



Byrsonima sericea Ethanol Extract Protected PC12 Cells from the Oxidative Stress and Apoptosis Induced by 6-Hydroxydopamine

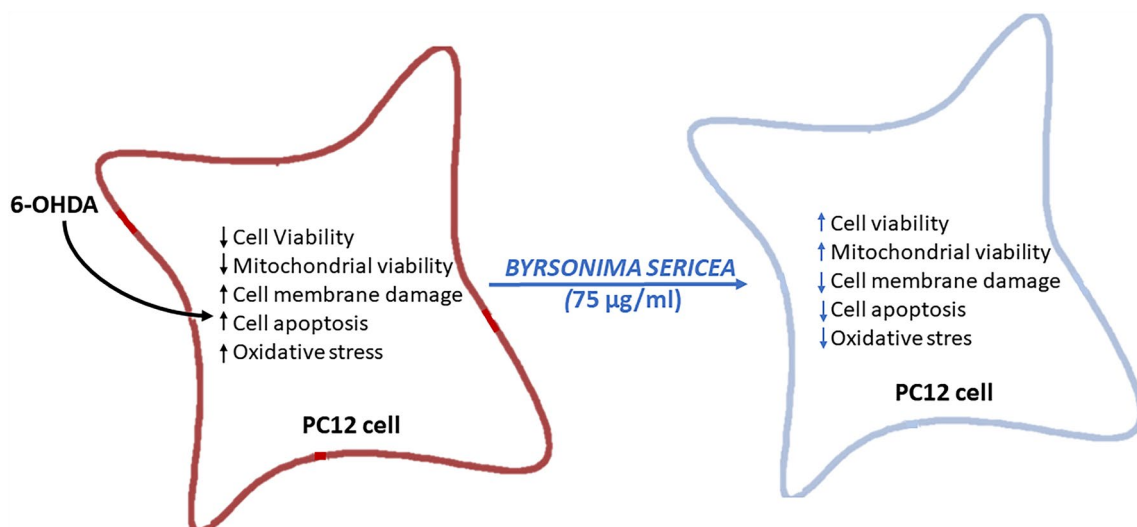
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Abstract

Parkinson's disease is characterized by the progressive loss of dopaminergic neurons in the nigrostriatal pathway and oxidative stress is one of the main mechanisms that lead to neuronal death in this disease. Previous studies have shown antioxidant activity from the leaves of *Byrsonima sericea*, a plant of the Malpighiaceae family. This study aimed to evaluate the cytoprotective activity of the *B. sericea* ethanolic extract (BSEE) against the cytotoxicity induced by 6-hydroxydopamine (6-OHDA) in PC12 cells, an in vitro model of parkinsonism. The identification of phenolic compounds in the extract by HPLC-DAD revealed the presence of geraniin, rutin, isoquercetin, kaempferol 3-O- β -rutinoside, and quercetin. The BSEE (75–300 μ g/mL) protected PC12 cells from toxicity induced by 6-OHDA (25 μ g/mL), protected cell membrane integrity and showed antioxidant activity. BSEE was able to decrease nitrite levels, glutathione depletion, and protect cells from 6-OHDA-induced apoptosis. Thus, we suggest that the BSEE can be explored as a possible cytoprotective agent for Parkinson's disease due to its high antioxidant capacity and anti-apoptotic action.

Graphical Abstract



Keywords *Byrsonima sericea* ethanolic extract · Parkinson's Disease · 6-OHDA · Cytoprotection · Anti-apoptotic

Extended author information available on the last page of the article

Introduction

Parkinson's disease (PD) is the second most common age-related degenerative neurological disorder in humans [1] and affects more than 10 million people worldwide (*Parkinson's Disease Foundation* 2017). PD is identified by the progressive and irreversible loss of myelinated dopaminergic neurons in the substantia nigra, leading to a significant reduction in striatal dopamine levels [2, 3]. Cytoplasmic inclusions such as α -synuclein are also observed [4–6], involving a dysfunction in multiple monoaminergic systems [7, 8]. Among the main factors that lead to cell death in PD are inflammation, apoptosis, oxidative stress and glutamatergic excitotoxicity that lead to the degeneration of dopaminergic cells [9–11]. However, the causes of neuronal death that occur in PD are not yet fully understood.

6-Hydroxydopamine (6-OHDA) is a dopamine analogue and was the first model of Parkinson's disease employed. 6-OHDA is transported by the dopamine transporter (DAT) into the interior of dopaminergic neurons where it inhibits complex I and IV of the mitochondrial electron transport chain, leading to oxidative stress, glutamatergic excitotoxicity and inflammation, and selective death of dopaminergic neurons [12].

The *Byrsonima* genus is commonly used in folk medicine in Brazil to treat skin infections, anti-diarrheal [13], snake bites and gastrointestinal dysfunction [14–18]. *Byrsonima sericea* DC. belongs to the family of Malpighiaceae (the same family as acerola), popularly known as “beach murici”, widely distributed in the Northeastern states of Brazil. The methanolic and ethanolic of *Byrsonima* species demonstrated the presence of glycoside flavonoids mainly derived from quercetin, quinic acid derivatives, gallic acid derivatives, galloylquinic acids and proanthocyanidins., catechin, galloylquercetin, Kaempferol-O-hexoside and the presence of these chemical compounds may be associated with its increased antioxidant actions [19]. This study aimed to evaluate the cytoprotective effect of the ethanolic extract of the leaf of *B. sericea* (BSEE) in a PC12 cell model of parkinsonism caused by 6-OHDA.

Materials and Methods

Plant Collection and Preparation of the Extract

Fresh leaves of *B. sericea* were collected in the Itaperi Campus of the State University of Ceará, Fortaleza, Ceará, Brazil, with the following geographical coordinates: – 3.7930862; – 38.5574677. The plant was identified by

the botanist Dr. Afrânio G. Fernandes and a voucher specimen of the plant was deposited in the Herbarium Prisco Bezerra of Federal University of Ceará (#39451). Fresh leaves of *B. sericea* (1.24 kg) were macerated with 70% ethanol at room temperature for 7 days. The resulting ethanolic solution was filtered using a buchner funnel under vacuum and evaporated to dryness at 50 °C on a rotary evaporator to yield 153 g of crude ethanolic extract.

High-Performance Liquid Chromatography (HPLC-DAD) of Plant Extract

The analytical standards rutin, geranin, isoquercitrin, kaempferol 3-O- β -rutinoside and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents used for extraction were of analytical grade (Vetec®), and in HPLC they were HPLC grade solvents (J.T. BAKER®) used. The water was purified with a system Milli-Q (UV Direct3). All samples (BSEE) and solutions prepared for HPLC analysis were filtered through a 0.45 μ m nylon membrane and 0.22 μ m membrane filter (Millipore), respectively, before use.

High-performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20 A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT plunger pumps connected to a DGU 20A5 degasser with an integrator CBM 20 A, SPD-M20A diode array detector, and LC 1.22 SP1 software. Chromatographic analyzes were performed using a column of reversed-phase (Phenomenex®) Luna C18 (4.6 \times 250 mm, 5 μ m). Mobile phases C and D were of acetonitrile and Milli-Q water, acidified to pH 2.8 with phosphoric acid, correspondingly. The solvent gradient was used as follows: 0–12 min, one elution isocratic with C:D (20:80 v/v); 17–23 min, linear variation to C:D (40:60 v/v); 25–4040 min, an isocratic elution with C:D (20:80 v/v). The flow rate was 1.0 mL/min, with an injection volume of 20 L and wavelengths of 350 nm. Stock solutions of standard references were prepared in HPLC methanol at concentrations of 0.00032–1.0 mg/mL for rutin; 0.000064–0.2 mg/mL for quercetin; 0.008–1.0 mg/mL for isoquercitrin; 0.032–0.5 mg/mL for geranin and 0.008–1.0 mg/mL for 3-O- β -rutinoside of kaempferol. The sample was analyzed in three replicates.

PC12 Cell Culture

The PC12 cell line was obtained from the cell bank (APAB-CAM, Rio de Janeiro, RJ, BRA). They were grown in plastic culture bottles (75 cm², 250 mL volume) growing in HAM medium: F12 supplemented with 15% equine serum, 2.5% fetal bovine serum and 1% antibiotic (streptomycin and penicillin). After 80% confluence, cells were trypsinized and

subsequently subcultured in 96-well multi-well plates at a concentration of 2.5×10^4 cells/well or in 24-well plates at a concentration of 1×10^5 cells/plates. After 24 h after plating, the experiments were performed.

Evaluation of 6-OHDA Cytotoxicity- MTT Test

The (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-MTT test was used (MOSMANN, 1983). The BSEE was diluted in dimethyl sulfoxide (DMSO), not exceeding 0.01% and subsequently prepared at concentrations of 300, 150, 100, 75 and 50 $\mu\text{g}/\text{mL}$. The 6-OHDA 25 $\mu\text{g}/\text{mL}$ (concentration that reduce cell viability in about 50%) was added 15 min after BSEE, and the cells were incubated. After 24 h, 100 μL of the medium was used for the assessment of nitrite levels, and the remainder of the medium was removed. Then the cells were incubated for 3 h with a fresh medium (200 μL) containing MTT (0.5 mg/mL in HAM F12 medium) in each well. After this period, the supernatant was discarded, and 150 μL of dimethylsulfoxide (DMSO $\geq 99.7\%$ - Sigma-Aldrich) was added for cell lysis and formazan solubilization, after which the plate was shaken for 15 min, and the absorbance was measured in a microplate reader at 540 nm. The inhibition of MTT reduction indicates decreased cell viability. The experiments were performed in triplicate and repeated on three different days.

Cell Viability Assay-Lactate Dehydrogenase (LDH)

Cell membrane damage was evaluated by measuring the amount of cytoplasmic lactate dehydrogenase (LDH) released into the medium. LDH activity is determined according to the conversion of pyruvate to lactate, catalyzed by LDH, in the presence of NADH. The decrease in absorbance at 340 nm due to the oxidation of NADH is proportional to the activity of LDH in the sample. After the incubation period with BSEE (75 $\mu\text{g}/\text{mL}$), 20 μL of the supernatant was collected to measure the amount of LDH. Enzyme analysis was performed in triplicate according to kit instructions (Liquiform LDH Kit).

Membrane Integrity by Flow Cytometry

The test is based on the ability of propidium iodide (PI), which is hydrophilic, to penetrate only cells whose membrane is ruptured. After binding to DNA, PI emits high fluorescence when excited by the argon laser (488 nm). The cell with an integrated membrane, therefore, emits low fluorescence (Macklis and Madison 1990). PC12 cells were plated in 24-well plates and incubated with BSEE (75 $\mu\text{g}/\text{mL}$). After the incubation period, the cells were trypsinized and 500 μL of HAM:F12 medium was added. Then, a 100 μL aliquot was taken and incubated with 100 μL of PI solution

(diluted with PBS) in the absence of light. After 5 min, the samples were analyzed by flow cytometry (EasyCyte, Guava Technologies, USA) and information on the percentage of viable cells (integral membranes) was obtained using the red spectrum filter. The experiments were conducted in triplicate.

Determination of Nitrite Concentration

After the incubation period, the concentration of nitrite was determined according to the method of Green et al., [20], which is based on revealing the presence of nitrite in a sample (urine, plasma, tissue homogenate and cell supernatant) by a reaction of diazotization that forms a pink color chromophore, with an absorbance peak of 560 nm. For this experiment 100 μL of the Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)-ethylenediamine hydrochloride/1% H_3PO_4 /distilled water, in the proportion of 1:1:1:1) were added. to 100 μL of cell culture supernatant and incubated at room temperature for 10 min, after which time, the absorbance reading of the samples was performed. A standard curve was prepared with various concentrations of NaNO_2 (ranging from 0.75 to 100 μM) under the same conditions. Blanks were prepared by adding 100 μL of the Griess reagent to 100 μL of the culture medium and the absorbance was measured in a microplate reader at 560 nm. Assays were performed in triplicate.

Determination of Reduced Glutathione (GSH) Concentration

The cells were plated (7×10^6 cells/well) and, after the incubation period, they were transferred to eppendorfs and centrifuged for 5 min at 400 g. After centrifugation the supernatant was discarded, and the precipitate was resuspended in 400 μL of lysis buffer (0.2% Triton X-100). Subsequently, 100 μL were removed for protein dosage, and 75 μL of 0.5 μM perchloric acid were added to the 300 μL of the lysate and centrifuged for 5 min at 500 g. In a 96-well plate, 75 μL of the supernatant, 75 μL of phosphate-EDTA buffer and 37.5 μL of 2 mM DTNB were added and the absorbance was measured in a microplate reader at 412 nm. The tests were carried out in triplicate.

Quantification of SOD by Western Blot

To prepare the lysate, the medium was removed from the bottle and the cells were washed with PBS. After washing, 1ml of PBS was added, and the cells were scraped from the bottom of the bottle. Then, it was aspirated into an eppendorf and centrifuged for 3 min at 300 g at room temperature. After centrifugation, the supernatant was discarded, and 50 μL of buffer (0.1% SDS, 1% sodium deoxyalate, 10mM

Tris-HCL pH 7.5, 150mM NaCl, 2 µg/mL of aprotinin, 1 µg/ml leupeptin µg/ml, 100 µg PMSF and 0.5 mM EDTA) with protease inhibitor was added and left on ice for 20 min. After 20 min, the lysate was centrifuged for 15 min at 14,000 g at 4 °C and the protein concentration of the supernatant was determined using the Lowry method. Samples were heated at 95 °C for 5 min (25 µg/per well) and separated by SDS-PAGE (12%) and electrically transferred to PVDF membrane (BioRad, Hercules, Ca, USA). The membrane was blocked with 5% skim milk in TBS, with 0.1% Tween 20 at 4 °C, and incubated with appropriate primary antibody diluted in TBS-Tween (anti-SOD 1:100, Santa Cruz Biotechnology, USA), anti- α -tubulin 1:4000 (Sigma ST Louis Missouri USA). After successive washings with TBS-Tween buffer the membranes were incubated with secondary antibodies (1:2000 Sigma ST Louis Missouri, USA) conjugated to a suitable horseradish peroxidase enzyme for one hour. Revelation was performed using the ECL chemiluminescence kit (Clarity Western, Bio-Rad) for about 2 min and were analyzed and quantified by the ChemiDoc Imager (Bio Rad) and by the molecular software Image Studio lite 4.0 (LI-COR, USA).

Determination of Apoptotic stage by PE Annexin-V and Amino-Actinomycin (7-AAD) labeling

PE Annexin V is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. In apoptotic cells, membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment. Amino-Actinomycin (7-AAD) is a standard flow cytometry viability marker and is used to distinguish viable from non-viable cells. PC12 cells were plated in 24-well plates and incubated with the drugs. After the incubation period, the cells were trypsinized and 500 µl of HAM:F12 medium was added and the cells were centrifuged at 1500 rpm and resuspended in 100 µl in the Annexin-V binding buffer present in the kit (Kit I BD Pharmingen) and 5 µl of PE Annexin V and 5 µl of 7-AAD were added and incubated for 15 min in the dark. After the incubation time, 400 µl of the buffer was added and the reading was performed on the flow cytometer. Cells that label PE Annexin V positive and 7-AAD negative are undergoing apoptosis. Cells that stain positively for both PE Annexin V and 7-AAD are in the final stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both PE Annexin V and 7-AAD are alive and do not undergo measurable apoptosis. Assays were performed in triplicate.

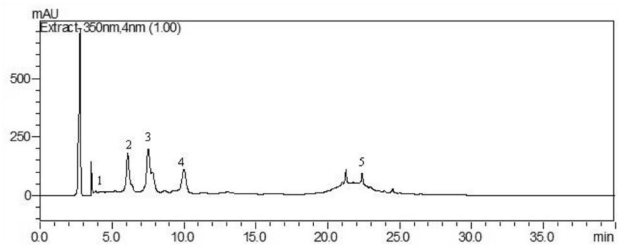


Fig. 1 Analysis of the ethanolic extract of *B. sericea* (BSEE) by high performance liquid chromatography (HPLC-DAD)

Statistical Analysis

Statistical analysis was performed using the Graph Pad Prism 6.0 program. To compare the means, an analysis of variance (ANOVA) was performed, followed by the Student-Newman-Keuls test. Differences were considered statistically significant when $p < 0.05$. Values were expressed as Mean \pm S.E.M.

Results

Analysis of the BSEE by High Performance Liquid Chromatography (HPLC-DAD)

The high-performance liquid chromatography (HPLC) analysis was conducted on the active extract of BSEE to identify its primary components. The resulting chromatographic profile of BSEE is presented in Fig. 1, which reveals the presence of five distinct peaks. These peaks correspond to the following molecules: Geraniin (peak 1, with a retention time of 3.91 min, 8.503 ± 0.082 mg/g), Rutin (peak 2, with a retention time of 6.12 min, 7.836 ± 0.133 mg/kg), Isoquercetin (peak 3, with a retention time of 7.53 min, 9.080 ± 0.145 mg/g), Kaempferol 3-O- β -rutinosid, the majoritarian compound, (peak 4, with a retention time of 9.90 min, 29.98 ± 0.542 mg/g), and Quercetin (peak 5, with a retention time of 22.35 min, 2.125 ± 0.0113 mg/g) (Fig. 2).

Effect of BSEE on Mitochondrial Viability of PC12 Cells Exposed to 6-OHDA

According to the results shown in Fig. 3, various concentrations of BSEE (75, 100, 150, and 300 µg/mL) were effective in protecting PC12 cells from reduced viability caused by 6-OHDA (25 µg/mL). Since, the concentration of 75 µg/mL provided significant cell protection, this concentration was used for the further testing.

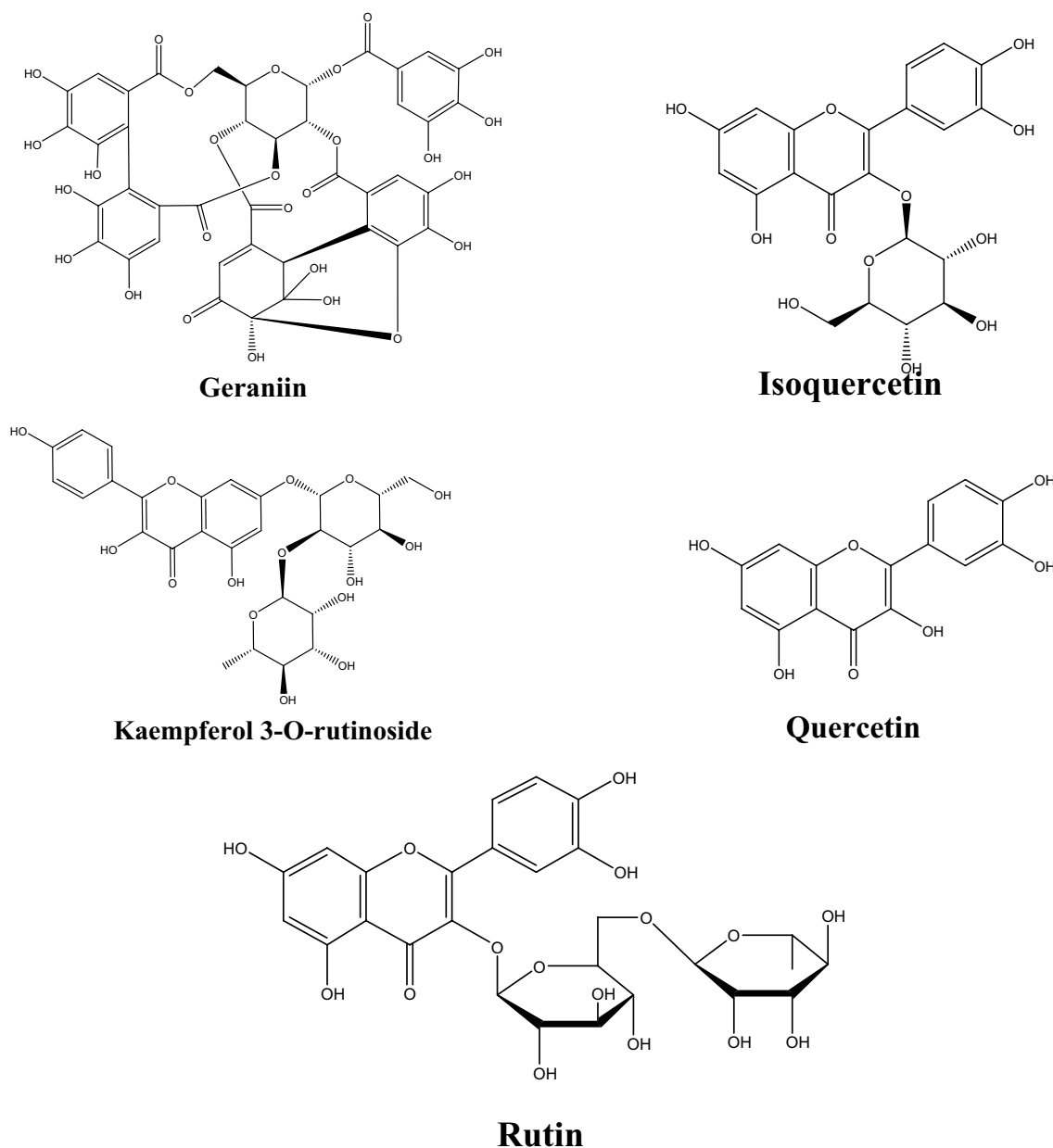


Fig. 2 Chemical structure of phenolic compounds found in BSEE

Effect of BSEE on Cell Viability in PC12 Exposed to 6-OHDA

According to our experimental data, the cells treated with 6-OHDA (25 $\mu\text{g}/\text{mL}$) showed a significant increase in LDH activity compared to the control cells ($p < 0.05$). However, we found that the administration of BSEE (75 $\mu\text{g}/\text{mL}$) had a remarkable protective effect against this harmful increase ($p < 0.05$). As depicted in Fig. 4, the use of BSEE effectively mitigated the negative effects of 6-OHDA treatment on cellular function. This highlights the potential of BSEE as a protective agent in future treatments.

Effect of BSEE on Membrane Integrity of PC12 Cells Exposed to 6-OHDA

The results show a significant decrease in the number of healthy cells after being exposed to 6-OHDA ($p < 0.05$). However, the group that received BSEE (75 $\mu\text{g}/\text{mL}$) had a positive effect on cell viability compared to the group exposed to 6-OHDA, resulting in a notable increase ($p < 0.05$), as shown in Fig. 5.

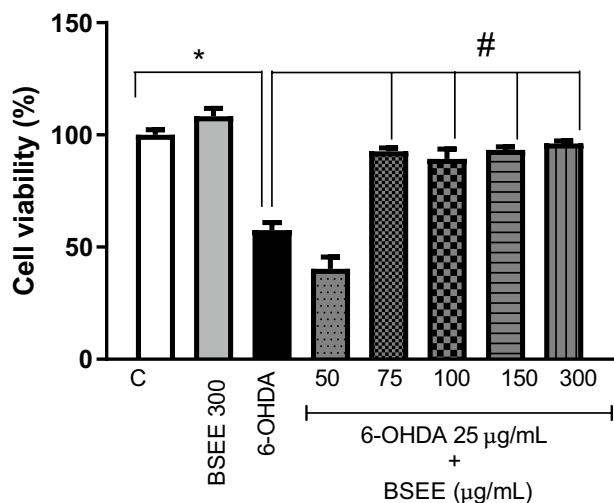


Fig. 3 Effect of *B. sericea* ethanolic extract (BSEE) on mitochondrial viability of PC12 cells exposed to 6-OHDA (25 µg/mL). * $p < 0.05$ compared to control cell, and # $p < 0.05$ compared to 6-OHDA exposed cells. C- Control, vehicle-treated cells

Effect of BSEE on Oxidative Stress Parameters in PC12 Cells Exposed to 6-OHDA

The nitrite levels were increased when PC12 cells were exposed to 6-OHDA compared to control cells ($p < 0.05$). The BSEE treatment protected against 6-OHDA-induced increase in nitrite levels (Fig. 6). Furthermore, 6-OHDA reduced GSH levels, and BSEE (75 µg/mL) protected cells from 6-OHDA-induced decrease in GSH levels ($p < 0.05$) (Fig. 6). No significant changes were found in SOD levels between groups (Fig. 6).

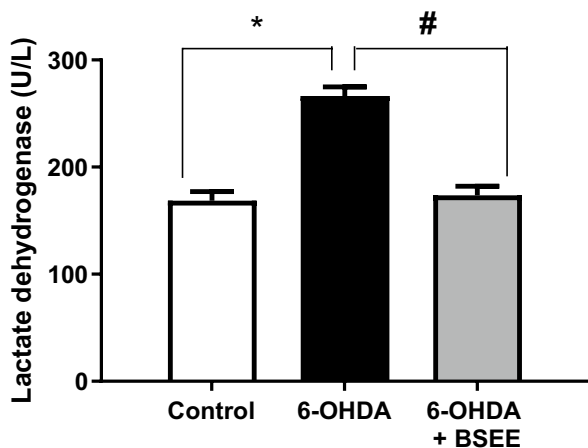


Fig. 4 Effect of *B. sericea* ethanolic extract (BSEE, 75 µg/mL) on LDH activity of PC12 cells exposed to 6-OHDA (25 µg/mL). * $p < 0.05$ compared to control cell, and # $p < 0.05$ compared to 6-OHDA exposed cells. C- Control, vehicle-treated cells

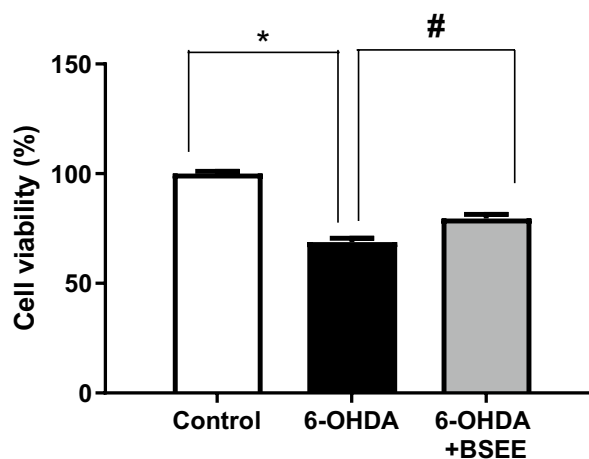


Fig. 5 Effect of *B. sericea* ethanolic extract (BSEE, 75 µg/mL) on membrane integrity of PC12 cells exposed to 6-OHDA (25 µg/mL). * $p < 0.05$ compared to control cell, and # $p < 0.05$ compared to 6-OHDA exposed cells. C- Control, vehicle-treated cells

BSEE Protected PC12 Cells from 6-OHDA-induced Apoptosis

The exposure of PC-12 cells to 6-OHDA resulted in a significant decrease in the percentage of viable cells and a significant increase in the percentage of cells undergoing total apoptosis ($p < 0.05$). However, the administration of BSEE (75 µg/mL) effectively protected PC-12 cells against 6-OHDA-induced apoptosis ($p < 0.05$), as shown in Fig. 7.

Discussion

The pathophysiology of PD is complex, multifactorial and still not completely understood. However, it is well established that oxidative stress plays a crucial role in the development and evolution of PD. At the same time, the treatment of PD is still quite limited [21–24]. In this study, it was demonstrated that BSEE can protect PC12 cells against 6-OHDA-induced toxicity by decreasing oxidative stress and cell apoptosis.

The BSEE showed the presence of quercetin, isoquercetin, rutin, geraniin and kaempferol 3-O-β-rutinoside, as the main secondary metabolites. Rodrigues et al. [18] previously identified these compounds in the ethanolic extract of leaves of *B. sericea*, and showed gastroprotective effects against ethanol-induced lesions, which was attributed to the strong antioxidant activity of the flavonoids present in the extract. Boscolo et al. [25] found that *B. sericea* demonstrated antioxidant activity with low concentration ($IC_{50} = 1 \mu\text{g/mL}$), and Fraige et al. [19] showed that the methanolic extract of the leaf of *B. sericea* could scavenge the peroxy radical ($ROO\cdot$) ($IC_{50} = 1.6 \mu\text{g/mL}$). The flavonoids present in the

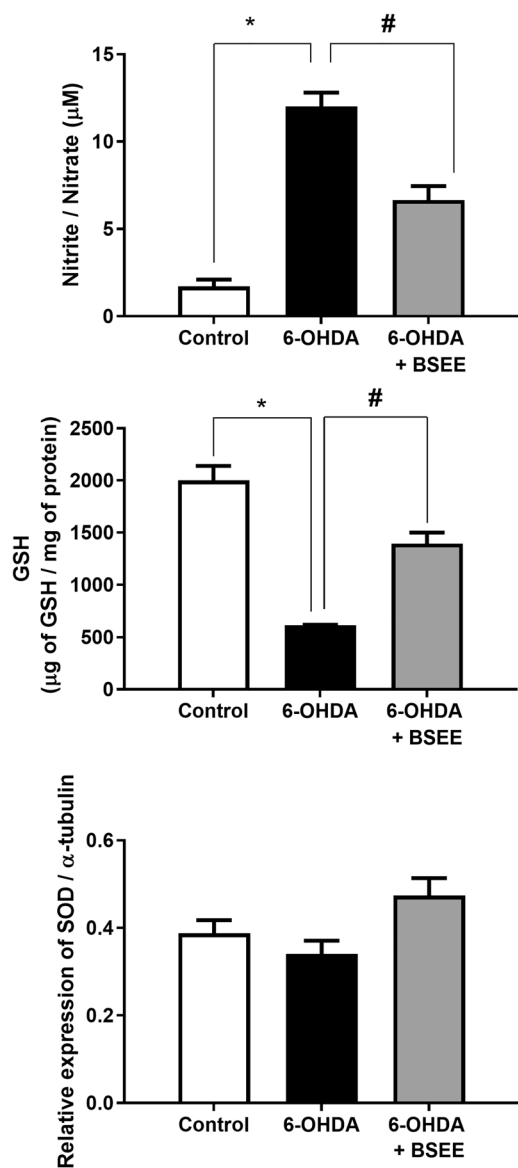


Fig. 6 Effect of *B. sericea* ethanolic extract (BSEE, 75 µg/mL) on nitrite concentration, reduced glutathione (GSH) levels and SOD expression of PC12 cells exposed to 6-OHDA (25 µg/mL). * $p < 0.05$ compared to control cell, and # $p < 0.05$ compared to 6-OHDA exposed cells. C- Control, vehicle-treated cells

extract probably act in synergism, enhancing the antioxidant effects. Also, importantly, BSEE showed no cytotoxic effect at the highest concentration tested.

In vitro studies using PC12 cells for several models of neurodegenerative diseases have demonstrated the protective effects of the isolated quercetin (100–1000 µg/mL) [26], isoquercetin (25–100 µg/mL) [27], kaempferol (40–80 µg/mL) [28], rutin (25 µg/mL) [29] and geraniin (0.1, 1, and 10 µmol/L) [30]. However, it should be noted that, in general, the concentrations at which the compounds alone exert protective effects are one-third or even higher

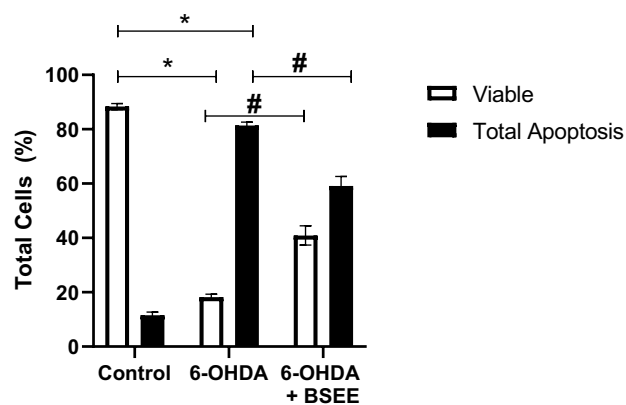


Fig. 7 Effect of *B. sericea* ethanolic extract (BSEE, 75 µg/mL) on apoptosis of PC12 cells exposed to 25 µg/mL 6-OHDA. * $p < 0.05$ compared to control cell, and # $p < 0.05$ compared to 6-OHDA exposed cells. C- Control, vehicle-treated cells

than the concentration used in the present BSEE study. This data demonstrates that the use of the crude extract of BSEE becomes an interesting target of study, since a lower concentration is required, yet reducing the risk of cytotoxic effects for healthy cells. Another interesting point is that, in general, it is recommended to use natural products in unmodified form, such as concentrated herbal extracts. For example, green vegetables and fruits have been found to greatly reduce the risk of cancer, largely due to the action of a combination of polyphenols [31].

Interactions may occur between antioxidants in general and these interactions seem to be important for the biological activity of these compounds. Synergy effects are also reported for mixtures of flavonoids, which are a significant increase of antioxidant activity with the combinations of kaempferol with myricetin, quercetin or quercetin-3-glucoside [32]. Taken together, these evidences confirm the importance of the synergistic effect of flavonoids in BSEE extract.

The PC12 cell have very similar characteristics to dopaminergic neurons, and when these cells are exposed to the neurotoxin 6-OHDA are used as an in vitro model of PD for screening of new drugs [33, 34]. In this study, 6-OHDA was able to reduce mitochondrial enzyme activity, release LDH, and decrease cell viability. The 6-OHDA is capable of inducing oxidative changes in PC12 cells [35] as well as in in vivo [36] studies, similar to those found in dopaminergic neurons in PD. The BSEE protected PC12 cells from inhibition of mitochondrial dysfunction, membrane lysis and cell death. Several studies have also reported the ability of flavonoids, as found in the BSEE, to reduce the loss of dopaminergic neurons in models of neurodegenerative diseases, including PD [37–39].

Interestingly, quercetin, has already shown to have important promising effects against PD models [40]. Wan et al.,

[41] demonstrated that quercetin protect the contents and integrity of the mitochondria and reverse the increased reactive oxygen species (ROS) release in PC12 cells treated with 6-OHDA. The authors of the study also noted that quercetin enhances the neurochemical profile and reduces the parkinsonian disability score in PD rats with 6-OHDA lesions. Furthermore, previous research has indicated that quercetin activates the Nrf2 protein, thereby inhibiting ferroptosis [42].

As can be seen in this study, *B. sericea* ethanolic extracts which present the antioxidant compounds geraniin, rutin, isoquercetin, kaempferol-3-O-rutinoside and quercetin were able to protect PC12 cells from oxidative damage induced by 6-OHDA at very low concentrations compared to studies with these compounds isolated [43–45] showing that probably the synergism between antioxidant compounds is more effective in relation to isolated compounds. In the present study, the action of isolated flavonoid compounds was not evaluated, which would be important to prove their synergistic effect on the BSEE extract. However, in view of the identification and quantification of flavonoid compounds in the BSEE extract, this synergistic effect must be considered.

The involvement of nitric oxide (NO) in the degeneration of dopaminergic neurons in the nigrostriatal pathway is reported in the literature. Gouda and Cho [46], demonstrated an increase in the expression of neuronal nitric oxide synthase (nNOS) and NF- κ B in PC12 cells exposed to 6-OHDA. In this work, 6-OHDA increased the nitrite levels, however when cells were pre-treated with BSEE, nitrite levels were decreased. This action may have occurred due to a regulation in the expression of the genes that encode NOS, for example, via NF- κ B and/or its free radical scavenging activity, which culminated in the NO reduction [47]. The availability of antioxidant compounds containing free hydroxyl groups enables BSEE to donate electrons to RNS, and subsequently leading to the reduction of nitrite levels [48].

GSH reduction is observed in the brain of post mortem PD patient, so, considering that GSH is the main intracellular antioxidant compound [49], the GSH reduction favors oxidative stress, mitochondrial dysfunction and consequently cell death [50, 51]. In this study 6-OHDA decreased GSH levels, possibly due to the increase ROS. When cells were pretreated with BSEE, GSH levels increased 2-fold. Corroborating this data, it has already been demonstrated that quercetin is able to increase the levels of GSH in the brain of animals submitted to an experimental model of PD [52, 53].

Overproduction of ROS can trigger both the intrinsic mitochondrial pathway and the extrinsic death receptor pathway of apoptosis [54, 55]. In our study was possible to verify that, at least in part, 6-OHDA-induced cell death occurred by apoptosis. The pretreatment with BSEE decreased the number of apoptotic cells. The BSEE cytoprotection may be associated with the presence of the phenolic compounds,

which are capable of scavenging free radicals, as well as inducing the activation of anti-apoptotic proteins as Bcl-2 and decrease pro-apoptotic protein as Bax [45, 55, 56]. BSEE cytoprotective effects can be explained by antioxidant mechanisms associated with the flavonoids present in the BSEE such as, the modulation of inducible nitric oxide synthase (iNOS) and NADPH oxidase 2, ROS scavenging activity, modulation of the antioxidant enzymes. Another important mechanism is the oxidation of flavonoids by ROS and reactive nitrogen species (RNS) which form electrophilic quinone that can activate the Nrf2 transcription [57, 58].

Conclusion

Through this study, it can be stated that the BSEE exert a cytoprotective action in the experimental cell model of PD, protecting cells from damage caused by oxidative stress, and apoptosis induced by 6-OHDA. The *Byrsonia sericea* extract has potential to be used as an adjuvant therapy in the treatment of PD. However, the effectiveness of the extract and its active compounds should be investigated using in vivo models of PD in the future.

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Author Contributions Assis ALC, Gomes JMP, Nascimento TC and Oliveira AV: Cells treatment, biochemical analysis, statistical analysis, results interpretation, discussion, and manuscript preparation. Rodrigues PA, Morais SM and Rodrigues ALM: Plant collection, preparation of the extract and HPLC analysis. Andrade GM, Aguiar MSS, Morais SM: Experimental design, statistical analysis, results interpretation, discussion, and manuscript preparation.

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Data Availability Supporting data are available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

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