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Neuroprotective Effects of *Salvia splendens* Extracts Against Rotenone-Induced Oxidative Stress and Cell Death in SH-SY5Y Cells

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ABSTRACT

Numerous studies in literature indicate that rotenone (ROT) has a cytotoxic effect on humans or animals, and it causes a neurodegenerative disease as Parkinson's Disease (PD). Our study showed that ROT can trigger oxidative damage and cause neuron cell loss in SH-SY5Y cells, an *in vitro* model of dopaminergic neuronal cells. *Salvia splendens* (*S. splendens*) can exhibit antioxidant, anti-inflammatory, and cholinergic effects on neuronal cells thanks to the phenolic compounds it contains. This study focused on investigating the neuroprotective impacts of ethanol extracts prepared from aerial and root parts of *S. splendens* (AE and RE, respectively) against ROT-induced oxidative stress and neuron cell loss in SH-SY5Y cells. In the current study, ROT dose dependently increased neuron cell loss in SH-SY5Y cells, and the IC_{50} value of ROT was determined as 40 μM. The optimum concentrations of AE and RE, which showed the maximum protective effect on SH-SY5Y viability, were determined as 195.3125 ng/mL in the presence of 40 μM ROT. AE and RE, at a specified concentration of 195.3125 ng/mL, in the presence of 40 μM ROT significantly increased the total antioxidant status (TAS) and did not create a profound difference in the total oxidant status (TOS). Additionally, AE and RE significantly decreased the oxidative stress index (OSI) value, as evidence of total cellular oxidative stress, compared to the ROT group in the presence of ROT. In summary, AE and RE obtained from *S. splendens* protect the SH-SY5Y cells from ROT-induced damage through reducing oxidative stress and preventing neuronal cell loss.

Keywords: *Salvia splendens*, Rotenone, SH-SY5Y Neuroblastoma Cells, Oxidative Stress, Cell Death, Neuroprotective Effect

Introduction

Rotenone (ROT) is a naturally occurring compound obtained from plants of the *Leguminosae* family. It has a highly cytotoxic effect and is also used as an insecticide and a pesticide (1). It inhibits mitochondrial complex I by blocking the electron transport system, and this compound has been widely used in experimental models to induce oxidative stress *in vitro* and *in vivo* in PD-like neurodegenerative diseases (2, 3). Parkinson's disease (PD) is a chronic and advancing neurodegenerative disorder defined via the progressive loss of dopaminergic neuron cells in the substantia nigra pars compacta. PD also causes motor and cognitive impairments (4). Worldwide, approximately 10 million people are affected by this neurodegenerative disease, and the rate of patients diagnosed is anticipated to increase twofold by 2040 (5). Environmental (paraquat, toxic solvents, mitochondrial dysfunction, inflammation, epigenetic methylation and changes in the microbiome, chlorinated solvents such as trichloroethylene, a toxic chemical that can present in air, soil, water and food) and genetic factors cause the development and progression of the disease (6, 7). In addition, ROT increases oxidative stress by inhibiting mitochondrial

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complex I in the cells. At the cellular level, oxidative stress induces cellular dysfunction and eventually cell death. Although the cause and pathophysiology of Parkinson's disease are not yet fully understood, the loss of dopaminergic neurons in this disease is thought to be an important cause of oxidative stress. Oxidative stress is among the major factors that play a crucial role in the pathogenesis of neurodegenerative diseases including PD, and this oxidative stress arises from the instability among the generation of ROS (reactive oxygen species) and cellular antioxidant defense mechanisms. ROS are continuously generated *in vivo* via whole body tissues, as well as oxidants and superoxide radicals, which are produced as products of oxidative phosphorylation, assembling mitochondria the major site of ROS production in the cell (8). In recent years, ROT has been used to create a Parkinson's model. The interest in investigating natural compounds with antioxidants and neuroprotective properties as potential therapeutic agents during the management of neurodegenerative diseases has increased in recent years. *S. splendens*, usually known as red sage or red colored sage, belongs to the *Lamiaceae* family and is known for its rich phytochemical components such as phenolic acids, flavonoids and terpenoids. These bioactive compounds have shown significant antioxidant, anti-inflammatory and cytoprotective effects in various experimental models (9, 10). However, the neuroprotective properties of *S. splendens* against ROT-induced oxidative stress and neuronal cell death studies have not been studied. The aim of this study is to explore the neuroprotective effect of ethanol extracts of aerial and root parts obtained from *S. splendens*, which has high antioxidant and anti-inflammatory function, against ROT-induced oxidative stress and cell death in SH-SY5Y cell line. It is thought that the outcome of this research will form the basis of the studies about *S. splendens* against neurodegenerative diseases associated with ROT-induced oxidative stress and dopaminergic neuron loss.

Materials and Methods

***S. splendens* Procurement and Extract Preparation:** *S. splendens* was purchased from a florist shop in the central district of Bingöl province during the growing season. The aerial and root parts of the *S. splendens* were carefully separated and dried in the shade at room temperature. Extracts of the aerial and root component of the *S. splendens* were obtained using the maceration method. Ethyl alcohol (Merck, Darmstadt, Germany) was used as the extraction solvent. Each 15 g sample of the aerial and root parts of the *S. splendens* was subjected to maceration in 200 mL of ethyl alcohol for 24 hours by shaking in a shaker incubator at 40 °C and 150 rpm. The obtained aerial extract (AE) and root extract (RE) were filtered and ethyl alcohol was evaporated using a rotary evaporator. All obtained extracts were freeze-dried and stored at 4 °C until the experiment was performed (11).

Cell Culture and Treatment: Human neuroblastoma SH-SY5Y (ATCC: CRL-2266) cells were cultured in T75 flask using basic medium (RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotic mixture of penicillin/streptomycin) as described in Dervişoğlu (12). Cells were seeded in a 37 °C incubator in a humidified chamber containing 5% CO_2 and subcultured every two days. For each experiment, extracts of aerial (AE) and root (RE) parts of the *S. splendens* and rotenone (ROT) were freshly prepared in dimethylsulfoxide (0.05%). After 24 h of cell attachment to microplates or cell culture dishes, the cells were randomly divided into 6 different groups as control group, ROT group, AE group, RE group, AE + ROT group and RE + ROT group. In order to fully determine the effects of ROT, AE, and RE on SH-SY5Y cells, the FBS ratio in the medium was reduced to 1% during the application of these substances to SH-SY5Y cells. All active substances were applied to the cells in RPMI 1640 medium containing 1% fetal bovine serum and 1% penicillin/streptomycin antibiotic cell culture mixture. The cells were pretreated for 12 hours in media containing, respectively, untreated (control group), 0 μM ROT (ROT group), 195.3125 ng/mL AE (AE group), 195.3125 ng/mL RE (RE group), 195.3125 ng/mL AE + 0 μM ROT (AE + ROT group), and 195.3125 ng/mL RE + 0 μM ROT (RE + ROT group). Consequently, the cell culture medium was renewed, and the cells were cultured for an additional 12 hours in culture media containing, respectively, untreated (control group), 40 μM ROT (ROT group), 195.3125 ng/mL AE (AE group), 195.3125 ng/mL RE (RE group), 195.3125 ng/mL AE + 40 μM ROT (AE + ROT group), and 195.3125 ng/mL RE + 40 μM ROT (RE + ROT group). All biochemical experiments were performed after the end of incubation and three replicates were made for each treatment.

Cell Viability (WST-8) Assay: The effects of AE, RE, and ROT on SH-SY5Y cells, along with the neuroprotective effect of AE and RE against ROT-induced reduction in cell viability in SH-SY5Y cells, were determined using the Cell Viability Detection Kit-8 (WST-8 / CVDK-8) as described in the study by Duman, Dervişoğlu (13). Briefly, 1×10^6 SH-SY5Y cells were added into 96-well plates and incubated for a day. After various treatments with the active substances, 5 µl of water-soluble WST-8 tetrazolium salt was applied to each well and incubated at 37 °C in 5% CO_2 for up to 3 hours. Absorbance was calculated at 450 nm with a reference at 690 nm using an Elisa Reader device (Rel Assay Diagnostics, BK-EL10C, Mega Tıp, Gaziantep, Türkiye). Cell viability was determined using the following formula:

Cell viability (%control) = $[(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank})] \times 100$

Cell viability and cell death (%100 – %cell viability) were determined as a percentage of the control. The IC_{50} Calculator software (AAT Bioquest, Inc.) was used to calculate the IC_{50} value. Each experiment was triplicated, and the outcomes are presented as the mean ± SD of independent experiments.

Determination of Total Antioxidant Status (TAS): The effects of ROT and AE and RE in the presence/absence of ROT on the total antioxidant levels of SH-SY5Y cells were investigated using the Rel Assay Diagnostics Total Antioxidant Status (TAS) kit (Cat no: RL0017, Mega Tıp, Gaziantep, Türkiye) via the manufacturer's handbook. This experiment is based on the principle of cellular antioxidants reducing the ABTS radical, which is present in the kit contents, and the color fading. The fading of the color is an indicator of cellular antioxidant levels. Briefly, 3×10^5 SH-SY5Y cells were added into a 96-well plate at 37 °C and incubated for a day. After different treatments with the active substances and the final incubation, cell culture media from the control and experimental groups were collected into Eppendorf tubes according to the respective groups. 12 µL from each sample (samples collected in Eppendorf tubes, standards, and dH_2O) was transferred to the respective wells of the 96-well plate. Then, 200 µL of reagent 1 was applied and mixed thoroughly, maintained in the absence of light at room temperature for half minute, and spectrophotometric absorbance measurement was performed at a wavelength of 660 nm. The initially obtained absorbance values were recorded as A1. Subsequently, 30 µL of reagent 2 was added, mixed thoroughly, and incubated at room temperature in the dark for 10 minutes. In the final step, spectrophotometric measurement was performed at a wavelength of 660 nm, and the second absorbance values were recorded as A2. The results were calculated using the following formula and expressed in µmol Trolox Equiv./L. All data were obtained from three separate experiments.

TAS (µmol Trolox Equiv./L) = $([\Delta Abs_{H2O} - \Delta Abs_{Sample}] / [\Delta Abs_{H2O} - \Delta Abs_{Standard}]) \times 1000$

ΔAbs of standard or sample or H_2O = A2– A1

Determination of Total Oxidant Status (TOS): The effects of ROT and AE/RE in the absence/presence of ROT on the total oxidant levels of SH-SY5Y cells were investigated using the Rel Assay Diagnostics Total Oxidant Status (TOS) kit (Cat no: RL0024, Mega Tıp, Gaziantep, Türkiye) via the manufacturer's handbook. This experiment is based on the principle that cellular oxidants oxidize ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) present in the kit, and the ferric ions then combine with xylenol orange to form a colored complex. The color intensity of the complex is an indicator of the total cellular oxidant level. Briefly, 3×10^5 SH-SY5Y cells were added in a 96-well plate and incubated at 37 °C for a day. After various treatment procedures and the final incubation, the cell culture media from the control and experimental groups were collected into Eppendorf tubes according to the respective groups. From each sample (samples collected in Eppendorf tubes and standards), 30 µL was taken and transferred to the corresponding wells. Then, 200 µL of reagent 1 was applied, thoroughly mixed, and maintained in the absence of light at room temperature for 30 seconds, followed by spectrophotometric absorbance measurement at a wavelength of 530 nm. The initial absorbance values were recorded as A1. Subsequently, 10 µL of reagent 2 was added, thoroughly mixed, and placed in the dark at room temperature for 10 minutes. In the final step, spectrophotometric measurement was performed at a wavelength of 530 nm, and the second absorbance values were recorded as A2. The results were assessed with the help of the following formula and expressed as µmol H_2O_2 Equiv./L. All data were obtained from three separate experiments.

TOS (µmol H_2O_2 Equiv./L) = $(\Delta Abs_{Sample} / \Delta Abs_{Standard}) \times 10$

ΔAbs of standard or sample= A2– A1

Determination of Oxidative Stress: The intracellular total oxidative stress level is measured by the oxidative stress index (OSI). OSI is the quantity of the total oxidant amount to the total antioxidant amount in the cell and is calculated using the formula as described in the study by Dervişoğlu (12);

Statistical Analysis: All measurements were performed in triplicate, and data from three independent experiments (n = 3) are expressed as mean ± standard deviation (SD). Normality was assessed using the Shapiro–Wilk test in GraphPad Prism 8.0.2. The majority of groups showed p-values>0.05, indicating no significant deviation from a Gaussian distribution, while a few groups exhibited only mild departures from normality. Given the approximately symmetric distributions, absence of extreme outliers, and comparable standard deviations across groups, the assumptions for parametric testing were considered sufficiently met. Accordingly, between group comparisons were performed using unpaired two-tailed Student's t-tests, and $p<0.05$ was considered statistically significant.

Results

Treatment of SH-SY5Y cells with ROT for half day resulted in a dose-dependent, statistically significant increase ($^{**}p<0.01$ and $^{****}p<0.0001$ vs. 0 µM) in cell death (Figure 1). Specifically, cell death rates were approximately 4.89%, 8.62%, 7.60%, 10.71%, 21.32%, 51.74%, and 43.03% at ROT concentrations of 1.25, 2.5, 5, 10, 20, 40, and 80 µM, respectively. Furthermore, using the IC_{50} Calculator software (AAT Bioquest, Inc.), the IC_{50} value of ROT was determined to be 40 µM for a 12-hour treatment.

To observe ROT-induced damage, SH-SY5Y cells were pretreated with RPMI 1640 medium (containing 1% FBS and 1% antibiotic mixture) without AE and RE of *S. splendens* for 12 h, followed by exposure to 40 µM ROT without AE and RE of *S. splendens* for an additional 12 h. After the treatments, it was observed that ROT significantly reduced cell viability in both groups compared to the control, decreasing it to 38.8504% and 39.1004%, respectively ($^{\#\#\#\#}p<0.0001$ vs. 0 µM) (Figure 2A and B). To determine the appropriate doses of AE and RE of *S. splendens* to protect against ROT-induced damage, SH-SY5Y cells were pretreated with AE and RE of *S. splendens* at concentrations ranging from 3.051758 to 390.625 ng/mL for 12 h, followed by exposure to 40 µM ROT in the presence of the same concentrations of AE and RE for an additional 12 h. Following the treatments, AE demonstrated a slight attenuation of ROT-induced cell viability reduction only at a concentration of 195.3125 ng/mL, resulting in a cell viability of 42.3086% ($^{**}p<0.01$ vs. 40 µM ROT). In contrast, RE significantly mitigated the ROT-induced decrease in cell viability across a concentration range of 6.103516 to 390.625 ng/mL, yielding cell viabilities of 44.7354%, 50.5931%, 48.7424%, 45.5342%, 42.7320%, 51.3744%, and 48.8253%, respectively (with statistical significance of $^{**}p<0.01$, $^{****}p<0.0001$, $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$, $^{****}p<0.0001$, and $^{****}p<0.0001$ vs. 40 µM ROT) (Figure 2A and B). The highest protective effect of both AE and RE against ROT-induced cell damage was observed at a concentration of 195.3125 ng/mL, and this concentration was selected for subsequent experiments.

As shown in Figure 3A and B, the viability rates of SH-SY5Y cells in the AE and RE groups were found to be 96.9518% and 98.5176%, respectively. When each group was compared separately with the control group, no statistically significant difference in cell viability was observed ($^{ns}p>0.05$ vs. 0 µM). The viability rates for SH-SY5Y cells treated with ROT alone for 12 h were 40.6821% and 40.2904% respectively compared with the control group ($^{****}p<0.0001$ vs. 0 µM). However, treatment with 195.3125 ng/mL of AE and RE significantly increased the viability of SH-SY5Y cells to 46.3086% and 52.7720%, respectively, compared to the ROT group ($^{\#\#\#\#}p<0.0001$ vs. 40 µM ROT). These obvious differences suggest that the AE and RE of *S. splendens* are capable of effectively mitigating cell damage induced by ROT.

We measured the levels of TAS and TOS in SH-SY5Y human neuroblastoma cells to determine whether AE and RE of *S. splendens* protect against ROT-induced oxidative stress. TAS of the control group was determined to be 217.8153 µmol Trolox equivalent/L, while in the AE and RE groups, these values were measured as 263.7191 and 261.3784 µmol Trolox equivalent/L, respectively. A statistically significant increase in TAS was found in the AE and RE groups compared to the control group ($^{\#\#\#\#}p<0.0001$ and $^{\#\#\#}p<0.001$ vs. 0 µM, respectively) (Figure 4). However, the TAS of the ROT group, AE + ROT group, and RE + ROT group was determined as 113.1339, 153.7191, and 248.6996 µmol Trolox equivalent/L, respectively. Compared to the control group, ROT significantly decreased the TAS in a statistically significant manner ($^{\#\#\#\#}p<0.0001$ vs. 0 µM). In contrast,

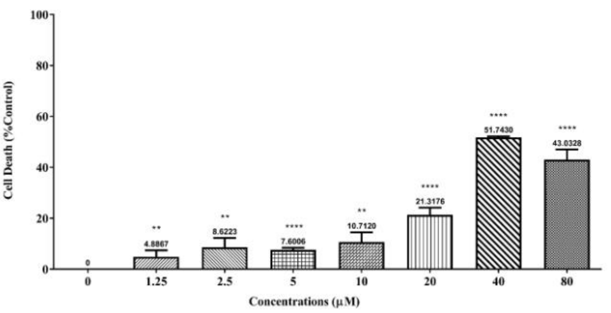

Fig. 1. Cell death in SH-SY5Y human neuroblastoma cells (% of control) after incubation with ROT. Data are presented as the mean ± standard deviation (SD) of three independent experiments (n=3). Analyses were performed using an unpaired two-tailed Student's t-test ($p<0.05$ was considered significant). $^{**}p<0.01$ and $^{****}p<0.0001$, versus the control (0 μM)

when ROT was compared separately with all extract groups, all extract groups significantly increased the TAS in a statistically significant manner ($^{****}p<0.0001$ vs. 40 μM ROT) (Figure 4).

TOS of the control group was determined to be 8.5415 μmol H_2O_2 equivalent/L, while in the AE and RE groups, these values were measured as 10.8923 and 9.5547 μmol H_2O_2 equivalent/L, respectively. A statistically significant increase in TOS was found in the AE and RE groups compared to the control group ($^{\#\#\#\#}p<0.0001$ and $^{\#\#}p<0.01$ vs. 0 μM, respectively) (Figure 5). Furthermore, the TOS of the ROT group, AE + ROT group, and RE + ROT group was determined as 8.7783, 8.7967, and 8.7783 μmol H_2O_2 equivalent/L, respectively. Compared to the control group, ROT significantly increased the TOS in a statistically significant manner ($^{\#}p<0.05$ vs. 0 μM). However, when ROT was compared separately with all extract groups, no statistically significant difference in TOS was observed ($^{ns}p>0.05$ vs. 40 μM ROT) (Figure 5).

OSI is the relative amount of TOS and TAS markers produced in the cell, and this ratio is used as an indicator of cellular oxidative stress. The OSI of the control group was determined to be 3.9203%, while in the AE and RE groups, these values were measured as 4.1310% and 3.5939%, respectively. A statistically slight increase in OSI was observed in the AE group, while a statistically significant decrease in OSI was observed in the RE group compared to the control group ($^{\#}p<0.05$ and $^{\#\#}p<0.01$ vs. 0 μM, respectively) (Figure 6). However, the OSI of the ROT group, AE + ROT group, and RE + ROT group was determined as 7.7617%, 5.7199%, and 3.1232%, respectively. Compared to the control group, ROT significantly increased the OSI in a statistically significant manner ($^{\#\#\#}p<0.001$ vs. 0 μM). In contrast, when ROT was compared separately with all extract groups, the AE + ROT and RE + ROT groups significantly decreased the OSI in a statistically significant manner ($^{**}p<0.01$ and $^{****}p<0.0001$ vs. 40 μM ROT, respectively) (Figure 6). These results suggest that the aerial part and root extracts (AE and RE) of *S. splendens* are effective in alleviating oxidative damage induced by ROT in SH-SY5Y cells.

Discussion

When SH-SY5Y cells were administered with ROT at concentrations ranging between 1.25 to 80 μM for 12 h, the death rate of SH-SY5Y cells increased in a dose dependent manner. In recent *in vitro* studies on ROT neurotoxicity in SH-SY5Y cells, the ROT dose typically ranged from 200 nM to 100 μM, and our found IC_{50} value of 40 μM was consistent with these previous reports (14-16) and used in subsequent experiments. In the absence of ROT, cell viability of the AE and RE groups did not show statistically significant differences compared to the control group. To determine the optimal dose of *S. splendens* AE in mitigating ROT-induced neurotoxicity, SH-SY5Y cells were treated with a concentration range of 3.051758–390.625 ng/mL for 12 hours in the presence of 40 μM ROT, followed by a WST-8 assay. At 195.3125 ng/mL, AE-treated cells exhibited a slight decrease in viability (42.3086%), whereas RE at the same concentration improved cell viability to 51.3744%, suggesting a protective effect. These findings indicate that RE is particularly effective in reducing ROT-induced cytotoxicity. Both extracts demonstrated their highest neuroprotective activity at 195.3125 ng/mL.

Methanolic extracts of *Salvia officinalis* exhibited potent cytotoxic effects on lymphoma and leukemia cell lines, with IC_{50} values ranging from 200 to 300 μg/mL, according to a prior study by Valiyari, Baradaran (17). According to earlier *in vitro* research, ROT toxicity may also impair TAS (18, 19).

In our study, treatment of SH-SY5Y cells with AE and RE at 195.3125 ng/mL in the absence of 40 μM ROT significantly increased TAS levels compared with control ($p<0.0001$ and $p<0.001$). But the treatment of SH-SY5Y cells with 40 μM ROT alone significantly decreased TAS levels compared with control ($p<0.0001$). However,

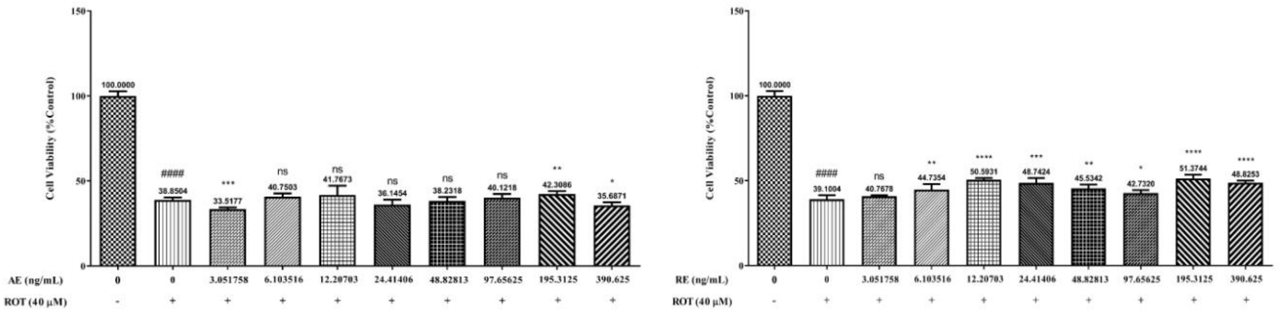

Fig. 2. The effects of (A) AE and (B) RE of *S. splendens* on cell viability (% of control) against ROT-induced cytotoxicity in SH-SY5Y human neuroblastoma cells. Data are presented as the mean ± standard deviation (SD) of three independent experiments (n=3). Analyses were performed using an unpaired two-tailed Student's t-test ($p<0.05$ was considered significant). ####$p<0.0001$ versus the control (0 μM); ns$p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$, versus rotenone

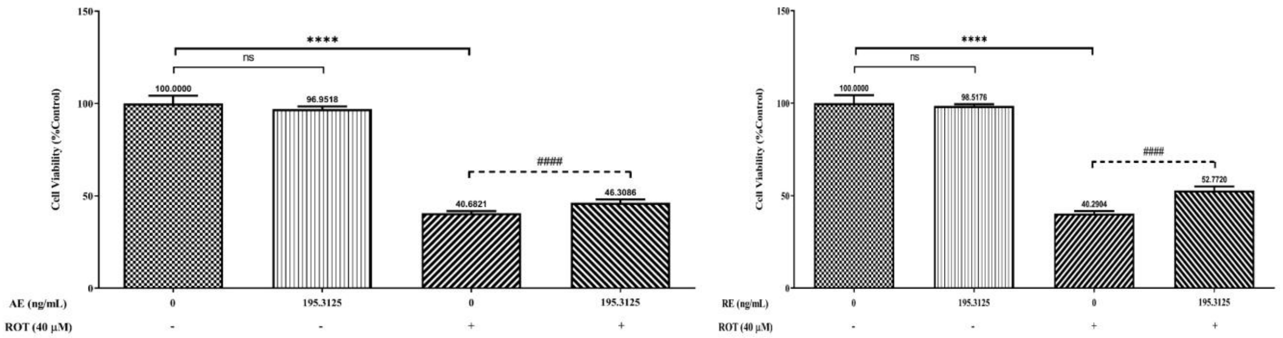

Fig. 3. Neuroprotective effects of (A) AE and (B) RE of *S. splendens* on cell viability (% of control) in SH-SY5Y human neuroblastoma cells in the absence and presence of ROT. Data are presented as the mean ± standard deviation (SD) of three independent experiments (n=3). Analyses were performed using an unpaired two-tailed Student's t-test ($p<0.05$ was considered significant). ns$p>0.05$ and ****$p<0.0001$, versus the control (0 μM); ####$p<0.0001$, versus rotenone

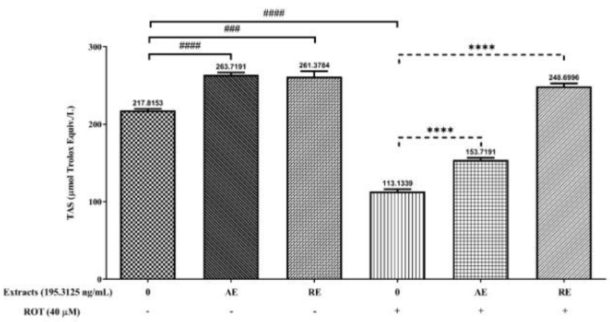

Fig. 4. Effects of AE and RE of *S. splendens* on the total antioxidant status (TAS) of SH-SY5Y human neuroblastoma cells in the absence and presence of ROT. Data are presented as the mean ± standard deviation (SD) of three independent experiments (n=3). Analyses were performed using an unpaired two-tailed Student's t-test ($p<0.05$ was considered significant). ###$p<0.001$ and ####$p<0.0001$, versus the control (0 μM); ****$p<0.0001$, versus rotenone.

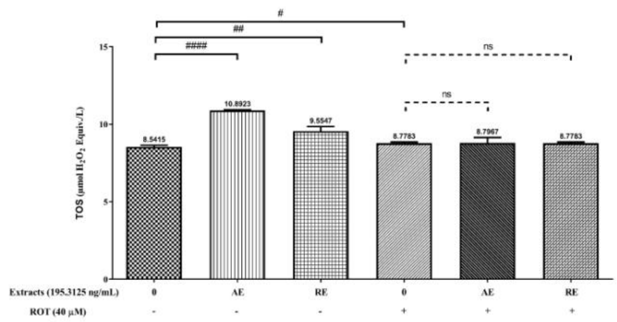

Fig. 5. Effects of AE and RE of *S. splendens* on the total oxidant status (TOS) of SH-SY5Y human neuroblastoma cells in the absence and presence of ROT. Data are presented as the mean ± standard deviation (SD) of three independent experiments (n=3). Analyses were performed using an unpaired two-tailed Student's t-test ($p<0.05$ was considered significant). #$p<0.05$, ##$p<0.01$ and ###$p<0.001$, versus the control (0 μM); ns$p>0.05$, versus rotenone

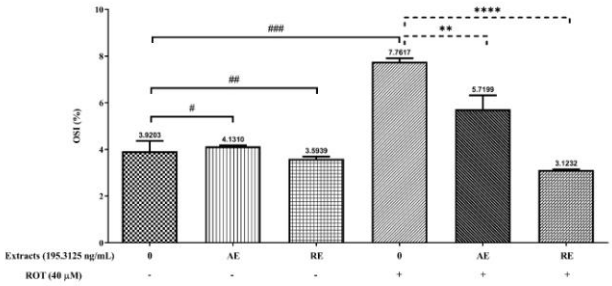

Fig. 6. Effects of AE and RE of *S. splendens* on the oxidative stress (OSI) of SH-SY5Y human neuroblastoma cells in the absence and presence of ROT. Data are presented as the mean ± standard deviation (SD) of three independent experiments (n=3). Analyses were performed using an unpaired two-tailed Student's t-test (p<0.05 was considered significant). $^{\#}p$<0.05, $^{\#\#}p$<0.01 and $^{\#\#\#}p$<0.001, versus the control (0 μM); $^{**}p$<0.01 and $^{****}p$<0.0001, versus rotenone

when cells treated with ROT alone were compared with the AE + ROT and RE+ ROT groups separately, both extracts significantly increased TAS levels (p<0.0001), indicating their antioxidant potential.

ROT treatment caused a slight but statistically significant increase in TOS compared to the control group (p<0.05). Nevertheless, no discernible variations in TOS were found when ROT-treated cells and extract-treated groups were evaluated independently (p>0.05).

Due to ATP depletion and oxidative stress caused by ROT's inhibition of mitochondrial complex I, excessive production of ROS and consequent damage to cells are encouraged (20-22). The OSI increased slightly but statistically significantly with AE therapy, whereas it decreased dramatically with RE treatment when compared to the control group ($^{\#}p$<0.05 and $^{\#\#}p$<0.01 vs. 0 μM, respectively). The OSI levels were considerably higher in the ROT, AE + ROT, and RE + ROT groups than in the control group ($^{\#\#\#}p$<0.001 vs. 0 μM). These findings suggest that *S. splendens* extracts (AE and RE) help counteract ROT-induced oxidative damage in SH-SY5Y cells.

ROT exposure significantly increased oxidative stress (OSI) compared to the control group. However, when ROT-treated cells were analyzed separately against the extract-treated groups, both AE and RE effectively reduced oxidative stress. Our findings align with previous studies (23), further supporting the antioxidative effects of *S. splendens*.

Overall, these results highlight that both the aerial and root extracts of *S. splendens* exhibit neuroprotective properties against ROT-induced in vitro neurodegeneration by mitigating oxidative stress.

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Conflict of interest: The authors of the article declare that they have no conflict of interest.

Summary of Declaration of Contribution of Researchers: Authors declare that they have contributed equally to the article.

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