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Cytoprotective–Antioxidant Effect of *Brunfelsia grandiflora* Extract on Neuron-like Cells

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Abstract: *Brunfelsia grandiflora* is a South American solanaceae widely used since long ago for its recognized medicinal properties. We have recently reported its chemical composition, showing a relevant number of bioactive compounds with antioxidant capacity, and proved the cytoprotective and antioxidative stress potential of *B. grandiflora* in cultured endothelial cells. Since *B. grandiflora* extracts have shown effects on the central nervous system, the present study was designed to show the potential cytoprotective capacity and the antioxidative stress potential of phenolic extracts from the plant on cultured neuron-like cells, as a model to reduce the presentation or effects of chronic diseases of the nervous system. To this end, we studied its reactive oxygen species (ROS)-reducing capacity, its antioxidant defense mechanisms, and some molecular markers involved in redox balance and apoptosis. The results show that cell survival and most changes in biomarkers related to oxidative status, ROS, reduced glutathione, glutathione peroxidase and reductase, malondialdehyde, and caspase 3/7 activity, and molecular expression of cell death-related genes (BAX, BNIP3, and APAF1), NFκB, SOD, and NRF2 (genes from oxidative stress—antioxidants) induced by oxidative stress were prevented by either co- or pretreatment of neuron-like cells with *B. grandiflora* extracts (25–200 µg/mL). The results demonstrate the chemoprotective potential of the plant and support its medicinal use.

Keywords: *Brunfelsia grandiflora*; neuronal cells; cytoprotection; antioxidative stress

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1. Introduction

The imbalance in the production and deactivation of reactive oxygen species (ROS) may lead to disproportionate concentrations of ROS and generate other reactive species and free radicals that are harmful and may react indiscriminately with cellular macromolecules with potentially dramatic consequences on the cell structure and function. Some of these consequences on cells in the central nervous system may lead to neurodegeneration diseases, characterized by progressive functional deterioration and neuronal cellular aging, with severe cytological and physiological damage of neurons [1–3].

Recent evidence suggests that neuronal oxidative stress, facilitated by microglia, leads to neuroinflammation, inducing neuronal necrosis and apoptosis [1–3]. Thus, protection of neuronal tissue from oxidative stress seems to be a promising strategy to prevent the onset

and development of neurodegenerative processes; consequently, protection of neuronal and glial cells against this difficult situation is one of the objectives of recent studies on nutritional and pharmacological prevention of aging and neurodegenerative diseases.

Since the culturing of primary neurons is quite difficult, the use of established cell lines is widely accepted to test the neuroregulatory effect of natural or synthetic active compounds at cellular and molecular levels. The most frequently used neuron-like cell culture is neuroblastoma SH-SY5Y, an established cell line derived from the SK-N-SH cell line. This cell line, suitable for biological, biochemical, and electrophysiological investigation, has been recently validated for studies about neuronal aging [4]. In fact, regarding the use of this cell line as a model of neuronal cell culture, we recently demonstrated its chemoprotective effect against an oxidative stress-induced neurodegeneration of an aqueous extract of cocoa [5] and an extract from the South American fabaceae *Desmodium tortuosum* rich in flavonoids [6].

The aqueous maceration of the root from the South American solanaceae *Brunfelsia glandiflora*, locally known as Chiric sanango, has been used in traditional Peruvian medicine as a drink protecting against autoimmune diseases, infections, somatic pain, and fatigue, and as an antipyretic [7]. The infusion of the leaves is used against arthritis and rheumatism and a decoction of the bark is applied to burns and areas of the body affected by leishmaniasis and as a healing agent [7,8]. The remarkable number of polyphenolic compounds may be responsible for most of these effects [9], but in particular the presence of scopoletin (coumarin with anti-inflammatory activity) would strengthen the effect of *B. grandiflora* against rheumatism, arthritis, body pain, headache, and joint and muscle pain [10]. More interestingly for the present study, extracts from the plant also show hallucinogenic and narcotic properties that might be associated with the presence of brunfelsamidine, cuscohygrin, scopolamine, scopoletin, and esculetin [11,12].

A recently published chemical characterization and description of the antioxidant capacity of *B. grandiflora* [9], shown in the Supplementary Materials (Table S1), revealed a substantial content of hydroxycinnamic acids/hydroxycinnamates that is sufficient to award an outstanding antioxidant capacity. Indeed, we have previously reported on the antioxidative potential of these phenolic compounds in cell culture models [13,14] as well as in experimental animals [15]. In addition to hydroxycinnamic derivatives, extracts from *B. grandiflora* also show relevant amounts of gallic acid, caffeic acid, catechin, and eriodictyol as well as dihydroxybenzoic, dihydroxyphenylacetic, and dihydroxy-75 phenylpropionic acids and their derivatives; all of them enhance the effect of this medicinal plant against oxidative stress.

Several studies have reported on the phytotherapeutic effect against chronic neurodegenerative conditions. Phytochemicals have been found to have anti-inflammatory, antioxidant, and anticholinesterase activities. Several neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and others share common features in their cellular and molecular pathology that induce the neuronal cell to undergo processes of oxidative stress, apoptosis, necroptosis, and inflammation. Widespread use of phytotherapy would offer a promising therapeutic alternative against neurodegenerative disorders. For example, *Withania somnifera* has been described as having memory-enhancing effects and the ability to reduce corticosterone release, activate choline-acetyltransferase, improve synapses, and regenerate axons and dendrites [16,17]. Neuroprotective effects of ginseng have also been observed through inhibition of voltage-dependent calcium channels, reduction of divalent metal transporter DMT1 expression, reduction of ROS production, inhibition of caspase 3 activity, and reduction of nitric oxide generation [18,19]. Curcumin has also been implicated in neuroprotective effects against Alzheimer's disease by stimulating macrophages to clear amyloid plaque and reducing cellular inflammatory processes [20]. Neuroprotective effects of the polyphenol resveratrol against Alzheimer's disease have also been observed [21].

Considering all of the above, the goal of the present study was to investigate whether realistic concentrations of extracts from *B. grandiflora* protect neuron-like cells from chemi-

cally induced oxidative stress. SH-SY5Y cells were used as an in vitro model of neuron-like cells and challenged with tert-butylhydroperoxide (t-BOOH), which was used to induce neuronal oxidative stress. After the antioxidative stress capacity of the plant extracts was asserted, the potential biochemical and molecular mechanisms through which *B. grandiflora* extracts protect neuronal cell viability and function were evaluated through parameters such as markers of redox status and other cell signals. The results seem to support the beneficial effects of the plant for medicinal use.

2. Materials and Methods

2.1. Reagents

Nicotine adenine dinucleotide phosphate reduced salt (NADPH), 2,4-dinitrophen ylhydrazine, antibiotics, 2′,7′-dichlorofluorescin-diacetate (DCFH-DA), 3-[4,5 di-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), t-BOOH, glutathione reductase, reduced and oxidized (GR, GSH, and GSSG, respectively), o-phthaldialdehyde, and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Sigma-Aldrich (Madrid, Spain). Dimethyl sulfoxide (DMSO), acetonitrile, methanol, and other reagents were acquired from Panreac Laboratory (Barcelona, Spain). Reagents (Nucleo-spin RNA purification, cDNA-synthesis and ICgreen) for PCR analysis and culture media were purchased from Cultek (Madrid, Spain). The Apo-ONE-Caspase-3/7 Assay was purchased from Promega (Madison, WI, USA). Bradford was purchased from BioRad Laboratories S.A.

2.2. Plant Extract

Brunfelsia grandiflora barks were obtained from the native community of Canaán de Cachiyacú, Contamana from the Ucayali region (Pucallpa, Peru). Barks were washed and dried in the open air and later reduced to a fine powder. The powered material was submitted to decoction by being placed in distilled water (10:1, *v*:*v*) and heated to boiling for 20 min. The extract was separated via filtration, concentrated, and lyophilized for preservation.

2.3. Cell Culture

Human neuroblastoma SH-SY5Y cells were a kind gift from Prof. Carlos Guillén (see Acknowledgments). A mixture of DMEM-F12, 10% FBS, and 50 mg/L of antibiotics was used for cell culture and passage. Cell incubation conditions were a humid environment, CO_2 (5%), air (95%), and temperature at 37 °C. *B. grandiflora* extract concentrations (1, 10, 25, 50, 100, and 200 µg/mL) were dissolved in culture media and added to the cells. Two different conditions, co- and pretreatments, were assayed to systematically evaluate the antioxidant cytoprotection of the *B. grandiflora* extract. The cotreatment assay was performed for 22 h with 100 µM t-BOOH plus the different extract concentrations of *B. grandiflora*, and the pretreatment assay was performed with concentrations of extract for 18 h, which were then washed and submitted to a new medium containing 200 µM t-BOOH for 4 h [6,14]. The reason for using two different concentrations of t-BOOH, 100 and 200 µM, is the distinct length of the treatment with the prooxidant, 22 h in the case of the cotreatment and 4 h in the case of the pretreatment. Previously, we determined that the optimal t-BOOH concentrations to test potential cytoprotection in both conditions, co- and pretreatment, were 100 and 200 µM t-BOOH, respectively [6].

2.4. Cell Viability Evaluation (MTT)

In order to check whether the mitochondria are capable of reducing tetrazolium (MTT) to formazan, 0.5 mg/mL MTT was added to each well for 2 h after co- and pretreatments, and 150 µL of DMSO was added to solubilize the desired reaction before the supernatant was removed. Only metabolically active mitochondrial cells changed the yellow-MTT to purple formazan [22]. The optical density was obtained at 540 nm (SPECTROstar BMG microplate reader, BMG LABTECH, Ortenberg, Germany) and cell viability was normalized with respect to the control.

2.5. Intracellular ROS Production

Intracellular ROS production can be assessed as a measure of oxidative stress by using a DCFH-DA-probe assay [23]. Once DCFH-DA is within the cell, esterases hydrolyze the compound and produce the cleavage of DCFH and its binding to ROS to generate detectable fluorescent activity. Briefly, DCFH-DA (10 µM) was added to each well (2×10^5 cells/well) in a black multiwell plate for 30 min and measured in a spectrofluorimeter (FLx800 Fluorimeter, BioTek, Winooski, VT, USA) at 485 excitation nm/530 emission nm. The values obtained are expressed as arbitrary fluorescence units, which are higher the higher the oxidative activity.

2.6. Caspase 3/7 Activity Assay

A total of 15×10^3 SH-SY5Y cells/well were used for this assay in black plates. Apo-ONE® Caspase-3/7 was used according to the manufacturer's instructions. We removed 96-well plates containing treated cells from the incubator, added 100 µL of homogeneous Caspase-3/7 reagent to 100 µL of the culture medium containing the cells previously treated in each well, and incubated them at room temperature for 60 min in the dark. Fluorescence was measured at 485 excitation/528 emission nm with a fluorimeter. Data were normalized with respect to the control group [24].

2.7. MDA Concentration

Cell concentration of MDA was performed using high-performance liquid chromatography (HPLC) as its DNPH derivative [25]. In brief, cells were treated in 100 mm plates (6×10^6), collected in PBS, and centrifuged (220 g/5 min/4 °C). Afterwards, pellets were resuspended in PBS and submitted to ultrasounds. After centrifugation (3500 g/5 min), 125 µL of supernatant was mixed with 25 µL of 6 M NaOH and heated to 60 °C/30 min, to yield hydrolysis of protein-bound MDA. Then, 62.5 µL of 35% (*v*/*v*) perchloric acid was added to precipitate protein and samples were centrifuged for 10 min at 2800× *g*. After, 12.5 µL DNPH from a 5 mM solution in 2M hydrochloric acid was mixed with 125 µL of supernatant. Lastly, this mixture was injected onto an Agilent 1100 Series HPLC-DAD with a Fortis C18 column (4.6 mm × 250 mm, 5 µm particle size, Fortis Technologies Ltd., Neston, UK). The adducts were detected at 310 nm and quantification was calculated based on a calibration chromatogram performed with a standard solution of MDA prepared via acid hydrolysis [25]. MDA values are expressed as nmol of MDA/mg protein; protein concentration was evaluated using the Bradford reagent.

2.8. Antioxidant Defenses

2.8.1. Reduced Glutathione (GSH)

GSH levels were determined via fluorometry [14], and based on the reaction of GSH with o-phthalaldehyde at pH 8.0. The fluorescence probe was detected at 340 excitation nm/460 emission nm. Data were fitted to a pure GSH standard curve (5–1000 ng).

2.8.2. Antioxidant Enzymes

Determination of GPx and GR activity has been previously described and was based on the decrease in absorbance due to the oxidation of NADPH utilized in the reduction of oxidized glutathione by GR [14]. Briefly, cells were seeded, treated, washed once with PBS, and collected in 3 mL PBS. After centrifugation at 1350 rpm/5 min/4 °C, pellets were resuspended in PBS and submitted to ultrasounds for 7 min. Afterwards, samples were centrifuged at 5000 rpm for 15 min at 4 °C, and enzyme activities were measured in the supernatants. Protein concentration was evaluated using the Bradford reagent that measures the protein concentration by adding Coomassie dye to the sample under acidic conditions and reading absorbance at 595 nm.

2.9. Real-Time PCR Assay

Cell pellets were collected after cotreatment and pretreatment, and total RNA was purified using the NucleoSpin kit (Macherey-Nagel, Germany). Total RNA and A260/A280 ratios (1.9–2.1) for all samples were determined using a nano-spectrophotometer (Microdigital, Seoul, Korea). The cDNA synthesis was obtained from 1 µg of total RNA via retro-transcription using the cDNA Synthesis Kit (PCRBiosystems, Wayne, PA, USA), and it was stored at −80 °C for further analysis. Real-time qPCR assays for BAX, BNIP3, APAF1 (genes from cell death), NFκB, SOD, and NRF2 (genes from oxidative stress—antioxidants) (Supplementary Materials, Table S2) were carried out with BioRad system (BioRad CFX, Hercules, CA, USA) and the Mastermix ICgreen (Nippon Genetics, Duren, Germany). For the expression, it was necessary to use specific primers (400 nM). The following thermocycling protocol was used: 95 °C/2 min, and 40 cycles of 95 °C/5 s–60 °C/30 s. GAPDH was used as a housekeeping gene, and the efficiencies were calculated using LinRegPCR software (V.2021.2) [26].

2.10. Statistical Analysis

To evaluate statistical differences between the data of the different treatments, a one-way ANOVA test was performed, followed by a Tukey's post hoc test, starting at $p < 0.05$. GraphPad Prism version 8.0 software was used.

3. Results

3.1. Cell Viability

In a previous study, we ensured that the concentrations of the *B. grandiflora* extract of 1, 10, 25, 50, 100, and 200 µg/mL were not toxic to cultured endothelial cells in a 22 h period [27], but neuroblastoma SH-SY5Y might show a different sensibility and, consequently, we tested the same concentrations for 22 h in this cell line; no concentration showed any measurable damage on cell viability (Figure S1). Thus, this range of doses could be safe to be assayed for their potential cytoprotection against an oxidative insult. Exposure of SH-SY5Y to 100 µM t-BOOH for 18 h (cotreatment) or 200 µM t-BOOH for 4 h (pretreatment) evoked a considerable decrease in cell viability of around 39% (Figure 1). Concentrations of the extract of and over 50 µg/mL evoked a significant recovery of neuron-like cell viability in both pre- and cotreatment, although this rescue was dose-dependent in pretreatment and dose-independent in cotreatment. The highest recovery of cell viability from the oxidative insult in both conditions was observed at 100–200 µg/mL, especially in pretreatment, although both were partial, and no treatment evoked a complete recovery of cell viability (Figure 1).

3.2. Assessment of Oxidative Stress

In this study, ROS concentration and oxidative damage to lipids, such as that caused by MDA, and to neuronal survival, such as that caused by apoptotic mediators, were determined to establish neuronal functionality in a condition of oxidative insult. For the evaluation of ROS generation and the apoptotic mediators caspases 3/7, the same range of extract concentrations (1–200 µg/mL) was assayed, whereas just the most effective doses, 50–200 µg/mL, were used for MDA.

3.2.1. ROS Production

Addition of either 100 µM t-BOOH for 18 h or 200 µM t-BOOH for 4 h to SH-SY5Y cells induced a notable generation of ROS of 62% that was similar for both co- and pre-treatments, ensuring a comparative model for oxidative damage (Figure 2). A significant dose-dependent decrease in ROS production was found from 25 µg/mL onwards in both pre- and cotreatments, but a complete reduction in ROS was not achieved compared with the control group with any treatment, even with the highest dose of *B. grandiflora* extract (200 µg/mL). These results clearly indicate that *B. grandiflora* extract (25–200 µg/mL, co- and pretreatment) significantly prevents the increased ROS generation induced by

t-BOOH. This remarkable ROS-reducing effect of plant extracts may be instrumental in the chemoprotection observed in neuron-like cells.

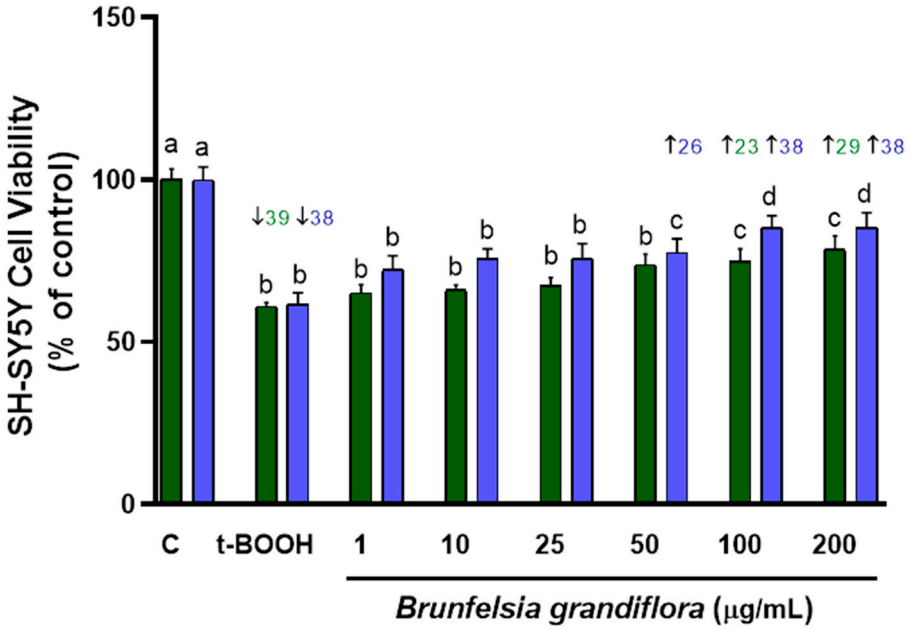

Figure 1. Cytoprotection effects of *Brunfelsia grandiflora* on SH-SY5Y cells after 22 h coincubation (green bars) and preincubation (blue bars). Cell viability data are % of control. Data bars indicate the mean ± SEM of 6 replicates. Different letters (a, b, c, d) show significance ($p < 0.05$) between groups. ↓ represents decrease (%) compared with control; ↑ represents increase (%) compared with t-BOOH. Statistical comparison was made between the groups including pretreatment or between those including cotreatment, never between pretreatment and cotreatment groups.

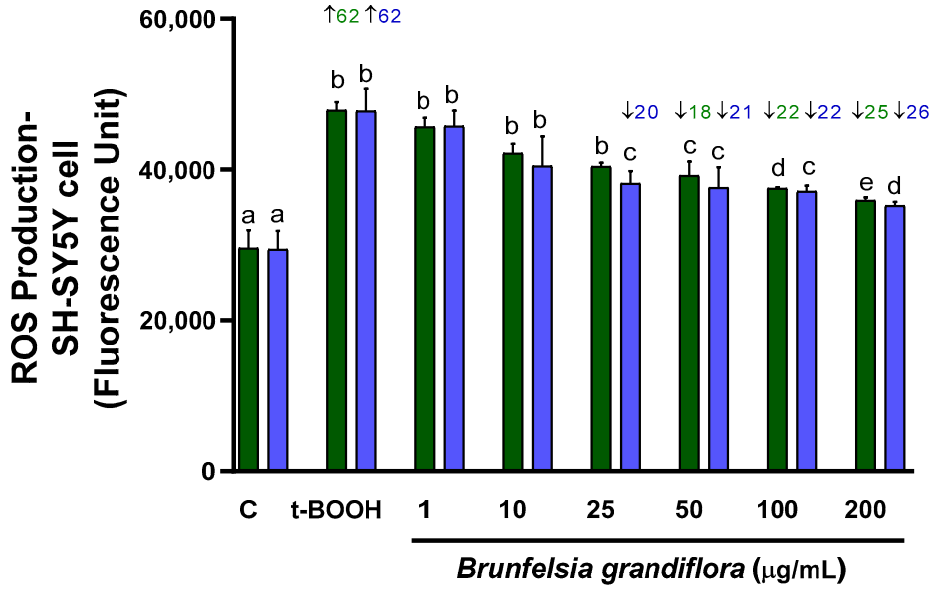

Figure 2. Effect of *Brunfelsia grandiflora* extract on ROS generation produced by t-BOOH on SH-SY5Y cells after 22 h coincubation (green bars) and preincubation (blue bars). ROS generation data were measured as fluorescence units (mean ± SEM) of 6 replicates. Different letters (a, b, c, d, e) show significance ($p < 0.05$) between groups. ↑ represents increase (%) compared with control; ↓ represents decrease (%) compared with t-BOOH. Statistical comparison was made between the groups including pretreatment or between those including cotreatment, never between pretreatment and cotreatment groups.

3.2.2. Caspase 3/7 Activity

In unprotected cells, the response to an oxidant insult by t-BOOH provokes apoptosis activation and cell death. As a measure of the apoptotic process, the activity of caspase 3/7 is an excellent marker. Caspase 3/7 activity increased by almost 100% compared with the control in both treatment conditions, indicating a comparable induction of the apoptotic process and consequent cell damage (Figure 3). *B. grandiflora* extract (25–200 µg/mL) was also found to significantly reduce caspase activity, even reaching control-like levels at the highest dose (Figure 3). These findings indicate a significant restriction of apoptosis and subsequent cell mortality.

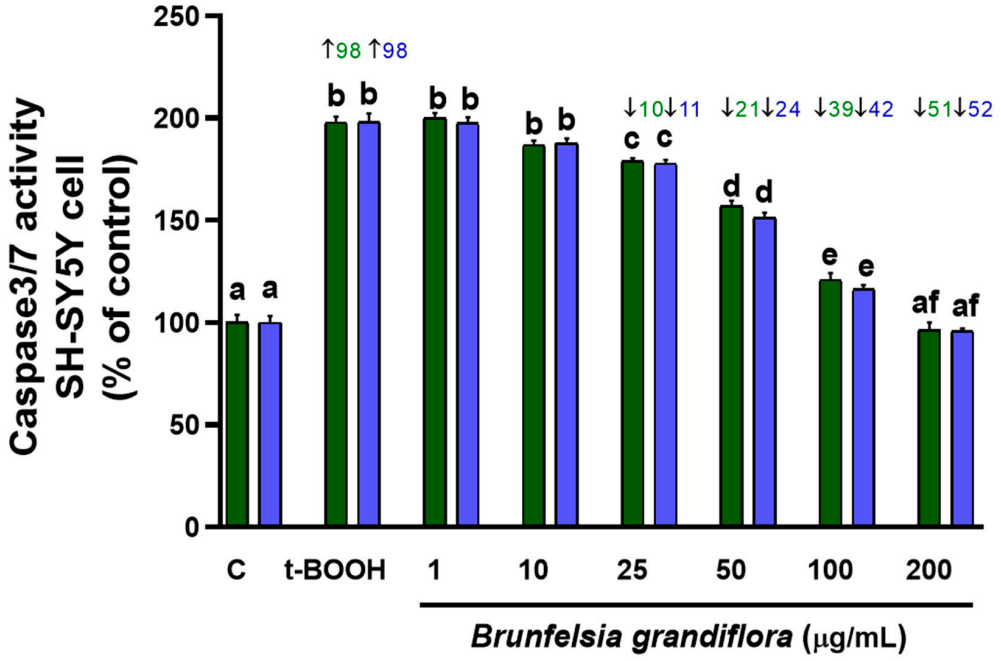

Figure 3. Antiapoptotic effects of *Brunfelsia grandiflora* on caspase 3/7 activity induced by t-BOOH on SH-SY5Y cells after 22 h coincubation (green bars) and preincubation (blue bars). Caspase 3/7 activity data were measured as % of control (mean ± SEM) of 6 replicates. Different letters (a, b, c, d, e, f) show significance ($p < 0.05$) between groups. ↑ represents increase (%) compared with control; ↓ represents decrease (%) compared with t-BOOH. Statistical comparison was made between the groups including pretreatment or between those including cotreatment, never between pretreatment and cotreatment groups.

3.2.3. MDA Concentration

Only 50, 100, and 200 µg/mL of the *B. grandiflora* extract were used for MDA protection against the t-BOOH challenge in both the co- and pretreatment approaches since they were the most efficient doses for rescuing previous parameters. A significant increase of around 50% in MDA concentration was found in SH-SY5Y cells submitted to either co- or pretreatment. Since MDA concentration is considered a consistent index of oxidative damage to lipids, the comparable increase in both treatment conditions indicates an equivalent damage to cell lipids in both co- and pretreatment (Table 1). Surprisingly, all three doses of *B. grandiflora* extract (50–200 µg/mL) completely reduced MDA to values well below those of control cells not submitted to the oxidative stress. This result shows that the three concentrations of *B. grandiflora* extract totally reduced t-BOOH-induced lipid peroxidation (Table 1).

Table 1. Effect of different concentrations of *B. grandiflora* extract (BG) in co- and pretreatment on MDA levels (nmol MDA/mg protein).

	MDA Levels (Mean ± SD)	
	Cotreatment	**Pretreatment**
Control	5.18 ^{a} ± 0.12	5.18 ^{a} ± 0.12
t-BOOH	8.64 ^{b} ± 0.31	7.63 ^{b} ± 0.29
50 µg/mL BG + t-BOOH	0.28 ^{c} ± 0.11	0.50 ^{c} ± 0.21
100 µg/mL BG + t-BOOH	2.22 ^{d} ± 0.20	0.68 ^{d} ± 0.17
200 µg/mL BG + t-BOOH	3.16 ^{e} ± 0.15	0.88 ^{e} ± 0.18

Data are from 3 replicates. Superscripts (a, b, c, d, e) indicate statistically significant ($p < 0.05$) differences between groups.

3.3. GSH

Tripeptide GSH is the most abundant nonenzymatic antioxidant defense and the main intracellular reducing power. Assessing GSH concentration offers the most reliable information on the cell redox condition. Since bioactive compound-rich plant extracts administered for long treatments could induce changes in the basal GSH concentration that could alter its future reaction to an impending oxidant insult, a direct treatment (22 h) with *B. grandiflora* concentrations was carried out on SH-SY5Y cells. Table 2A shows that *B. grandiflora* extract (50–200 µg/mL) did not produce changes in the GSH levels of neuron-like cells.

Table 2. (**A**) Direct effect of different concentrations of *B. grandiflora* extract (BG) for 22 h on GSH levels in SH-SY5Y cells. (**B**) Effects of *B. grandiflora* extract (co- and pretreatment) on GSH levels in SH-SY5Y cells.

(A)		
	GSH Levels	
	nmol/mg Prot ± SD	
Control	0.47 ± 0.01	
50 µg/mL BG	0.48 ± 0.04	
100 µg/mL BG	0.53 ± 0.05	
200 µg/mL BG	0.51 ± 0.01	
(B)		
	GSH Levels	
	nmol/mg Prot ± SD	
	Cotreatment	**Pretreatment**
Control	0.46 ^{a} ± 0.01	0.47 ^{a} ± 0.01
t-BOOH	0.38 ^{b} ± 0.02	0.40 ^{b} ± 0.01
50 µg/mL BG + t-BOOH	0.41 ^{b} ± 0.02	0.41 ^{b} ± 0.01
100 µg/mL BG + t-BOOH	0.41 ^{b} ± 0.01	0.43 ^{c} ± 0.01
200 µg/mL BG + t-BOOH	0.45 ^{ac} ± 0.01	0.44 ^{c} ± 0.01

Data are from 3 replicates. Superscripts (a, b, c) indicate statistically significant ($p < 0.05$) differences between groups.

Later, we assessed the protective capacity of the extracts on neuron-like cells submitted to the two different conditions of oxidative stress. Thus, t-BOOH (co- and pretreatment) on neuron-like cells evoked around a 20% decrease in the cell GSH concentration (Table 2B). When neuron-like cells were pretreated with the three *B. grandiflora* concentrations before exposure to t-BOOH, there was a significant dose-dependent recovery of the diminished GSH, whereas only the highest extract concentration of 200 µg/mL produced a significant recovery of GSH when cotreated with t-BOOH (Table 2B). These results indicate a partial protection of the depleted GSH induced by oxidative stress via a co- or pretreatment of the cells with the three tested *B. grandiflora* extracts. These results also point out that co- or

pretreatment with the selected doses of *B. grandiflora* extract, which effectively restrained cell death and ROS overproduction, significantly averted the risky loss of GSH induced by t-BOOh in SH-SY5Y cells.

3.4. Antioxidant Enzymes

The standard response to a stressful challenge with overproduction of ROS involves effective activation of the antioxidant system, particularly of GPx and GR, two major antioxidant enzymes that guarantee the quenching of excessive ROS. Long treatments with plant extracts with a high content of bioactive antioxidants might alter the basal GPx and GR activities, which may alter their subsequent effects on upcoming oxidative stress. Therefore, prior to testing the effects of the co- and pretreatment, a plain direct treatment with the extracts with no stress was also assayed.

3.4.1. GPx Activity

When SH-SY5Y cells were cotreated with the three *B. grandiflora* concentrations, no changes in GPx activity were determined with 50 and 100 µg/mL, but a significant increase in GPx activity was found with 200 µg/mL extract (Table 3A). As a predicted response to the overproduction of ROS induced by a prooxidant condition in neuron-like cells, pretreatment of SH-SY5Y cells produced an increase of over 100% in GPx activity (Table 3B), whereas the cotreatment provoked around 40% enhancement of enzyme activity (Table 3B). The reported increase in GPx activity was dose-dependently palliated when stressed cells were cotreated with the three concentrations of *B. grandiflora*, whereas all three doses fully recovered GPx activity to basal prestress values when cells were pretreated with the extracts before the 4 h stress (Table 3B).

Table 3. (**A**) Direct effect of different concentrations of *B. grandiflora* extract on GPx levels in SH-SY5Y cells after 22 h treatment period. (**B**) Effect of *Brunfelsia grandiflora* extract (co- and pretreatment) on GPx concentration (mU GPx/mg protein) in SH-SY5Y cells.

(A)		
	GPx Levels	
	mU GPx/mg Prot. ± SD	
Control	158.85 ^{a} ± 2.81	
50 µg/mL BG	154.56 ^{a} ± 4.87	
100 µg/mL BG	168.05 ^{a} ± 4.63	
200 µg/mL BG	1922.70 ^{b} ± 3.49	
(B)		
	mU GPx/mg Prot. (Mean ± SD)	
	Cotreatment	**Pretreatment**
Control	158.85 ^{a} ± 2.81	158.85 ^{a} ± 2.81
t-BOOH	305.84 ^{b} ± 4.73	204.24 ^{b} ± 4.31
50 µg/mL BG + t-BOOH	185.34 ^{c} ± 4.93	153.24 ^{c} ± 11.41
100 µg/mL BG + t-BOOH	181.39 ^{d} ± 3.61	172.35 ^{d} ± 4.63
200 µg/mL BG + t-BOOH	203.48 ^{b} ± 4.73	155.79 ^{d} ± 3.83

Data are from 3 replicates. Superscripts (a, b, c, d) indicate statistically significant ($p < 0.05$) differences between groups.

3.4.2. GR Activity

A slight dose-dependent increase in GR activity was determined when SH-SY5Y cells were exposed to 50–200 µg/mL, reaching around 30% with the highest dose (Table 4A). As was found for GPx, GR activity was also enhanced by over threefold by t-BOOH (100 µM/22 h) (Table 4B) and around fourfold by t-BOOH (200 µM/4 h) (Table 4B). This intensified response of GR ensures a proper recovery of GSSG to GSH for reutilization. As in the case of GPx, cotreatment with the three *B. grandiflora* extract concentrations exerted a

significant dose-dependent return of the changed GR activity in SH-SY5Y cells, whereas all three doses produced a partial recovery of the GR activity, with that of 100 µg/mL being the most effective (Table 4B). These results suggest that doses of the *B. grandiflora* extract that protect cell viability, compromise ROS overexpression, and rescue decreased GSH also prevent the hyperstimulation of antioxidant enzyme defenses produced by oxidative stress in SH-SY5Y cells.

Table 4. (**A**) Direct effect of different concentrations of *B. grandiflora* extract on GR levels in SH-SY5Y cells after 22 h treatment period. (**B**) Effect of *Brunfelsia grandiflora* extract (co- and pretreatment) on GR concentration (mU GR/mg protein) in SHSY-5Y cells.

(A)	
	GR Levels
	mU GR/mg Prot. ± SD
Control	$3.94^{a} \pm 0.29$
50 µg/mL BG	$4.57^{b} \pm 0.53$
100 µg/mL BG	$4.92^{b} \pm 0.67$
200 µg/mL BG	$5.27^{b} \pm 0.26$

(B)		
	mU GR/mg Prot. (Mean ± SD)	
	Cotreatment	**Pretreatment**
Control	$3.94^{a} \pm 0.29$	$3.94^{a} \pm 0.29$
t-BOOH	$13.69^{b} \pm 0.78$	$7.71^{b} \pm 1.02$
50 µg/mL BG + t-BOOH	$9.35^{c} \pm 0.49$	$5.06^{c} \pm 0.31$
100 µg/mL BG + t-BOOH	$7.00^{d} \pm 0.30$	$3.72^{a} \pm 0.10$
200 µg/mL BG + t-BOOH	$5.25^{e} \pm 0.44$	$4.79^{ac} \pm 0.42$

Data are from 3 replicates. Superscripts (a, b, c, d, e) indicate statistically significant ($p < 0.05$) differences between groups.

3.5. Molecular Assay

In our study, we evaluated the mRNA expression of cell death-related genes (BAX, BNIP3, and APAF1) and oxidative stress-related genes (NRF2, SOD, and NFκB1). We observed that the expression of these genes was altered by the effect of the oxidative compound, and this effect was partially or completely reduced by the highest concentration of *B. grandiflora* extract. The expression of these genes was not altered by exposure to *B. grandiflora* extract alone (Figure S2).

3.5.1. Biomarkers of Cell Death

The prooxidant compound t-BOOH increased BAX gene expression by more than 2.5-fold in both the cotreatment and pretreatment groups, while *B. grandiflora* extract was able to reduce this effect at the dose of 200 µg/mL (Figure 4A). The gene expression levels of BNIP3 increased by more than 2.5-fold by the effect of t-BOOH, which was reduced by 100 and 200 µg/mL of *B. grandiflora* extract (Figure 4B). Finally, we observed that t-BOOH increased APAF1 expression by more than 1.5-fold, an effect partially reversed by the higher concentration of *B. grandiflora* (Figure 4C).

3.5.2. Biomarkers of Oxidative Stress

In Figure 5, an induction effect of the expression of the "master regulator" NFκB caused by t-BOOH was determined to be greater than 3.5-fold (Figure 5A); this effect was significantly reduced with concentrations of 100 and 200 µg/mL of *B. grandiflora*. On the other hand, the antioxidant biomarkers SOD (Figure 5B) and NRF2 (Figure 5C) were significantly reduced by t-BOOH, and the molecular expression of these biomarkers was fully recovered at the highest concentration of *B. grandiflora* extract.

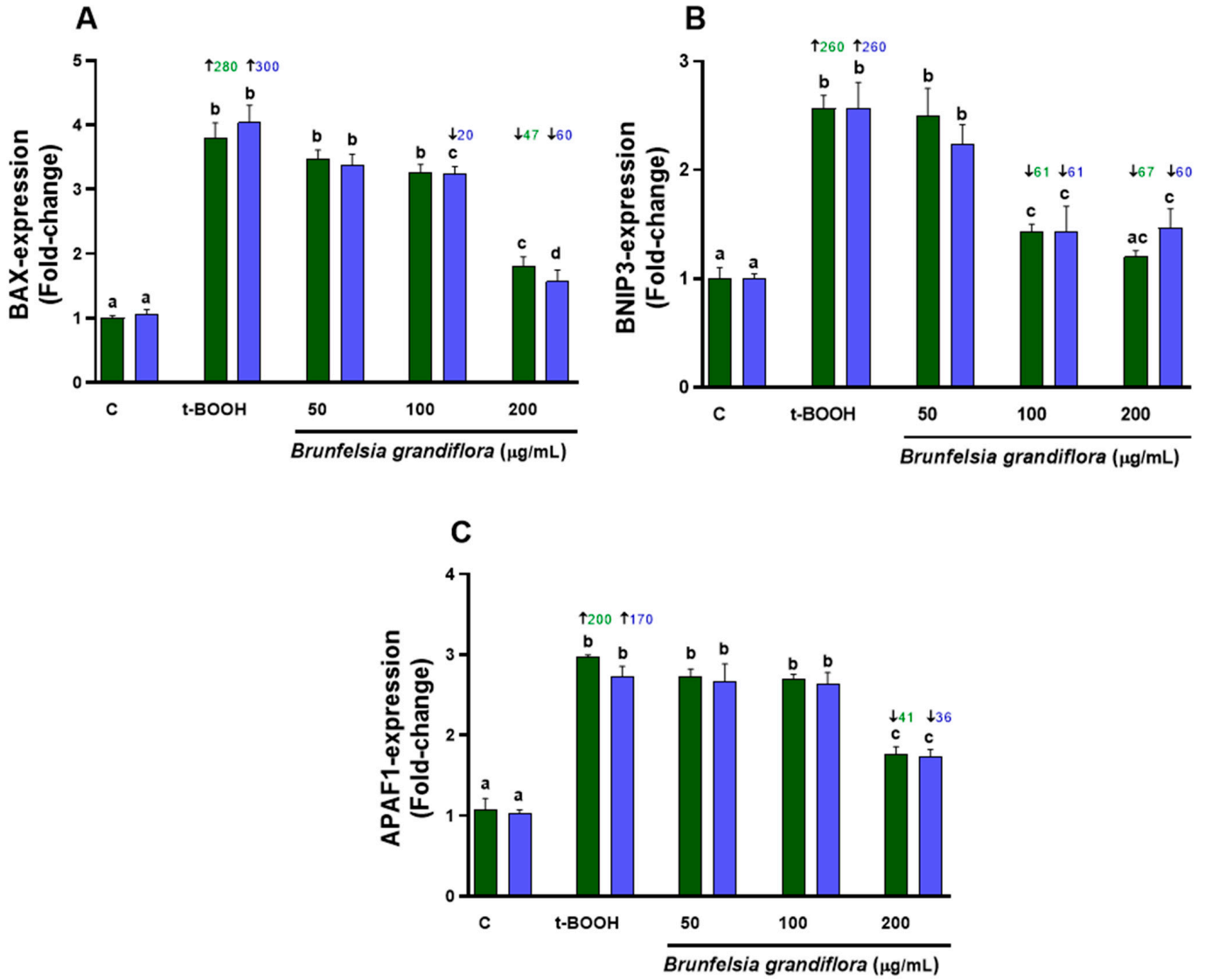

Figure 4. Effects of *B. grandiflora* extract (50–200 μM) on cell death-related mRNA levels (**A**) BAX, (**B**) BNIP3, and (**C**) APAF1 in SH-SY5Y cells after co- (green bars) and pretreatment (blue bars). mRNA levels were normalized with respect to control (mean ± SEM) of three replicates. Different letters (a, b, c, d) show significance ($p < 0.05$) between groups. ↑ represents increase (%) compared with control and ↓ represents decrease (%) compared with t-BOOH.

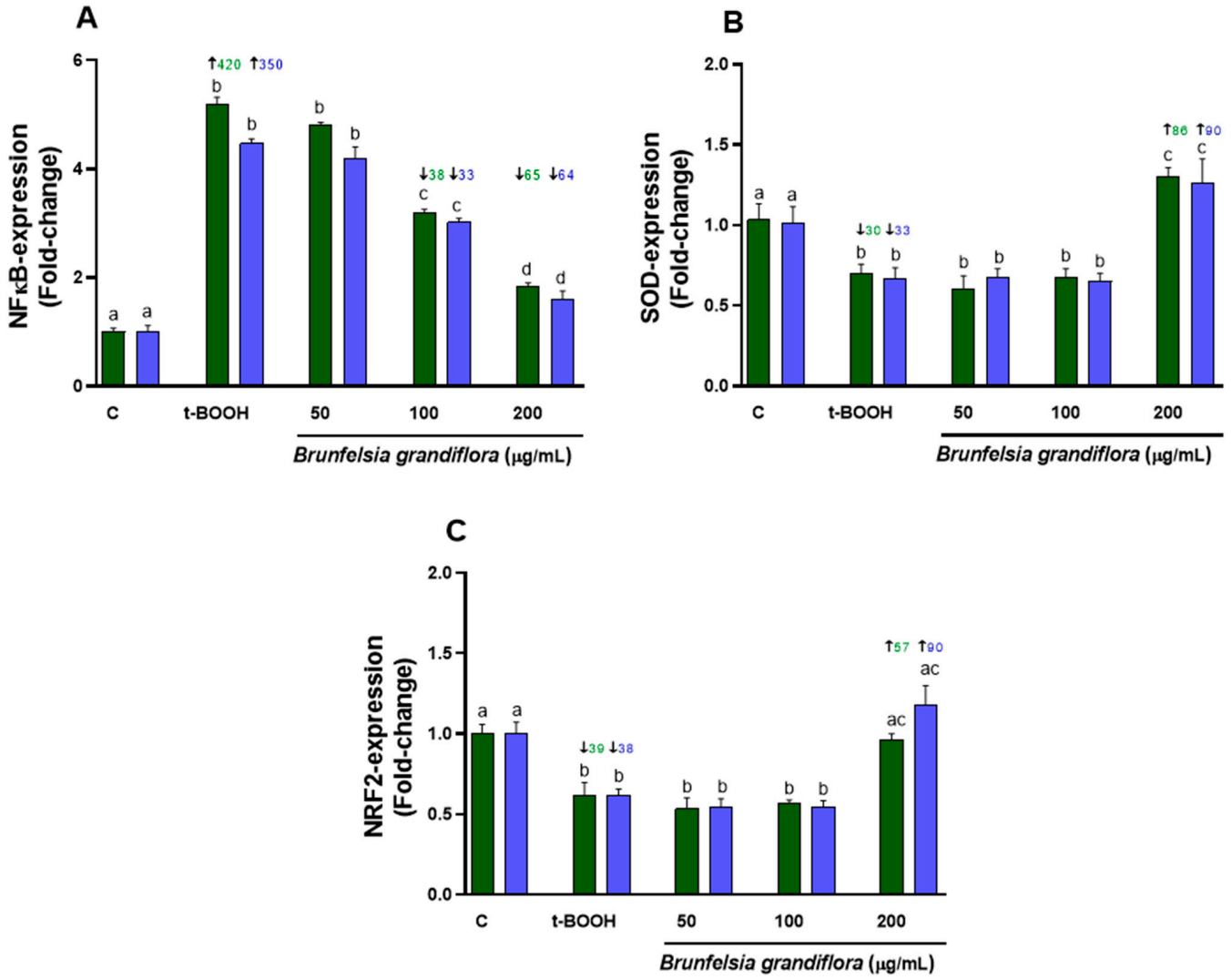

Figure 5. Effects of *B. grandiflora* extract (50–200 μM) on the mRNA levels of oxidative stress-related genes (**A**) NFκB, (**B**) SOD, and (**C**) NRF2 in SH-SY5Y cells after co- (green bars) and pretreatment (blue bars). mRNA expression was normalized with respect to control (mean ± SEM) of three replicates. Different letters (a, b, c, d) show significance ($p < 0.05$) between groups. ↑ represents increase (%) and ↓ represents decrease (%) compared with control and compared with t-BOOH, respectively.

4. Discussion

A very recent chemical characterization of *Brunfelsia grandiflora* has revealed that its main phenolic components are found in high amounts and that all these bioactive compounds support the traditional use of this solanacea as a medicinal therapy in the Peruvian Amazon [9]. In the same study, the in vitro antioxidant capacity of *B. grandiflora* extracts was reported [9], and data from our laboratory suggest the antioxidative stress capacity of the extracts in cultured endothelial cells [27]. In the present study, *Brunfelsia grandiflora* extract was prepared and its cytoprotective and antioxidative stress potential was evaluated in a culture of neuron-like cells. Realistic concentrations of the extract showed significant cytoprotection of neuron-like cells in culture, including the restriction of ROS generation and apoptotic activity as well as a remarkable antioxidant effect and molecular regulation of oxidative stress/cell death-related genes.

The selection of the range of extract doses of *B. grandiflora* to assay the antioxidant capacity was based on data from preceding studies testing the neuroprotective activity of plant extracts and other plant-derived products. In the last few years, a range of doses of different plant extracts like that used in this study has proved effective in neuronal

and glial protection against induced challenges. Thus, 10–20 μg/mL of extracts from *Tinospora cordifolia* showed neuroprotective and neurodegenerative potential against glutamate-mediated excitotoxicity [28]. Likewise, 10–100 μg/mL concentrations of *Danggui Buxue Tang*, an ancient Chinese herbal decoction, protected against β-amyloid-induced cell death in cultured cortical neurons [29]. Similarly, 1–50 μg/mL extracts from *Lithospermum erythrorhizon* showed significant cytoprotection of rat embryonic precursor neuroretinal cells in culture [30]. Concentrations of *Gynostemma pentaphyllum* extract from 10 to 100 μg/mL showed positive effects on injured neuroblastoma–spinal cord hybrids NSC-34 and activated BV-2 microglial cells [31]. In the same year, 5–50 μg/mL extract from *Graptopetalum paraguayense* inhibited β-amyloid-induced damage in SH-SY5Y cells [32], and similar extract concentrations from two other medicinal plants were found to be neuroprotective in primary cultures of retinal ganglion cells [33]. Finally, last year, 10 μg/mL of a *Melissa officinalis* extract showed significant cytoprotection and reduced neuroinflammation in microglial cells [34].

We have recently standardized an oxidative stress assay in neuron-like cells by challenging them with the potent oxidative inducer t-BOOH [5,6]. In a reaction assisted by metal ions, organic peroxide t-BOOH decomposes to other peroxyl and alkoxyl groups that may produce ROS [35]. In a situation of long-lasting overproduction of ROS, namely oxidative stress, there might be excessive damage to cellular macromolecules that can irrevocably compromise cell viability, such as when t-BOOH induces cellular stress [36]. In this model of oxidative stress in SH-SY5Y cells, we report that concentrations of 25–200 μg/mL of phenolic compounds of cocoa [5], and of *Desmodium tortuosum* extract (25–200 μg/mL) [6], protect neuron-like cells from oxidative stress.

Therefore, the *B. grandiflora* extract concentrations used in the present study should be considered realistic and comparable to those of previous studies focused on neuroprotection with natural compounds. Indeed, when SH-SY5Y cells were exposed to *B. grandiflora* extracts at the selected range of doses, there was a dose-dependent reduction in cytotoxicity induced by t-BOOH, indicating that the stressed cells' integrity was significantly protected against the oxidative insult. Although the chemoprotective effect of the *B. grandiflora* extract seemed to be slightly more prominent in the pretreatment condition, an important quantity of the bioactive phenolic compounds in the extract [9] had enough capacity for fractional but substantial dose-dependent cell protection, comparable to that previously reported for other phenolic extracts in similar cell culture conditions [5,6].

Recently, the higher adequacy of differentiated neuroblastoma as a neuron-like cell culture model has been reported [37–39], and, indeed, we have previously used SH-SY5Y differentiated with retinoic acid submitted to stress conditions similar to those utilized in the present study [5]. Interestingly, in from that study that were obtained simultaneously from undifferentiated SH-SY5Y cells, very similar results regarding ROS concentration (determined via two methods, flow cytometry and in situ fluorescence with a microplate reader) were observed. Furthermore, the present study was carried out in parallel to a similar approach that was used for testing the cytoprotective effect of an extract from another Peruvian medicinal plant, *Desmodium tortuosum*, where the same cell line, undifferentiated SH-SY5Y, was used, in order to facilitate stimulating comparisons [6].

As stated above, a substantial overproduction of ROS that might be responsible for the increased cell death was observed when t-BOOH was added to SH-SY5Y cells in both treatment conditions. Both co- and pretreatment of stressed cells with the *B. grandiflora* extracts evoked a remarkable dose-dependent inhibition of ROS, which plainly confirms the antioxidant capacity of the phenolic compounds in the extract and offers a first reason for the reported cell protection. This chemoprotective capacity of *B. grandiflora* does not seem specific to this particular neuron-like cell type since we have observed a comparable ROS-quenching capacity of this plan in cultured endothelial cells [31]. In addition, we have recently reported a similar protective effect of extracts from *D. tortuosum* in both endothelial and neuron-like cells [6], as well as of phenolic extracts from other medicinal plants in

hepatic cells [13,14]. All these results clearly indicate that the chemoprotective effect is the result of the antioxidant capacity of natural compounds.

In addition to their ROS-quenching capacity, plant extracts rich in bioactive phenolics may balance cell redox status by regulating antioxidant defenses such as GSH, the major reducing power in eukaryotic cells. GSH concentration is strongly regulated within the cell and its diminution denotes an increase in intracellular oxidation and hazardous oxidative status, whereas a stable GSH level permits the cell to handle in good condition an impending oxidative challenge [36]. As recently reported in our model of neuron-like cells, the addition of t-BOOH to any treatment condition resulted in a substantial decrease in GSH concentration, which is indicative of oxidative stress that might provoke severe damage to cellular macromolecules [6]. SH-SY5Y cells pretreated with *B. grandiflora* extracts prior to the stress prevented this dangerous situation in a dose-dependent pattern, whereas only 200 µg/mL of *B. grandiflora* caused a significant recovery of GSH concentration in cotreated stressed cells. As in the case of cell viability protection, the condition of pretreatment with extracts prior to the stress seems to be more effective than that of cotreatment with both prooxidants and extracts. In fact, we have recently reported a similar outcome for another South American medicinal fabaceae, *Desmodium tortuosum*, in identical cell model conditions [6], suggesting that the constant presence of the potent prooxidant in the cotreatment condition seems more severe for the cell culture, reducing the efficiency of the extract compounds in rescuing the depleted GSH to suitable levels in the cells. Nevertheless, the positive recovery of GSH in stressed neuron-like cells treated with *B. grandiflora* extracts agrees with previous studies of other phenolic antioxidant-rich extracts in neuron-like cells [5,6]. This recovery of GSH concentration above a suitable threshold while fighting a stressful insult is critical for cell survival [35,36].

As we have reported for an SH-SY5Y cell culture, the addition of t-BOOH (co- and pretreatment) produced a substantial rise in GPx and GR activities in order to face the stress-induced overproduction of ROS [5,6]. GPx, with the help of GSH, catalyzes the reduction of peroxide species and forms oxidized glutathione, whereas GR catalyzes oxidized glutathione to GSH to maintain the steady state of cellular reducing power [35,36]. Thus, the stimulation of the antioxidant enzyme defenses observed after exposure to t-BOOH unequivocally shows an adequate response of the antioxidant defense system to an oxidative challenge [13,14,35]. The significant decrease in GPx and GR activities when stressed neuron-like cells were co- or pretreated with the *B. grandiflora* extracts clearly indicates an efficient quenching of ROS, followed by a return to a basal condition that decreases cellular damage and prepares the cell to cope with impending oxidative insults. This enzymatic antioxidant response by antioxidant-rich phenolic extracts agrees with our previous data on the same neuron-like cell culture model [5,6], as well as on other cell lines [13,14]. Thus, the chemoprotective mechanism of exogenous antioxidants that modulates the antioxidant enzyme response to oxidative stress is an overall means of cellular protection. On the other hand, we also molecularly evaluated three biomarkers related to oxidative status (NFκB, NRF2, and SOD). In the present study, we have shown that NFκB, NRF2, and SOD return to control-like levels in SH-SY5Y neuronal cells due to *B. grandiflora* extract after their levels were altered by t-BOOH, both in cotreatment and pretreatment, as reported in other studies [6,40–42].

The exacerbation of cellular oxidative stress is closely related to the activation of cell death biomarkers such as caspase-3/7 enzymes, the Bax/BNIP3/APAF1 regulator, and others [36]. In our study, we observed that oxidative stress increased due to the effect of the compound t-BOOH, and it is possible that this generated an increase in the activity of caspase-3/7 and apoptosis mediators (BAX, BNIP3, and APAF1) in SH-SY5Y cells. This effect, in turn, was reversed in a dose-dependent manner by *B.grandiflora* extract; this effect is closely related to the phenolic compound content of this plant [9]. Similar effects were reported in other studies, showing that plant extracts or plant-derived compounds have the potential to reduce apoptosis through the reduction of caspase-3/7 activity [6].

Peroxidation of polyunsaturated fatty acids generates MDA, which is used as an index of oxidative damage to lipids in biomedicine [25]. Since MDA has been reported to be elevated in diseases caused by oxidative stress, the remarkable decline in MDA in stressed neuron-like cells co- or pretreated with the *B. grandiflora* extracts indicates a decrease in lipid peroxidation and cellular damage. This outcome also points to a potential therapeutic effect in the prevention of pathologies resulting from oxidative stress, such as neurodegenerative diseases. Again, a similar chemoprotective mechanism of lipid peroxidation has been previously reported in hepatic [13,14], colonic [43], and endothelial cells [44], but interestingly, never in neuron-like SH-SY5Y cells. This novel finding strongly suggests that this chemoprotective mechanism of natural extracts that contain high amounts of natural antioxidants is independent of cell type and tissue and can also be applied to neuron-like cultures.

In general, in chemically induced oxidative stress, both co- and pretreatment of neuron-like cells with realistic concentrations of *B. grandiflora* extract rich in antioxidant compounds significantly protect cells from damage by reducing ROS and recovering GSH and antioxidant enzymes. Although the cytoprotective effect of the selected doses of *B. grandiflora* extract was better in pretreatment than in cotreatment, under both conditions it was observed that there is a systematic biochemical and molecular reaction of the antioxidant defenses that decreases the number of molecules that induce cell damage/death. The relevant quantity of bioactive compounds with antioxidant regulatory capacity in the tested extract could naturally explain this inclusive response and subsequent neuron-like cytoprotection. Although in vivo studies in mammals are needed to ensure the cytoprotective/medicinal activity of *B. grandiflora* extracts, the effects reported in this study support the medicinal use of this plant.

5. Conclusions

B. grandiflora bark extract at 25, 50, 100, and 200 μg/mL concentrations was able to significantly reduce the deleterious effects of t-BOOH on SH-SY5Y neuronal cells. Cell viability, ROS, MDA, caspase-3/7, GSH, GPx, GR, BAX, BNIP3, APAF1, NFκB, SOD, and NRF2 values were significantly altered by t-BOOH compared with the control, and these effects were reversed, reaching even control-like levels, in both pre- and cotreatment due to the effect of the *B. grandfilora* extract, which is due to the high content of polyphenolic compounds in this potential phytotherapeutic agent. However, complementary in vivo studies are needed to better evaluate the mechanisms of action of the biological activity of *B. grandiflora*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app132212233/s1.

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References

1. Ionescu-Tucker, A.; Cotman, C.W. Emerging roles of oxidative stress in brain aging and Alzheimer's disease. *Neurobiol. Aging* **2021**, *107*, 86–95. [CrossRef]
2. Niedzielska, E.; Smaga, I.; Gawlik, M.; Moniczewski, A.; Stankowicz, P.; Pera, J.; Filip, M. Oxidative Stress in Neurodegenerative Diseases. *Mol. Neurobiol.* **2016**, *53*, 4094–4125. [CrossRef] [PubMed]
3. Rivas, F.; Poblete-Aro, C.; Pando, M.E.; Allel, M.J.; Fernandez, V.; Soto, A.; Nova, P.; Garcia-Diaz, D. Effects of Polyphenols in Aging and Neurodegeneration Associated with Oxidative Stress. *Curr. Med. Chem.* **2022**, *29*, 1045–1060. [CrossRef] [PubMed]
4. Strother, L.; Miles, G.B.; Holiday, A.R.; Cheng, Y.; Doherty, G.H. Long-term culture of SH-SY5Y neuroblastoma cells in the absence of neurotrophins: A novel model of neuronal ageing. *Neurosci. Methods* **2021**, *362*, 109301. [CrossRef] [PubMed]
5. Carballeda Sangiao, N.; Chamorro, S.; de Pascual-Teresa, S.; Goya, L. Aqueous Extract of Cocoa Phenolic Compounds Protects Differentiated Neuroblastoma SH-SY5Y Cells from Oxidative Stress. *Biomolecules* **2021**, *11*, 1266. [CrossRef] [PubMed]
6. Rodríguez, J.L.; Berrios, P.; Clavo, Z.M.; Marin-Bravo, M.; Inostroza-Ruiz, L.; Ramos-Gonzalez, M.; Quispe-Solano, M.; Fernández-Alfonso, M.S.; Palomino, O.; Goya, L. Chemical Characterization, Antioxidant Capacity and Anti-Oxidative Stress Potential of South American Fabaceae *Desmodium tortuosum*. *Nutrients* **2023**, *15*, 746. [CrossRef]
7. Plowman, T. Brunfelsia in ethnomedicine. *Bot. Mus. Leafl. Harv. Univ.* **1977**, *25*, 289–320. [CrossRef]
8. Polesna, L.; Polesny, Z.; Clavo, M.Z.; Hansson, A.; Kokoska, L. Ethnopharmacological inventory of plants used in Coronel Portillo Province of Ucayali Department, Peru. *Pharm. Biol.* **2011**, *49*, 125–136. [CrossRef]
9. Mateos, R.; Ramos-Cevallos, N.; Castro-Luna, A.; Ramos-Gonzalez, M.; Clavo, Z.M.; Quispe-Solano, M.; Goya, L.; Rodríguez, J.L. Identification, Quantification, and Characterization of the Phenolic Fraction of *Brunfelsia grandiflora*: In vitro Antioxidant Capacity. *Molecules* **2022**, *27*, 6510. [CrossRef]
10. Moon, P.D.; Lee, B.H.; Jeong, H.J.; An, H.J.; Park, S.J.; Kim, H.R.; Ko, S.G.; Um, J.Y.; Hong, S.H.; Kim, H.M. Use of Scopoletin to Inhibit the Production of Inflammatory Cytokines through Inhibition of the IB/NF-KB Signal Cascade in the Human Mast Cell Line HMC-1. *Eur. J. Pharmacol.* **2007**, *555*, 218–225. [CrossRef]
11. Bennett, B.C. Hallucinogenic plants of the shuar and related indigenous groups in amazonian Ecuador and Peru. *Brittonia* **1992**, *44*, 483–493. [CrossRef]
12. Schultes, R.E.; Hofmann, A.; Rätsch, C. Plants of the Gods. In *Their Sacred, Healing and Hallucinogenic Powers*; Healing Arts Press: Rochester, VT, USA, 1998.
13. León-González, A.; Mateos, R.; Ramos, S.; Martín, M.A.; Sarriá, B.; Martín-Cordero, C.; López-Lázaro, M.; Bravo, L.; Goya, L. Chemo-protective activity and characterization of phenolic extracts from Corema album. *Food Res. Int.* **2012**, *49*, 728–738. [CrossRef]
14. Martín, M.A.; Ramos, S.; Mateos, R.; Marais, J.; Bravo, L.; Khoo, C.; Goya, L. Chemical characterization and chemo-protective activity of cranberry phenolic extracts in a model cell culture. Response of the antioxidant defenses and regulation of signaling pathways. *Food Res. Int.* **2015**, *71*, 68–82. [CrossRef]
15. Bravo, L.; Mateos, R.; Sarriá, B.; Baeza, G.; Lecumberri, E.; Ramos, S.; Goya, L. Hypocholesterolaemic and antioxidant effects of yerba mate (*Ilex paraguariensis*) in high-cholesterol fed rats. *Fitoterapia* **2014**, *92*, 219–229. [CrossRef]
16. Jayaprakasam, B.; Zhang, Y.; Seeram, N.P.; Nair, M.G. Growth inhibition of human tumor cell lines by with anolides from Withania somnifera leaves. *Life Sci.* **2003**, *74*, 125–132. [CrossRef]
17. Khan, S.; Malik, F.; Suri, K.A.; Singh, J. Molecular insight into the immune up-regulatory properties of the leaf extract of Ashwagandha and identification of Th1 immunostimulatory chemical entity. *Vaccine* **2009**, *27*, 6080–6087. [CrossRef]
18. Kim, Y.C.; Kim, S.R.; Markelonis, G.J.; Oh, T.H. Ginsenosides Rb1 and Rg3 protect cultured rat cortical cells from glutamate-induced neurodegeneration. *J. Neurosci. Res.* **1998**, *53*, 426–432. [CrossRef]
19. Chen, X.C.; Zhu, Y.G.; Zhu, L.A.; Huang, C.; Chen, Y.; Chen, L.M.; Fang, F.; Zhou, Y.C.; Zhao, C.H. Ginsenoside Rg1 attenuates dopamine-induced apoptosis in PC12 cells by suppressing oxidative stress. *Eur. J. Pharmacol.* **2003**, *473*, 1–7. [CrossRef]
20. Zhang, L.; Fiala, M.; Cashman, J.; Sayre, J.; Espinosa, A.; Mahanian, M.; Zaghi, J.; Badmaev, V.; Graves, M.C.; Bernard, G.; et al. Curcuminoids enhance amyloid-beta uptake by macrophages of Alzheimer's disease patients. *J. Alzheimers Dis.* **2006**, *10*, 1–7. [CrossRef]
21. Anekonda, T.S. Resveratrol—A boon for treating Alzheimer's disease? *Brain Res. Rev.* **2006**, *52*, 316–326. [CrossRef]
22. Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **1986**, *89*, 271–277. [CrossRef] [PubMed]
23. Wang, H.; Joseph, J.A. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic. Biol. Med.* **1999**, *27*, 612–616. [CrossRef] [PubMed]
24. Miret, S.; De Groene, E.M.; Klaffke, W. Comparison of in vitro assays of cellular toxicity in the human hepatic cell line HepG2. *J. Biomol. Screen.* **2006**, *11*, 184–193. [CrossRef] [PubMed]
25. Esterbauer, H.; Lang, J.; Zadravec, S.; Slater, T.F. Detection of malonaldehyde by high-performance liquid chromatography. *Methods Enzymol.* **1984**, *105*, 319–328.
26. Ramakers, C.; Ruijter, J.M.; Deprez, R.H.; Moorman, A.F. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci. Lett.* **2003**, *339*, 62–66. [CrossRef]
27. Rodríguez, J.L.; Clavo, Z.M.; Ramos-Gonzalez, M.; De La Cruz, M.; Inostroza-Ruiz, L.; Bada-Laura, W.; Palomino, O.; Fernández-Alfonso, M.S.; Mateos, R. Cytoprotective and Anti-Oxidative Stress Capacity of South American Medicinal Plant B. grandiflora in Endothelial Cells. *J. Biol. Regul. Homeost. Agents* **2023**, *unpublished*.

28. Sharma, A.; Kaur, G. Tinospora cordifolia as a potential neuroregenerative candidate against glutamate induced excitotoxicity: An in vitro perspective. *BMC Complement. Altern. Med.* **2018**, *18*, 268. [CrossRef]
29. Gong, G.; Qi, B.; Liang, Y.T.; Dong, T.T.X.; Wang, H.Y.; Tsim, K.W.K.; Zheng, Y. Danggui Buxue Tang, an ancient Chinese herbal decoction, protects β-amyloid-induced cell death in cultured cortical neurons. *BMC Complement. Altern. Med.* **2019**, *19*, 9. [CrossRef]
30. Kang, T.K.; Le, T.T.; Kim, K.A.; Kim, Y.J.; Lee, W.B.; Jung, S.H. Roots of Lithospermum erythrorhizon promotes retinal cell survival in optic nerve crush-induced retinal degeneration. *Exp. Eye Res.* **2021**, *203*, 108419. [CrossRef]
31. Keilhoff, G.; Ludwig, C.; Pinkernelle, J.; Lucas, B. Effects of Gynostemma pentaphyllum on spinal cord motor neurons and microglial cells in vitro. *Acta Histochem.* **2021**, *123*, 151759. [CrossRef]
32. Chen, Y.X.; Le, P.T.N.; Tzeng, T.T.; Tran, T.H.; Nguyen, A.T.; Cheng, I.H.; Huang, C.F.; Shiao, Y.J.; Ching, T.T. *Graptopetalum paraguayense* Extract Ameliorates Proteotoxicity in Aging and Age-Related Diseases in Model Systems. *Nutrients* **2021**, *13*, 4317. [CrossRef] [PubMed]
33. Aldbass, A.; Amina, M.; Al Musayeib, N.M.; Bhat, R.S.; Al-Rashed, S.; Marraiki, N.; Fahmy, R.; El-Ansary, A. Cytotoxic and anti-excitotoxic effects of selected plant and algal extracts using COMET and cell viability assays. *Sci. Rep.* **2021**, *11*, 8512. [CrossRef] [PubMed]
34. Borgonetti, V.; Pressi, G.; Bertaiola, O.; Guarnerio, C.; Mandrone, M.; Chiocchio, I.; Galeotti, N. Attenuation of neuroinflammation in microglia cells by extracts with high content of rosmarinic acid from in vitro cultured *Melissa officinalis* L. cells. *J. Pharm. Biomed. Anal.* **2022**, *220*, 114969. [CrossRef]
35. Alía, M.; Ramos, S.; Mateos, R.; Granado-Serrano, A.B.; Bravo, L.; Goya, L. Quercetin protects human hepatoma HepG2 against oxidative stress induced by tert-butyl hydroperoxide. *Toxicol. Appl. Pharmacol.* **2006**, *212*, 110–118. [CrossRef]
36. Myhrstad, M.C.; Carlsen, H.; Nordström, O.; Blomhoff, R.; Moskaug, J.Ø. Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. *Free Radic. Biol. Med.* **2002**, *32*, 386–393. [CrossRef]
37. Şahin, M.; Öncü, G.; Yılmaz, M.A.; Özkan, D.; Saybaşılı, H. Transformation of SH-SY5Y cell line into neuron-like cells: Investigation of electrophysiological and biomechanical changes. *Neurosci. Lett.* **2021**, *745*, 135628. [CrossRef] [PubMed]
38. Simões, R.F.; Ferrão, R.; Silva, M.R.; Pinho, S.L.C.; Ferreira, L.; Oliveira, P.J.; Cunha-Oliveira, T. Refinement of a differentiation protocol using neuroblastoma SH-SY5Y cells for use in neurotoxicology research. *Food Chem. Toxicol.* **2021**, *149*, 111967. [CrossRef]
39. Ioghen, O.C.; Ceafalan, L.C.; Popescu, B.O. SH-SY5Y Cell Line In Vitro Models for Parkinson Disease Research-Old Practice for New Trends. *J. Integr. Neurosci.* **2023**, *22*, 20. [CrossRef]
40. Sies, H.; Belousov, V.V.; Chandel, N.S.; Davies, M.J.; Jones, D.P.; Mann, G.E.; Murphy, M.P.; Yamamoto, M.; Winterbourn, C. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat. Rev. Mol. Cell Biol.* **2022**, *23*, 499–515. [CrossRef]
41. Yang, C.L.; Wang, S.B.; He, W.P.; Liu, J.J. Anti-oxidant and Anti-inflammatory Effects of Ethanol Extract from Polygala sibirica L. var megalopha Fr. on Lipopolysaccharide-Stimulated RAW264.7 Cells. *Chin. J. Integr. Med.* **2023**, *29*, 905–913. [CrossRef]
42. Puppala, E.R.; Yalamarthi, S.S.; Aochenlar, S.L.; Prasad, N.; Syamprasad, N.P.; Singh, M.; Nanjappan, S.K.; Ravichandiran, V.; Tripathi, D.M.; Gangasani, J.K.; et al. Mesua assamica (King&Prain) kosterm. Bark ethanolic extract attenuates chronic restraint stress aggravated DSS-induced ulcerative colitis in mice via inhibition of NF-κB/STAT3 and activation of HO-1/Nrf2/SIRT1 signaling pathways. *J. Ethnopharmacol.* **2023**, *301*, 115765. [PubMed]
43. Taghour, M.S.; Elkady, H.; Eldehna, W.M.; El-Deeb, N.; Kenawy, A.M.; Abd El-Wahab, A.E.; Elkaeed, E.B.; Alsfouk, B.A.; Metwaly, A.M.; Eissa, I.H. Discovery of new quinoline and isatine derivatives as potential VEGFR-2 inhibitors: Design, synthesis, antiproliferative, docking and MD simulation studies. *J. Biomol. Struct. Dyn.* **2023**, *8*, 1–16. [CrossRef] [PubMed]
44. Wang, Y.J.; Wang, X.Y.; Hao, X.Y.; Yan, Y.M.; Hong, M.; Wei, S.F.; Zhou, Y.L.; Wang, Q.; Cheng, Y.X.; Liu, Y.Q. Ethanol Extract of *Centipeda minima* Exerts Antioxidant and Neuroprotective Effects via Activation of the Nrf2 Signaling Pathway. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 9421037. [CrossRef] [PubMed]

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