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Antioxidative Effects of *Parnassia palustris* L. Extract on Ferrous Sulfate-Induced Cellular Injury in Cultured C6 Glioma Cells

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Abstract

This study sought to evaluate the mechanism of cellular injury caused by ferrous sulfate ($FeSO_4$) and the protective effects of *Parnassia palustris* L. (PP) extract against $FeSO_4$-induced cytotoxicity of cultured C6 glioma cells. $FeSO_4$ is known to cause neurotoxicity and induce Parkinson's disease. The antioxidative effects of PP, such as superoxide dismutase (SOD)-like and superoxide anion-radical (SAR)-scavenging activities, as well as effects on cell viability, were studied. $FeSO_4$ significantly decreased cell viability in a dose-dependent manner and the XTT_{50} value, the concentration of $FeSO_4$ which reduced the cell viability by half, was measured at 63.3 μM in these cultures. $FeSO_4$ was estimated to be highly cytotoxic by the Borenfreund and Puerner toxicity criteria. Quercetin, an antioxidant, significantly improved the cell viability damaged by $FeSO_4$-induced cytotoxicity. While evaluating the protective effects of the PP extract on $FeSO_4$-induced cytotoxicity, it was observed that the extract significantly increased cell viability compared to the $FeSO_4$-treated group. Additionally, the PP extract showed SOD-like and SAR-scavenging activities. Based on these findings, it can be concluded that $FeSO_4$ induced oxidative stress-related cytotoxicity, and the PP extract effectively protected against this cytotoxicity via its antioxidative effects. In conclusion, natural antioxidant sources such as PP may be useful as agents to prevent oxidative stress-related cytotoxicity induced by heavy metal compounds such as $FeSO_4$, which is a known Parkinsonism inducer.

Keywords

Antioxidative effect
Ferrous sulfate
Oxidative stress
Parkinsonism

1. Introduction

As the world is rapidly becoming an aging society, the incidence of neurodegenerative diseases such as stroke and dementia, including Parkinson's disease, is increasing along with the number of elderly people. According to the World Health Organization, the number of people living with Parkinson's disease increased from 3 million in 1990 to 6.2 million in 2015, and is expected to increase to 14.2 million by 2040 [1]. However, there is still a lack of understanding of the toxic mechanisms, appropriate treatments, and effective therapies for the disease. In addition, the morbidity rate of the disease is increasing due to external factors such as fine dust, heavy metals, and water pollution caused by environmental pollution, and heavy metal ferrous sulfate ($FeSO_4$) is known to cause Parkinson's disease [2]. Parkinsonism is a neurodegenerative disease similar to dementia or Lou Gehrig's disease, which is an intractable brain lesion with three main symptoms such as tremor, rigidity, and akinesia, and it was discovered and named by British physician James Parkinson in 1817 [3]. Parkinson's disease is directly caused by the loss of dopaminergic neurons in the substantia nigra due to internal factors, and indirectly resulted from the damage or degeneration of dopaminergic neurons due to environmental factors [4]. Neurodegenerative diseases, such as Parkinson's disease, are challenging to treat because there are currently no effective treatments or medications. Surgical treatments for Parkinson's disease include placement of electrodes in the subthalamic nucleus and thalamotomy, both of which have the disadvantage of severe sequelae. Therefore, pharmacotherapy is the mainstay of treatment, with anticholinergic agents such as levodopa and trihexyphenidyl currently approved by the US Food and Drug Administration (FDA) [5]. In recent years, therapeutic agents have been developed from compounds, antibodies, stem cells, and genes [6]. However, these agents have severe side effects due to toxicity when administered for a long period of time, thus there is an urgent need to develop alternative substances that have high therapeutic efficacy without toxicity. Recently, iron (Fe), a heavy metal, has been recognized as a trigger of Parkinson's disease. It has been suggested that iron compounds cause oxidative damage to neurons in the dopaminergic system, causing them to degenerate, thereby leading to Parkinson's disease [2]. Therefore, in this study, we investigated the toxicity of iron compounds using a cultured C6 glioma cell line, a type of neuroglial cell, to determine whether the neurotoxicity caused by iron compounds is related to oxidative damage in neurons other than dopaminergic cells. It has been suggested that some heavy metals, such as lead, cadmium, and aluminum, decay to produce free radicals, and that the toxicity of the resulting free radicals is related to oxidative damage [7]. On the other hand, various plants have been found to contain a large amount of antioxidant, antiviral, antibacterial, and antitoxic active ingredients, and therapeutic approaches using these ingredients have been attempted [8]. Among the plants, *Parnassia palustris* L. (abbreviated as PP) is also called grass of Parnassus or bog star. PP is a perennial grass belonging to the Saxifragaceae family that inhabits the mountains and forests throughout Korea, and its leaves are egg-shaped, with a blunt tip and smooth, non-serrated edges. The white flowers bloom from July to September, and the fruit is egg-shaped and contains many small seeds, and the herb is collected in summer and dried in the sun [9]. PP is cold in nature and bitter in flavor, but it contains several components of the flavonoid family such as rutin, quercetin, kaempferol, alkaloid, and hyperin, which have been used for a long time to treat diseases such as jaundice, hepatitis, gangrene, carbuncle, and arteritis, including antitoxicity [10]. In particular, curcumin, kaempferol, rutin, quercetin, tannin, and hyperin are members of the phenolic flavonoid family, and it is well-known that they have excellent antioxidant activity [11]. Most of the research on PPs has been focused on genetic studies of differences in PP species or on their constituents, with little research on anti-inflammatory, antimicrobial, antitoxic, and antioxidant properties [12]. In recent

years, with the development of cell culture methods, suitable lesion models have been created and used for various quantitative analyses, such as investigating the mechanisms of diseases, the efficacy of substances, and the safety of chemical agents [13]. In this study, we investigated the neurotoxicity of ferrous sulfate ($FeSO_4$), one of the triggers of Parkinson's disease, in terms of oxidative damage using C6 glioma cells, a neuronal cell line. In addition, the antioxidative effect of PP extract on the toxicity of $FeSO_4$ was analyzed to identify alternative natural substances that can effectively reduce the pathological toxicity of heavy metal compounds associated with oxidative damage.

2. Materials and methods

2.1. Pharmaceutical preparation

The reagents used in this experiment were $FeSO_4$, quercetin, alcohol, pyrogallol, nitroblue tetrazolium (NBT), phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), xanthine, Tris-HCl buffer, and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, disodium salt (XTT), which were purchased from Sigma (St Louis, MO, USA). Furthermore, the preparation of $FeSO_4$ was carried out using minimum essential medium (MEM) without fetal bovine serum (FBS, Gibco, USA) to make 50, 100, 150, and 200 μM of each stock solution. The XTT (50 μg/mL) stock solution was diluted in PBS.

2.2. *Parnassia palustris* L. (PP) harvesting and extraction

The plant was collected from August to September in Yasan, Iksan, Jeollabuk-do, dried in the sun, and the samples were frozen for use. For extraction, 80.6 g of the sample and about 280 mL of distilled water were placed in a 1,000 mL reflux flask and heated for 3 hours. The liquid obtained by repeating the process three times was collected, centrifuged at 3,000 rpm for 30 minutes, and concentrated under reduced pressure to obtain 2.3 g of sample with a yield of 2.9%. The sample was diluted as necessary to meet the experimental purpose.

2.3. Cell culture and cell viability assay

Cultures of the C6 glioma cell line (ATCC, CCL-107) were isolated using trypsin enzyme, and the isolated cells were mixed well in serum-containing culture medium and then seeded into 96-well culture plates at a density of 1×10^5 cells/well. After seeding, cells were incubated at 36°C, 5% CO_2 for 72 hours in a constant temperature chamber, and cell viability was analyzed after treating the cultured cells with the agents by concentration. For cell viability assay, 10 μL of XTT (50 μg/mL) was added to each well of drug-treated cells using the method described by Mosmann [14] and incubated in a thermostat for 4 hours. After the incubation was completed, the cells were treated with DMSO and the absorbance was measured at 450 nm in an ELISA reader (SpectraMax 250, Molecular Devices, Sunnyvale, USA) to compare with the control.

2.4. Treatment of $FeSO_4$

Based on the results of Pyo *et al.* [15], cultured C6 glioma cells were treated with $FeSO_4$ at concentrations ranging from 30 to 70 μM for 48 hours, respectively, and then the XTT_{50} values for cell viability were determined.

2.5. Determination of antioxidant capacity of quercetin (QU) and its effect on $FeSO_4$

To investigate the antioxidant capacity of QU based on the results of Chung *et al.* [16], the cultured cells were treated with 25 μM H_2O_2 before treatment, and QU at 35 μM and 45 μM concentrations were each treated for 2 hours to investigate the effect on cell viability. In addition, to investigate the effect of QU on $FeSO_4$, the cells were treated with 35 μM and 45 μM of QU for 2 hours before treatment with $FeSO_4$ at the concentration of XTT_{50}, and the cell survival rate was investigated.

2.6. Cytotoxicity determination of PP extracts and extract treatment against $FeSO_4$

To investigate the toxicity of the PP extract, cultured cells were treated with 90 and 150 μg/mL of the extract for 48 hours, and the cell viability was investigated. In addition, to investigate the effect of PP extract on $FeSO_4$, cultured cells were treated with 110 μg/mL and 130 μg/mL extracts for 2 hours before treatment with $FeSO_4$ at the concentration of XTT_{50}, and the effect of each was investigated by cell viability.

2.7. Composition analysis of PP extract

The polyphenol analysis was performed according to the method by the Association of Official Analytical Chemists [17], where 0.2 mL extract and 0.2 mM of phenol reagent were reacted together for 3 minutes and then treated with 0.4 mL sodium carbonate for 1 hour. The absorbance was measured at 725 nm with an ELISA reader. Tannic acid was used as the standard reagent and a calibration curve was prepared. Flavonoid analysis was performed according to the method by Nieva Moreno *et al.* [18], in which 0.1 mL of sample solution, 10% aluminum nitrate, 4.7 mL of ethanol, and 0.2 mL of 1 M potassium acetate mixture were reacted at 25℃ for 40 minutes, and the absorbance was measured at 415 nm with an ELISA reader. A calibration curve was prepared using rutin as the standard.

2.8. Determination of superoxide dismutase (SOD)-like activity

The SOD-like activity was determined by the method of Marklund and Marklund [19], in which samples were treated with 10 mM pyrogallol and Tris-HCl buffer and reacted for 10 minutes at 25℃. After the reaction, the samples were treated with HCl and the absorbance was measured at 420 nm with an ELISA reader. The SOD-like activity was expressed as a percentage of the control, and the SOD-like activity was expressed as SOD-like activity (%) = [(absorbance of sample added / absorbance of no sample added) × 100] – 100, with QU as the positive control.

2.9. Determination of superoxide anion-radical (SAR)-scavenging activity

The determination of SAR-scavenging activity was performed using the method of nitroblue tetrazolium (NBT) by adding NBT to 0.1 mL of sample solution, 0.4 mL potassium phosphate buffer (pH 7.5), and 0.4 mM xanthine, and reacting at 37℃ for 20 minutes. Upon completion of the reaction, 1 mL of 1 N HCl was added and the absorbance was measured at 560 nm using an ELISA reader. SAR-scavenging activity was expressed as a percentage of the control. The SAR-scavenging activity was expressed as SAR-scavenging activity (%) = 100 – [(absorbance of sample added / absorbance of no sample added) × 100], with QU as the positive control.

2.10. Statistical analysis

The results were expressed as mean ± standard deviation (SD) using SPSS/WIN (20.0) program, the comparisons between groups were analyzed by one-way ANOVA, and post-hoc analysis was performed by Tukey's Honest Significant Difference (HSD). The level of significance was adopted at $P < 0.05$.

3. Result

3.1. Determination of cytotoxicity of $FeSO_4$

To determine the toxicity of $FeSO_4$, cultured C6 glioma cells were incubated in culture media containing $FeSO_4$ at concentrations ranging from 30 to 70 μM for 48 hours. The results showed a significant decrease in cell viability in a dose-dependent manner, indicating toxicity ($P < 0.001$). $FeSO_4$ at concentrations of 30 μM, 50 μM, and 70 μM resulted in 73.8%, 64.3%, and 42.9% cell viability compared to the control, respectively, and the XTT_{50} value was found at the treatment of 63.3 μM (**Table 1**). Post-hoc analysis of cytotoxicity showed that $FeSO_4$ at

Table 1. The cytotoxicity of FeSO4 by XTT assay

Concentrations of $FeSO_4$ (μM)	XTT assay (450 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.42 ± 0.02	100	208.53	< 0.001	a > b > c > d
30[b]	0.31 ± 0.02	73.8			
50[c]	0.27 ± 0.02	64.3			
70[d]	0.18 ± 0.01	42.9			

The data indicate the mean ± SD for triplicate experiments.
Abbreviation: $FeSO_4$, ferrous sulfate

Table 2. The antioxidative ability of QU on the H_2O_2 in cultured C6 glioma cells

Concentrations of QU (μM)	XTT assay (450 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.50 ± 0.03	100	417.61	< 0.001	a > d > c > b
25 H_2O_2[b]	0.19 ± 0.01	38.0			
35/25 H_2O_2[c]	0.36 ± 0.02	72.0			
45/25 H_2O_2[d]	0.38 ± 0.02	76.0			

The data indicate the mean ± SD for triplicate experiments.
Abbreviations: QU, quercetin; H_2O_2, hydrogen peroxide

Table 3. The effect of QU on the cytotoxicity induced by $FeSO_4$ in cultured C6 glioma cells

Concentrations of QU (μM)	XTT assay (450 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.57 ± 0.03	100	393.15	< 0.001	a > d, c > b
$FeSO_4$ (XTT_{50})[b]	0.20 ± 0.03	35.1			
35/$FeSO_4$ (XTT_{50})[c]	0.27 ± 0.01	47.4			
45/$FeSO_4$ (XTT_{50})[d]	0.34 ± 0.02	59.6			

The data indicate the mean ± SD for triplicate experiments.
Abbreviations: QU, quercetin; $FeSO_4$, ferrous sulfate

concentrations of 70 μM, 50 μM, 30 μM, and control were highly cytotoxic.

3.2. Determination of antioxidant capacity of quercetin (QU)

To investigate the antioxidant capacity of QU, the cultured cells were treated with 25 μM H_2O_2 for 2 hours before treatment with QU at concentrations of 35 μM and 45 μM, respectively. As a result, the cell survival rate of H_2O_2 treated cells was 38.0% (0.19 ± 0.01) compared to the control, while the cell survival rate of cells treated with 35 μM and 45 μM QU was 72.0% (0.36 ± 0.02) and 76.0% (0.38 ± 0.02), respectively. Unlike H_2O_2 alone, all treatments significantly increased the cell viability ($P < 0.001$) (**Table 2**). Post-hoc analysis of the antioxidant capacity of QU showed that cell viability was the highest in the control, 45 μM QU, 35 μM QU, and 25 μM H_2O_2, in that order.

3.3. Effect of QU on the toxicity of $FeSO_4$

The effect of QU, an antioxidant, on the cytotoxicity of $FeSO_4$ was investigated by treating cultured cells with 35 μM and 45 μM QU for 2 hours before treating them with $FeSO_4$ at the concentration of XTT_{50}. The results showed that the cell viability was 35.1% (0.20 ± 0.03) in the treatment of $FeSO_4$ compared to the control, but it

Table 4. The cytotoxicity of PP extract on cultured C6 glioma cells by XTT assay

Concentrations of PP extract (μg/mL)	XTT assay (450 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.39 ± 0.03	100	5.07	0.002	a > e
90[b]	0.38 ± 0.02	97.4			
110[c]	0.37 ± 0.02	94.9			
130[d]	0.36 ± 0.03	92.3			
150[e]	0.35 ± 0.01	89.7			

The data indicate the mean ± SD for triplicate experiments.
Abbreviation: PP, *Parnassia palustris* L.

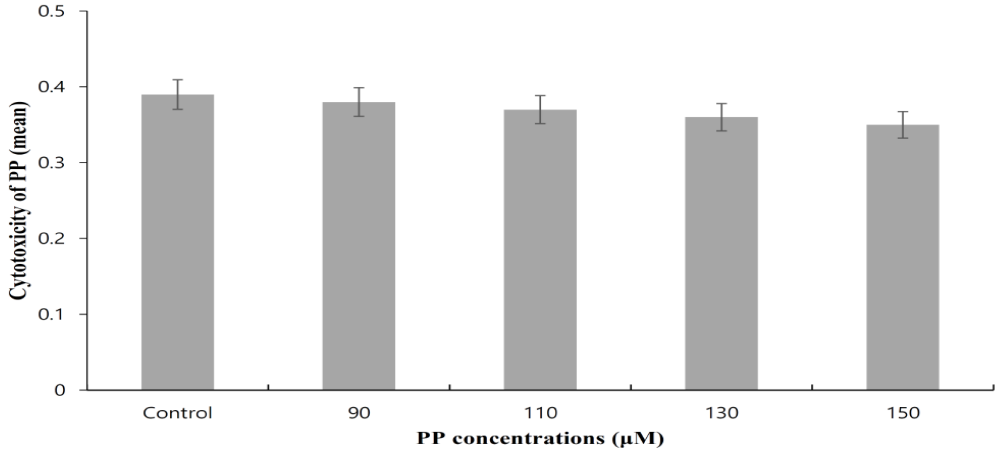

Figure 1. The cytotoxicity of *Parnassia palustris* L. (PP) extract on cultured C6 glioma cells. Cultured cells were incubated with PP extract at concentrations of 90, 110, 130, and 150 μg/mL. The data indicate the mean ± SD for triplicate experiments.

was 47.4% (0.27 ± 0.01) and 59.6% (0.34 ± 0.02) in the treatment of 35 μM and 45 μM QU, respectively ($P < 0.001$) (**Table 3**). Post-hoc analysis of the effect of QU on the cytotoxicity of $FeSO_4$ showed that cell viability was the highest in the control, followed by 45 μM QU, 35 μM QU, and $FeSO_4$ (XTT_{50}).

3.4. Cytotoxicity of PP extract

The toxicity analysis of PP extract was performed by exposing the cells in the culture media containing 90 and 150 μg/mL of PP extract, respectively. Based on **Table 4** and **Figure 1**, the cell survival rate was 97.4% (0.38 ± 0.02) and 94.9% (0.37 ± 0.02) at 90 μg/mL and 110 μg/mL concentrations, respectively, and 92.3% (0.36 ± 0.03) and 89.7% (0.35 ± 0.01) at 130 μg/mL and 150 μg/mL concentrations, respectively ($P < 0.001$). The post-hoc analysis of the toxicity of PP extract showed that there was no statistical difference between 110 μg/mL, 130 μg/mL, and 150 μg/mL, but cell viability was higher at 110 μg/mL, 130 μg/mL, and 150 μg/mL, in that order. 90 μg/mL was not statistically different from the control, 110 μg/mL, 130 μg/mL, but was statistically different from 150 μg/mL. Since there was a statistical difference between the control group and 150 μg/mL, it was confirmed that the maximum tolerable concentration at which cell viability was significantly reduced compared to the control was above 150 μg/mL. Therefore, PP extract with a concentration of 150 μg/mL or less was used in this experiment.

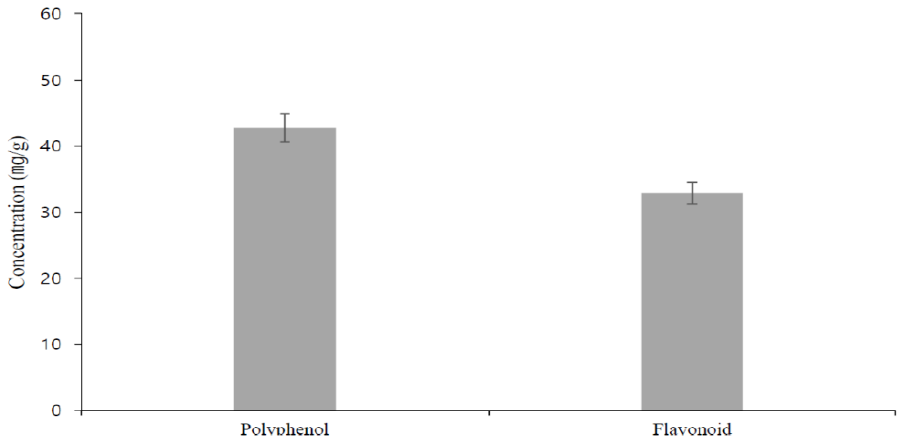

Figure 2. The component of *Parnassia palustris* L. (PP) extract. The data indicate the mean ± SD for triplicate experiments.

Table 5. The protective effect of PP extract on the cytotoxicity induced by $FeSO_4$ in cultured C6 glioma cells

Concentrations of PP extract (μg/mL)	XTT assay (450 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.33 ± 0.002	100	293.71	< 0.001	a > d > c > b
$FeSO_4$[b]	0.13 ± 0.001	39.4			
110/$FeSO_4$ (XTT_{50})[c]	0.17 ± 0.01	51.5			
130/$FeSO_4$ (XTT_{50})[d]	0.20 ± 0.001	60.6			

The data indicate the mean ± SD for triplicate experiments.
Abbreviations: PP, *Parnassia palustris* L.; $FeSO_4$, ferrous sulfate

3.5. Composition analysis of PP extract

The content of polyphenols in the PP extract was 42.8 mg/g, and the content of flavonoids was 32.9 mg/g (**Figure 2**).

3.6. Effect of PP extract on cytotoxicity of $FeSO_4$

The effect of PP extracts on the cytotoxicity of $FeSO_4$ was investigated by treating cultured cells with 110 μg/mL and 130 μg/mL of PP extracts, respectively, before treating them with $FeSO_4$ at the concentration of XTT_{50}. The cell survival rate of $FeSO_4$-treated cells was 39.4% (0.13 ± 0.01) in the 110 μg/mL extract treatment, 51.5% (0.17 ± 0.01) in the 130 μg/mL extract treatment, and 60.6% (0.20 ± 0.01) in the 130 μg/mL extract treatment, which showed a significant increase ($P < 0.001$) in contrast to the treatment with $FeSO_4$ (**Table 5**). Post-hoc analysis showed that the cell viability was decreasing in the order of control, 130 μg/mL PP extract, 110 μg/mL PP extract, and $FeSO_4$.

3.7. Measurement of SOD-like activity

To measure the SOD-like activity, 110 μg/mL and 130 μg/mL concentrations of PP extract samples were treated and analyzed. The treatment of 110 μg/mL concentration resulted in an activity of 113.6% compared to the control, while the 130 μg/mL treatment resulted in an activity of 118.4% (**Table 6**). The SOD-like activity at 110 μg/mL and 130 μg/mL concentrations was 13.6% and 18.4%, respectively, which both showed a significant increase in SOD-like activity over the control. In particular, the treatment of 130 μg/mL concentration resulted in almost 30% of

Table 6. The SOD-like activity of PP extract determined at a wavelength of 420 nm

Concentrations of PP extract (μg/mL)	SOD-like activity (420 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.38 ± 0.02	100	241.19	< 0.001	a > c, d > b
45 μM QU[b]	0.62 ± 0.02	163.2			
110[c]	0.43 ± 0.02	113.6			
130[d]	0.45 ± 0.02	118.4			

The data indicate the mean ± SD for triplicate experiments.
Abbreviations: PP, *Parnassia palustris* L.; QU, quercetin; SOD, superoxide dismutase

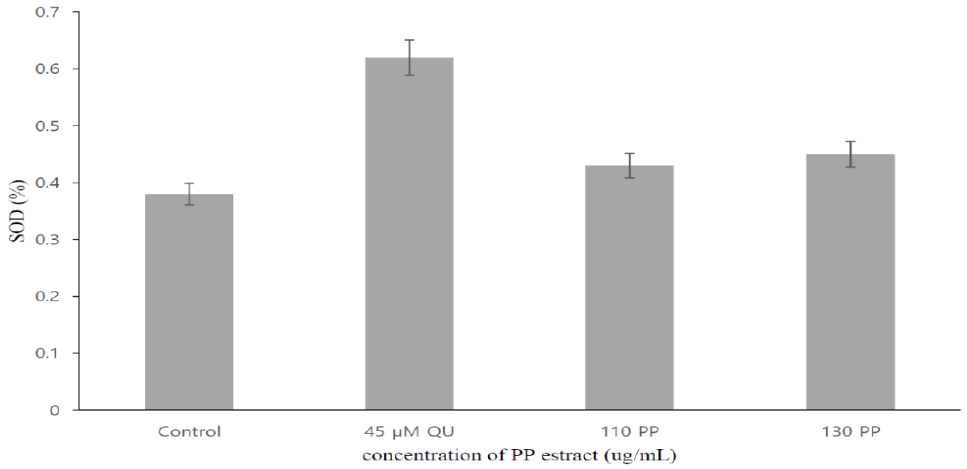

Figure 3. The superoxide dismutase (SOD)-like ability of *Parnassia palustris* L. (PP) extract determined at a wavelength of 420 nm. The data indicate the mean ± SD for triplicate experiments.

the scavenging capacity of the positive control, QU, of 63.2 ($P < 0.001$) (**Figure 3**). Post-hoc analysis of SOD-like activity showed that QU had the highest activity, followed by 130 μg/mL PP extract, 110 μg/mL PP extract, and the control.

3.8. SAR-scavenging activity measurement

To measure the extent of SAR-scavenging activity of PP extract, 110 μg/mL and 130 μg/mL extract samples were analyzed, and the results showed that the SAR-scavenging activity was 80.4% in the 110 μg/mL extract treatment and 67.4% in the 130 μg/mL extract treatment (**Table 7**). Thus, the SAR-scavenging capacity was 19.6% ($P < 0.01$) and 32.6% at 110 μg/mL and 130 μg/mL, respectively, both of which showed higher inhibition than the control ($P < 0.001$). In particular, at a concentration of 130 μg/mL, the clearance was more than 45% of the positive control QU's clearance of 69.6% ($P < 0.001$) (**Figure 4**). Post-hoc analysis showed that the scavenging capacity was increasing in the order of QU, 130 μg/mL PP extract, 110 μg/mL PP extract, and the control.

4. Discussion

Parkinson's disease, like dementia, not only makes it difficult for patients to live a normal life, but also threatens the continuation of human life. In particular, as the environment becomes more polluted, incurable diseases such as Parkinson's disease are occurring at a higher frequency due to fine dust, heavy metals,

Table 7. The SAR scavenging activity of PP extract at a wavelength of 560 nm

Concentrations of PP extract (μg/mL)	SAR-scavenging activity (560 nm) Mean ± SD	%	*F*	*P*	Tukey's HSD
Control[a]	0.46 ± 0.01	100	622.49	< 0.001	a > c > d > b
45 μM QU[b]	0.14 ± 0.02	30.4			
110[c]	0.37 ± 0.01	80.4			
130[d]	0.31 ± 0.02	67.4			

The data indicate the mean ± SD for triplicate experiments.
Abbreviations: PP, *Parnassia palustris* L.; QU, quercetin; SAR, superoxide anion radical

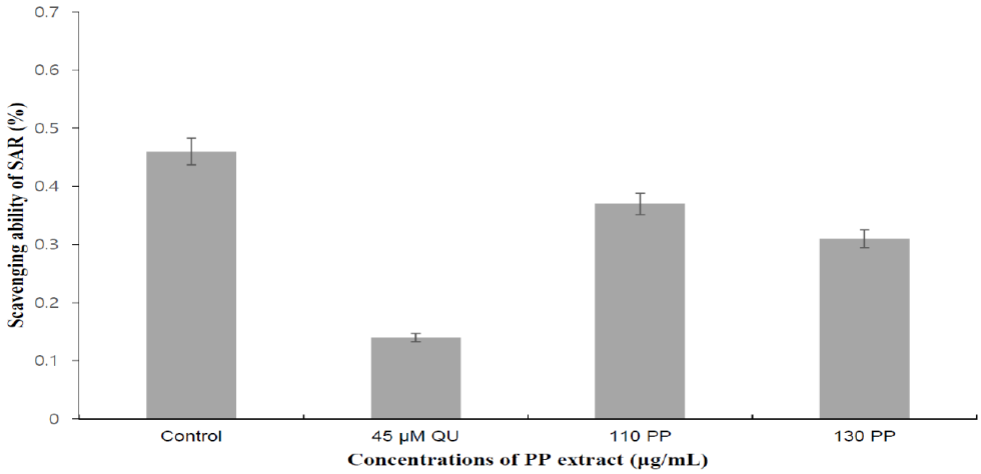

Figure 4. The superoxide anion radical (SAR)-scavenging ability of *Parnassia palustria* L. (PP) extract determined at a wavelength of 560 nm. The data indicate the mean ± SD for triplicate experiments.

and polluted air and water [20]. Among heavy metals, ferrous sulfate ($FeSO_4$) is known to be a trigger of Parkinson's disease, and much attention has been focused on its neurotoxicity. Therefore, in this study, we investigated the neurotoxicity of $FeSO_4$ in terms of oxidative damage by culturing C6 glioma cells. For this purpose, cultured cells were treated with $FeSO_4$ at concentrations ranging from 30 to 70 μM for 48 hours each, and showed cytotoxicity by significantly decreasing cell viability in a concentration-dependent manner. In addition, the XTT_{50} value was found at 63.3 μM, indicating that it was highly cytotoxic according to the toxicity criteria of Borenfreund and Puerner [21]. This result was consistent with the cytotoxicity of $FeSO_4$ on skin cells reported by Pyo *et al.* [15] and on fibroblasts reported by Jung [13]. The neurotoxicity of $FeSO_4$ on C6 glioma cells may be due to $FeSO_4$ interfering with intracellular deoxyribonucleic acid (DNA) synthesis, similar to cadmium compounds [22], or affecting the intracellular protein synthesis system [23], but it is more likely to be due to oxidative damage caused by $FeSO_4$. Therefore, in this study, to investigate whether oxidative damage was involved in the toxicity of $FeSO_4$, the cultured cells were treated with QU, an antioxidant, at concentrations of 35 μM and 45 μM before treatment with $FeSO_4$, and the cell viability was significantly increased to 47.4% and 59.6%, respectively, compared to 35.1% in the case of $FeSO_4$ alone. These results indicate that the antioxidant QU protected the cells against the toxicity of $FeSO_4$, which proves that the toxicity of $FeSO_4$ is related to oxidative damage. On the other hand, in the investigation of the protective effect of PP

extract against the toxicity of $FeSO_4$, when the cultured cells were pretreated with 110 μg/mL and 130 μg/mL concentrations of PP extract for 2 hours before treatment with $FeSO_4$, the cell survival rate was 51.5% and 60.6%, respectively, which was significantly increased compared to 39.4% in the treatment with $FeSO_4$ alone. These results showed that the PP extract protected against the toxicity of $FeSO_4$, which was consistent with the report that *Smilax china* L. (SC) extract, which contains flavonoids like PP extract, protected against the toxicity of $FeSO_4$. This phenomenon is thought to be due to the antioxidant activity of phenolic flavonoids such as kaempferol, quercetin, rutin, and tannin contained in PP and SC extracts, either alone or in combination. Therefore, in this study, we investigated the total flavonoid and polyphenol contents in PP extracts. The reason for investigating the total content is that the antioxidant activity of each of the above components is already well known, thus the analysis was performed in terms of total content rather than the potency of each component. The results showed that the total polyphenol and total flavonoid amounts were 42.8 mg/g and 32.9 mg/g, respectively. These results were somewhat lower than the 43.5 mg/g and 23.8 mg/g of cattail (*Typha orientalis* L., TO) extracts, which contain components of the flavonoid system such as isorhamnetin and quercetin, which show high antioxidant activity [24]. Therefore, in this study, the antioxidant activity of PP extracts was investigated through the analysis of SOD-like activity and SAR-scavenging activity. SOD-like activity is a measure of the ability of SOD enzyme, which is located in the intracellular antioxidant system and responsible for converting free radicals generated in the metabolic process into water together with catalase (CAT) to prevent any oxidative damage to the human body [19]. In this study, the SOD-like activity of 110 μg/mL and 130 μg/mL of PP extract samples was 13.6% and 18.4%, respectively, but both showed a significant increase in SOD-like activity compared to the control group. This result is somewhat lower than the 21.3% (120 ug/mL) of cattail (TO) extract reported by Yoon and Sohn [24], but almost comparable. On the other hand, in terms of SAR-scavenging capacity, the scavenging capacity of 110 μg/mL and 130 μg/mL of PP extract samples was 19.6% and 32.6%, respectively, indicating a significant increase in SAR-scavenging capacity compared to the control. This is almost comparable to the 35.8% (100 ug/mL) reported by Jung *et al.* [25] for *Lonicerae flos* extract. SAR-scavenging capacity refers to the ability to scavenge superoxide anion radicals, which is an assay that measures antioxidant capacity [26]. As shown above, the SOD-like and SAR-scavenging activities of PP extracts are thought to be the result of the interaction of antioxidant components contained in PP extracts. Therefore, it is thought that various analyses of natural products with high antioxidant capacity such as PP extracts can provide useful data for the development of alternative substances for the treatment of lesions by revealing the bioactivity of each component in more detail.

5. Conclusion

This study investigated the neurotoxicity of ferrous sulfate ($FeSO_4$), a trigger of Parkinson's disease, and the effect of *Parnassia palustria* L. (PP) extract on $FeSO_4$-induced cytotoxicity in cultured C6 glioma cells. For this purpose, cell viability and antioxidant effects such as SOD-like activity and SAR-scavenging activity were analyzed. In this study, $FeSO_4$ significantly decreased cell viability in a concentration-dependent manner, with an XTT_{50} value of 63.3 μM, indicating that it was highly toxic according to Borenfreund and Puerner's toxicity criteria. In addition, quercetin, a type of antioxidant, significantly increased the cell viability impaired by the toxicity of $FeSO_4$. On the other hand, PP extract significantly increased cell survival compared to $FeSO_4$ treatment alone, and at the same time exhibited antioxidant activities such as SOD-like and SAR-scavenging activities. From the above results, oxidative damage was involved in the cytotoxicity of $FeSO_4$, and PP extract effectively protected against

$FeSO_4$-induced cytotoxicity via its antioxidant effect. In conclusion, natural products such as *Parnassia palustria* extract are useful materials for treating, if not ameliorating, the toxicity caused by heavy metal compounds such as $FeSO_4$, that cause diseases related to oxidative damage.

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Disclosure statement

The authors declare no conflict of interest.

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