodamine 123.

JC-1 When added to live cells, the JC-1 dye enters mitochondria in an MMP-dependent manner where it forms red-fluorescing aggregates. Disaggregated JC-1 and cytosolic JC-1 fluoresce green following loss of MMP; hence, the ratio of red to green fluorescence can be used as a sensitive gauge of MMP (Smiley et al. 1991). Findings revealed a significant decrease in the red to green ratio in MPP^+-treated SH-SY5Y cells ($p < 0.0001$, Fig. 4), indicating loss of MMP and mitochondrial dysfunction. Conversely, pretreatment with rutin significantly prevented the decrease in MMP at 25 μM ($p < 0.0001$) and 100 μM ($p = 0.0010$), respectively. The increase in MMP following pretreatment with rutin at 50 μM was however not significant ($p = 0.2189$).

For Rhodamine 123, a decrease in fluorescence intensity indicates a loss of MMP (Wang and Xu 2005). Findings further confirmed the observation of lower MMP in MPP^+-treated cells, as the fluorescence intensity in MPP^+-treated SH-SY5Y cells was significantly decreased ($p < 0.0001$, Fig. 5), thus confirming the loss of MMP and possible mitochondrial dysfunction. However, the MMP was significantly increased following pretreatment with rutin at 25 μM ($p = 0.0057$), 50 μM ($p = 0.0458$), and 100 μM ($p = 0.0179$), respectively.

Rutin Improves Basal and Maximal Respiration as well as Spare Respiratory Capacity in SH-SY5Y Cells Treated with MPP^+

The mitochondrial stress test was measured under four conditions: (i) basal respiration (basal measurement without the oligomycin, FCCP, rotenone/antimycin A response); (ii) ATP-coupled respiration (reduction in OCR after the injection of oligomycin); (iii) maximal respiration (OCR response after the injection of FCCP); and (iv) spare respiratory capacity (difference between maximal respiration and basal respiration). Non-mitochondrial respiration (OCR response after the injection of rotenone and antimycin A) was subtracted from all other parameters. The overall mitochondrial respiration results for SH-SY5Y cells are shown in Fig. 6.

Basal respiration indicates the energetic need of the cell under baseline conditions. Findings showed that MPP^+ treatment resulted in a significant decrease in basal respiration in SH-SY5Y cells ($p < 0.0001$, Fig. 7), whereas all concentrations of rutin significantly increased basal respiration ($p < 0.0001$) in pretreated SH-SY5Y cells. ATP-coupled respiration indicates the ATP generated by the mitochondria that contributes to the energetic demand of the cell. Our findings revealed that treatment of SH-SY5Y cells with MPP^+ resulted in a significant decrease ($p < 0.0001$) in ATP-coupled respiration; however, there were no significant changes ($p > 0.05$) following pretreatment with rutin.

Also, maximal respiration indicates the maximum capacity of the electron transport chain when provided with sufficient substrates. Following treatment and after the addition of FCCP (stimulates the respiratory chain to function at maximum capacity), it was observed that MPP^+ caused a significant decrease ($p < 0.0001$), whereas rutin pretreatment resulted in a significant increase ($p < 0.0001$) in maximal respiration in SH-SY5Y cells. Furthermore, spare respiratory capacity indicates the ability of the cell to respond to an energetic need. After addition of FCCP to treated SH-SY5Y cells, MPP^+ treatment caused a significant decrease ($p < 0.0001$) in spare respiratory capacity. However, this reduction in spare respiratory capacity was significantly increased ($p < 0.05$) in SH-SY5Y cells pretreated with rutin. These findings indicate a potential protective effect of rutin on impaired mitochondria.

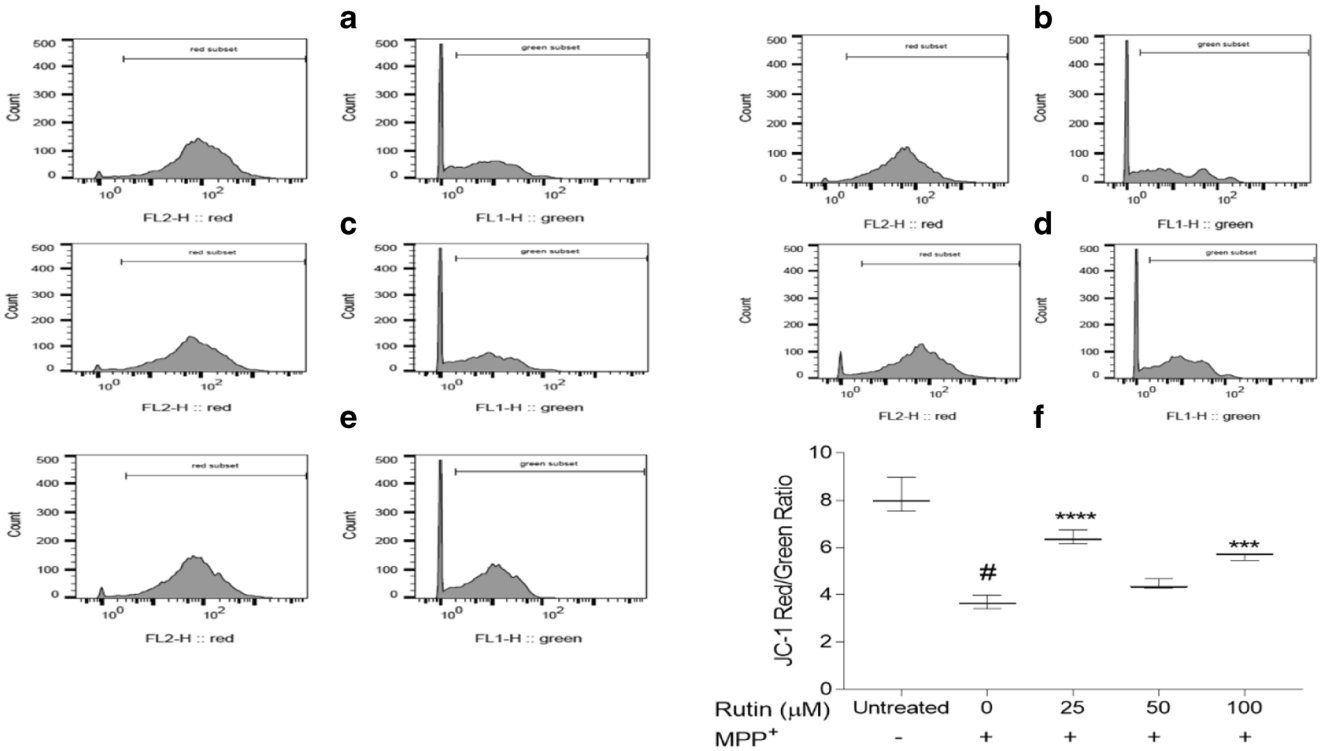

Fig. 4 Mitochondrial membrane potential shown by a ratio of red to green fluorescence intensity of JC-1. (**A**) Control SH-SY5Y cells. (**B**) SH-SY5Y cells treated with MPP^+ only. (**C**) SH-SY5Y cells treated with 25 μM rutin and then treated with MPP^+. (**D**) SH-SY5Y cells treated with 50 μM rutin and then treated with MPP^+. (**E**) SH-SY5Y cells treated with 100 μM rutin and then treated with MPP^+. (**F**) Box and whisker plot showing the mitochondrial membrane potential in untreated and treated SH-SY5Y cells. Bars represent the mean ± SEM from three independent experiments. $^{\#}p < 0.0001$ vs untreated SH-SY5Y cell; $^{***}p < 0.0005$ and $^{****}p < 0.0001$ vs SH-SY5Y cells treated with MPP^+ only

Rutin Improves Basal and Compensatory Glycolysis in SH-SY5Y Cells Treated with MPP^+

OXPHOS is primarily responsible for the production of ATP through a process of mitochondrial respiration. However, when OXPHOS is impaired, glycolysis becomes primarily responsible for the production of ATP (Keuper et al. 2014; Ozawa et al. 2015). It is thus essential to examine glycolytic function for an in-depth understanding of cellular energy demands. The glycolytic rate assay assessed basal glycolysis and compensatory glycolysis in treated and untreated SH-SY5Y cells. Our findings show that treatment of SH-SY5Y cells with MPP^+ reduced basal ($p = 0.2439$, Fig. 8) and compensatory glycolysis ($p < 0.0001$), while rutin pretreatment significantly improved basal glycolysis and compensatory glycolysis ($p < 0.0001$).

Discussion

There is currently renewed interest in the search for potential neuroprotective compounds from plant materials. We report that rutin is a promising neuroprotective agent that prevents cell toxicity in a toxin-induced model of PD, in agreement with previous publications (Magalingam et al. 2013, 2016; Park et al. 2014). In order to investigate the mechanism(s) underlying this protective effect, we investigated the ability of rutin to protect against MPP^+-induced dysregulation of Ca^{2+} homeostasis, ER stress, and bioenergetic deficits in SH-SY5Y cells. Ca^{2+} is a common second messenger that regulates an array of cellular signaling pathways (Petersen et al. 2005). For optimal survival and functioning of dopaminergic neurons, it is essential for Ca^{2+} levels to be sustained within a low range of concentrations (Michel et al. 2013). Our current study showed that MPP^+ induced a significant increase in Ca^{2+} levels in the cells, and further demonstrated that rutin significantly reduced Ca^{2+} levels, thus preventing the deleterious effects associated with excessive Ca^{2+}. We hypothesize that the observed dysregulation of Ca^{2+} is driven by MPP^+-induced impairments of mitochondrial function and ATP generation, as maintenance of neuronal Ca^{2+} homeostasis is energetically demanding (Surmeier and Schumacker 2013). MMP is essential to the organelle's several functions (Nicholls and Ferguson 2013) and is responsible for driving ATP synthesis by OXPHOS. The magnitude of MMP is determined by the equilibrium between its production and utilization by processes such as ATP synthesis. The quantification of changes in MMP enables mechanistic insights into mitochondrial and

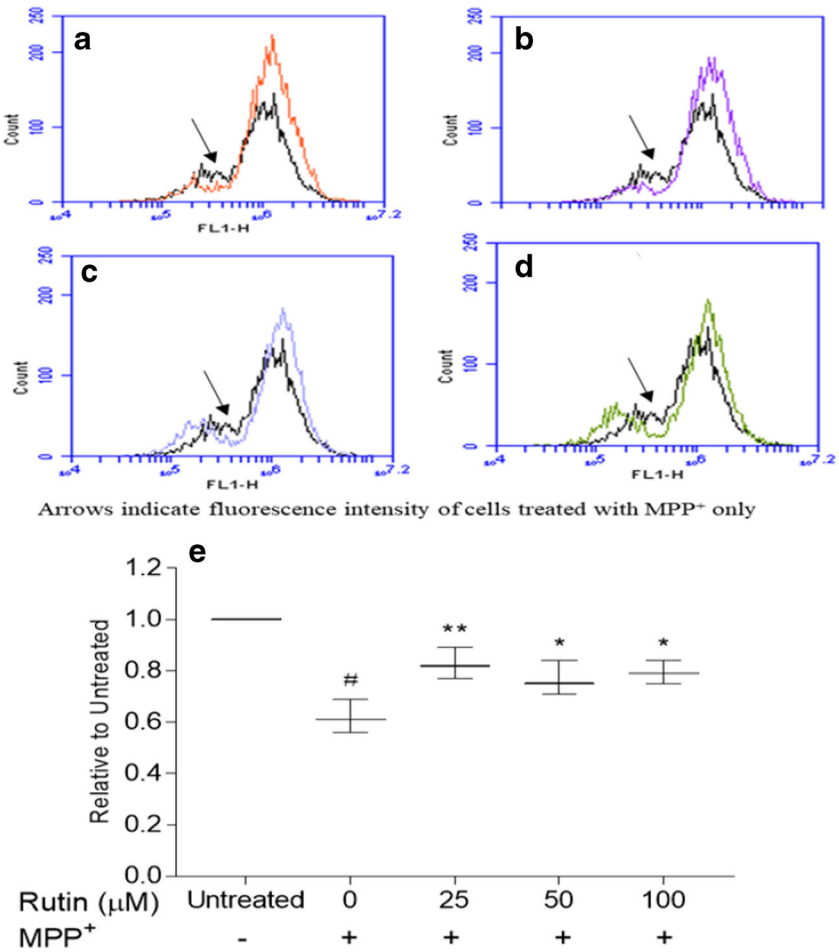

Fig. 5 Mitochondrial membrane potential shown by the fluorescence intensity of Rhodamine 123. Histogram showing SH-SY5Y cells treated with MPP^+ only vs (**A**) untreated SH-SY5Y cells. (**B**) SH-SY5Y cells pretreated with 25 μM rutin. (**C**) SH-SY5Y cells pretreated with 50 μM rutin. (**D**) SH-SY5Y cells pretreated with 100 μM rutin. (**E**) Box and whisker plot showing the MMP in untreated and treated SH-SY5Y cells. Bars represent the mean ± SEM from three independent experiments. $^{\#}p < 0.0001$ vs untreated SH-SY5Y cells; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs SH-SY5Y cells treated with MPP^+ only

cellular function. In our study, we observed that MPP^+ caused a significant reduction in MMP in SH-SY5Y cells, indicating mitochondrial dysfunction. However, this loss in MMP was significantly attenuated by rutin, suggesting that rutin was able to prevent the harmful activities associated with a decline in MMP.

The present study also provides the first research evidence on the ability of rutin to inhibit ER stress in SH-SY5Y cells treated with MPP^+. BiP is activated in response to ER stress (Ghribi et al. 2003; Kaufman 1999), and its activation during the UPR protects neurons against metabolic/excitotoxic damage (Lowenstein et al. 1991; Yu et al. 1999) helps in sustaining proper ER function and enables proper protein folding (Ghribi et al. 2003). Accordingly, reports show that the UPR is a therapeutic target for proper protein homeostasis during inhibition of neurodegeneration. For example, the drug valproate increases BiP levels and other ER chaperones (Bown et al. 2000; Wang et al. 1999), while MPP^+ resulted in a down-regulation of BiP mRNA in MN9D cells (Holtz and O'Malley 2003). We hypothesized that BiP is responsible for the main chaperone action in our PD model and that its upregulation would possibly improve proper protein folding, attenuate ER stress, and protect against apoptosis in SH-SY5Y cells. Our results show that rutin significantly increased the expression of BiP, thus suggesting that a "stress response" may trigger the protective activity of rutin in this model of PD, thereby enabling proper function of the UPR and alleviating ER stress.

Upregulation of BiP is linked to the inhibition of ER stress-induced activation of CHOP (Oyadomari et al. 2001; Wang et al. 1996). CHOP/GADD153 is a 29-kDa protein that has been associated with the control of processes necessary for cellular differentiation, proliferation, expression, and energy metabolism of specific genes (Birkenmeier et al. 1989; Umek et al. 1991). Normally, the expression of CHOP is extremely low; however, it is upregulated in the nucleus following ER stress where it contributes to apoptosis (Lu et al. 2014). Reports show that MPP^+ increases the expression of CHOP in SH-SY5Y cells (Conn et al. 2002; Zhao et al. 2016), and authors have observed a correlation between cell death induced by 6-OHDA and CHOP induction (Ryu et al. 2002). The present study found comparable upregulation

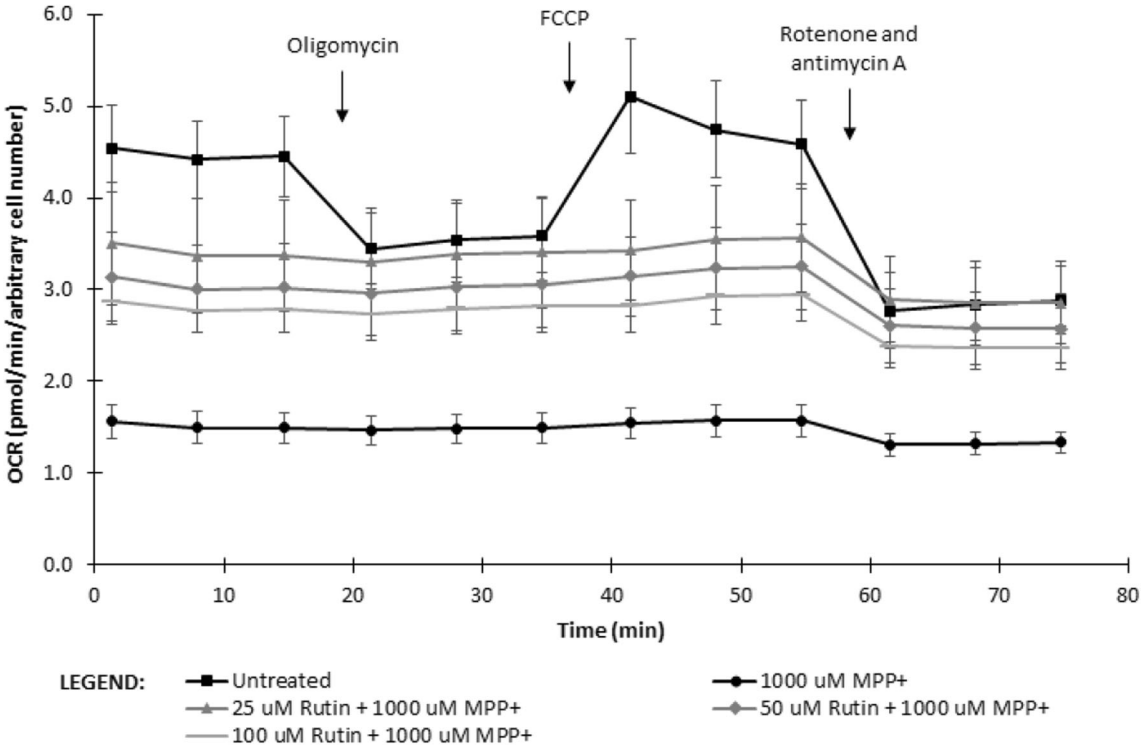

Fig. 6 Seahorse XF Mito stress test line graph depicting the oxygen consumption rate (OCR), as determined by the Seahorse XF analyzer, following the injection of oligomycin, FCCP, and rotenone/antimycin A with three measurements per condition (3 min measure and 3 min mix per measurement)

of CHOP following MPP^+ exposure. Additionally, rutin pretreatment prevented this MPP^+-induced CHOP accumulation, possibly via the upregulation of BiP. These findings suggest that neuroprotective agents aimed at targeting proper regulation of these proteins would be a worthwhile therapeutic option in PD.

In our study, different aspects of cellular bioenergetic status were investigated in SH-SY5Y cells following treatment with MPP^+. A range of human pathologies associated with mitochondrial dysfunction frequently arises as a result of OXPHOS impairment (Koopman et al. 2013; Koopman et al. 2012). A dysfunction in OXPHOS initiates adaptive cellular responses that may inhibit cell death or alternatively contribute to further dysfunction (Benard et al. 2013; Germain et al. 2012; Hao et al. 2010). The adaptive responses may include alterations in redox state and antioxidant reactions, upregulation of glycolysis, and initiation of mitochondrial biogenesis (Koopman et al. 2010). Thus, evaluation of bioenergetic status provides critical insight into the energetic demands and metabolic flexibility achieved by the cells in normal and stressed conditions.

Findings from this study show a significant reduction in the basal respiration in SH-SY5Y cells treated with MPP^+. We also observed that rutin did not improve ATP-coupled respiration but significantly improved spare respiratory capacity and maximal respiration in pretreated SH-SY5Y cells when compared to those treated with MPP^+ only. These findings are important because maximal respiration indicates the maximum capacity operated by the respiratory chain arising from substrate oxidation to meet its metabolic challenges; thus, a reduction in maximal respiration is a strong measure of an impairment to the mitochondria (Brand and Nicholls 2011). Additionally, the mitochondrial spare respiratory capacity is considered as a vital aspect of mitochondrial function, and its measurement signifies the cell's ability to respire maximally as well as its capability to react to an energetic demand (Yamamoto et al. 2016). When cells are exposed to stress conditions, more ATP is needed to maintain normal functions. Thus, cells with higher spare respiratory capacity are capable of producing more ATP to overcome stress (Hill et al. 2009). Consequently, our observation that rutin significantly increases maximal respiration and spare respiratory capacity confirms its role in protecting mitochondrial function.

Inhibition of OXPHOS is often linked with the stimulation of glycolysis as a compensatory response to damages in mitochondrial ATP synthesis (Keuper et al. 2014; Ozawa et al. 2015). While neurons have a limited capacity for glycolysis, increase in glycolysis is known to attenuate mitochondria-associated energy failure and cell death (Chaudhuri et al. 2015; Hong et al. 2016). Reports show that sustaining cellular ATP concentrations through glycolysis inhibits toxicity induced by MPP^+ (Chalmers-Redman et al. 1999; Maruoka et al. 2007; Mazzio and Soliman 2003). This is in line with our

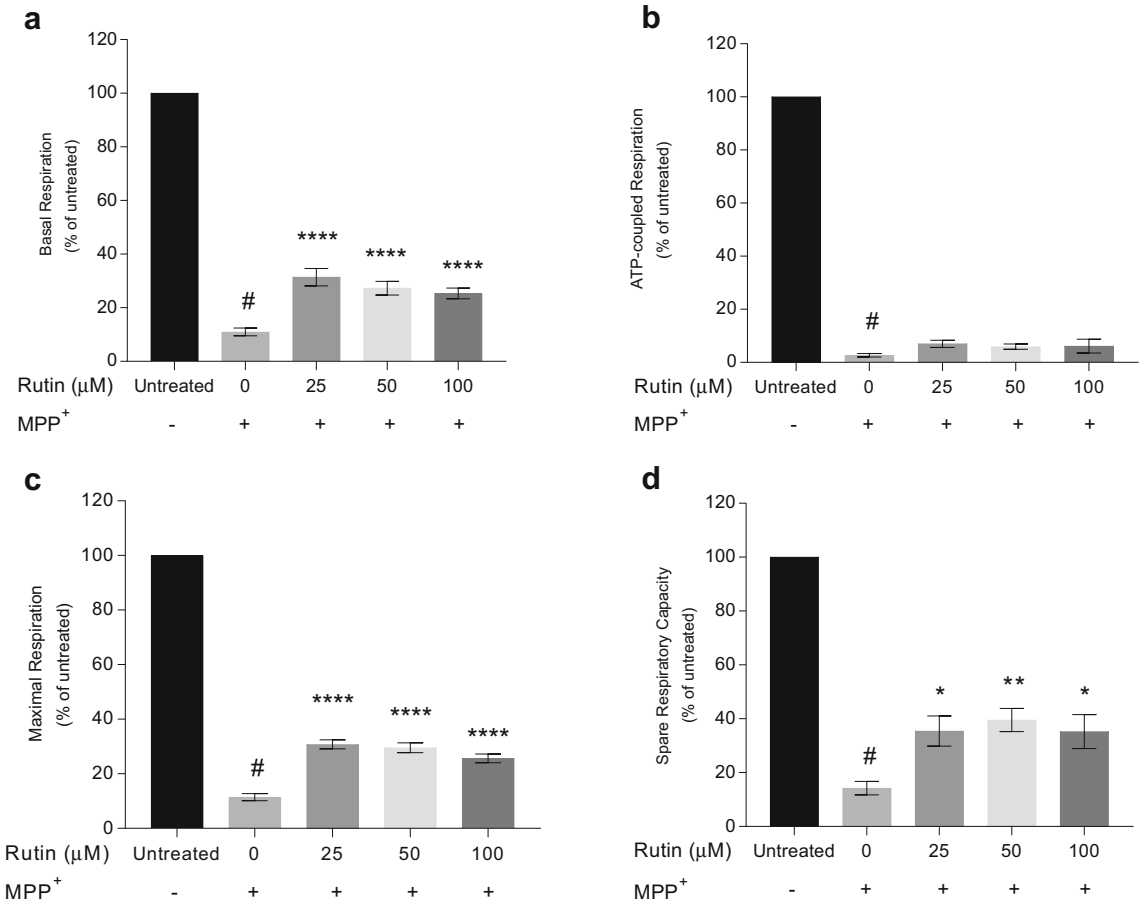

Fig. 7 Rutin mitigates impaired oxidative phosphorylation in SH-SY5Y cells treated with MPP^+. (**A**) Basal respiration. (**B**) ATP-coupled respiration. (**C**) Maximal respiration. (**D**) Spare respiratory capacity. Each bar represents mean ± SEM ($n = 15$). $^{\#}p < 0.0001$ vs untreated SH-SY5Y cells; $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{****}p < 0.0001$ vs SH-SY5Y cells treated with MPP^+ only

observations that rutin significantly increased basal and compensatory glycolysis in the SH-SY5Y cells treated with MPP^+, and to our knowledge, the current study provides the first research evidence on the activity of rutin on cellular bioenergetic status in SH-SY5Y cells.

In conclusion, our findings demonstrate the ability of rutin to maintain Ca^{2+} homeostasis, inhibit ER stress, and protect the mitochondria in MPP^+-treated SH-SY5Y cells, thus highlighting its potential as a promising neuroprotective compound in the treatment of PD.

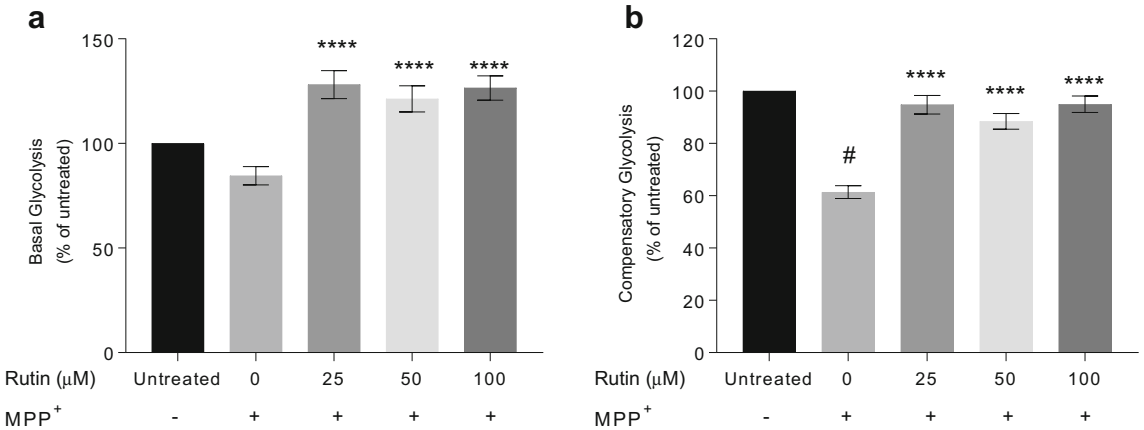

Fig. 8 Glycolytic parameters in treated and untreated SH-SY5Y cells. (**A**) Basal glycolysis. (**B**) Compensatory glycolysis. Each bar represents mean ± SEM ($n = 15$). $^{\#}p < 0.0001$ vs untreated SH-SY5Y cells; $^{****}p < 0.0001$ vs SH-SY5Y cells treated with MPP^+ only

Acknowledgements We are grateful to the Parkinson's Disease Research Group, Division of Molecular Biology and Human Genetics, Stellenbosch University, for providing some laboratory materials and workspace needed for this study.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Altman SA, Randers L, Rao G (1993) Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. Biotechnol Prog 9:671–674

Benard G, Trian T, Bellance N, Berger P, Lavie J, Espil-Taris C, Rocher C, Eimer-Bouillot S, Goizet C, Nouette-Gaulain K (2013) Adaptative capacity of mitochondrial biogenesis and of mitochondrial dynamics in response to pathogenic respiratory chain dysfunction. Antioxid Redox Signal 19:350–365

Bezprozvanny I (2009) Calcium signaling and neurodegenerative diseases. Trends Mol Med 15:89–100

Birkenmeier EH, Gwynn B, Howard S, Jerry J, Gordon JI, Landschulz WH, McKnight SL (1989) Tissue-specific expression, developmental regulation, and genetic mapping of the gene encoding CCAAT/enhancer binding protein. Genes Dev 3:1146–1156

Blandini F, Armentero MT (2012) Animal models of Parkinson's disease. FEBS J 279:1156–1166

Bown CD, Wang J-F, Young LT (2000) Increased expression of endoplasmic reticulum stress proteins following chronic valproate treatment of rat C6 glioma cells. Neuropharmacology 39:2162–2169

Boyce M, Yuan J (2006) Cellular response to endoplasmic reticulum stress: a matter of life or death. Cell Death Differ 13:363–373

Brand MD, Nicholls DG (2011) Assessing mitochondrial dysfunction in cells. Biochem J 435:297–312

Cai P, Ye J, Zhu J, Liu D, Chen D, Wei X, Johnson NR, Wang Z, Zhang H, Cao G (2016) Inhibition of endoplasmic reticulum stress is involved in the neuroprotective effect of bFGF in the 6-OHDA-induced Parkinson's disease model. Aging Dis 7:336–449

Chalmers-Redman RM, Fraser ADM, Carlile GW, Pong A, Tatton WG (1999) Glucose protection from MPP+-induced apoptosis depends on mitochondrial membrane potential and ATP synthase. Biochem Biophys Res Commun 257:440–447

Chaudhuri AD, Kabaria S, Choi DC, Mouradian MM, Junn E (2015) MicroRNA-7 promotes glycolysis to protect against 1-methyl-4-phenylpyridinium-induced cell death. J Biol Chem 290:12425–12434

Choi AY, Choi JH, Yoon H, Hwang K-Y, Noh MH, Choe W, Yoon K-S, Ha J, Yeo E-J, Kang I (2011) Luteolin induces apoptosis through endoplasmic reticulum stress and mitochondrial dysfunction in Neuro-2a mouse neuroblastoma cells. Eur J Pharmacol 668:115–126

Conn KJ, Gao WW, Ullman MD, McKeon-O'Malley C, Eisenhauer PB, Fine RE, Wells JM (2002) Specific up-regulation of GADD153/CHOP in 1-methyl-4-phenyl-pyridinium-treated SH-SY5Y cells. J Neurosci Res 68:755–760

Couch JF (1943) Rutin for the capillaries. Yearbook of agriculture, pp 711–715

Egawa N, Yamamoto K, Inoue H, Hikawa R, Nishi K, Mori K, Takahashi R (2011) The endoplasmic reticulum stress sensor, ATF6α, protects against neurotoxin-induced dopaminergic neuronal death. J Biol Chem 286:7947–7957

Germain M, Nguyen AP, Khacho M, Patten DA, Screaton RA, Park DS, Slack RS (2012) LKB1-regulated adaptive mechanisms are essential for neuronal survival following mitochondrial dysfunction. Hum Mol Genet 22:952–962

Ghorbani A (2017) Mechanisms of antidiabetic effects of flavonoid rutin. Biomed Pharmacother 96:305–312

Ghribi O, Herman MM, Pramoonjago P, Savory J (2003) MPP+ induces the endoplasmic reticulum stress response in rabbit brain involving activation of the ATF-6 and NF-κB signaling pathways. J Neuropathol Exp Neurol 62:1144–1153

Gorbatyuk MS, Gorbatyuk OS (2013) The molecular chaperone GRP78/BiP as a therapeutic target for neurodegenerative disorders: a mini review. J Genet Syndr Gene Ther 4:128

Guardia T, Rotelli AE, Juarez AO, Pelzer LE (2001) Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. II Farmaco 56:683–687

Hang L, Thundyil J, Lim KL (2015) Mitochondrial dysfunction and Parkinson disease: a Parkin–AMPK alliance in neuroprotection. Ann N Y Acad Sci 1350:37–47

Hao W, Chang C-PB, Tsao C-C, Xu J (2010) Oligomycin-induced bioenergetic adaptation in cancer cells with heterogeneous bioenergetic organization. J Biol Chem 285:12647–12654

Harding HP, Calfon M, Urano F, Novoa I, Ron D (2002) Transcriptional and translational control in the mammalian unfolded protein response. Annu Rev Cell Dev Biol 18:575–599

Hill BG, Dranka BP, Zou L, Chatham JC, Darley-Usmar VM (2009) Importance of the bioenergetic reserve capacity in response to cardiomyocyte stress induced by 4-hydroxynonenal. Biochem J 424:99–107

Hiller MM, Finger A, Schweiger M, Wolf DH (1996) ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. Science 273:1725–1728

Holtz WA, O'Malley KL (2003) Parkinsonian mimetics induce aspects of unfolded protein response in death of dopaminergic neurons. J Biol Chem 278:19367–19377

Hong CT, Chau K-Y, Schapira AH (2016) Meclizine-induced enhanced glycolysis is neuroprotective in Parkinson disease cell models. Sci Rep 6:25344

Ito K, Eguchi Y, Imagawa Y, Akai S, Mochizuki H, Tsujimoto Y (2017) MPP+ induces necrostatin-1-and ferrostatin-1-sensitive necrotic death of neuronal SH-SY5Y cells. Cell Death Dis 3:17013

Kaufman RJ (1999) Molecular chaperones and the heat shock response: Sponsored by Cold Spring Harbor Laboratory, 6–10 May 1998. Biochim Biophys Acta Rev Cancer 1423:R13–R27

Keuper M, Jastroch M, Yi C-X, Fischer-Posovszky P, Wabitsch M, Tschöp MH, Hofmann SM (2014) Spare mitochondrial respiratory capacity permits human adipocytes to maintain ATP homeostasis under hypoglycemic conditions. FASEB J 28:761–770

Koopman WJ, Nijtmans LG, Dieteren CE, Roestenberg P, Valsecchi F, Smeitink JA, Willems PH (2010) Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. Antioxid Redox Signal 12:1431–1470

Koopman WJ, Willems PH, Smeitink JA (2012) Monogenic mitochondrial disorders. N Engl J Med 366:1132–1141

Koopman WJ, Distelmaier F, Smeitink JA, Willems PH (2013) OXPHOS mutations and neurodegeneration. EMBO J 32:9–29

Korecka JA, van Kesteren RE, Blaas E, Spitzer SO, Kamstra JH, Smit AB, Swaab DF, Verhaagen J, Bossers K (2013) Phenotypic characterization of retinoic acid differentiated SH-SY5Y cells by transcriptional profiling. PLoS One 8:e63862

Koyano F, Okatsu K, Ishigaki S, Fujioka Y, Kimura M, Sobue G, Tanaka K, Matsuda N (2013) The principal PINK1 and Parkin cellular events triggered in response to dissipation of mitochondrial membrane potential occur in primary neurons. Genes Cells 18:672–681

La Casa C, Villegas I, De La Lastra CA, Motilva V, Calero MM (2000) Evidence for protective and antioxidant properties of rutin, a natural

flavone, against ethanol induced gastric lesions. J Ethnopharmacol 71:45–53
Li X-x, He G-r, Mu X, Xu B, Tian S, Yu X, Meng F-r, Xuan Z-h, Du G-h (2012) Protective effects of baicalein against rotenone-induced neurotoxicity in PC12 cells and isolated rat brain mitochondria. Eur J Pharmacol 674:227–233
Lopes FM, Schröder R, da Frota Júnior MLC, Zanotto-Filho A, Müller CB, Pires AS, Meurer RT, Colpo GD, Gelain DP, Kapczinski F (2010) Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies. Brain Res 1337:85–94
Lowenstein DH, Chan PH, Miles MF (1991) The stress protein response in cultured neurons: characterization and evidence for a protective role in excitotoxicity. Neuron 7:1053–1060
Lu T-H, Tseng T-J, Su C-C, Tang F-C, Yen C-C, Liu Y-Y, Yang C-Y, Wu C-C, Chen K-L, Hung D-Z (2014) Arsenic induces reactive oxygen species-caused neuronal cell apoptosis through JNK/ERK-mediated mitochondria-dependent and GRP 78/CHOP-regulated pathways. Toxicol Lett 224:130–140
Magalingam KB, Radhakrishnan A, Haleagrahara N (2013) Rutin, a bioflavonoid antioxidant protects rat pheochromocytoma (PC-12) cells against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity. Int J Mol Med 32:235–240
Magalingam KB, Radhakrishnan A, Haleagrahara N (2016) Protective effects of quercetin glycosides, rutin, and isoquercetrin against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in rat pheochromocytoma (PC-12) cells. Int J Immunopathol Pharmacol 29: 30–39
Maruoka N, Murata T, Omata N, Takashima Y, Fujibayashi Y, Wada Y (2007) Biphasic mechanism of the toxicity induced by 1-methyl-4-phenylpyridinium ion (MPP+) as revealed by dynamic changes in glucose metabolism in rat brain slices. Neurotoxicology 28:672–678
Mazzio E, Soliman K (2003) D-(+)-glucose rescue against 1-methyl-4-phenylpyridinium toxicity through anaerobic glycolysis in neuroblastoma cells. Brain Res 962:48–60
McCoy MK, Cookson MR (2012) Mitochondrial quality control and dynamics in Parkinson's disease. Antioxid Redox Signal 16:869–882
Michel PP, Toulorge D, Guerreiro S, Hirsch EC (2013) Specific needs of dopamine neurons for stimulation in order to survive: implication for Parkinson disease. FASEB J 27:3414–3423
Nicholls D, Ferguson S (2013) Bioenergetics, vol 4. Academic, Norwell
Oyadomari S, Takeda K, Takiguchi M, Gotoh T, Matsumoto M, Wada I, Akira S, Araki E, Mori M (2001) Nitric oxide-induced apoptosis in pancreatic β cells is mediated by the endoplasmic reticulum stress pathway. Proc Natl Acad Sci 98:10845–10850
Ozawa S, Ueda S, Imamura H, Mori K, Asanuma K, Yanagita M, Nakagawa T (2015) Glycolysis, but not mitochondria, responsible for intracellular ATP distribution in cortical area of podocytes. Sci Rep 5:18575
Park A, Stacy M (2009) Non-motor symptoms in Parkinson's disease. J Neurol 256:293–298
Park S-E, Sapkota K, Choi J-H, Kim M-K, Kim YH, Kim KM, Kim KJ, Oh H-N, Kim S-J, Kim S (2014) Rutin from Dendropanax morbifera Leveille protects human dopaminergic cells against rotenone induced cell injury through inhibiting JNK and p38 MAPK signaling. Neurochem Res 39:707–718
Paschen W, Mengesdorf T (2005) Endoplasmic reticulum stress response and neurodegeneration. Cell Calcium 38:409–415
Pelletier M, Billingham LK, Ramaswamy M, Siegel RM (2014) Extracellular flux analysis to monitor glycolytic rates and mitochondrial oxygen consumption. In: Methods Enzymol. Elsevier, Amsterdam, pp 125–149
Perk AA, Shatynska-Mytsyk I, Gerçek YC, Boztaş K, Yazgan M, Fayyaz S, Farooqi AA (2014) Rutin mediated targeting of signaling machinery in cancer cells. Cancer Cell Int 14(1):124
Petersen OH, Michalak M, Verkhratsky A (2005) Calcium signalling: past, present and future. Cell Calcium 38:161–169
Pringsheim T, Jette N, Frolkis A, Steeves TD (2014) The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord 29:1583–1590
Ravid R, Ferrer I (2012) Brain banks as key part of biochemical and molecular studies on cerebral cortex involvement in Parkinson's disease. FEBS J 279:1167–1176
Requejo-Aguilar R, Bolaños JP (2016) Mitochondrial control of cell bioenergetics in Parkinson's disease. Free Radic Biol Med 100:123–137
Ryu EJ, Harding HP, Angelastro JM, Vitolo OV, Ron D, Greene LA (2002) Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. J Neurosci 22: 10690–10698
Schapira A, Cooper J, Dexter D, Clark J, Jenner P, Marsden C (1990) Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54:823–827
Smiley ST, Reers M, Mottola-Hartshorn C, Lin M, Chen A, Smith TW, Steele G, Chen LB (1991) Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. Proc Natl Acad Sci 88:3671–3675
Song J-X, Shaw P-C, Sze C-W, Tong Y, Yao X-S, Ng T-B, Zhang Y-B (2010) Chrysotoxine, a novel bibenzyl compound, inhibits 6-hydroxydopamine induced apoptosis in SH-SY5Y cells via mitochondria protection and NF-κB modulation. Neurochem Int 57: 676–689
Surmeier DJ, Schumacker PT (2013) Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. J Biol Chem 288:10736–10741
Surmeier DJ, Guzman JN, Sanchez J, Schumacker PT (2012) Physiological phenotype and vulnerability in Parkinson's disease. Cold Spring Harb Perspect Med 2:a009290
Umek RM, Friedman AD, McKnight SL (1991) CCAAT-enhancer binding protein: a component of a differentiation switch. Science 251: 288–292
Van der Merwe C, Van Dyk HC, Engelbrecht L, van der Westhuizen FH, Kinnear C, Loos B, Bardien S (2017) Curcumin rescues a PINK1 knock down SH-SY5Y cellular model of Parkinson's disease from mitochondrial dysfunction and cell death. Mol Neurobiol 54:2752–2762
Wang X-J, Xu J-X (2005) Salvianic acid A protects human neuroblastoma SH-SY5Y cells against MPP+-induced cytotoxicity. Neurosci Res 51:129–138
Wang X-Z, Lawson B, Brewer JW, Zinszner H, Sanjay A, Mi L-J, Boorstein R, Kreibich G, Hendershot LM, Ron D (1996) Signals from the stressed endoplasmic reticulum induce C/EBP-homologous protein (CHOP/GADD153). Mol Cell Biol 16:4273–4280
Wang J-F, Bown C, Young LT (1999) Differential display PCR reveals novel targets for the mood-stabilizing drug valproate including the molecular chaperone GRP78. Mol Pharmacol 55:521–527
Wang B, Su C-J, Feng Y, Zhou Y, Huang Y, Wang Z-H, Luo W-F, Chen L-H, Liu T, Liu T-T (2018) The neuroprotection of low-dose morphine in cellular and animal models of Parkinson's disease through ameliorating endoplasmic reticulum (ER) stress and activating autophagy. Front Mol Neurosci 11:120
Webster R, Gawde M, Bhattacharya R (1996) Protective effect of rutin, a flavonol glycoside, on the carcinogen-induced DNA damage and repair enzymes in rats. Cancer Lett 109:185–191
Xie H-r, Hu L-s, Li G-y (2010) SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. Chin Med J 123:1086–1092
Yamamoto H, Morino K, Mengistu L, Ishibashi T, Kiriyama K, Ikami T, Maegawa H (2016) Amla enhances mitochondrial spare respiratory capacity by increasing mitochondrial biogenesis and antioxidant

systems in a murine skeletal muscle cell line. Oxidative Med Cell Longev 2016:1–11

Yang J, Guo J, Yuan J (2008) In vitro antioxidant properties of rutin. LWT-Food Science and Technology 41:1060–1066

Yoo H, Ku S-K, Baek Y-D, Bae J-S (2014) Anti-inflammatory effects of rutin on HMGB1-induced inflammatory responses in vitro and in vivo. Inflamm Res 63:197–206

Yu Z, Luo H, Fu W, Mattson MP (1999) The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. Exp Neurol 155:302–314

Zaichick SV, McGrath KM, Caraveo G (2017) The role of Ca2+ signaling in Parkinson's disease. Dis Model Mech 10:519–535

Zhao Q, Yang X, Cai D, Ye L, Hou Y, Zhang L, Cheng J, Shen Y, Wang K, Bai Y (2016) Echinacoside protects against MPP+-induced neuronal apoptosis via ros/atf3/chop pathway regulation. Neurosci Bull 32:349–362