Neuroprotective effects of *Geranium Robertianum* *L*. *Aqueous* extract on the cellular Parkinson’s disease model

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**Abstract.** – **OBJECTIVE:** In recent years, botanical medicines alone or in conjunction with existing therapies have attracted considerable popularity as an alternative treatment for Parkinson’s Disease (PD). For instance, *Geranium robertianum* L. (Geraniaceae family) has been used in folk medicine for its antioxidant and anti-inflammatory properties. However, its neuroprotective potential has not been well demonstrated.

**MATERIALS AND METHODS:** Herein and for the first time, we have investigated the in vitro neuroprotective effects of leaf extract of *G. Robertianum* over a wide dose range (0-200 µg/mL) on the PD model using retinoic acid (RA)-differentiated SHSY-5Y cells and 1-methyl-4-phenylpyridinium (MPP+) induced neurotoxicity. The neuroprotective effects were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays. The antioxidant activity of the extract was measured by total antioxidant status (TAS) and total oxidant status (TOS). The effect of leaf aqueous extracts on acetylcholinesterase (AChE) activity was also determined. Finally, cell death mechanisms were analyzed by flow cytometry.

**RESULTS:** Our results showed that *G. Robertianum* leaf extract ameliorated cytotoxicity and oxidative damage by MPP+. Moreover, *G. Robertianum* extract exhibited a protective activity against MPP+ induced apoptosis.

**CONCLUSIONS:** The current findings could lead to a promising new candidate for a possible cure of Parkinson’s disease through neuroprotective mechanisms with respect to antioxidant and apoptosis inhibitory properties of *G. Robertianum* water extracts.

**Key Words:** Antiapoptotic, In vitro, *Geranium Robertianum*, MPP+ toxicity, neurotoxicity, Parkinson’s disease.

**Introduction**

Parkinson’s disease (PD), which is one of the most common neurodegenerative disorders, was first described by James Parkinson (nearly 200 years ago) as “shaky paralysis”. James Parkinson defined the symptoms of the disease as involuntary tremors, decreased muscle strength, and poor balance without mental and sensory disorders1,2. The disease was coined by Jean-Martin Charcot, who disagreed with the name “shaky paralysis”. While studying the clinical spectrum of the disease with his students, Charcot discovered that tremors were not a necessary symptom for all individuals with PD3. The PD incidence around the world is approximately 10-50 people per 100,000 people, and the prevalence is estimated to be 250-570 people per 100,000 people. The incidence and prevalence trend of the disease increases with age. When the aging population is considered, it is expected that the number of individuals with PD will double in densely populated countries by 20304,5. Motor symptoms associated with PD include akinesia, bradykinesia, tremor, rigidity, and postural instability (primary) as well as gait and speech disorders (secondary)6. However, non-motor symptoms, such as anxiety and depression are common in PD as well. Anxiety disorders associated with PD may include generalized anxiety disorder, agoraphobia, panic disorder, obsessive-compulsive disorder, and social or specific phobia7,8.

The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin, which degenerates dopaminergic neurons in substantia nigra (SN) causing a Parkinson-equivalent syndrome9, accelerated research on PD pathogenesis related to environmental factors. MPTP is a small, lipophilic compound that can pass through the blood-brain barrier (BBB). This small compound is transformed into the toxic form called MPP+ in astrocytes by the glial monoamine oxidase B (MAO-B) enzyme and up-taken to dopaminergic neurons through the organic cation transporter (OCT-3) enzyme. Among dopaminergic neurons, MPP+ acts as a complex inhibitor and
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prevents oxidative phosphorylation. This inhibition decreases adenosine triphosphate (ATP) levels in the cell and increases ROS production, and dopaminergic neuronal death by causing lipid peroxidation. Thus, MPP+ is considered the most effective toxin for designing in vitro PD models. Since there is no known definitive cure, therapeutic approaches for PD have focused on symptomatic remedies. The medications used in the treatment of PD are aimed at increasing the dopamine level in the brain and simulating the effects of dopamine deficiency. Treatment of PD is based on the control of motor symptoms with levodopa supplementation. However, long-term exposure to a high dose of levodopa is associated with the development of motor complications, characterized by levodopa-induced dyskinesia.

The Geraniaceae family includes 750 species that are cultivated for their economic importance in aromatherapy and cosmetics perfumery. *Geranium Robertianum*, also known as “Red Robin” or “Herb-Robert”, has been used in several countries as an herbal medicine with therapeutic benefits for different types of lesions, such as ulcers, diarrhea, and wounds. *G. Robertianum* is native to Europe, Asia, North America, and North Africa and exhibits good antioxidant and free radical scavenging properties. Furthermore, multiple studies have reported the anticarcinogenic activity of *G. Robertianum*.

Today, herbal medicine therapy is particularly common in the treatment of PD to reduce the side effects of existing medicines as well as drug dependence. Various herbs have been investigated for the treatment of PD including the herbs traditionally used by local people for many years to treat different diseases. The strategy of such herbal approaches is to reduce oxidative stress, which plays a crucial role in the pathogenesis of PD. For this purpose, isolates obtained from natural variegated plants have been tested in vivo and in vitro PD models which also reported their neuroprotective actions. The current literature shows that the neuroprotective effects of *G. Robertianum* have not been fully identified in the cellular PD model. Collapsed oxidative stress machinery in the brain was claimed to be one of the most important reasons for the PD emergence and the potential antioxidant properties of *G. Robertianum* were suggested through several scientific investigations. Therefore, this study aims to examine the neuroprotective potentials of leaf aqueous extracts obtained from *G. Robertianum* in vitro PD model.

**Materials and Methods**

**Plant Extract Preparation**

*G. Robertianum* samples were collected from the Yedigöller region of Erzurum Province in Turkey and morphological identifications of the plant species were performed by Prof. Dr. Meryem Sengül Koseoglu. After plant identifications were completed, air-dried *G. Robertianum* leaves were grounded by using a tissue lyser (Qiagen®, Hilden, Germany) to obtain a powder after being frozen at -80°C and incubated in ultra-pure distilled water at room temperature overnight. The supernatant was obtained by centrifugation followed by lyophilization. The concentrations of the lyophilized aqueous extracts were determined with an analytical balance (Shimadzu®, Kyoto, Japan). The aqueous extracts were filtered with a 0.40 µm membrane filter (Mec MilliporeTm, Burlington, MA, USA) for application to SHSY-5Y cell cultures.

**Cell Culture**

SHSY-5Y cell line (ATCC, CRL-2266) was used to establish the Parkinson’s disease toxicity model. The growth culture media consists of 1% penicillin/streptomycin, 5% fetal bovine serum and 94% DMEM/F12. The prepared medium was heated to 37°C temperature, 5 mL was collected and transferred into a T25 flask and SHSY-5Y cells were seeded. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO2, until achieving approximately 80% confluent. Every two days, the old medium in the flask was removed, cell debris washed with phosphate buffered saline (PBS) and incubation continued with a fresh medium.

**Neuronal Differentiation of SHSY-5Y Cells**

When the cells reached about 80% confluence, the old medium was removed, and the cell debris was washed three times with 2 ml of PBS. Afterward, 10 µM retinoic acid (RA) was added to the culture and the cells were incubated at 37°C and 5% CO2. After 11 days of culture, neuronal cell differentiation can be achieved by exposing the cells to a medium containing RA one day and a medium containing no RA the other day, respectively. Cell culture analysis was examined under an inverted microscope after each medium change. Also, cellular differentiation was further confirmed by using flow cytometry cell cycle analysis.

**In Vitro PD Model**

At the end of the 11th day, differentiated SHSY-5Y cells were harvested with trypsin and seeded...
into a 96-well plate with approximately $10^4$ cells per well. MPP+ iodide was applied to the cells (in triplicate, $n=3$) at a concentration range of 12, 24, 47, 94, 188, 375, 750, and 1,500 µg/mL, with a total volume of 200 µL for each well. While negative control wells contained only medium and cells, 1% Triton X-100 was added to positive control wells in addition to cells and medium. After 24 hours of incubation period, the cells were tested for viability, and the IC$_{50}$ concentration of MPP+ toxic substance in differentiated SHSY-5Y cells was determined$^{29}$.

Cytotoxicity Screening of Plant Extract

Differentiated SHSY-5Y cells were harvested with trypsin and inoculated into a 96-well plate with 104 cells per well. *G. Robertianum* extract was applied to the cells (in triplicates) at a concentration range of 3, 6, 12, 25, 50, 100, 200, and 400 µg/mL, with a total volume of 200 µL in each well. While negative control wells contained only medium and cells, 1% Triton X-100 was added to positive control wells in addition to cells and medium. After 24 hours of the incubation period, the cells were tested for viability and the cytotoxicity was measured.

Application of Aqueous Extracts to In Vitro PD Model

Differentiated SHSY-5Y cells were harvested with trypsin and inoculated into a 96-well plate with approximately 104 cells per well. MPP+ iodide was added to the cells at a predetermined inhibitory concentration (IC$_{50}$). Next, extract at a concentration range of 3, 6, 12, 25, 50, 100 and 200 µg/mL were added (in triplicate), with a total volume of 200 µL in each well. While negative control wells contained only medium and cells, 1% Triton X-100 was employed as a positive control in addition to cells and medium. After 24 hours of the incubation period, the cells were tested for viability and the cytotoxicity was measured.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay

The MTT solution used in viability tests was prepared by dissolving the compounds in PBS to yield a 5 mg/mL concentration rate. After 24 hours of experimental incubation, prepared MTT solution was added to each sample with up to 10% of the total volume in the well. The cells were incubated again at 37°C and 5% CO$_2$ for 3 hours. Afterward, the supernatant was removed, and the formazan crystals attached to the bottom of the well were dissolved by using dimethyl sulfoxide (DMSO). The wells were measured at 570 and 630 nm wavelengths with a microplate reader$^{30}$.

Lactate Dehydrogenase (LDH) Release Assay

The LDH solution used in viability tests was prepared according to the manufacturer’s protocols (Thermo Fisher Scientific, Waltham, MA, USA). At the end of 24 hours of incubations, 50 µL supernatant was transferred from each well to a new plate and a 50 µL reaction mixture was added. The plates were incubated in the dark at room temperature for 30 min. Then, 50 µL of stop solution was added to each well, and measurements were recorded at 490 and 680 nm wavelengths with a microplate reader$^{31}$.

Acetylcholinesterase (AChE) Activity Assay

AChE activity was determined by using an assay kit (Abcam, Cambridge, UK) and analyses were conducted based on the manufacturer’s instructions. Briefly, cell lysates were prepared by incubating with lysis buffer at room temperature. Then, 50 µL acetylthiocholine reaction buffers were added to each sample to reach 100 µL/well volume and incubated for 30 min at room temperature. After the incubation period, samples were analyzed by a microplate reader at 410 nm. Experimental samples were compared with the kit’s standards to assess the activity levels$^{32}$.

Total Antioxidant Status (TAS) And Total Oxidative Status (TOS) Analysis

Total Antioxidant Status (TAS) and Total Oxidative Status (TOS) were investigated by using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). For TAS analysis, cell culture supernatants were used and culture samples, kit standard (equivalent to 1 mmol/L of Trolox), and dH2O were mixed with Reagent 1 in 96-well plates and incubated for 5 min according to the manufacturer’s protocols. Then, Reagent 2 was added, and mixtures were incubated at 37°C for 5 min; absorbance was monitored by a microplate reader at 660 nm. Each sample data was calculated via the kit’s standard (equivalent to 1 mmol/L of Trolox). For TOS analysis, Reagent 1 was added to each well, and absorbances were measured at 530 nm. Next, Reagent 2 was added, and sam-
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Samples were incubated for 5 min at 37°C. Finally, absorbance was monitored at 530 nm by using a microplate reader. Oxidative status values were calculated by using the kit’s standard (µM H₂O₂ Equiv./L)³³,³⁴.

**Flow Cytometry Analysis**

SHSY-5Y cells treated with *G. Robertianum* extract and MPP⁺ iodide for were harvested 24 hours with trypsin and precipitated by centrifugation. After the cells were suspended with 500 µL binding buffer, 5 µL Annexin V-FITC and propidium iodide were added. Samples were incubated at room temperature for 5 min and analyzed by flow cytometry³⁵.

**Statistical Analysis**

The obtained data were analyzed by the GraphPad Prism (GraphPad Software®, La Jolla, CA, USA) statistical program. Statistical evaluations were performed by applying one-way ANOVA followed by the Dunnett comparison test. The maximum statistical significance level was accepted as *p*<0.05.

**Results**

SHSY-5Y cell cultures were incubated with all-trans RA for 11 days to induce cell differentiation into mature neuron-like cells. After the differentiation period, SHSY-5Y cells (initially deltoid-shaped) began to develop elongated body structures and avoid clustering behavior after 11 days of RA exposure. In addition, an increasing number of dendrite-like structures were observed between cells. Moreover, flow cytometry cell cycle analysis was utilized for further validation of differentiation. Prior to differentiation, SHSY-5Y cell culture was presented as S phase (47.15 ± 2.25%) and G2 phase (11.88 ± 1.05%). After differentiation, cell cultures were more likely to be in the G1 phase (75.07 ± 2.94%) as shown in Table I.

To determine the IC₅₀ concentration of MPP⁺ compound, spectrum wide range of concentrations (12, 25, 47, 94, 188, 375, 750, and 1,500 µg/mL) were applied to differentiated SHSY-5Y cell culture. After viability tests, a logarithmic plot of MPP⁺ concentrations were constructed and the IC₅₀ concentration value for MPP⁺ was

**Table I.** 40x image of normal and differentiated SHSY-5Y cells. (A), Undifferentiated SHSY 5Y cells; (B), differentiated SHSY-5Y cells, and (C), Cell cycle analysis of differentiated SH-SY5Y cells after 11 days of retinoic acid application (RA: Retinoic acid).

<table>
<thead>
<tr>
<th>Group</th>
<th>G1 phase</th>
<th>G2 phase</th>
<th>S phase</th>
<th>G2/G1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.41 ± 1.77</td>
<td>11.88 ± 1.05</td>
<td>47.15 ± 2.25</td>
<td>1.74 ± 0.07</td>
</tr>
<tr>
<td>RA treated</td>
<td>75.07 ± 2.94*</td>
<td>1.42 ± 0.07*</td>
<td>25.61 ± 0.92*</td>
<td>1.92 ± 0.05</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± standard deviation. Symbol (*) indicates statistically significant difference at *p*<0.01 as compared with the negative control.
found to be 313.9 µg/mL. After determination of IC\textsubscript{50} concentration of MPP\textsuperscript{+} compound on differentiated SHSY-5Y cell culture, \textit{G. Robertianum} extract was applied at different concentration ranges of 6, 12, 25, 50, 100, 200, and 400 µg/mL to identify toxicological properties of isolates on the differentiated SHSY-5Y cell culture for determining suitable extract concentrations. We found that the highest concentrations (200 and 400 µg/mL) caused significant decreases in cell viabilities, whereas concentrations lower than 200 µg/mL did not induce any neurotoxicity (Figure 1).

Flow cytometry was used to assess cell death in the \textit{in vitro} PD model. Herein, applications of MPP\textsuperscript{+} at IC\textsubscript{50} concentration for 24 hours mainly increased apoptotic cell deaths (early apoptosis = 32.98\% and necrosis = 14.78\%) in the differentiated SHSY-5Y cell culture. After the cells were incubated for 24 hours with MPP\textsuperscript{+} at IC\textsubscript{50} concentra-
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Concentration with *G. Robertianum* extract at 12 µg/mL, apoptosis-related death levels were reduced when compared to the MPP+ group (from 32.98% to 24.30%) as shown in Figure 2.

Decreased AChE activity, one of the most common causes of PD, was used to verify that the MPP+ applied *in vitro* PD model is a usable system for anti-PD compound administration. Our results revealed that AChE activity was significantly decreased after the application of MPP+ into differentiated SHSY-5Y cell culture. On the other hand, there were significant changes in AChE activity compared to the negative control when *G. Robertianum* extract was applied to cell culture, and a significant ameliorative effect of *G. Robertianum* extract when applied with MPP+ (Table II) was detected.

*G. Robertianum* extract was applied to differentiated SHSY-5Y cells with MPP+ (IC50 concentration) to evaluate the oxidant-antioxidant effects. We have found that MPP+ significantly decreased antioxidant levels (from 1.62 to 1.05 mM) and increased oxidant levels compared to the negative control (from 5.61 to 8.28 µM H2O2 Equiv./L). Additionally, we have seen that the application of *G. Robertianum* extract with MPP+ compound significantly elevates the antioxidant level (from 1.05 to 1.54 mM) enough to reach the total antioxidant status of the negative control. Furthermore, the application of *G. Robertianum* extract with MPP+ compound was found to decrease oxidative status (from 8.28 to 5.76 µM H2O2 Equiv./L) and the regulated oxidative state had a similar level to the negative control (Table III).

**Figure 2.** Flow cytometry analysis of *in vitro* Parkinson’s Disease model. (A), Negative control; (B), MPP+ application; (C), *G. Robertianum* + MPP+ application; (D), only *G. Robertianum* application and (E), summary of flow cytometry analysis.

**Table II.** Acetylcholinesterase activity (1 µmol/min) of *in vitro* Parkinson’s disease model in respect to the application of *G. Robertianum* + MPP+.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acetylcholinesterase Activity (µmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>445.75 ± 5.67a</td>
</tr>
<tr>
<td>MPP+</td>
<td>346.85 ± 7.44b</td>
</tr>
<tr>
<td><em>G. Robertianum</em></td>
<td>435.27 ± 8.36c</td>
</tr>
<tr>
<td><em>G. Robertianum</em> + MPP+</td>
<td>358.32 ± 9.12c</td>
</tr>
</tbody>
</table>

Means with the same letter in the column are not significantly different at level of p<0.05. Same superscripted letters (a,b) define statistically similar results.
Discussion

Although the underlying pathological mechanism of PD has not been fully elucidated, previous studies\textsuperscript{36,37} have concluded that different mechanisms, such as mitochondrial disorders, oxidative stress, lipid peroxidation, and heavy metal accumulation could contribute to the disease by accelerating neuronal cell death. At this point, certain plant aqueous extracts exhibited crucial anti-apoptotic roles in Parkinsonism\textsuperscript{38}. In this study, we investigated the neuroprotective effects of aqueous extracts obtained from \textit{G. Robertianum} leaves in an \textit{in vitro} PD model induced by MPP+. Furthermore, AChE activity, TAS and TOS levels, and flow cytometry were performed to reveal the neuroprotective mechanism of \textit{G. Robertianum} extract against MPP+ toxicity. The neurotoxicity induced by the MPP+ in SHSY-5Y cells has been of use to mimic the PD model\textsuperscript{39-41}. In this study, we aimed to differentiate SHSY-5Y cells into primary neuronal cells using retinoic acid, which is in line with other studies\textsuperscript{42-44} in the literature. After 11 days of all-trans RA application, the cells that initially had a deltoid body structure and tended to clump together started to move away from each other by moving into a thin-long body structure and also form axon-dendrite-like cellular structures between each other. Furthermore, flow cytometry analysis of the cell cycle showed that SHSY-5Y cells that were in the S phase progressed to the G1 intermediate phase. These results proposed that the RA application could differentiate cancerous cells into stable and undivided neuron-like cell cultures.

According to the MPP+ toxicity tests, the IC\textsubscript{50} concentration of the MPP+ was found to be approximately 300 µg/mL; the finding is in line with others\textsuperscript{45,46}. It has to be noted that \textit{G. Robertianum} leaves aqueous extracts up to a concentration rate of 100 µg/mL did not show any toxic effects on differentiated SHSY-5Y cells as compared to the negative control. Moreover, \textit{G. Robertianum} extract (12 µg/mL) resulted in elevations in cell viability in differentiated SHSY-5Y cell culture exposed to MPP+ compared to MPP+ treated group. Present results revealed that MPP+ would inhibit the activity of AChE in differentiated SHSY-5Y cell culture. This finding indicates that differentiated cell cultures after MPP+ could stimulate PD-like neurotoxicity and might be used as a disease model for drug discovery and toxicity studies\textsuperscript{30,47,48}. However, \textit{G. Robertianum} extract has a significant effect on the AChE activity against MPP+ toxicity. It could be concluded that the underlying mechanism behind the neuroprotective effect of the plant extract was most probably due to the regulation of AChE activity.

In previous tissue culture studies\textsuperscript{11,49} and animal experiments, MPP+ has been reported to inhibit oxidative phosphorylation by acting as a mitochondrial complex I protein inhibitor. As a result, it activates the apoptotic pathway through ROS-induced lipid peroxidation. Herein, we have found that MPP+ could contribute to oxidative stress by decreasing the total antioxidant level and increasing the total oxidant level in differentiated SHSY-5Y cells, significantly. The application of \textit{G. Robertianum} aqueous extracts with MPP+ tends to increase the antioxidant status and decrease oxidative stress in cell cultures. The survival-enhancing properties of \textit{G. Robertianum} extract against MPP+ toxicity on differentiated SHSY-5Y culture could be attributed to oxidative stress ameliorative effects of aqueous extracts.

MPP+ application was found to induce apoptosis in different cell cultures and some of them were proven to have resulted from the increase of oxidative stress\textsuperscript{50,51}. Also, this study correlated with the literature by flow cytometry and antioxidant analyses that cell treated with MPP+ at IC\textsubscript{50} concentration would activate the apoptotic pa-

<table>
<thead>
<tr>
<th>Groups</th>
<th>TAS (mM Trolox Equiv./L)</th>
<th>TOS (µM H\textsubscript{2}O\textsubscript{2} Equiv./L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>1.62 ± 0.11\textsuperscript{b}</td>
<td>5.61 ± 0.39\textsuperscript{a}</td>
</tr>
<tr>
<td>MPP+</td>
<td>1.05 ± 0.07\textsuperscript{a}</td>
<td>8.28 ± 0.58\textsuperscript{b}</td>
</tr>
<tr>
<td>\textit{G. Robertianum}</td>
<td>2.45 ± 0.25\textsuperscript{c}</td>
<td>5.32 ± 0.45\textsuperscript{a}</td>
</tr>
<tr>
<td>\textit{G. Robertianum} + MPP+</td>
<td>1.54 ± 0.27\textsuperscript{b}</td>
<td>5.76 ± 0.40\textsuperscript{a}</td>
</tr>
</tbody>
</table>

TAS: Total Antioxidant Status, TOS: Total Oxidative Status, NC: Negative control, MPP+: MPP+ treated cells. Means with the same letter in the column are not significantly different at level of \(p<0.05\). Same superscripted letters (\textsuperscript{a,b,c}) define statistically similar results.

Table III. TAS and TOS levels in an experimental \textit{in vitro} Parkinson model treated with \textit{G. Robertianum} and MPP+ compound for 24 hours.
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Moreover, *G. Robertianum* extract has shown anti-apoptotic properties against MPP+ induced toxicity in the *in vitro* PD model. Antioxidants were reported to block oxidative stress-mediated cellular damages and suppress apoptosis. Besides, antioxidants executed remarkable cell protection and apoptosis inhibition. Again, some naturally occurring compounds, such as pioglitazone and melatonin, are being considered for clinical studies on PD therapy in humans. In this context, the observed anti-apoptotic effect could be attributed to the most abundant components in *G. Robertianum*, including linalool, α-terpineol, geraniol, α-caryophyllene, germacrene D, phytol acetate as well as oxidized alkenes like 2-(E)-hexenal and 3-(Z)-hexen-1-ol that exhibited more or less antioxidative properties.

**Conclusions**

*In vitro* PD model constituted of SHSY-5Y cell culture differentiation and MPP+ application could be a useful model to investigate PD-like conditions for drug discovery and toxicological analyses. Furthermore, *G. Robertianum* was shown to exhibit neuroprotective features against MPP+ induced toxicity through regulating oxidative stress machinery and modulating anti-apoptotic systems. Additionally, it was observed that the application of *G. Robertianum* plant aqueous extracts alter AChE enzyme activity. The current findings suggest that *G. Robertianum* extracts could be a promising candidate for the treatment of PD pathology.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

**Ethics Approval**

Not applicable.

**Informed Consent**

Not applicable.

**Availability of Data and Materials**

All the data used to support the findings of this study are included within the article and are available from the corresponding author by a reasonable request.

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**Authors’ Contribution**

Conceptualization: M.E.A.; methodology: A.Y. and M.E.A.; writing, original draft preparation: M.E.A. and A.Y.; writing, review and editing: M.E.A.; supervision: M.E.A. All authors have read and agreed to the published version of the manuscript.

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