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Antioxidant Properties and Protective Effects of Aerial Parts from *Cnidium officinale* Makino on Oxidative Stress-Induced Neuronal Cell Death

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ABSTRACT: The rhizomes of *Cnidium officinale* Makino have been used as a traditional medicine for many purposes, however, use of its aerial parts is very limited. We investigated the antioxidant properties and protective effects of the aerial parts (leaves and stems) from *C. officinale* on H_2O_2-induced toxicity in SH-SY5Y neuroblastoma. *C. officinale* methanol extracts (70%) were sequentially fractionated using hexane (non-polar fraction, NF), ethyl acetate (intermediate polar fraction, IF), and water (polar fraction, PF). Total phenolics and flavonoids contents were highest in IF, followed by PF. IF also showed the strongest radical scavenging activities against 2,2-diphenyl-2-picrylhydrazyl and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), as well as superoxide, with the half maximal inhibitory concentrations of 13.2, 23.2, and 12.8 μg/mL, respectively. Furthermore, all fractions significantly inhibited linoleic acid peroxidation induced by the Fenton reaction or by UV irradiation. Both PF and IF protected against H_2O_2-induced SH-SY5Y neuronal cell death by increasing the cell survival by 22.1～47.7 and 35.9～50.3% at concentrations of 25～100 and 25～400 μg/mL, respectively, whereas NF was toxic to the cells at these concentrations. IF also significantly decreased intracellular levels of reactive oxygen species by 7.72～47.47% at a concentration of 25～200 μg/mL. Our results indicate that compounds from the aerial parts of *C. officinale* have potent antioxidant activities, which may help rescue neuronal cells from oxidative stress-induced injury. Therefore, the aerial parts, as well as the rhizomes, of *C. officinale* may have medicinal applications.

Keywords: aerial part, antioxidant, *Cnidium officinale* Makino, neuroprotective, oxidative stress

INTRODUCTION

Reactive oxygen species (ROS) are generated in all aerobes, mainly through the respiration process. Excessive ROS beyond the capacity of chemical and enzymatic defense systems in the body could cause damage to various cellular components and modify cellular structures and functions (Rajendran et al., 2014). In particular, oxidative stress induced by ROS and other oxidizing agents is a major factor that induces dysfunction or death of neuronal cells, which can cause initiation and progression of degenerative neuronal diseases such as Alzheimer's and Parkinson's diseases (Singh et al., 2019). Therefore, there has been growing interest in the therapeutic usage of dietary supplements from natural sources with antioxidant properties to reduce oxidative stress-induced damage associated with the neurodegenerative diseases (Lau et al., 2005).

Many phenolic compounds from plants have been reported to show antioxidant potential. In food industries, antioxidants can be utilized as food additives that are able to prevent various types of lipid oxidation (Robards et al., 1999). Artificial antioxidants, including butylated hydroxyanisole or butylated hydroxytoluene, have been reported to be toxic and to induce cancer and other diseases (Branen, 1975). Therefore, studies identifying natural and safe functional dietary antioxidants such as catechin, curcumin, resveratrol, and isoflavone are increasing in popularity.

Cnidium officinale Makino, is a perennial plant of the family Umbelliferae. Its rhizome has long been used in a traditional medicine for analgesic treatment of colds, headaches, rheumatic, and traumatic pain (Kwon and Ahn, 2003). Furthermore, the leaves of *C. officinale* may be consumed as a rice-wrapping or as seasoned greens. The leaves and stems of *C. officinale* may also be used as

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herb-bathing materials to help prevent feelings of coldness and improve blood circulation (Kim et al., 2010).

Several physiological effects of *C. officinale* have been previously investigated, including its function as a muscle relaxant (Ozaki et al., 1989), and its anti-inflammatory (Ningsih et al., 2020), anti-angiogenic (Kwak et al., 2002), and anti-diabetic (Jeong et al., 2005) activities. The preventive effects of *C. officinale* on several types of cancers have also been investigated (Lee et al., 2013; Hong et al., 2017; Cha et al., 2018).

Compounds, including ligustilide, cnidilide, sekyunolide (Ozaki et al., 1989), falcarindiol (Kim et al., 2003), cnidilide, (Z)-ligustilide, (3S)-butylphthalide, and neocnidilide (Tsukamoto et al., 2005), have been isolated from the rhizome of *C. officinale*. The rhizome has also been reported to contain 1∼2% oil compounds, including cnidilide, ligustilide, neocnidilide, butylidenephthalide, butylphthalide, senkyunolide A∼M, chlorogenic acid, ligustilidiol, pregnenolone, and coniferyl ferulate (Yamagishi and Kaneshima, 1977; Kobayashi et al., 1984; Kano et al., 1985). Yi et al. (2007) estimated that the amount of senkyunolide A in *C. officinale* is important to determine its quality. However, since most studies have focused on the rhizome of *C. officinale*, research focused on physiological actions of the aerial parts of the plant is limited, with only one study investigating its antioxidant activity (Oh et al., 2010).

In this study, we prepared sequential solvent fractions from 70% methanolic extracts of *C. officinale* aerial parts, including nonpolar hexane, intermediate polar ethyl acetate (EA), and polar water fraction (NP, IF, and PF, respectively), and analyzed their radical scavenging activities and inhibitory effects against two types of lipid peroxidation. In addition, we investigated the protective effects of the *C. officinale* fractions against ROS-induced SH-SY5Y neuronal cell damage.

MATERIALS AND METHODS

Materials and reagents

Aerial parts of *C. officinale* was obtained from Bonghwa Alpine Medicinal Plant Experiment Station of Gyeongbuk Agricultural Technology Administration (Bongwha, Korea). Human neuroblastoma SH-SY5Y cells were obtained from ATCC (Manassas, VA, USA). Methanol, *n*-hexane, and EA for sample preparation were purchased from Duksan Co. (Ansan, Korea). For cell culture experiments, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FSB), trypsin, and penicillin/streptomycin were purchased from Hyclone Laboratories, Inc. (Logan, UT, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide (MTT) was purchased from Amresco Inc. (Solon, OH, USA). All other chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA).

Sample fractionation

The harvested *C. officinale* was washed and freeze-dried (Labconco Corporation, Kansas City, MO, USA). Dried aerial parts (80 g) were then soaked twice in 70% methanol (2 L and 1.6 L, respectively) at 60°C in a water bath for 2 and 4 h, respectively. Samples was stirred every 10 min to ensure a well-mixed extraction. After the extract was filtered using Whatman No. 2 paper (Whatman Inc., Clifton, NJ, USA), the methanolic phase was evaporated (N-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan); serial solvent fractionation was performed on the remaining aqueous phase. The aqueous phase was then extracted three times by equal volumes of *n*-hexane, and the residual aqueous phase was extracted three times with the equal volume of EA. The *n*-hexane and EA extracts were dried completely using a rotary evaporator, and NF and IF were prepared, respectively. The residual water fraction (PF) was prepared using a freeze-dryer. The dried fractions were dissolved in dimethyl sulfoxide (DMSO) and stored at −80°C. The fractionation scheme is shown in Fig. 1.

Analysis of polyphenol and flavonoid contents

Total polyphenol content was determined using Folin-Ciocalteu's phenol reagent, as previously published (Hong et al., 2011), with the amount of total polyphenol determined in tannic acid equivalents. Total flavonoid content was analyzed using the method described by Jia et al.

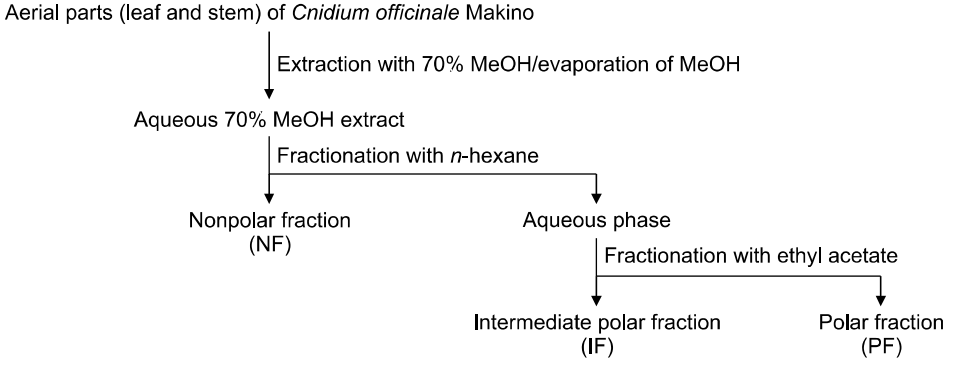

Fig. 1. The extraction and fractionation scheme of the aerial parts of *Cnidium officinale* using a series of different solvents.

(1999), with slight modification. Briefly, each fraction was dissolved in water (50 μL), reacted with 30 μL of 5% $NaNO_2$ for 5 min and mixed with 60 μL of 2% $AlCl_3$. After 6 min, 100 μL of 4% NaOH was added to the mixture and the mixture was left to stand for 11 min. Color development was measured at 510 nm using a microplate reader (Spectra Max 250, Molecular Devices, Sunnywale, CA, USA). The amount of total flavonoid was determined in (+)-catehchin equivalents.

Radical and nitrite scavenging activity assays

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the fractions was determined by the method described by Blois (1958). Fraction solutions (100 μL) were added to 100 μL of 600 μM DPPH dissolved in 99% methanol. After incubation for 30 min in a dark, absorbance was measured at 517 nm.

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was measured following the method described by Re et al. (1999). First, ABTS radical stock solution containing 7.4 mM ABTS and 2.45 mM potassium persulfate was incubated at 37°C overnight in a dark place to form $ABTS^+$ solutions, and diluted in phosphate buffered saline (PBS) to adjust to control absorbance of 0.90 (±0.05) at 734 nm. Then, 50 μL of each fraction was added to 150 μL of the ABTS radical solution and, after 30 min incubation, absorbance was measured at 517 nm.

Superoxide radical scavenging activity was also determined using the pyrogallol-luminol system (Li, 2012). Briefly, 50 μL of each fraction was mixed with 100 μL of 50 μM luminol diluted with 10 mM carbonate buffer. The mixture was then added to 50 μL of 200 μM pyrogallol, and chemiluminescence intensity recorded using a multimode reader (Triad LT, Dynex Technologies Inc., Chantilly, VA, USA).

Nitrite scavenging activities were measured by following the method of Kosem et al. (2007), with a slight modification. Briefly, 100 μL of 100 μM $NaNO_2$ solution in 0.1 N HCl was mixed with 50 μL of each fraction, and the mixture was incubated for 1 h in the dark. The mixture was then added to 50 μL of 5% H_3PO_4 in 1% sulfanilamide for 10 min and subsequently to 50 μL of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (NED) solution. After 10 min incubation, absorbance was measured at 540 nm.

Measurement of lipid peroxidation

The effect of each fraction on Fenton reaction-induced lipid peroxidation was evaluated according to the method of Rao et al. (2006), which measures formation of thiobarbituric acid (TBA)-reactive substances (TBARS). Reaction mixtures (1 mL) consisted of 2% linoleic acid, 0.4 % sodium dodecyl sulfate, 100 μM ascorbic acid, and 10 μM $Fe_2(SO_4)_3$ in 20 mM sodium phosphate buffer (pH 7.4) with or without sample fractions. To analyze the effects on the ultraviolet (UV)-induced lipid peroxidation, reaction mixtures were prepared without ascorbic acid and $Fe_2(SO_4)_3$. The reaction was initiated by adding $Fe_2(SO_4)_3$ or irradiating UV-C (254 nm) at a distance of 8 cm. After incubation or irradiation for 1 h, reaction mixtures were added to 250 μL of 0.5 M trichloroacetic acid, followed by 250 μL of 0.8% TBA and 20 μL of 5% butylated hydroxytoluene (w/v). Mixtures were then heated at 100°C for 5 min, and the colors developed from TBARS complexes were analyzed at 532 nm.

Ferrous ion chelating assays

The chelating effects of each fraction on ferrous ions were estimated follwoing the method of Riemer et al. (2004). Briefly, 50 μL of each fraction was incubated with 100 μL of 100 μM $FeCl_2$ for 10 min. Then, 50 μL of 1 mM ferrozine was added, and fractions were incubated for a further 10 min. The absorbance was measured at 550 nm with a microplate reader.

Analysis of cytotoxicity and cellular ROS levels

Human neuroblastoma SH-SY5Y cells were grown at 37°C with 5% CO_2 in DMEM medium supplemented with 10% heat-inactivated FSB, and 50 units/mL of penicillin/streptomycin. MTT assays were used to evaluate cytotoxicity and preventive effects on H_2O_2-induced SH-SY5Y cell death. Cells were grown in 96 well plates until 60~70% confluency, then replaced with serum free growth medium containing each fraction or vehicle. After 2 h incubation, the medium containing the fractions were removed and cells were exposed to 300 μM H_2O_2 in serum free medium for 2 h. Then, the medium was replaced with fresh complete medium and cells were allowed to grow for a further 18 h. Cells were incubated with 0.5 mg/mL MTT in serum free media for 2 h. MTT formazan formed was solubilized with 100 μL of DMSO, and absorbance was detected at 550 nm.

To analyze levels of intracellular ROS, confluent SH-SY5Y cells were incubated with each fraction in PBS for 30 min, then collected by centrifugation at 1,000 *g* for 5 min. Cells were resuspended in medium, treated with 10 μM 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) for 15 min, and exposed to 300 μM H_2O_2 for 30 min. The fluorescence intensity was detected by a multimode reader at 485 nm excitation and 535 nm emission (Triad LT, Dynex Technologies Inc.).

Statistical analysis

Statistical analysis was performed using two-tailed Student's *t*-tests and one-way analysis of variance (ANOVA) for determining statistical significance. Tukey's honestly significant difference (HSD) tests were used to compare

multiple results. Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Extraction yields, and total polyphenol and flavonoid contents

In this study, 70% methanol/30% water (v/v) capable of extracting both hydrophilic and hydrophobic compounds was used for initial solvents. Samples were fractionated with different solvents according to their polarity (*n*-hexane, NF; EA, IF; water, PF). The total yield of 70% methanolic extracts from the dried aerial parts of *C. officinale* was 27.7%. Final yields of PF, IF, and NF after solvent fractionation were 21.88%, 1.80%, and 4.03%, respectively. The yield of PF obtained was much higher than those of the other fractions (Table 1).

The total polyphenol and flavonoid contents of each fraction were both in order of IF> PF> NF, calculated based on tannic acid and catechin equivalents (Table 1). Polyphenols possess more than one aromatic ring bearing one or more hydroxyl groups. Flavonoids, characterized by a benzo-γ-pyrone structure, are the most abundant polyphenols and are widely distributed in plants. Flavonoids are mainly responsible for the antioxidant activities of most plant-derived dietary materials (Pietta, 2000). IF and PF fractions were both rich in polyphenols (containing 37.2 and 21.7%, respectively), and were expected to show strong antioxidant activity. IF fractions also contained significant amounts of flavonoids, therefore, were expected to possess various other physiological functions.

Antioxidant activities

Antioxidant activities of the aerial parts of *C. officinale* were evaluated based on scavenging activities of DPPH, ABTS, superoxide anion radicals, and nitrite. DPPH colorimetry assays are based on detecting reductions in DPPH radicals of a dark-purple color. DPPH is reduced by antioxidants and radical scavengers to yellow, which shows decreased absorbance at 517 nm (Blois, 1958). Of the fractions investigated, IF showed the highest scavenging activities against DPPH radicals, followed by PF then NFs (Fig. 2A).

Table 1. Yields after solvent fractionation of the aerial part of *Cnidium officinale*, and total polyphenols, and total flavonoids contents in solvent fractions of different polarities

Fractions	Extraction yield (%)	Total polyphenols[1)]	Total flavonoids[2)]
Hexane (NF)	4.03	31.06±1.60	22.07±4.04
EA (IF)	1.80	372.77±1.88	283.48±14.07
Water (PF)	21.88	216.67±2.61	90.89±16.59

Data indicate mean±SD (n=3).
[1)]μg of total polyphenol content/mg of extracts based on tannic acid equivalent.
[2)]μg of total flavonoids content/mg of extracts based on (+)-catechin equivalent.
NF, non-polar fraction; EA, ethyl acetate; IF, intermediate polar fraction; PF, polar fraction.

ABTS radicals are produced by the reaction between ABTS and potassium persulfate. Blue-green ABTS radicals are reduced in the presence of antioxidants, which can be detected at 734 nm. ABTS radical scavenging assays are considered to be more versatile than DPPH radical scavenging assays since DPPH assays show limited ability to evaluate activities of hydrophilic compounds. Furthermore, scavenging activities of both the polar and non-polar samples can be evaluated in ABTS assays (Re et al., 1999). The order of ABTS radical scavenging activities of each fraction was IF> PF> NF, which is consistent with DPPH radical scavenging activity (Fig. 2B).

Superoxide anion radicals can be generated during oxidative respiration, and are converted to H_2O_2 and hydroxyl radicals thorough reactions, including dismutation. Superoxide and its derivatives can cause damage to DNA and cell membranes, and can act as major causes of aging, cancer, and neurodegenerative diseases (Singh et al., 2019). The protection from superoxide ROS is regarded as an important strategy for biological defense in the body. Superoxide radical scavenging activity of the fractions from *C. officinale* was evaluated in the pyrogallol self-oxidation system. IF exhibited the most potent scavenging activities against superoxide, followed by PF and NF (Fig. 2C).

Sodium nitrite ($NaNO_2$) is used as a food additive for developing color in meat and controlling microbial growth. However, sodium nitrite may cause acute toxicity and produce nitrosamine to induce cancers (Crowe et al., 2019). Nitrite (NO_2^-) can also be reduced to nitric oxide (NO), which can be further reduced or nitrous oxide (N_2O) and nitrogen gas. NO is an important signaling molecule for inducing blood vessel relaxation and lowering blood pressure (Pacher et al., 2007). Either scavenging or reducing nitrite can be considered as possessing a useful function in the body. In this experimental system, nitrite reacted with sulfanilamide and NED produced an Azo compound, which can be monitored at 520~550 nm. All fractions of the aerial parts of *C. officinale* reduced the residual levels of nitrite; the scavenging effects on nitrite was most potent with IF [half maximal inhibitory concentration (IC_{50}), 24.1 μg/mL] (Fig. 2D and 2E). Kim et al. (2003) reported that fractions of *C. officinale* rhizomes decrease inflammatory NO production in LPS-stimulated cells through inhibiting expression of inducible nitric oxide synthase. However, the present result indicated that fractions from *C. officinale* aerial parts can scavenge nitrite and may produce NO from nitrite during the chemical reduction.

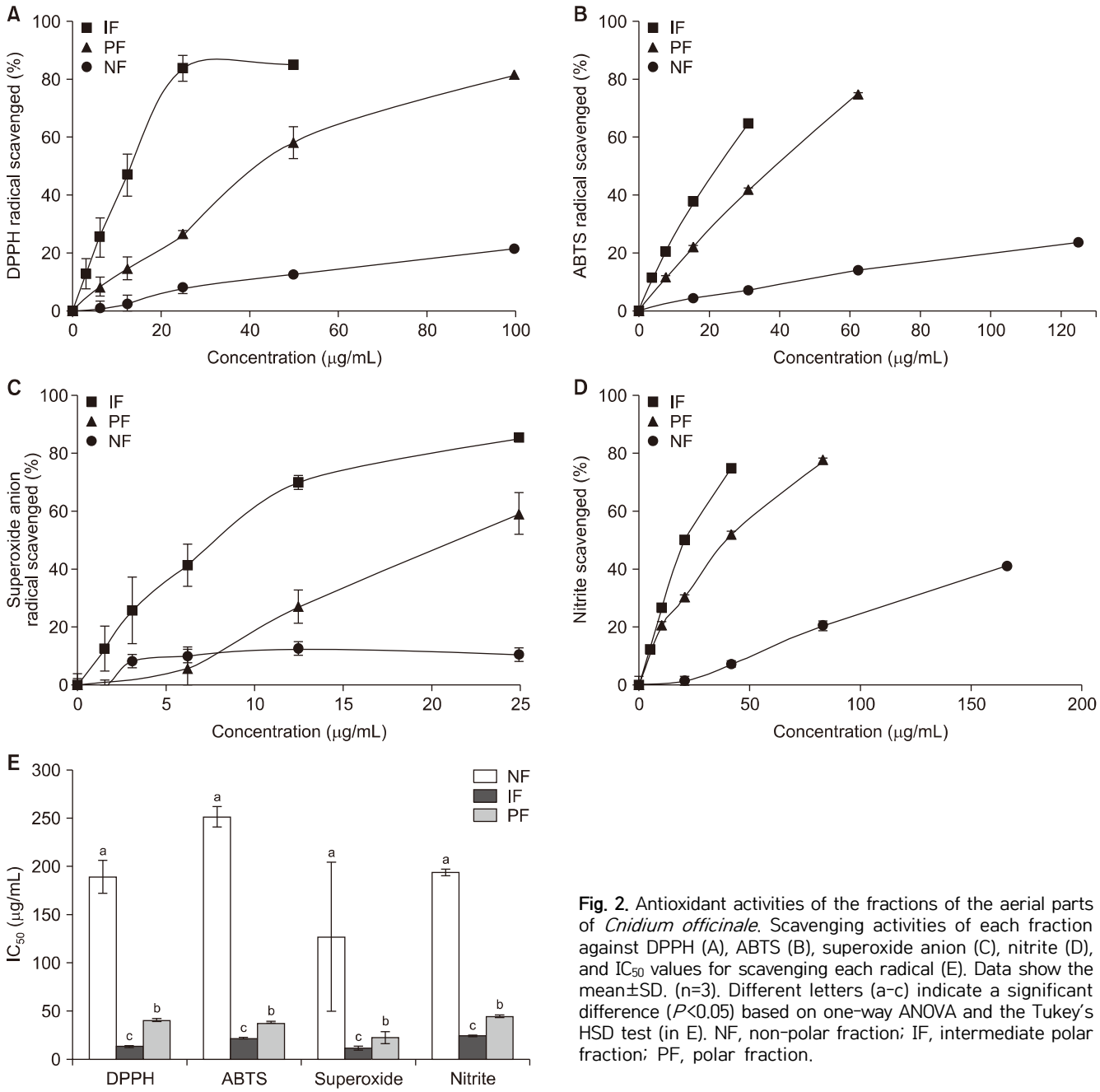

Fig. 2. Antioxidant activities of the fractions of the aerial parts of *Cnidium officinale*. Scavenging activities of each fraction against DPPH (A), ABTS (B), superoxide anion (C), nitrite (D), and IC_{50} values for scavenging each radical (E). Data show the mean±SD. (n=3). Different letters (a-c) indicate a significant difference ($P<0.05$) based on one-way ANOVA and the Tukey's HSD test (in E). NF, non-polar fraction; IF, intermediate polar fraction; PF, polar fraction.

The scavenging effects of each fraction against different radicals and nitrite were compared by calculation their IC_{50} values (Fig. 2E). IF showed the strongest scavenging activities against all radicals examined, followed by PF. These results are positively correlated with the polyphenol and flavonoid contents of the fractions (Table 1), which are believed to play a critical role in scavenging various radicals.

Inhibitory effect on lipid peroxidation

Lipid peroxidation is a major deterioration factor in lipid-containing foods (Sharififar et al., 2009), and can cause oxidative damage in the body. During the propagation of lipid peroxidation, malondialdehyde (MDA), a product of lipid peroxidation, is formed. MDA is a highly reactive compound that can react with various biomolecules, such as proteins and DNA, and can react with TBA to produce TBARS, a red colored product (Ghani et al., 2017). In the present study, the effects of the fractions from *C. officinale* arial parts on lipid peroxidation were evaluated in two different oxidation systems induced by the Fenton reaction and UV-C irradiation.

The amount of TBARS formed from linoleic acid oxidation induced by the Fenton reaction (Fe-ascorbate) decreased by 85% in the presence of IF (200 μg/mL). IF was significantly more effective than NF and PF for inhibiting Fenton reaction-induced lipid peroxidation (Fig. 3A). In addition, IF showed the highest ability to inhibit linoleic acid oxidation induced by UV radiation. At 400 μg/mL, IF inhibited TBARS formation by 94.8%. In both

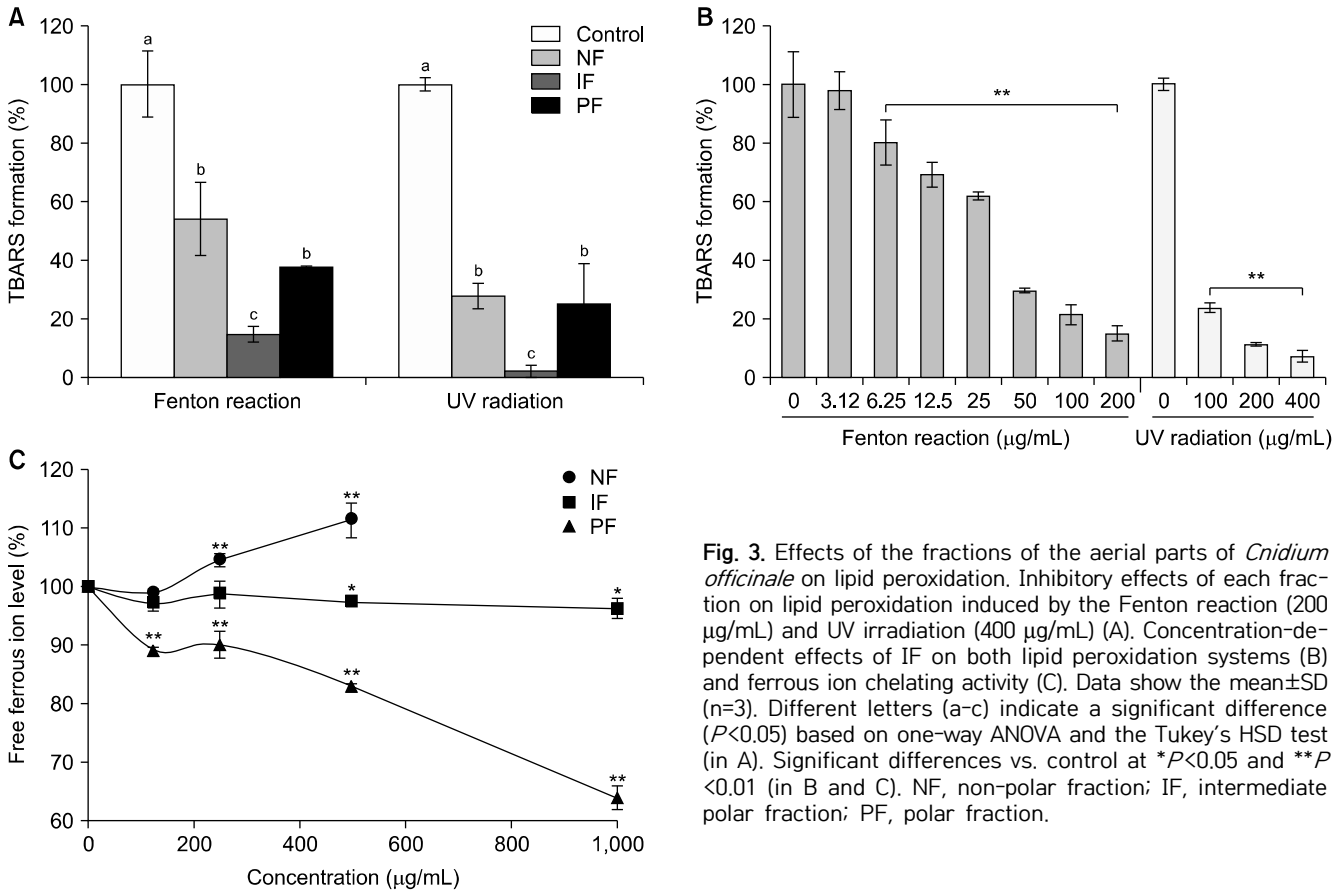

Fig. 3. Effects of the fractions of the aerial parts of *Cnidium officinale* on lipid peroxidation. Inhibitory effects of each fraction on lipid peroxidation induced by the Fenton reaction (200 μg/mL) and UV irradiation (400 μg/mL) (A). Concentration-dependent effects of IF on both lipid peroxidation systems (B) and ferrous ion chelating activity (C). Data show the mean±SD (n=3). Different letters (a-c) indicate a significant difference ($P<0.05$) based on one-way ANOVA and the Tukey's HSD test (in A). Significant differences vs. control at $*P<0.05$ and $**P<0.01$ (in B and C). NF, non-polar fraction; IF, intermediate polar fraction; PF, polar fraction.

of oxidation processes, IF inhibited lipid peroxidation in a concentration-dependent manner, with a concentration of 6.25 μg/mL able to inhibit Fenton reaction-induced lipid peroxidation (Fig. 3B). PF showed comparable inhibitory ability to NF on lipid peroxidation, but much stronger radical scavenging activities than NF. This may be because polar antioxidant compounds in PF did not function effectively in the current emulsion system for evaluating lipid peroxidation (Laguerre et al., 2015). Hydroxyl radicals, one of the most reactive ROS, plays a key role in Fenton reaction-induced oxidation (Carocci et al., 2018). Formation of hydroxyl radical through the Fenton reaction commonly occurs in body systems and can be a reason for neurodegenerative diseases (Carocci et al., 2018; Singh et al., 2019). In this study, IF strongly inhibited Fenton-like oxidation, showing it could help protect against oxidative neuronal damage and neurodegenerative diseases. In a previous experiment using lipid homogenates from mouse brains, IF showed much higher inhibitory activity against Fenton reaction-induced brain lipid peroxidation (IC_{50}, 5.54 μg/mL) (data not shown). The results suggest that synergistic interactions of IF with various biomolecules might induce much stronger antioxidant activities and cause potent effects for inhibiting lipid peroxidation in the body.

Ferrous ion (Fe^{2+}) plays an important role in generating hydroxyl radicals in the Fenton reaction (Carocci et

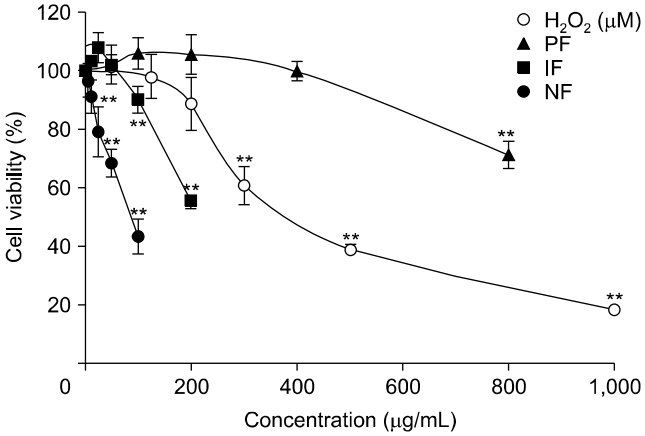

Fig. 4. Cytotoxic activity of H_2O_2 and the fractions of the aerial parts of *Cnidium officinale*. SH-SY5Y cells were treated with different concentrations of each fraction or H_2O_2. Cell viability was analyzed using MTT assays. Data show the mean±SD (n=8). Significant differences vs. control at $**P<0.01$. NF, non-polar fraction; IF, intermediate polar fraction; PF, polar fraction.

al., 2018). Accordingly, chelating Fe^{2+} is an important approach for protecting against Fenton reaction-induced oxidation. In the present study, the chelating effects of the fractions on Fe^{2+} ions were evaluated using ferrozine reagent. Ferrozine forms a red colored complex with Fe^{2+}, which can be detected by a colorimetric method, the intensity of which allows estimation of the ability of Fe^{2+} chelation (Riemer et al., 2004). PF significantly reduced

the levels of free Fe^{2+} in the range of 125~1,000 μg/mL ($P<0.05$), whereas other fractions did not demonstrate iron chelating activity (Fig. 3C). These results suggest that Fe^{2+} chelation is not involved in the inhibitory effect of IF and NF on lipid peroxidation, but plays a role in the effect of PF.

Cytotoxicity and effect on H_2O_2-induced cell damage

SH-SY5Y neuroblastoma cells have neuron-like characteristics and are widely used for studying events in neuronal cells (Biedler et al., 1973). Therefore, we next evaluated the cytotoxic effects of *C. officinale* aerial fractions on SH-SY5Y neuroblastoma cells. Cells were treated with each fraction or H_2O_2 for 2 h and allowed to regrow with complete medium for 18 h. Cell viability decreased according to treatment concentration of each fraction or H_2O_2. NF showed the strongest cytotoxic effect, followed by IF (IC_{50}, 85.4 and 241.8 μg/mL respectively). Exposure to H_2O_2 for 2 h also decreased cell viability (IC_{50}, 420 μM) (Fig. 4).

The protective effects of *C. officinale* on H_2O_2-induced oxidative damage in SH-SY5Y cell were next analyzed. H_2O_2 treatment decreased SH-SY5Y neuronal cell viability concentration dependent manner (Fig. 4). At a concentration of 300 μM, H_2O_2 induced 40~50% cell death, and this concentration was selected for further studies. Treatment with 300 μM H_2O_2 decreased SH-SY5Y cell viability to 60.7%. Pretreatment with PF or IF for 2 h before H_2O_2 exposure, showed a protective effect against neuronal cell death. PF and IF significant protected against H_2O_2-induced SH-SY5Y cell damage, increasing cell survival by 22.1~47.7% at a concentration of 12.5~100 μg/mL and by 35.9~50.3% at a concentration of 25~400 μg/mL (Fig. 5B and C). However, pretreatment with NF rather enhanced cellular cytotoxicity (Fig. 5A). These results indicate that IF and PF fractions from the aerial parts of *C. officinale* could protect neuronal cells from H_2O_2-induced oxidative damage. It is believed that the high antioxidant activities of the fractions induced these effects due to their high polyphenol content.

DCFH-DA fluorescent dyes were used to determine levels of intracellular ROS. DCFH-DA can cross cell membranes where they may be converted into DCFH by hydrolysis of acetyl groups by intracellular esterases. Liberated DCFH can react with various ROS and be oxidized to DCF, which emits high fluorescence (Marchesi et al., 1999). Treatment of SH-SY5Y cells with 300 μM H_2O_2 induced ~3 fold-increase in DCF fluorescence. Pretreatment with NF did not markedly affect fluorescence, whereas IF decreased ROS in cells in a concentration dependent manner (by 7.72~47.47% at concentrations of 25~200 μg/mL) (Fig. 6A and B). In addition, PF signifi-

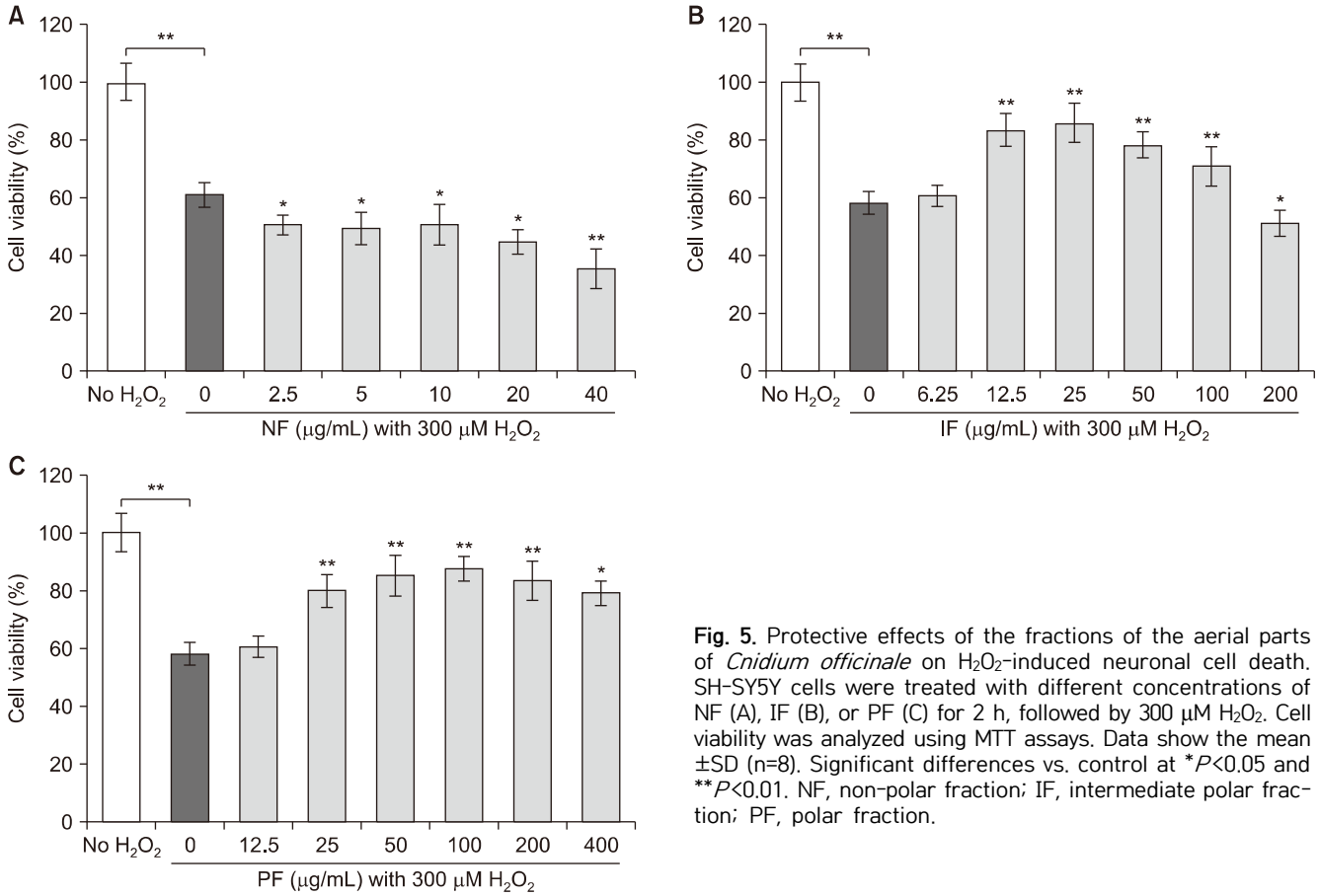

Fig. 5. Protective effects of the fractions of the aerial parts of *Cnidium officinale* on H_2O_2-induced neuronal cell death. SH-SY5Y cells were treated with different concentrations of NF (A), IF (B), or PF (C) for 2 h, followed by 300 μM H_2O_2. Cell viability was analyzed using MTT assays. Data show the mean ±SD (n=8). Significant differences vs. control at $^*P<0.05$ and $^{**}P<0.01$. NF, non-polar fraction; IF, intermediate polar fraction; PF, polar fraction.

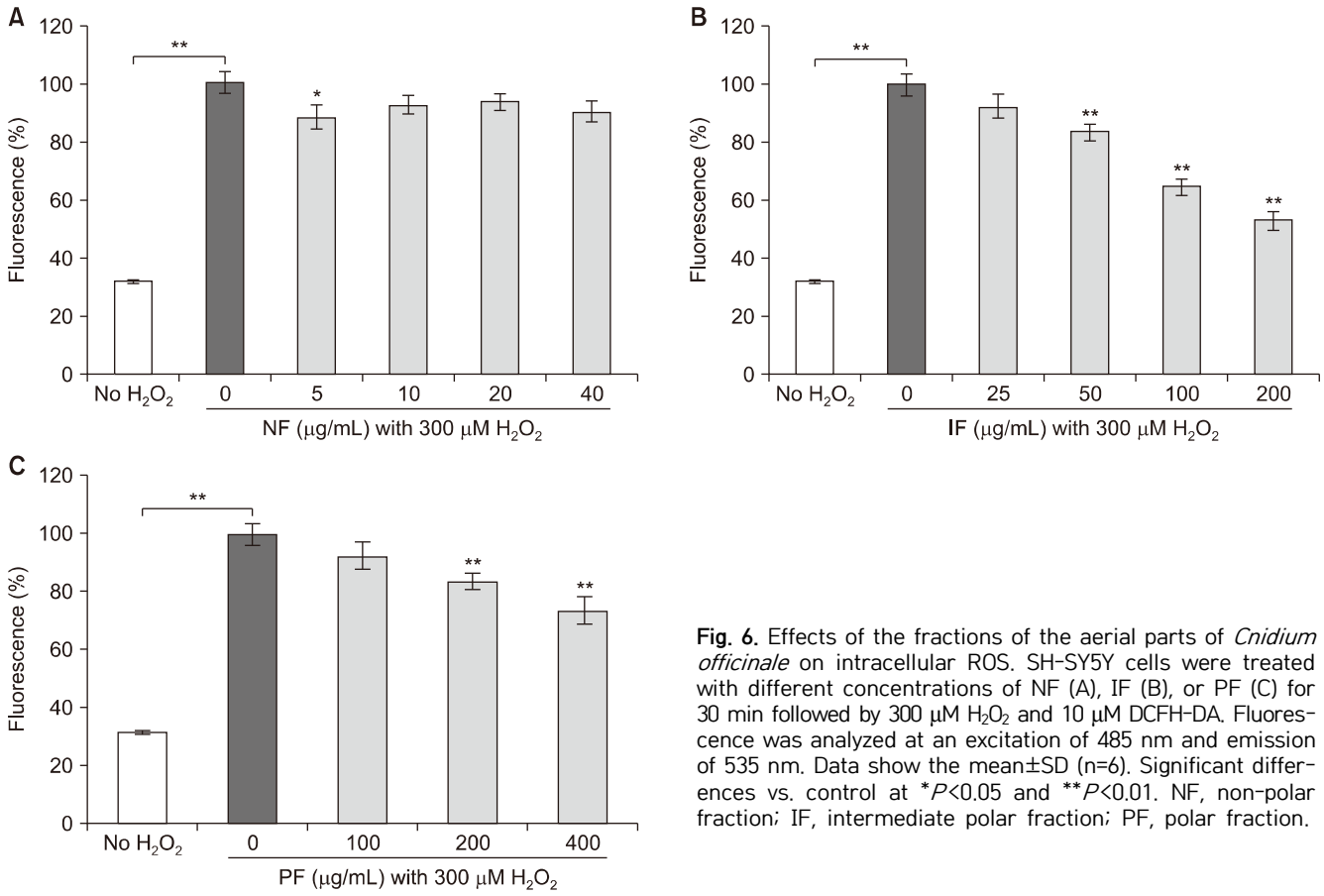

Fig. 6. Effects of the fractions of the aerial parts of *Cnidium officinale* on intracellular ROS. SH-SY5Y cells were treated with different concentrations of NF (A), IF (B), or PF (C) for 30 min followed by 300 μM H_2O_2 and 10 μM DCFH-DA. Fluorescence was analyzed at an excitation of 485 nm and emission of 535 nm. Data show the mean±SD (n=6). Significant differences vs. control at $^{*}P<0.05$ and $^{**}P<0.01$. NF, non-polar fraction; IF, intermediate polar fraction; PF, polar fraction.

cantly reduced intracellular ROS by 26.36% at 800 μg/mL (Fig. 6C). These results demonstrate the fractions of *C. officinale* may exhibit a preventive effect against H_2O_2-induced cell damage and decrease intracellular ROS, an effect which is also attributable to their antioxidant properties.

To conclude, the results of this present indicate possible uses for aerial parts of *C. officinale*, of which only the root part has been previously used. Furthermore, they suggest that consumption of the aerial parts may have various beneficial physiological effects, including prevention of oxidative neuronal damage due to their antioxidant activity.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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