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# Impact of Rapeseed Pomace (RSP) extract on markers of oxidative stress and DNA damage in human SH-SY5Y cells

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## Abstract

With increased longevity and subsequent rise in people with age-related neurodegenerative diseases, protection of neurons from oxidative stress damage has become an important field of study. For the first time, we highlight the neuroprotective properties of rapeseed pomace (RSP) extract in SH-SY5Y human neuroblastoma cells. We used resazurin to determine cell metabolism, 2,2'-dichlorofluorescein diacetate (H<sub>2</sub>DCFDA) to assess the potential of RSP extracts to shield cells from reactive oxygen species (ROS) induced by H<sub>2</sub>O<sub>2</sub> using flow cytometry, HPLC to analyze for malondialdehyde (MDA) as a lipid peroxidation marker and the COMET assay to assess DNA strand breakage. Protein stress arrays were used to investigate the cellular pathways affected by RSP extract. No effect on cell metabolism in SH-SY5Y cells was observed after RSP extract treatment (up to 1.5 mg/mL). Pretreatment (24 hrs) with RSP extract (1 mg/mL), before H<sub>2</sub>O<sub>2</sub>-induced stress, alleviated ROS production and DNA strand breakage by 68%, and 38%, respectively. At protein level, the RSP extract increased the levels of FABP-1, HIF-1 $\alpha$ , SOD2 and Cytochrome c proteins. Under H<sub>2</sub>O<sub>2</sub>-induced stress, however, it helped to downregulate p38 $\alpha$  levels, a protein kinase which is receptive to stress impulse (mitogen-activated). RSP extract shows very promising cell protective properties in relation to oxidative stress.

## Practical applications

Oxidative stress has been associated with numerous diseases for example cancer, diabetes, and many neurological disorders including Parkinson's and Alzheimer's diseases. Hence there is acceptance among the scientific community of antioxidant therapy and the quest for effective, low cost and readily available sources of natural antioxidants is paramount. Rapeseed plantations are abundant around the world due to the use of rapeseed oil in cooking and as a biofuel. The resulting rapeseed pomace (by-product), specifically its extract, contains high levels of phytochemicals that protect cells against oxidative stress. Therefore, RSP extract can potentially be used/developed as functional food and nutraceuticals in the prevention of many complex neurodegenerative diseases.

## Keywords

Rapeseed; plant extract; SH-SY5Y neuroblastoma cells; cytoprotection; comet assay; ROS

## 43 1 INTRODUCTION

44 Over the last decades, constant growth and advancement of medical services and higher standards of living, especially  
45 in the developed countries, have led to a continuous increase in human life expectancy (Dimitriadi & Hart, 2010).  
46 This positive outcome however comes as a double-edged sword, as the number of patients with age-related diseases,  
47 for example neurodegenerative diseases, has increased significantly.

48 Gradual and progressive degradation and loss of neurons are linked to neurodegenerative diseases with the final  
49 consequence resulting in nervous system dysfunction (Brown, Lockwood, & Sonawane, 2005). The latter can lead to  
50 either symptoms of movement deficiencies (e.g. Parkinson's disease (PD), Spinocerebellar ataxia (SCA) and Motor  
51 Neuron Disease (MND or ALS)) and/or problems with mental function (Alzheimer's disease (AD), Prion diseases).  
52 Many of these neurodegenerative diseases are well researched, however, causation and mechanisms of their etiology  
53 are not completely understood, and cures are nonexistent. Current available treatments only alleviate some of the  
54 symptoms. The main pathological landmark for many neurodegenerative diseases involves misfolded proteins that  
55 can lead to their aggregation and deposition in the central nervous system (Skovronsky, Lee, & Trojanowski, 2006).  
56 In addition, they are often associated with oxidative stress (Farooqui, 2015; Gilgun-Sherki, Melamed, & Offen, 2001;  
57 Liu, Zhou, Ziegler, Dimitrion, & Zuo, 2017; Uttara, Singh, Zamboni, & Mahajan, 2009). Whether this oxidative stress  
58 is part of the causation or a downstream effect of other factors such as protein aggregations, is not well understood.  
59 Excessive levels of cellular reactive oxygen species (ROS) can initiate protein oxidation, increased DNA damage,  
60 lipid peroxidation and mitochondrial dysfunction, all of which can be fatal to the function of neurons if not prevented  
61 (Emerit, Edeas, & Bricaire, 2004; Pohl & Kong Thoo Lin, 2018; Pollari, Goldsteins, Bart, Koistinaho, & Giniatullin,  
62 2014).

63 However, several antioxidants, those obtained from natural sources, have received significant interest for their  
64 potential to treat/prevent neurodegeneration. Previous *in vitro* and *in vivo* studies had demonstrated positive outcomes  
65 in a number of different neurodegenerative disease models (Pohl & Kong Thoo Lin, 2018).

66 Rapeseed pomace (RSP) extracts, characterized for secondary metabolite composition as well as their *in vitro*  
67 and *in vivo* activities (Pohl et al., 2018, 2019; Yates et al., 2019), have been studied in our laboratory. After the  
68 production of edible oil from rapeseed (*Brassica napus*), RSP is obtained as the by-product. In our previous work  
69 (Pohl et al., 2018, 2019; Yates et al., 2019) we studied the potential to increase the value of RSP by determining  
70 whether it can be used to prevent and/or treat neurodegenerative disease. Studies on the RSP extract and its secondary  
71 metabolites, had identified sinapine as the main component together with several phenolic acids,  
72 flavonoids/coumarins, benzaldehydes, indoles and amines (Pohl et al., 2018; Yates et al., 2019). Furthermore, we  
73 showed that antioxidant property of the RSP extract was mainly due to sinapine and other phenolic acids (caffeic,  
74 syringic, sinapic and ferulic acids). The RSP extract also showed acetylcholinesterase inhibition activity and the  
75 capacity to protect pBR22 plasmid DNA that had been inflicted with oxidative stress initiated by 2,2'-Azobis(2-  
76 amidinopropane) dihydrochloride (AAPH) (Pohl et al., 2018; Yates et al., 2019).

77 Here we report for the first time, the antioxidant protective effects of RSP extract in the human neuroblastoma  
78 cell line SH-SY5Y. This cell line, notwithstanding its limitations, has been studied regularly as an experimental model  
79 in studies that needed neuron-like cells for research into Parkinson's- and Alzheimer's diseases as well as ALS  
80 (Koriyama, Furukawa, Muramatsu, Takino, & Takeuchi, 2015; Krishna et al., 2014; Xicoy, Wieringa, & Martens,  
81 2017; Yu et al., 2012). Previous research in the field of neurodegeneration using this cell line and natural plant extracts  
82 include, amongst others, the study of *Liriope platyphylla* (Lily turf) (Park et al., 2015), four medicinal plants (*Crinum*  
83 *bulbispermum*, *Scadoxus puniceus* *Zanthoxylum capense*, and *Lannea schweinfurthii*) (Seoposengwe, van Tonder, &  
84 Steenkamp, 2013), tea extracts (green and black tea) (Levites, Youdim, Maor, & Mandel, 2002) as well as Ginkgo  
85 biloba extract (EGb761) (Shi et al., 2009). After oxidative stress was induced, the potential of RSP extracts to prevent  
86 the induced cellular changes were determined. This included ROS production, cellular nuclear DNA strand breakage,  
87 lipid peroxidation and the expression of cell stress-related proteins.

88  
89

## 90 2 MATERIALS AND METHODS

### 91 2.1 Rapeseed pomace extract

92 RSP (harvest 2014) was supplied by Mackintosh of Glendaveny (Mains of Buthlaw, Glendaveny, Peterhead, Scotland,  
93 (www.macintoshofglendaveny.co.uk) and the extract prepared as previously described (Pohl et al., 2018; Yates et al.,  
94 2019). Briefly, 6 g ground RSP (125-710  $\mu\text{m}$ ) was weighed into a filtration extraction thimble (Fioroni S.A X25  
95 cellulose thimble 33 x 80 mm) in a Soxhlet extractor (Gerhardt; Soxtherm SE 416) and lipids were extracted using  
96 petroleum ether (140 mL) for 45 min at 150°C. That oil fraction was discarded. After overnight drying, the de-fatted  
97 pomace was subjected to another Soxhlet extraction with ethanol/water mixture (95:5, v:v, 140 mL, 45 min at 240°C).  
98 The resulting extract was evaporated to dryness (Büchi Rotavapor R-114) and freeze dried (Edwards, Freeze Dryer  
99 Modulyo)(Pohl et al., 2018). After several extractions, all the extracts were combined, homogenized and vacuum  
100 packed prior to storage at -80°C until further use (Pohl et al., 2019).

### 101 2.2. Cell culture

102 SH-SY5Y neuroblastoma cells (from ECACC, cat number 94030304, PHE, UK) were cultured as described before  
103 (Smith et al., 2005) with slight modifications. Supplemented media was prepared with DMEM medium (DMEM, high  
104 glucose, pyruvate), FBS (10%), non-essential amino acids (1%, NEAA) and Penicillin/Streptomycin (1%, Pen/Strep);  
105 all from Fisher Scientific, UK. All cell culture flasks and plates/flasks were kept at 37°C in a humidified incubator  
106 under 5% CO<sub>2</sub> atmosphere. Cells were passaged when the levels of confluency reached 80% to 90%. In all  
107 experiments, only adherent cells were used. The latter were washed with PBS and/or lysed prior to proceeding with  
108 the experiments described in sections 2.3 to 2.7.

### 109 2.3. Cell viability assay using Resazurin

110 In 96-well plates, SH-SY5Y cells (10<sup>4</sup>/100  $\mu\text{L}$ /well) were seeded and left to incubate for 24 hours. The cells were then  
111 exposed to RSP extract (0-5 mg/mL) for 24 hours. After the addition of resazurin (20  $\mu\text{L}$ , R&D systems, UK) to each  
112 well, the plate was incubated (37°C) for 4 hours before measuring fluorescence intensity at 530/25 nm (excitation)  
113 and 590/35 nm (emission). The experiment was carried out 3 times independently with 3 replicates for each treatment  
114 per plate. The fluorescence intensity (fl) means of the three experiments (exp) were added and divided by 3 ( $mean =$   
115  $(fl\ exp\ 1 + fl\ exp\ 2 + fl\ exp\ 3)/3$ ). The mean of untreated cells (0 mg/ml RSP extract) was set to 100% and the  
116 other wells normalized to the untreated control using GraphPad Prism software.

### 117 2.4. ROS detection

118 2,7'-dichlorofluoresceindiacetate (H<sub>2</sub>DCFDA, Invitrogen, Life Technologies, Fisher Scientific, UK) was used to  
119 determine ROS production in SH-SY5Y cells. Cells (7 x 10<sup>5</sup>) were cultured in T25 culture flasks and treated with RSP  
120 extract (1 mg/ml) for 24 hours. The RSP extract was then removed and cellular oxidative stress was triggered with  
121 H<sub>2</sub>O<sub>2</sub> (1 mM) for 30 minutes. The H<sub>2</sub>O<sub>2</sub> was removed followed by the addition of H<sub>2</sub>DCFDA (1  $\mu\text{M}$  in medium) and  
122 exposed for 45 min. The cells were collected after trypsinization. After resuspension of cell pellet (PBS), the latter  
123 was subjected by flowcytometric analysis on a Coulter Epics XL-MCL flow cytometer (EXPO32 ADC XL 4 color,  
124 Beckman Coulter, UK) at 525 nm fluorescence signal. To record and analyze the 10,000 events, the EXPO32 ADC  
125 software (Beckman Coulter, UK) was applied. The % of ROS production (% of events/cells after set threshold of 9<sup>th</sup>  
126 decade of 1<sup>st</sup> log) was determined from the FL-1 plots (**Supplementary Information**, Figure 1). The experiment was  
127 carried out 4 times independently (n=4) with each treatment in duplicate and means ( $mean = (mean\ \% \ exp\ 1 +$   
128  $mean\ \% \ exp\ 2 + mean\ \% \ exp\ 3 + mean\ \% \ exp\ 4)/4$ ) and standard deviations calculated using GraphPad Prism  
129 software.

### 130 2.5. Lipid peroxidation

131 To determine malondialdehyde (MDA), cells were cultured, treated (1 mg/mL RSP extract) and harvested as described  
132 above for ROS detection. Following cell lysis (~200  $\mu$ L, 150 mM NaCl, 50 mM Tris pH 8.0, 1.0% Triton-X100) for  
133 30 minutes on ice and sonication for a further 15 minutes (4°C), the MDA assay was performed following the method  
134 of Domijan *et al.* (Domijan, Ralić, Radić Brkanac, Rumora, & Žanić-Grubišić, 2015). Chromatographic analysis was  
135 carried out on a Shimadzu Prominence liquid chromatography system (LC-20AD) with degasser, fitted with a  
136 Phenomenex Hyperclone ODS (C18) 120 column (150 x 4.6 mm; 3  $\mu$ m) and maintained at 40°C. The HPLC was  
137 coupled to an SPD-M20A diode array detector (DAD) and a Shimadzu RF-10A XL fluorescence detector. The DAD  
138 was set at 532 nm whilst the fluorescence detector was operated at wavelengths 527 nm (excitation) and 551 nm  
139 (emission). Quantification was carried out by external standard calibration.

140 The mobile phase conditions included buffers A (20 mM ammonium acetate in water) and B (20 mM ammonium  
141 acetate in methanol) (Fisher Scientific, UK). The chromatographic conditions were flow rate of 1 mL/min over 20  
142 min and an injection volume of 10  $\mu$ L. The gradient programme was: 90% A – 10% B initially, changed to 5% A –  
143 95% B over 10 min, held for 5min then changed to 90% A – 10% B over 5 min. There was an equilibrium step after  
144 each run.

145

## 146 2.6. Comet assay (alkaline)

147 SH-SY5Y cells were incubated with RSP extract (1 mg/mL) or vehicle control for 24 hours, followed by removal of  
148 the RSP extract and exposure to H<sub>2</sub>O<sub>2</sub> (300 and 1000  $\mu$ M) in medium for 30 minutes; 37°C. Cells were harvested after  
149 trypsinization and DNA strand breakage was analyzed using single-cell gel electrophoresis (SCGE, comet assay) as  
150 reported by Barron *et al.* (Barron *et al.*, 2015) and Duthie *et al.* (Duthie, Narayanan, Blum, Pirie, & Brand, 2009). To  
151 determine the concentration of H<sub>2</sub>O<sub>2</sub> that induced DNA strand breakage, initially a concentration gradient (100-1000  
152  $\mu$ M H<sub>2</sub>O<sub>2</sub>) was tested. Concentrations of 300 and 1000  $\mu$ M H<sub>2</sub>O<sub>2</sub> were chosen for subsequent study of the RSP extract.  
153 All the comets were manually scored following the method of Heuser *et al.* (Heuser, Erdtmann, Kvitko, Rohr, & da  
154 Silva, 2007). Briefly, a fluorescence microscope (Leica DMRB (Germany), 200x magnification) with wavelengths  
155 340-380 nm (excitation) and 425 nm (emission) was used to score a total number of 100 comets with arbitrary numbers  
156 based on 5 recognizable classes of comet, from class 0 (undamaged, no discernible tail) to class 4 (almost all DNA in  
157 tail, insignificant head) as described by Duthie *et al.* (Duthie, Ma, Ross, & Collins, 1996) and is shown in  
158 **Supplementary Information**, Figure 2. 100 comets are recorded from each gel giving a score between 0-400 for each  
159 scored gel. Three gels were scored for each experimental condition per experiment and the mean value determined  
160 ( $mean = (score\ gel1 + score\ gel2 + score\ gel3)/3$ ). Statistical analysis was applied on three independent  
161 experiments (n=3) using GraphPad Prism software.

## 162 2.7. Protein array (cell stress)

163 The relative levels of selected human cell stress proteins was studied with the proteome profiler (Cat# ARY018, R&D  
164 Systems, UK) and carried out following the instructions from the manufacturer (R&D Systems Inc., 2014). Briefly  
165 the cells were seeded (1 x 10<sup>6</sup>) in T25 flasks followed by RSP extract treatment (1 mg/mL) for 24 hours, then H<sub>2</sub>O<sub>2</sub> (1  
166 mM in medium) for 30 minutes after the RSP extract had been removed. The cells were trypsinized, collected and  
167 lysed according to the proteome profiler kit instructions (R&D Systems Inc., 2014). Each treatment was performed in  
168 duplicate and the protein contents were deduced with the DC protein assay (BIO-RAD, UK) according to the  
169 manufacturer's instructions [22]. The membranes were exposed to X ray films (CL Xposure, ThermoScientific, UK)  
170 for 10 to 15 min. After the development of the films (Developer and Fixer solution from Sigma, UK, **Supplementary**  
171 **Information**, Figure 3), spot analysis was performed as described in (Jaul & Barron, 2017) using a FUSION FX7™  
172 imaging instrument with Fusion 1 and BIO-1D™ imaging software (PeqLab; VWR International Ltd., UK). The data  
173 for the same treatment were pooled together prior to calculating the ratio of Treatment/Control.

174

## 175 2.8. Statistical analysis

176

177 All experiments, except the protein array, were carried out at least 3 times independently. Statistical analysis was  
178 undertaken using GraphPad Prism 7. Data was checked for normal distribution and outliers, and comparisons were  
179 made using one-way ANOVA followed by Bonferroni post-hoc test (multiple comparison).

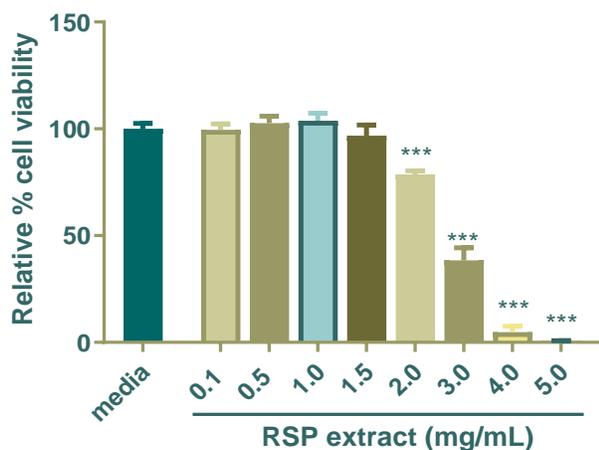
### 180 3 RESULTS AND DISCUSSION

#### 181 3.1. RSP extract toxicity towards SH-SY5Y cells

182 Several plant extracts have previously been tested for their toxicity and *in vitro* properties, and have therefore  
183 been suggested for potential use as treatments for neurodegenerative diseases (Mohd Sairazi & Sirajudeen, 2020; Pohl  
184 & Kong Thoo Lin, 2018). Here in this study, for the first time, we studied the effect of RSP extract on human SH-  
185 SY5Y neuroblastoma cells for its toxicity and cytoprotective properties.

186 Undifferentiated human SH-SY5Y cells were chosen as the cellular system to study the protective properties of  
187 RSP on markers of oxidative stress and DNA damage due to their many advantages over other models as reviewed by  
188 Xicoy *et al.* (Xicoy *et al.*, 2017), Kumar Dubey *et al.* (Dubey *et al.*, 2019) and Kovalevich and Langford (Kovalevich  
189 & Langford, 2013). Nevertheless, they have a number of limitations, such as their cancer properties, as they are derived  
190 from SH-N-SH, uncloned neuroblastoma cells (Biedler, Helson, & Spengler, 1973). However, they are easy to culture  
191 and hence have been used in many publications as a first study model for testing protective properties of natural  
192 products in neuron like cells (Koriyama *et al.*, 2015; Krishna *et al.*, 2014; Morán-Santibañez *et al.*, 2019; Xicoy *et al.*,  
193 2017; Yu *et al.*, 2012).

194 SH-SY5Y cells showed high tolerability towards the RSP extract with concentrations up to 1.5 mg/mL exhibiting  
195 no significant impact on cellular metabolism (toxicity). Only concentrations above 2 mg/mL of RSP extract had a  
196 significant effect on cell viability (Figure 1).



197

198 **Fig. 1** Cell viability assay of RSP extract (0.1-5.0 mg/mL, 24 h treatment) using Resazurin. One-way ANOVA  
199 and Bonferroni's multiple comparison analysis was used to assess statistical significance relative to media  
200 control: \*\*\* $p \leq 0.001$ ,  $n=3$

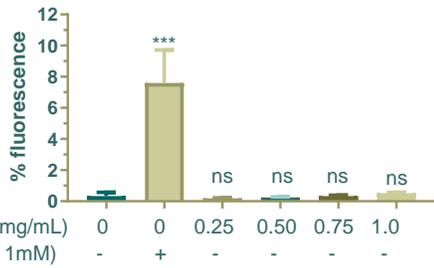
201 The SH-SY5Y cells exhibited higher tolerance to the RSP extract in comparison to other natural product extracts  
202 published previously. For example, H.E. Moore (corozo fruit) reported lower SH-SY5Y cell viability with an ethanol  
203 extract of *Bactris guineensis* (L.) at a concentration of 0.15 mg/mL (López *et al.*, 2017), meaning that the latter plant  
204 extract is 10 times more toxic than our the RSP extract. Similarly, even lower concentrations (0.05 and 0.10 mg/mL)  
205 were found to cause significant decrease in cell viability with *Agaricus blazei* (almond mushroom) extract (Venkatesh  
206 Gobi *et al.*, 2018). An elevated tolerance towards the RSP extract is advantageous, since higher extract concentrations  
207 can be used to study cells protection from oxidative stress in the current study.

208

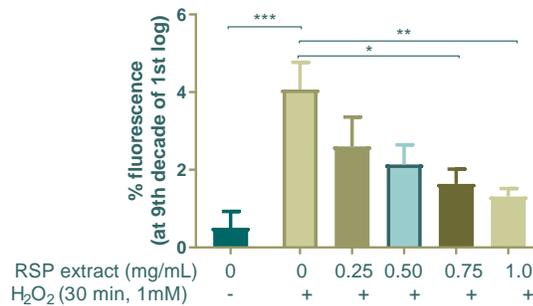
209 3.2. Protection of SH-SY5Y cells with RSP extract from induced H<sub>2</sub>O<sub>2</sub> ROS production

210 Previously, plant extracts have demonstrated *in vitro* protection from ROS. In the case of RSP extract, treatment of  
211 SH-SY5Y cells with extracts at 0.25-1.0 mg/mL did not induce ROS production; however, significant increase  
212 ( $p \leq 0.001$ ) in ROS levels was observed with H<sub>2</sub>O<sub>2</sub> (1 mM, 30 min, Figure 2a, **Supplementary Information**, Figure  
213 1). However, the pre-treatment of cells with RSP extract (0.25-1.0 mg/mL) for 24 hours followed by H<sub>2</sub>O<sub>2</sub> (1 mM)  
214 incubation for 30 minutes, resulted in significant suppression of ROS production by 60% ( $p \leq 0.05$ ) and 68% ( $p \leq 0.01$ )  
215 for 0.75 and 1.0 mg/mL RSP extract, respectively (Figure 2b, **Supplementary Information**, Figure 1).  
216

217 **a**



**b**



218

219 **Fig. 2** (a) Generation of ROS in SH-SY5Y cells treated with H<sub>2</sub>O<sub>2</sub> (1 mM, 30 min) at varying RSP extract  
220 concentrations (0.25-1.0 mg/mL); (b) ROS generation after 24 h RSP extract pre-treatment followed by exposure  
221 to H<sub>2</sub>O<sub>2</sub> (1 mM, 30 minutes); One-way ANOVA and Bonferroni's multiple comparison analysis was used to  
222 assess statistical significance relative to media control: ns: not significant; \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ ,  $n \geq 3$

223 Of note, Park *et al.* (Park et al., 2015) reported that SH-SY5Y cells stressed with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) after  
224 pretreatment with *Liriope platyphylla* extract (0.5-50  $\mu$ g/mL) for 6 hours, resulted in a decrease of ROS  
225 concentrations. In another study, phenolic extracts of *Inula helenium* (0.5, 5  $\mu$ g/mL and 1 hour cellular pretreatment)  
226 significantly reduced ROS production after 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> treatment (Wang, Zhao, Zhang, & Guo, 2015). In contrast  
227 to the latter two papers in which extracts' pre-treatment was carried out for one and six hours respectively, in our study  
228 pre-treatment with RSP extract was for 24 hours. Direct comparison among previous studies to determine which  
229 extract showed better protection are difficult due to the different concentrations of H<sub>2</sub>O<sub>2</sub> and extract employed. ROS  
230 are well accepted to be the root of oxidative stress within a cellular system. This is because oxidative stress can lead  
231 to DNA strand breaks (nuclear) and lipid peroxidation. Therefore, here we studied the ability of RSP extract to prevent  
232 or reduce these consequences as a result of increased ROS levels.

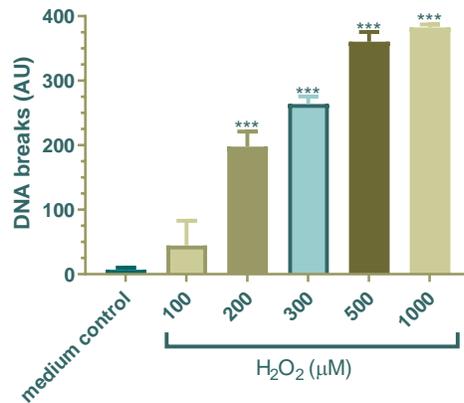
233 3.3. H<sub>2</sub>O<sub>2</sub> (1 mM) does not induce measurable levels of MDA as result of lipid peroxidation in SH-SY5Y cells

234 The degree of lipid peroxidation induced by treating SH-SY5Y cells with H<sub>2</sub>O<sub>2</sub> (1 mM) for 30 minutes was very  
235 minimal and below the limit of detection (data not shown). Interestingly similar observation was reported when H<sub>2</sub>O<sub>2</sub>  
236 was utilised as the stressor against Jurkat T cells in a previous work by Erba *et al.* (Erba, Riso, Criscuoli, & Testolin,  
237 2003).

238 3.4. Prevention of DNA strand breaks induced by H<sub>2</sub>O<sub>2</sub> (300 mM) with RSP extract pretreatment

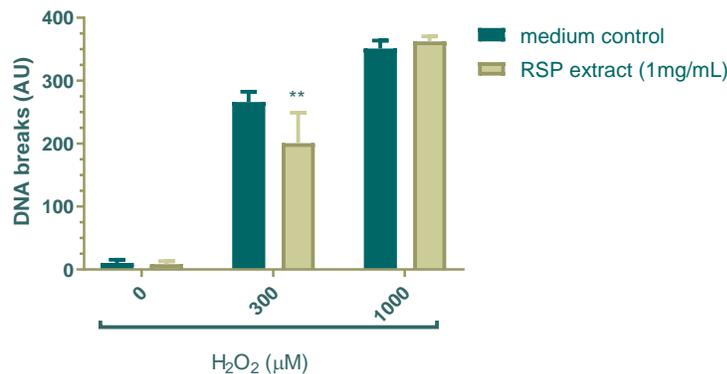
239 In previous studies we demonstrated that RSP extract prevented plasmid DNA damage (cuts from supercoiled  
 240 to circular and linear forms) *in vitro* induced by 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) (Pohl et al.,  
 241 2018; Yates et al., 2019). To determine whether DNA damage can be reduced/prevented in a cellular environment,  
 242 the comet assay (single cell gel electrophoresis) was carried out on SH-SY5Y cell line. Increasing H<sub>2</sub>O<sub>2</sub> concentrations  
 243 (100-1000 μM) showed the expected increase in DNA breaks (Figure 3a). However, when cells were pre-treated with  
 244 only RSP extract, no increase in DNA strand breaks was observed (Figure 3a), thus confirming the safe RSP extract  
 245 concentration (1 mg/mL) towards SH-SY5Y cells used in this study.  
 246

a



247  
 248

b



249

250 **Fig. 3** (a) DNA strand breaks induced by H<sub>2</sub>O<sub>2</sub> (30 minutes) in a concentration-dependent manner; (b) Cellular  
 251 DNA protection from H<sub>2</sub>O<sub>2</sub> (300 but not 1000 μM) induced DNA strand breaks by the RSP extract (1 mg/mL,  
 252 24 h preincubation); One-way ANOVA and Bonferroni's multiple comparison analysis was used to assess  
 253 statistical significance relative to media control: \*\*p<0.01\*\*\*p<0.001, n≥3; AU-arbitrary units

254 In addition, RSP extract (1 mg/mL) pretreated cells were able to partially prevent H<sub>2</sub>O<sub>2</sub>-induced DNA strand  
 255 breaks (Figure 3b). This effect further supports the RSP extracts protective properties at molecular level *in vitro*. We  
 256 would like to highlight that this is the first reported study where RSP extract has been used to protect cellular DNA  
 257 from H<sub>2</sub>O<sub>2</sub>-induced DNA strand breaks. There are only a handful of similar studies in the literature where natural  
 258 compounds/extracts had previously been used to prevent DNA strand breakage in SH-SY5Y cells. For example, in a  
 259 report by Kim *et al.* (H. S. Kim, Lee, Kang, Lee, & Hyun, 2012), they showed similar positive results with the comet  
 260 assay. They applied a pre-treatment with phloroglucinol (1,3,5-trihydroxybenzene), a phenolic (phlorotannin)  
 261 commonly found in *Ecklonia cava* (brown algae). The latter prevented high ROS levels, induced by H<sub>2</sub>O<sub>2</sub> (0.8 mM)  
 262 and showed DNA protective properties (10 μg/mL). Furthermore, boysenberry and blackcurrant extracts (rich in

263 phenolic or/and anthocyanin) exhibited DNA protective properties in HL-60 cells with the comet assay (Ghosh,  
264 McGhie, Zhang, Adaim, & Skinner, 2006).

### 265 3.5 Cell stress protein levels show changes upon RSP extract and H<sub>2</sub>O<sub>2</sub> treatment

266 Protein, DNA and lipid damage to neuron cells is the main contributory factor to the development of many  
267 neurodegenerative diseases. Those compounds with the ability to significantly reduce ROS levels and protect DNA  
268 strand breakage when under oxidative conditions, could lead to candidates that can further be developed to prevent,  
269 manage, and treat these neurological disorders. Hence, further future studies to decipher the mechanism of how RSP  
270 extract protects DNA from damage is required. However, a possible explanation for the protection properties of the  
271 RSP extract on SH-SY5Y cells, may arise directly from its inherent antioxidant properties. Furthermore, the RSP  
272 extract could also impart indirect cellular protection *via* the modulation of cellular signaling pathways. From the  
273 literature there has been several reports showing that many extracts and isolated compounds activate the Nrf2 pathway  
274 both *in vitro* and *in vivo* models. For example, curcumin, sulforaphane and resveratrol are a selection of compounds  
275 that are known to trigger antioxidant and detoxification enzymes after the upregulation Nrf2 expressions (Hodges &  
276 Minich, 2015; Stefanson & Bakovic, 2014). Here further *in vitro* investigation to the effect of the RSP extract on the  
277 cell stress protein expression was carried out with protein arrays analysis.  
278

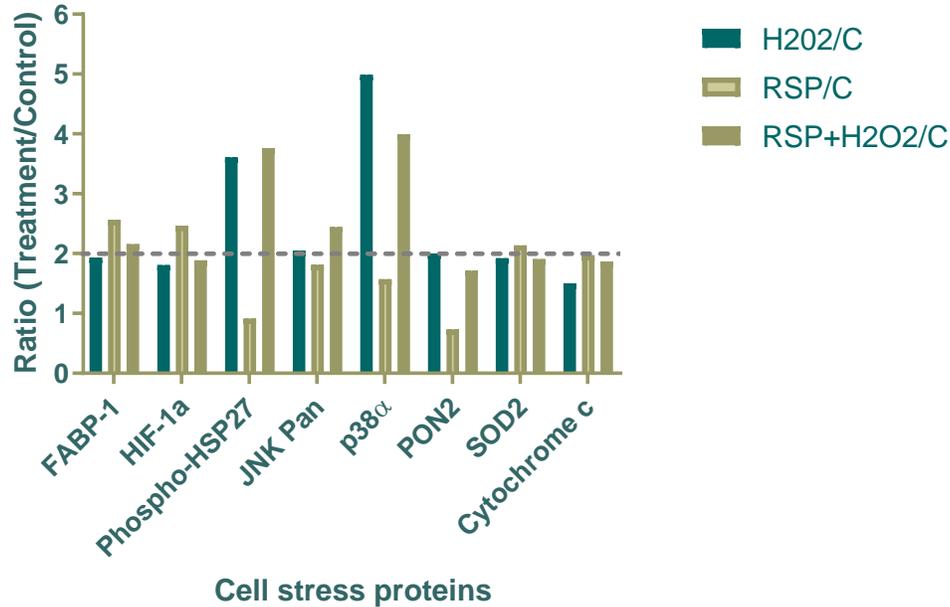
279 Protein arrays were used to study the potential stress related pathways affected by pre-treating SH-SY5Y cells  
280 with RSP extract followed by H<sub>2</sub>O<sub>2</sub>- induced stress (**Supplementary Information**, Figure 3). For the array analysis,  
281 a cut-off of 2-fold increase and/or 0.5-fold decrease was set. Sixteen out of 26 cell stress related proteins studied  
282 showed no change after either RSP extract (24 h, 1 mg/mL) and/or hydrogen peroxide treatment (30 min, 1 mM)  
283 (Table 1) and were not studied further.

284 **Table 1** List of stress related proteins not affected by treatment with 1 mM H<sub>2</sub>O<sub>2</sub> for 30 min (H<sub>2</sub>O<sub>2</sub>/C), 1  
285 mg/mL RSP for 24 hr (RSP/C), or both treatments (RSP+H<sub>2</sub>O<sub>2</sub>/C), and their relative expression (treatment  
286 to control ratio between 0.5 and 2, the set cut-off points) in SH-SY5Y cells

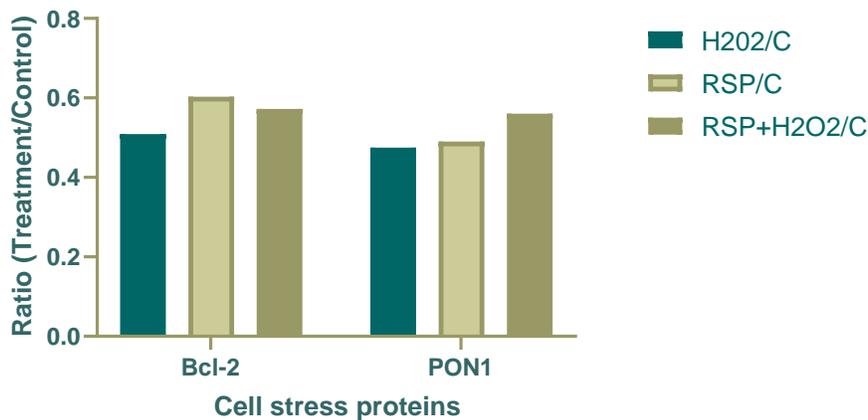
Cell stress protein	H <sub>2</sub> O <sub>2</sub> /C	RSP/C	RSP+H <sub>2</sub> O <sub>2</sub> /C
<b>ADAMTS1</b>	1.3	1.3	1.3
<b>Carb. Anhydrase</b>	1.2	1.0	1.1
<b>Cited-2</b>	1.3	1.8	1.5
<b>COX-2</b>	1.4	1.4	1.4
<b>Dkk-4</b>	1.5	1.8	1.6
<b>HIF-2a</b>	1.5	0.8	1.0
<b>HSP60</b>	1.7	1.5	1.6
<b>HSP70</b>	1.5	1.7	1.8
<b>IDO</b>	1.0	1.0	1.1
<b>NFkB1</b>	1.5	1.6	1.5
<b>p21</b>	1.6	1.8	1.4
<b>p27</b>	1.4	1.9	1.2
<b>Phospho-p53</b>	1.7	0.8	1.7
<b>PON3</b>	1.1	0.6	1.1
<b>Thioredoxin-1</b>	1.5	1.8	1.8
<b>SIRT2</b>	1.5	1.7	1.7

287  
288 On the other hand, protein array analysis showed upregulation of eight cell stress proteins (FABP-1, HIF-1 $\alpha$ ,  
289 phospho-HSP27, JNK Pan, p38 $\alpha$ , PON2, SOD2 and Cytochrome c). Of those, phospho-HSP27, p38 $\alpha$  and PON2 cell  
290 stress proteins were upregulated to  $\geq 2.0$  upon RSP extract and/or H<sub>2</sub>O<sub>2</sub> treatment (Figure 4) while only two proteins

291 (Bcl-2 and PON1) were down regulated to  $\leq 0.5$  (Figure 5). p38 $\alpha$  was upregulated 5-fold by H<sub>2</sub>O<sub>2</sub> treatment but upon  
 292 RSP extract pretreatment, its upregulation was decreased 4-fold (Figure 4). p38 $\alpha$  is a mitogen-activated protein kinase  
 293 (MAPK) known to be triggered by the presence of ROS and other physical and chemical stressors (Matsuzawa &  
 294 Ichijo, 2008). In our analysis, p38 $\alpha$  levels were highly increased (5-fold) by H<sub>2</sub>O<sub>2</sub> but only changed slightly (1.6-fold)  
 295 by RSP extract treatment compared to the control. Similarly to our observations in H<sub>2</sub>O<sub>2</sub> treated SH-SY5Y cells, Xia  
 296 *et al.* (Xia, Dickens, Raingeaud, Davis, & Greenberg, 1995) demonstrated in PC12 (neuron-like pheochromocytoma  
 297 cells) that withdrawal of nerve growth factors can lead to similar upregulation of p38 $\alpha$  and that this was required for  
 298 induction of apoptosis. This implies that a reduction in p38 $\alpha$  would reduce neuronal cell death, which is an important  
 299 factor in neuro-degenerative diseases.



300  
 301 **Fig. 4** Relative proteins' amount in SH-SY5Y cells treated with 1 mg/ml RSP for 24 hr and/or 1 mM H<sub>2</sub>O<sub>2</sub> for 30 min  
 302 with ratio treatment to control  $\geq 2.0$ ; experiment performed in duplicate



303  
 304 **Fig. 5** Relative proteins' amount in SH-SY5Y cells treated with 1 mg/ml RSP for 24 hr and/or 1 mM H<sub>2</sub>O<sub>2</sub> for  
 305 30 min with ratio treatment to control  $\leq 0.5$ , experiment performed in duplicate

306 Interestingly two other cell stress related proteins i.e. Bcl-2 and PON1 were down regulated after H<sub>2</sub>O<sub>2</sub> treatment  
307 (1 mM for 30 minutes) to 0.5 whereas only PON1 was down regulated by RSP extract (1 mg/mL) treatment to 0.5  
308 (Figure 5) and there were no further changes with RSP pre-treatment. It is known that the regulation of Bcl-2 is  
309 associated with apoptosis and cell death (Chi, Chang, & Sang, 2018) while PON-1 has previously shown to have  
310 protective properties (Borowczyk, Shih, & Jakubowski, 2012; M. J. Kim et al., 2015; Levy, Reichert, & Bydlowski,  
311 2019).

312 Importantly, most proteins in this study, were either not affected by the pretreatment with the RSP extract  
313 compared to untreated cells (10 proteins) nor there was a change in levels compared to the H<sub>2</sub>O<sub>2</sub> treatment alone (six  
314 proteins). It is noteworthy to mention that interpretation of the protein array is however limited due to the number of  
315 proteins up ( $\geq 2$ -fold) and/or down ( $\leq 0.5$ -fold) regulated (10 in total) and the complexity of their interactions in this  
316 cell system.

317

#### 318 **4.0 CONCLUSION**

319 Damage to biomolecules such as DNA, protein and lipid in neurons are well known to be responsible for the  
320 development of many neurodegenerative diseases. The ability of compounds to protect DNA strand breakage and  
321 reduce ROS levels when under oxidative attack, could provide potential therapeutic candidates in the prevention,  
322 management and treatment of these neurological disorders. Here we have demonstrated at cellular level that RSP  
323 extract at a non-toxic concentration reduces the levels of ROS and provide cellular protection by reducing oxidative  
324 DNA damage in the presence of an oxidizing agent (H<sub>2</sub>O<sub>2</sub>). Furthermore, the potential indirect antioxidant activity of  
325 the RSP extract through the modulation of antioxidant pathways within the cell system was exploited. We found in  
326 pretreated cells, the downregulation of p38 $\alpha$ , a mitogen-activated protein kinase amenable to stress stimuli. Taken  
327 together the protective properties of the RSP extract presented in this study while considering the promising effects  
328 showed *in vivo* studies in *C. elegans* (Pohl et al., 2019), warrant further studies to understand the full potential of the  
329 RSP extract on oxidative stress and in particular, in the context of neuro-degenerative diseases.

330

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335

#### 336 **CONFLICTS OF INTEREST**

337 The authors declare no conflict of interest.

#### 338 **REFERENCES**

- 339 Barron, G. A., Goua, M., Kuraoka, I., Bermano, G., Iwai, S., & Kong Thoo Lin, P. (2015). Bisnaphthalimidopropyl  
340 diaminodicyclohexylmethane induces DNA damage and repair instability in triple negative breast cancer cells  
341 via p21 expression. *Chemico-Biological Interactions*, 242, 307–315. <https://doi.org/10.1016/j.cbi.2015.10.017>
- 342 Biedler, J. L., Helson, L., & Spengler, B. a. (1973). Morphology and Growth , Tumorigenicity , and Cytogenetics of  
343 Human Neuroblastoma Cells in Continuous Culture Morphology and Growth , Tumorigenicity , and  
344 Cytogenetics of Human Neuroblastoma Cells in Continuous Culture1. *Cancer Research*, 33(NOVEMBER),  
345 2643–2652. <https://doi.org/10.1007/PL00000826>
- 346 Borowczyk, K., Shih, D. M., & Jakubowski, H. (2012). Metabolism and neurotoxicity of homocysteine thiolactone in  
347 mice: Evidence for a protective role of paraoxonase 1. *Journal of Alzheimer's Disease*, 30(2), 225–231.  
348 <https://doi.org/10.3233/JAD-2012-111940>
- 349 Brown, R. C., Lockwood, A. H., & Sonawane, B. R. (2005). Neurodegenerative diseases: an overview of  
350 environmental risk factors. *Environmental Health Perspectives*, 113(9), 1250–1256. Retrieved from  
351 <http://www.ncbi.nlm.nih.gov/pubmed/16140637>
- 352 Chi, H., Chang, H.-Y., & Sang, T.-K. (2018). Neuronal Cell Death Mechanisms in Major Neurodegenerative Diseases.  
353 *International Journal of Molecular Sciences*, 19(10). <https://doi.org/10.3390/ijms19103082>
- 354 Dimitriadi, M., & Hart, A. C. (2010). Neurodegenerative disorders: insights from the nematode *Caenorhabditis*

355 elegans. *Neurobiology of Disease*, 40(1), 4–11. <https://doi.org/10.1016/j.nbd.2010.05.012>  
 356 Domijan, A.-M., Ralić, J., Radić Brkanac, S., Rumora, L., & Žanić-Grubišić, T. (2015). Quantification of  
 357 malondialdehyde by HPLC-FL - application to various biological samples. *Biomedical Chromatography*, 29(1),  
 358 41–46. <https://doi.org/10.1002/bmc.3361>  
 359 Dubey, S. K., Ram, M. S., Krishna, K. V., Saha, R. N., Singhvi, G., Agrawal, M., ... Alexander, A. (2019). Recent  
 360 Expansions on Cellular Models to Uncover the Scientific Barriers Towards Drug Development for Alzheimer's  
 361 Disease. *Cellular and Molecular Neurobiology*, 39(2), 181–209. <https://doi.org/10.1007/s10571-019-00653-z>  
 362 Duthie, S. J., Ma, A., Ross, M. A., & Collins, A. R. (1996). Antioxidant Supplementation Decreases Oxidative DNA  
 363 Damage in Human Lymphocytes. *Cancer Research*, 56(6).  
 364 Duthie, S. J., Narayanan, S., Blum, S., Pirie, L., & Brand, G. M. (2009). Folate Deficiency In Vitro Induces Uracil  
 365 Misincorporation and DNA Hypomethylation and Inhibits DNA Excision Repair in Immortalized Normal  
 366 Human Colon Epithelial Cells Folate Deficiency In Vitro Induces Uracil Misincorporation and DNA  
 367 Hypomethylation and, (November 2013), 37–41.  
 368 Emerit, J., Edeas, M., & Bricaire, F. (2004). Neurodegenerative diseases and oxidative stress. *Biomedicine &*  
 369 *Pharmacotherapy*, 58(1), 39–46. <https://doi.org/10.1016/J.BIOPHA.2003.11.004>  
 370 Erba, D., Riso, P., Criscuoli, F., & Testolin, G. (2003). Malondialdehyde production in Jurkat T cells subjected to  
 371 oxidative stress. *Nutrition (Burbank, Los Angeles County, Calif.)*, 19(6), 545–548.  
 372 [https://doi.org/10.1016/s0899-9007\(02\)01010-9](https://doi.org/10.1016/s0899-9007(02)01010-9)  
 373 Farooqui, A. A. (2015). *Inflammation and Oxidative Stress in Neurological Disorders. Statewide Agricultural Land*  
 374 *Use Baseline 2015* (Vol. 1). Springer. <https://doi.org/10.1017/CBO9781107415324.004>  
 375 Ghosh, D., McGhie, T. K., Zhang, J., Adaim, A., & Skinner, M. (2006). Effects of anthocyanins and other phenolics  
 376 of boysenberry and blackcurrant as inhibitors of oxidative stress and damage to cellular DNA in SH-SY5Y and  
 377 HL-60 cells. *Journal of the Science of Food and Agriculture*, 86(5), 678–686. <https://doi.org/10.1002/jsfa.2409>  
 378 Gilgun-Sherki, Y., Melamed, E., & Offen, D. (2001). Oxidative stress induced-neurodegenerative diseases: the need  
 379 for antioxidants that penetrate the blood brain barrier. *Neuropharmacology*, 40(8), 959–975.  
 380 [https://doi.org/10.1016/S0028-3908\(01\)00019-3](https://doi.org/10.1016/S0028-3908(01)00019-3)  
 381 Heuser, V. D., Erdtmann, B., Kvitko, K., Rohr, P., & da Silva, J. (2007). Evaluation of genetic damage in Brazilian  
 382 footwear-workers: Biomarkers of exposure, effect, and susceptibility. *Toxicology*, 232(3), 235–247.  
 383 <https://doi.org/10.1016/J.TOX.2007.01.011>  
 384 Hodges, R. E., & Minich, D. M. (2015). Modulation of Metabolic Detoxification Pathways Using Foods and Food-  
 385 Derived Components: A Scientific Review with Clinical Application. *Journal of Nutrition and Metabolism*,  
 386 2015, 760689. <https://doi.org/10.1155/2015/760689>  
 387 Jaul, E., & Barron, J. (2017). Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old  
 388 and Over Population. *Frontiers in Public Health*, 5, 335. <https://doi.org/10.3389/fpubh.2017.00335>  
 389 Kim, H. S., Lee, K., Kang, K. A., Lee, N. H., & Hyun, J. W. (2012). Phloroglucinol exerts protective effects against  
 390 oxidative stress-induced cell damage in SH-SY5Y cells. *J Pharmacol Sci*, 119(2), 186–192.  
 391 <https://doi.org/10.1254/jphs.12056FP>  
 392 Kim, M. J., Park, M., Kim, D. W., Shin, M. J., Son, O., Jo, H. S., ... Choi, S. Y. (2015). Transduced PEP-1-PON1  
 393 proteins regulate microglial activation and dopaminergic neuronal death in a Parkinson's disease model.  
 394 *Biomaterials*, 64, 45–56. <https://doi.org/10.1016/j.biomaterials.2015.06.015>  
 395 Koriyama, Y., Furukawa, A., Muramatsu, M., Takino, J., & Takeuchi, M. (2015). Glyceraldehyde caused Alzheimer's  
 396 disease-like alterations in diagnostic marker levels in SH-SY5Y human neuroblastoma cells. *Scientific Reports*,  
 397 5(13313), 1–7. <https://doi.org/10.1038/srep13313>  
 398 Kovalevich, J., & Langford, D. (2013). Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology.  
 399 In S. Amini & K. M. White (Eds.), *Neuronal Cell Culture: Methods and Protocols* (vol. 1078, pp. 9–21). New  
 400 York: Springer Science+Business Media. Retrieved from <http://link.springer.com/10.1007/978-1-62703-640-5>  
 401 Krishna, A., Biryukov, M., Trefois, C., Antony, P. M. A., Hussong, R., Lin, J., ... May, P. (2014). Systems genomics  
 402 evaluation of the SH-SY5Y neuroblastoma cell line as a model for Parkinson's disease. *BMC Genomics*, 15(1),  
 403 1–23. <https://doi.org/10.1186/1471-2164-15-1154>  
 404 Levites, Y., Youdim, M. B. H., Maor, G., & Mandel, S. (2002). Attenuation of 6-hydroxydopamine (6-OHDA)-  
 405 induced nuclear factor-kappaB (NF-κB) activation and cell death by tea extracts in neuronal cultures.  
 406 *Biochemical Pharmacology*, 63(1), 21–29. [https://doi.org/10.1016/S0006-2952\(01\)00813-9](https://doi.org/10.1016/S0006-2952(01)00813-9)  
 407 Levy, D., Reichert, C. O., & Bydlowski, S. P. (2019). Paraoxonases activities and polymorphisms in elderly and old-  
 408 age diseases: An overview. *Antioxidants*, 8(5), 1–24. <https://doi.org/10.3390/antiox8050118>

- 409 Liu, Z., Zhou, T., Ziegler, A. C., Dimitrion, P., & Zuo, L. (2017). Oxidative Stress in Neurodegenerative Diseases:  
410 From Molecular Mechanisms to Clinical Applications. *Oxidative Medicine and Cellular Longevity*, 2017, 1–11.  
411 <https://doi.org/10.1155/2017/2525967>
- 412 López, S., Martá, M., Sequeda, L. G., Celis, C., Sutachan, J. J., & Albarracín, S. L. (2017). Cytoprotective action  
413 against oxidative stress in astrocytes and neurons by *Bactris guineensis* (L.) H.E. Moore (corozo) fruit extracts.  
414 *Food and Chemical Toxicology*, 109, 1010–1017. <https://doi.org/10.1016/J.FCT.2017.04.025>
- 415 Matsuzawa, A., & Ichijo, H. (2008). Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP  
416 kinase pathway in stress signaling. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1780(11), 1325–  
417 1336. <https://doi.org/10.1016/J.BBAGEN.2007.12.011>
- 418 Mohd Sairazi, N. S., & Sirajudeen, K. N. S. (2020). Natural Products and Their Bioactive Compounds:  
419 Neuroprotective Potentials against Neurodegenerative Diseases. *Evidence-Based Complementary and*  
420 *Alternative Medicine*, 2020, 5–7. <https://doi.org/10.1155/2020/6565396>
- 421 Morán-Santibañez, K., Vasquez, A. H., Varela-Ramirez, A., Henderson, V., Sweeney, J., Odero-Marrah, V., ... Skouta,  
422 R. (2019). *Larrea tridentata* Extract Mitigates Oxidative Stress-Induced Cytotoxicity in Human Neuroblastoma  
423 SH-SY5Y Cells. *Antioxidants*, 8(10), 427. <https://doi.org/10.3390/antiox8100427>
- 424 Park, H. R., Lee, H., Park, H., Jeon, J. W., Cho, W.-K., & Ma, J. Y. (2015). Neuroprotective effects of *Liriope*  
425 *platyphylla* extract against hydrogen peroxide-induced cytotoxicity in human neuroblastoma SH-SY5Y cells.  
426 *BMC Complementary and Alternative Medicine*, 15, 171. <https://doi.org/10.1186/s12906-015-0679-3>
- 427 Pohl, F., Goua, M., Bermanno, G., Russell, W. R., Scobbie, L., Maciel, P., & Kong Thoo Lin, P. (2018). Revalorisation  
428 of rapeseed pomace extracts: An in vitro study into its anti-oxidant and DNA protective properties. *Food*  
429 *Chemistry*, 239, 323–332. <https://doi.org/10.1016/j.foodchem.2017.06.129>
- 430 Pohl, F., & Kong Thoo Lin, P. (2018). The Potential Use of Plant Natural Products and Plant Extracts with Antioxidant  
431 Properties for the Prevention/Treatment of Neurodegenerative Diseases: In Vitro, In Vivo and Clinical Trials.  
432 *Molecules*, 23(12), 3283. <https://doi.org/10.3390/molecules23123283>
- 433 Pohl, F., Teixeira-Castro, A., Costa, M. D., Lindsay, V., Fiúza-Fernandes, J., Goua, M., ... Lindsay, V. (2019). GST-  
434 4-Dependent Suppression of Neurodegeneration in *C. elegans* Models of Parkinson's and Machado-Joseph  
435 Disease by Rapeseed Pomace Extract Supplementation Strains and General Maintenance. *Frontiers in*  
436 *Neuroscience*, 13(1091), 1–13. <https://doi.org/10.3389/fnins.2019.01091>
- 437 Pollari, E., Goldsteins, G., Bart, G., Koistinaho, J., & Giniatullin, R. (2014). The role of oxidative stress in  
438 degeneration of the neuromuscular junction in amyotrophic lateral sclerosis. *Frontiers in Cellular Neuroscience*,  
439 8, 131. <https://doi.org/10.3389/fncel.2014.00131>
- 440 R&D Systems Inc. (2014). Proteome Profiler™ Array-Human Cell Stress Array Kit.
- 441 Seoposengwe, K., van Tonder, J. J., & Steenkamp, V. (2013). In vitro neuroprotective potential of four medicinal  
442 plants against rotenone-induced toxicity in SH-SY5Y neuroblastoma cells. *BMC Complementary and*  
443 *Alternative Medicine*, 13(353), 1–11. <https://doi.org/10.1186/1472-6882-13-353>
- 444 Shi, C., Zhao, L., Zhu, B., Li, Q., Yew, D. T., Yao, Z., & Xu, J. (2009). Protective effects of Ginkgo biloba extract  
445 (EGb761) and its constituents quercetin and ginkgolide B against  $\beta$ -amyloid peptide-induced toxicity in SH-  
446 SY5Y cells. *Chemico-Biological Interactions*, 181(1), 115–123. <https://doi.org/10.1016/J.CBI.2009.05.010>
- 447 Skovronsky, D. M., Lee, V. M.-Y., & Trojanowski, J. Q. (2006). Neurodegenerative Diseases: New Concepts of  
448 Pathogenesis and Their Therapeutic Implications. *Annual Review of Pathology: Mechanisms of Disease*, 1, 151–  
449 170. <https://doi.org/10.1146/annurev.pathol.1.110304.100113>
- 450 Smith, W. W., Margolis, R. L., Li, X., Troncoso, J. C., Lee, M. K., Dawson, V. L., ... Ross, C. A. (2005).  $\alpha$ -Synuclein  
451 Phosphorylation Enhances Eosinophilic Cytoplasmic Inclusion Formation in SH-SY5Y Cells. *Journal of*  
452 *Neuroscience*, 25(23), 5544–5552. <https://doi.org/10.1523/JNEUROSCI.0482-05.2005>
- 453 Stefanson, A. L., & Bakovic, M. (2014). Dietary regulation of Keap1/Nrf2/ARE pathway: focus on plant-derived  
454 compounds and trace minerals. *Nutrients*, 6(9), 3777–3801. <https://doi.org/10.3390/nu6093777>
- 455 Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and Neurodegenerative Diseases: A  
456 Review of Upstream and Downstream Antioxidant Therapeutic Options. *Current Neuropharmacology*, 7(1),  
457 65–74. <https://doi.org/10.2174/157015909787602823>
- 458 Venkatesh Gobi, V., Rajasankar, S., Ramkumar, M., Dhanalakshmi, C., Manivasagam, T., Justin Thenmozhi, A., ...  
459 Chidambaram, R. (2018). *Agaricus blazei* extract attenuates rotenone-induced apoptosis through its  
460 mitochondrial protective and antioxidant properties in SH-SY5Y neuroblastoma cells. *Nutritional*  
461 *Neuroscience*, 21(2), 97–107. <https://doi.org/10.1080/1028415X.2016.1222332>
- 462 Wang, J., Zhao, Y. M., Zhang, B., & Guo, C. Y. (2015). Protective Effect of Total Phenolic Compounds from *Inula*

463           helenium on Hydrogen Peroxide-induced Oxidative Stress in SH-SY5Y Cells. *Indian Journal of*  
464           *Pharmaceutical Sciences*, 77(2), 163–169. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/26009648>  
465 Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., & Greenberg, M. E. (1995). Opposing effects of ERK and JNK-p38  
466           MAP kinases on apoptosis. *Science (New York, N.Y.)*, 270(5240), 1326–1331.  
467           <https://doi.org/10.1126/science.270.5240.1326>  
468 Xicoy, H., Wieringa, B., & Martens, G. J. M. (2017). The SH-SY5Y cell line in Parkinson’s disease research: a  
469           systematic review. *Molecular Neurodegeneration*, 12(10), 1–11. <https://doi.org/10.1186/s13024-017-0149-0>  
470 Yates, K., Pohl, F., Busch, M., Mozer, A., Watters, L., Shiryayev, A., & Kong Thoo Lin, P. (2019). Determination of  
471           sinapine in rapeseed pomace extract: Its antioxidant and acetylcholinesterase inhibition properties. *Food*  
472           *Chemistry*, 276, 768–775. <https://doi.org/10.1016/J.FOODCHEM.2018.10.045>  
473 Yu, Z., Fan, D., Gui, B., Shi, L., Xuan, C., Shan, L., ... Wang, Y. (2012). Neurodegeneration-associated TDP-43  
474           interacts with fragile X mental retardation protein (FMRP)/Staufen (STAU1) and regulates SIRT1 expression  
475           in neuronal cells. *The Journal of Biological Chemistry*, 287(27), 22560–22572.  
476           <https://doi.org/10.1074/jbc.M112.357582>  
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**Supplementary Information**

**Impact of Rapeseed Pomace (RSP) extract on markers of oxidative stress and DNA damage in human SH-SY5Y cells**

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**Figure 1**

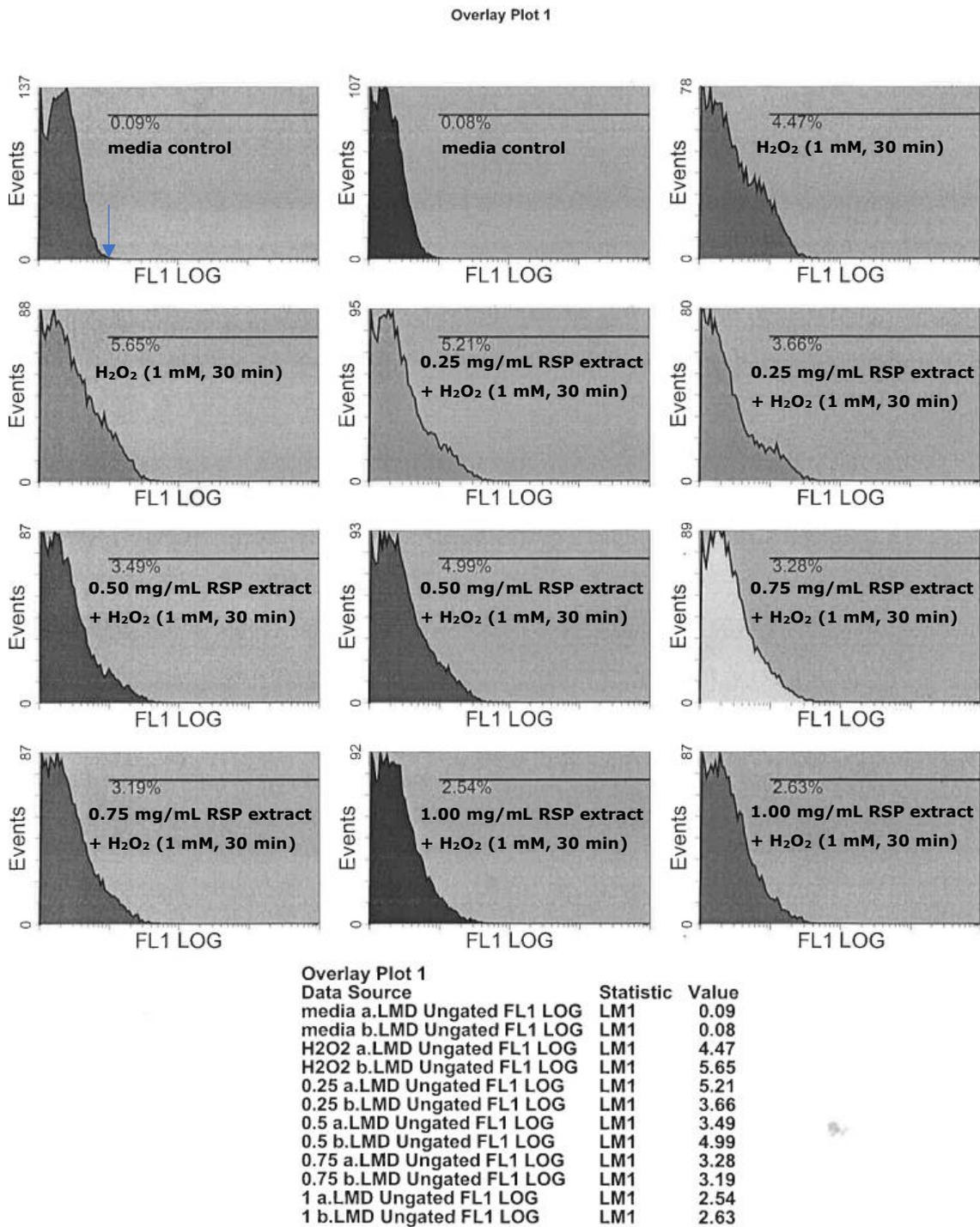


Figure 1. Raw data obtained from flowcytometry, showing Events (number of cells) related to their fluorescence intensity on a logarithmic scale (FL1 LOG) and percentage of events (cells) counted after threshold of 9<sup>th</sup> decade of 1st log (blue arrow)

**Figure 2**

Comet score	Schematic	Criteria
0	 <p>A bright, circular cyan comet head is shown against a dark blue background. A white line points to the head with the label "head".</p>	<ul style="list-style-type: none"> <li>• Bright big circular comet head</li> <li>• No indication of tail</li> </ul>
1	 <p>A bright, circular cyan comet head is shown. To its right is a tail composed of small, bright dots, resembling a sparkle. The tail extends to the right.</p>	<ul style="list-style-type: none"> <li>• Big and bright circular comet head (same as 0)</li> <li>• Sparkle like tail visible; at least the length of the head</li> </ul>
2	 <p>A bright, circular cyan comet head is shown. To its right is a larger, more diffuse tail. A white line points to the tail with the label "tail".</p>	<ul style="list-style-type: none"> <li>• Bright circular comet head, same size as 0 and 1</li> <li>• Comets show significant tail</li> <li>• Tail is less bright than head</li> <li>• Head still has a sharp outer contour</li> </ul>
3	 <p>The comet head and tail are shown as a single, elongated, bright cyan shape. The head is on the left and the tail is on the right, but they are very similar in brightness and have merged in shape.</p>	<ul style="list-style-type: none"> <li>• Big head, but fainter in brightness, similar to tail brightness</li> <li>• Tail and head begin to merge in shape</li> <li>• Head loses sharp circular contour</li> </ul>
4	 <p>A small, dim cyan comet head is shown on the left, and a large, dim cyan tail is shown on the right. The head is clearly separated from the tail.</p>	<ul style="list-style-type: none"> <li>• Small head with decreased brightness</li> <li>• Head is smaller in diameter than tail and looks separated from tail</li> </ul>

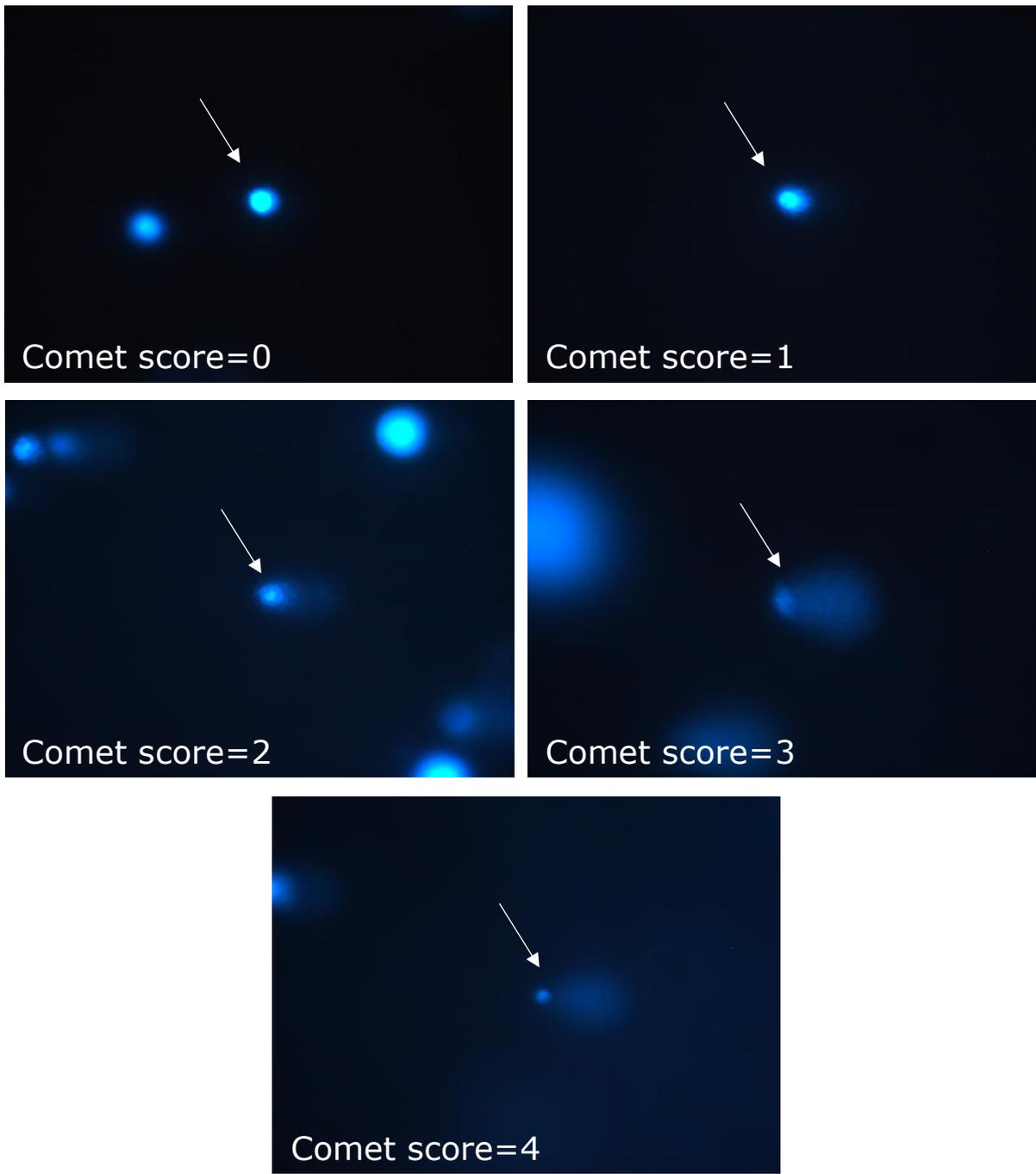


Figure 2: Comet scores and their criteria and SH-SY5Y examples



Coordinate	Colour	Analyte/Control	Alternative name
A1, A2, A21, A22, E1, E2		Reference Spot	/
B3, B4		ADAMTS1	/
C3, C4		HIF-2 $\alpha$	EPAS1
D3, D4		Phospho-p38 (T180/Y182)	
B5, B6		Bcl-2	
C5, C6		Phospho-HSP (S78/S82)	
D5, D6		Phospho-p53 (S46)	
B7, B8		Carbonic Anhydrase IX	CA9
C7, C8		HSP60	
D7, D8		PON1	
B9, B10		Cited-2	
C9, C10		HSP70	
D9, D10		PON2	
B11, B12		COX-2	
C11, C12		IDO	Indoleamine 2,3-dioxygenase
D11, D12		PON3	
B13, B14		Cytochrome c	
C13, C14		Phospho-JNK Pan (T183/Y182)	
D13, D14		Thioredoxin-1	
B15, B16		Dkk-4	
C15, C16		NF $\kappa$ B1	
D15, D16		SIRT2	Sirtuin 2
B17, B18		FABP-1	L-FABP

C17, C18		p21/CIP1	CDNK1A
D17, D18		SOD2	MnSOD
B19, B20		HIF-1 $\alpha$	
C19, C20		p27	Kip1
D19, C20		Negative control	Control (-)

Figure 3 Human cell stress proteins included in the protein array adapted from (R&D Systems Inc., 2014)

R&D Systems Inc. (2014). Proteome Profiler™ Array-Human Cell Stress Array Kit. 1–11.